資料２－２

Ａ　研究報告（詳細版）

平成２５年６月１２日
（平成２５年２月～平成２５年４月受理分）

別紙様式第２

医薬品　研究報告　調査報告書

識別番号・報告回数		報告日	第一報入手日 2013/01/16	新医薬品等の区分 該当なし	厚生労働省処理欄
一般的名称	フィブリノゲン配合剤	研究報告の公表状況	Hepatology2012:56(SUPPL.1)1100A	公表国 フランス	
販売名（企業名）	①タココンブ ②タココンブ組織接着用シート （CSLベーリング株式会社）				

研究報告の概要

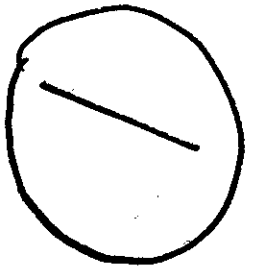
47歳肝移植患者は急性肝炎を発症し急性肝炎Eウイルス(HEV)感染と診断された。部分配列決定では中国のウサギから分離された株と高い相同性が示された。

患者の血清を子ブタおよびウサギに静脈内投与し、感染実験を実施した。陰性対照として、それぞれの第三種の動物を用いた。ブタはDay32まで、ウサギはDay39まで、血清学およびリアルタイムHEV RNA検査で感染をモニターした。

全配列は、ウサギ株と平均81%の相同性、人と動物のジェノタイプ3株と平均77%の相同性を示した。子ブタは感染しないままであった。接種ウサギは感染後Day7～14の間、抗HEV抗体の一過性の内分泌を示したが、HEV RNAは検出できないままであった。配列相同性およびウサギの抗体検出の変動は、ウサギ由来であるか議論がある。これらの動物はヒトのジェノタイプ3および4の株に容易に感染するので、ブタが非感染であることは、非定型的な特徴である。

患者は海外渡航しておらず、レストランでシェフとして働き、ウサギを含む複数の死亡動物と接触した。フランスのウサギのHEV感染のデータはない。しかし、中国はフランスの食用新鮮ウサギおよび冷凍ウサギの第一のサプライヤーであることは注目に値する。類似株がヒトで報告されていないので、ウサギからヒトへの感染が稀なのは確かである。免疫抑制によりウイルス感染が起こった可能性がある。

使用上の注意記載状況・その他参考事項等



報告企業の意見	今後の対応
本剤の原薬の製造工程中で用いられるトロンボプラスチンの原料としてウサギが用いられている。 原薬製造工程および製剤製造工程にウイルス除去/不活化工程（ナノフィルトレーション、γ線滅菌等）を含むことから、HEV に対する本剤の安全性への影響は取られていると考える。	今後とも新しい感染症に関する情報収集に努める所存である。

temic and neuroinflammation) was observed in saline-injected BDL, compared to sham pigs. In BDL pigs, brain water was increased with altered cerebral haemodynamics. in BDL pigs PDGFR-b expression was significantly increased and highly localised to surrounding small microvessels at the level of capillary beds, with no phosphorylated PDGFR-b detected in any sham pig. Conclusion: The results suggest that in our porcine model of cirrhosis, persistent pericyte activation within the capillary bed of the brain, may influence cerebral haemodynamics and play a pivotal role in the cirrhotic brain phenotype. Whether this is a compensatory mechanism to limit severe brain oedema, or causal, along with any interaction with inflammation and hyperammonaemia is the focus of ongoing study.

Disclosures:

Rajiv Jalan - Consulting: Ocera Therapeutics, Conatus

The following people have nothing to disclose: Gavin Wright, D. Blatti, P. Steigler, Vanessa Stadlbauer

1951

Myeloid cells require IL-6/gp130 signaling for protective anti-inflammatory functions during bacterial peritonitis

Sara D. Sackett, Leif E. Sander, Antje Mohs, Sonja Strauch, Daniela C. Kroy, Konrad L. Streetz, Christian Trautwein; RWTH University Hospital Aachen, Aachen, Germany

Background: Sepsis and sepsis related deaths represent a major complication of patients suffering from liver cirrhosis. In particularly, spontaneous bacterial peritonitis (SBP) is a severe bacterial complication of liver cirrhosis and the mechanisms involved in SBP are not completely understood. We recently reported that gp130, the signaling receptor for IL-6 family cytokines, is important for crosstalk between hepatocytes and myeloid derived suppressor cells to control inflammation during sepsis. *Objective*: We hypothesized that gp130 signaling plays a role in the differentiation of innate immune cells during bacterial peritonitis (BP) and is therefore involved in controlling the host inflammatory response during infections of the peritoneum. *Methods*: to elucidate the role of gp130/IL6 signaling in hematopoietic cells we generated bone marrow specific gp130 knockout mice and their respective controls by bone marrow transplantation. Following re-engraftment of the hematopoietic system, caecal ligation and puncture, a murine model of BP, was performed to evaluate survival, organ damage, cytokine production and regulation as well as immune cell response. *Results*: Deficiency of gp130 in hematopoietic cells caused increased liver apoptosis and kidney damage and rendered mice more susceptible to sepsis-induced mortality due to unrestrained inflammation. Gp130 deficient myeloid cells failed to induce the expression of arginase-1 and IL10, important immunosuppressive components, and instead express high levels TNF-α and IL-12 during peritonitis. Results from bone marrow derived macrophage (BMDM) experiments and gene expression analysis of FACS sorted exudate cells further demonstrate the defect in activation of anti-inflammatory programming. Furthermore, we show that the IL4 receptor, a downstream target of IL10, is reduced in BMDM. Additional in vivo and in vitro experiments show that this gene expression defect can be rescued by the exogenous addition of IL10 and significantly improves survival. *Conclusions*: Here we demonstrate a unique function of gp130 in promoting an anti-inflammatory phenotype and as a critical element for immune homeostasis in myeloid derived cells during BP. These results demonstrate that gp130 signals are required for efficient M2 skewing *in vitro* and *in vivo*. These data highlight the importance of gp130 regulation in the innate immune response during bacterial peritonitis and in macrophage activation and may provide a novel therapeutic approach for treatment of SBP.

Disclosures:

Christian Trautwein - Grant/Research Support: BMS, Novartis, BMS, Novartis; Speaking and Teaching: Roche, BMS, Roche, BMS

The following people have nothing to disclose: Sara D. Sackett, Leif E. Sander, Antje Mohs, Sonja Strauch, Daniela C. Kroy, Konrad L. Streetz

1952

First description of human infection due to a rabbit hepatitis E virus strain

Deborah Delaune[1], Nicole Pavio[2], Eric Marchadier[1], Olivier Chazouillères[3], Anne-Marie Roque-Afonso[1,4]; [1]AP-HP, Hopital Paul Brousse, National Reference Centre for HAV and HEV, Villejuif, France; [2]ANSES, Maison-Alfort, France; [3]AP-HP, Hopital Saint Antoine, Hepatology, Paris, France; [4]Inserm, Unité 785, Villejuif, France

A 47-years-old liver-transplant patient developed an acute hepatitis in June 2011. Acute hepatitis E virus (HEV) infection was diagnosed on the basis of positive anti-HEV IgM and detectable HEV RNA. Partial sequencing revealed a high homology with strains isolated from Chinese rabbits. To date, no human infections due to rabbit strains have been described. Full-length sequence was obtained and experimental infection of 2 piglets and 2 rabbits was performed by intravenous administration of patient's sera. A third animal of each species was used as a negative control. Infection was monitored by serology and real-time HEV RNA testing up to day 32 for pigs and day 39 for rabbits. The full length sequence presented a mean of 81 % homology with rabbit strains and a mean of 77 % homology with human and animal genotype 3 strains. Piglets remained uninfected as assessed by undetectable HEV RNA and negative serology. Inoculated rabbits presented a transitory secretion of anti-HEV antibodies between day 7 and 14 post infection, but HEV RNA remained undetectable in all samples. Sequence homology and transitory detection of antibodies in rabbits are arguments for its rabbit's origin. Absence of pig infection further confirms atypical characteristics since these animals are readily infected by human genotypes 3 and 4 strains. The patient did not travel abroad. He works as a chef in a restaurant and is in contact with several dead animals, including rabbits. No data are available on HEV infection of French rabbits. However, it is noteworthy that China is the first supplier of fresh and frozen rabbits used in catering in France. Rabbit to human transmission is certainly a rare event since no similar strains have been reported in humans. Virus transmission may have been favoured by immunosuppression.

Disclosures:

The following people have nothing to disclose: Deborah Delaune, Nicole Pavio, Eric Marchadier, Olivier Chazouillères, Anne-Marie Roque-Afonso

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
				2012. 10. 20	該当なし	

一般的名称	新鮮凍結人血漿	研究報告の公表状況	T Owada, M Kaneko, C Matsumoto, K Mio, S Uchida. M Satake, K Tadokoro. AABB Annual Meeting&CTTXPO 2012; October 6-9, 2012, BOSTON.	公表国 日本
販売名(企業名)	新鮮凍結血漿-LR「日赤」(日本赤十字社) 新鮮凍結血漿-LR「日赤」成分採血(日本赤十字社) 新鮮凍結血漿-LR「日赤」120(日本赤十字社) 新鮮凍結血漿-LR「日赤」240(日本赤十字社) 新鮮凍結血漿-LR「日赤」480(日本赤十字社)			

研究報告の概要

○ヒト血液由来E型肝炎ウイルス(HEV)ジェノタイプ3、4の培養系の確立ならびに本系を応用したHEV感染価定量系の構築について

背景:HEVは特に発展途上国や地域において経口感染すると考えられているが、近年先進国を含め世界中で拡がりを見せ、また輸血による感染が明らかとなり、懸念事項となっている。本研究はHEVの培養系を確立し、感染性因子低減化技術の有効性を評価するin vitroの感染価定量系を構築することを目的とした。HEVジェノタイプは4つに分類され、ジェノタイプ3(G3)が世界で最も多く見られる。G4は日本を含めアジアに多く、時に重症肝炎を引き起こす。また、血液から検出されたウイルスは、糞便から得たウイルスとは異なる表面構造を持つことが近年報告されている。それゆえ、培養系の確立と、それを応用した感染価定量系の構築において、日本のHEV RNA陽性血液検体から一般的に分離されるG3とG4を用いるのは妥当である。

方法:G3またはG4のHEVを含むHEV特異的IgM及びIgG陽性または陰性の血液検体14例を用い、ヒト肝癌細胞株(PLC/PRF/5)及びヒト肺腺癌細胞株(A549)に接種した。HEVに感染させた細胞は維持培地にて培養した。HEV RNAコピー数はRT-PCR法で測定した。HEVの感染性については培養3週間後に回収した培地における子孫ウイルスの検出により確認した。

結果:特異的IgMを含む血液検体由来のJRC-HE3株(G3)及びIgMとIgGを含むUA1株(G4)を用いた培地から子孫ウイルスが検出された。いずれの検体でも細胞変性は確認されなかった。極めて感染性の高いJRC-HE3株の感染効率(1組織培養感染量)としては、$10^{5.5}$RNAコピー数が必要であった。電子顕微鏡による観察で、感染細胞の培養上清中にHEVと見られる球形粒子が確認された。数回のウイルス継代と長期の培養を行っても、JRC-HE3にアミノ酸変異はほとんど認められなかった。本感染価定量系をMirasol感染性低減化技術に応用したところ、2log以上の感染性低減化が確認された。

結論:HEV RNA陽性血液検体から得たHEV G3とG4を用いてHEV培養系及びHEV感染性の力価測定を評価するin vitroシステムを確立した。このシステムはウイルス不活化技術の性能評価にも有用であろう。

使用上の注意記載状況・その他参考事項等

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

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
HEV RNA陽性血液検体から得たHEVジェノタイプ3と4を用いてHEV培養系を確立し、それを応用した感染価定量系を構築したとの報告である。	今後も引き続き情報の収集に努める。

(2)

別紙3

that in the *env* region, regardless of the results of WB (Table). **Conclusion:** It is speculated that the amino acid substitutions including the start-codon, stop-codon, and deletion mutations in the *env* region are associated with the indeterminate or negative WB results of HTLV-1-infected individuals.

Disclosure of Commercial Conflict of Interest

M. Kaneko: Nothing to disclose; C. Matsumoto: Nothing to disclose; M. Satake: Nothing to disclose; N. Shinohara: Nothing to disclose; R. Sobata: Nothing to disclose; K. Tadokoro: Nothing to disclose; R. Taira: Nothing to disclose; S. Uchida: Nothing to disclose

Disclosure of Grants Conflict of Interest

M. Kaneko: Nothing to disclose; C. Matsumoto: Nothing to disclose; M. Satake: Nothing to disclose; N. Shinohara: Nothing to disclose; R. Sobata: Nothing to disclose; K. Tadokoro: Nothing to disclose; R. Taira: Nothing to disclose; S. Uchida: Nothing to disclose

Frequencies of Amino Acid Substitutions

Results of WB	Total Number of Blood Donors	Observed Number of Amino Acid Substitutions	
		env	*gag*
Indeterminate	45	72	20
Negative	4	8	1
Positive	37	25	11

SP456

Establishment of Culture System for Hepatitis E Virus (HEV) Genotypes 3 and 4 Originating from Human Blood, and Application of this System to the Assessment of HEV Infectious Titer

T Owada[1] (t-owada@jrc.or.jp), M Kaneko[1], C Matsumoto[1], K Mio[2], S Uchida[1], M Satake[1], K Tadokoro[1]. *[1]Central Blood Institute, Japanese Red Cross Society, Tokyo, Japan; [2]Biomedicinal Information Research Center, National Institute of Advanced Industrial Science and Technology, Tokyo, Japan*

Background/Case Studies: The hepatitis E virus (HEV) has been considered to be orally transmitted particularly in developing countries and regions. However, recently, HEV has been found to be spreading worldwide including in industrialized nations and has also been demonstrated to be transmitted by blood transfusion. Thus, the possibility of HEV infection via transfusion is a major concern globally. The objectives of this study are to establish a culture system for HEV and an in vitro system for the evaluation of the efficacy of pathogen reduction technology. HEV is classified into four genotypes, among which genotype 3 (G3) is one of the most common worldwide, and G4 is mainly spreading in Asia, including Japan, and occasionally causes severe hepatitis. Moreover, it has recently been reported that the virus obtained from blood has a different composition of the envelope-like structure from that obtained from feces. Hence, it is reasonable to use G3 and G4 from HEV-RNA-positive blood specimens commonly isolated in Japan when we try to develop a culture system, and to expand this system to a methodology for evaluating HEV infectious titer referred to as tissue culture infectious dose (TCID). **Study Design/Methods:** Fourteen specimens containing HEV of the G3 or G4 were used, which were either positive or negative for HEV-specific IgM and IgG. The cell lines of human hepatoma cells (PLC/PRF/5) and human lung adenocarcinoma cells (A549) were inoculated with viral specimens for 2 h at 37ºC. The cells infected with HEV were incubated in a maintenance medium containing 30 mM Mg^{2+} and 2% FCS. The medium was collected and replaced with a fresh one every week. HEV RNA copies were determined by real-time RT-PCR analysis. The establishment of HEV infectivity was confirmed by the detection of viral progenies in recovered media after 3 weeks of incubation. **Results/Findings:** Viral progenies were detected in recovered media when the JRC-HE3 strain (G3) from blood specimens containing specific IgM or the UA1 strain (G4) containing IgM and IgG was used. No cytopathic effect was observed in any specimens. We found 1 TCID of the highly infectious strain JRC-HE3 corresponds to approximately $10^{5.5}$ RNA copies. Clear spherical particles that were likely to be HEV were found in the culture supernatant of infected cell lines by electron microscopy. Amino acid substitution in JRC-HE3 scarcely occurred after several viral passages and long-term cultivation. Using this titration system, we found a log reduction greater than 2 for HEV infectivity when the Mirasol Pathogen Reduction System (Terumo BCT) was used. **Conclusion:** We established an HEV culture system using G3 and G4 from HEV-RNA-positive blood specimens and an in vitro system for the titration of HEV infectivity. These systems could be useful for assessing the performance of a viral inactivation technology.

Disclosure of Commercial Conflict of Interest

M. Kaneko: Nothing to disclose; C. Matsumoto: Nothing to disclose; K. Mio: Nothing to disclose; T. Owada: Nothing to disclose; M. Satake: Nothing to disclose; K. Tadokoro: Nothing to disclose; S. Uchida: Nothing to disclose

Disclosure of Grants Conflict of Interest

M. Kaneko: Nothing to disclose; C. Matsumoto: Nothing to disclose; K. Mio: Nothing to disclose; T. Owada: Nothing to disclose; M. Satake: Nothing to disclose; K. Tadokoro: Nothing to disclose; S. Uchida: Nothing to disclose

SP457

Flow Cytometry Shows Biochemical Variations and Structural Changes in RBC Upon Binding of West Nile and Dengue Viruses

C Chancey[1] (caren.chancey@fda.hhs.gov), A Teixeira-Carvalho[1,2], G Añez[1], L M Espina[1], M Rios[1]. *[1]DETTD, FDA-CBER, Bethesda, MD, United States; [2]CPqRR, FIOCRUZ, Belo Horizonte, Brazil*

Background/Case Studies: West Nile (WNV) and Dengue virus (DENV) are enveloped positive-strand RNA viruses from the genus *Flavivirus*, family Flaviviridae. WNV and DENV are primarily transmitted to hosts by mosquito bites, but transmission can also occur from human-to-human by blood transfusion. In specimens from WNV- or DENV-positive blood donors, infectious virus bound to red blood cells (RBC) has been detected at levels equivalent to those found in plasma (for DENV) or up to one log higher than in plasma (WNV). We hypothesize that flavivirus-RBC adherence has a role in viral dissemination and pathogenesis, and the understanding of this adherence is important for development of improved diagnostics and therapeutics. In this study, we used flow cytometry to investigate the nature of WNV and DENV binding to RBC. **Study Design/Methods:** DENV-2 (New Guinea C strain) and WNV/DENV4-Ð30 chimera (attenuated virus containing structural proteins of WNV and non-structural proteins of DENV4-Ð30 vaccine construct) were grown in Vero cells, purified from culture supernatants, and labeled with a fluorescent probe. RBC were separated from whole blood collected in either ACD or EDTA, washed, and resuspended in 0.9% saline. Labeled virus was allowed to bind to RBC for 1 hour, RBC were washed, and data was collected using a digital flow cytometer. **Results/Findings:** Both DENV and WNV/DENV4-Ð30 bound to RBC under varying buffer and anti-coagulant conditions. DENV showed greater binding when EDTA was used as the anti-coagulant, and WNV/DENV4-Ð30 showed greater binding when ACD was used as the anti-coagulant and when Ca++/Mg++ were absent from the binding buffer. Approximately four times as much DENV was required to bind RBCs to yield the same mean fluorescence intensity (MFI) as observed when WNV/DENV4-Ð30 binds. Both viruses caused damage to RBC at virus:RBC ratios greater than 10:1, and WNV caused lysis of RBCs at 25:1. **Conclusion:** Flow cytometry is useful for investigating the frequency and intensity of WNV and DENV binding to RBC, and the consequent morphological alterations. The optimal conditions for WNV and DENV binding to washed RBC are slightly different, suggesting that there may be biochemical differences between the viruses and potential RBC ligands. The difference between DENV and WNV in amount of input virus required to reach the same MFI suggests that WNV binds RBC more efficiently than does DENV, which is consistent with previous studies on DENV and WNV distribution in blood donor samples. Although the amounts of virus used are higher than those that would be encountered in clinical samples, the damage to RBC caused by WNV and DENV is notable and future studies into the mechanism of membrane disruption are needed.

Disclosure of Commercial Conflict of Interest

G. Añez: Nothing to disclose; C. Chancey: Nothing to disclose; L. M. Espina: No Answer; M. Rios: Nothing to disclose; A. Teixeira-Carvalho: No Answer

Disclosure of Grants Conflict of Interest

G. Añez: Nothing to disclose; C. Chancey: Nothing to disclose; L. M. Espina: No Answer; M. Rios: Nothing to disclose; A. Teixeira-Carvalho: No Answer

SP458

Influenza A Virus (H5N1) Can Be Transmitted Through Blood Transfusion in Ferrets

X Wang[1,2] (xue.wang@fda.hhs.gov), J Tan[1,2], P Zhang[1,2], J Zhao[1,2], I Hewlett[1,2]. *[1]CBEER/DETTD, FDA, Bethesda, MD, United States; [2]FDA, Bethesda, MD, United States*

Background/Case Studies: Some highly pathogenic strains of influenza A virus can infect multiple organs other than the respiratory system. It has been reported that virus can be detected in blood during infection with both avian H5N1 viruses and pandemic H1N1 (swine) viruses, and is associated with severe case. It is unknown that virus infectivity in blood. **Study Design/Methods:** Using the susceptible ferret animal model, we studied the infectiv-

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
一般的名称	－		研究報告の公表状況	Br J Haematol. 2012 Nov;159(4):385-93.	公表国 フィンランド	
販売名(企業名)	－					

研究報告の概要

凝固因子濃縮製剤の複数の不活性化の導入は、実質的に過去 20 年間の重要な病原性に関連付けられているウイルスによる感染症を排除している。
現時点で導入可能なウイルス不活化の方法は、パルボウイルス B19 やプリオンを除去することができないことが知られており、感染の伝播に関する理論的な懸念が残っている。
血液製剤における新しいパルボウイルスであるヒトパルボ 4 (PARV4) や新しい遺伝子型のパルボウイルス B19 の発見後、懸念されている。
パルボウイルスは、ヒト免疫不全ウイルスやＣ型肝炎ウイルスのように慢性的な病原性はないが、免疫の抑制された患者では臨床症状を引き起こす可能性がある。
製造業者は既知のウイルスが含まれていないことを保証するために、ミニプールでのポリメラーゼ連鎖反応テストなどの対策を講じている。
これまでのところ、ヒトのボカウイルス、パルボウイルスは、分画された血液製剤で検出されておらず、それらの存在が実証されない限り、製造中のルーチン検査は必須ではない。
患者と血液製剤の安全性の継続的な監視は重要な事項である。

報告企業の意見	今後の対応
血漿分画製剤におけるパルボウイルスなどに関する懸念の情報である。 献血血漿については採血時に日本赤十字社でヒトパルボウイルス B19 の抗原検査(RHA 法)を輸入血漿については当社で NAT 検査を実施している。 また、当社血漿分画製剤は最終製品において NAT 検査を行い、パルボウイルス B19DNA 陰性であることを確認している。	今後ともパルボウイルスなどに関する安全性情報等に留意していく。

使用上の注意記載状況・その他参考事項等

慎重投与(次の患者には慎重に投与すること)
・溶血性・失血性貧血の患者［ヒトパルボウイルスＢ19 の感染を起こす可能性を否定できない。感染した場合には、発熱と急激な貧血を伴う重篤な全身症状を起こすことがある。］
・免疫不全患者・免疫抑制状態の患者［ヒトパルボウイルスＢ19 の感染を起こす可能性を否定できない。感染した場合には、持続性の貧血を起こすことがある。］
重要な基本的注意
(1)本剤の原材料となる‥［スクリーニング項目、不活化･除去工程］‥投与に際しては、次の点に十分注意すること。
1)血漿分画製剤の現在の製造工程では、ヒトパルボウイルス B19 等のウイルスを完全に不活化・除去することが困難であるため、本剤の投与によりその感染の可能性を否定できないので、投与後の経過を十分に観察すること。
妊婦、産婦、授乳婦等への投与
妊婦又は妊娠している可能性のある婦人には治療上の有益性が危険性を上回ると判断される場合にのみ投与すること。［妊娠中の投与に関する安全性は確立していない。本剤の投与によりヒトパルボウイルス B19 の感染の可能性を否定できない。感染した場合には胎児への障害（流産、胎児水腫、胎児死亡）が起こる可能性がある。］

bjh review

Parvovirus transmission by blood products – a cause for concern?

Päivi Norja,[1] Riitta Lassila[2] and Mike Makris[3,4]

[1]Department of Virology, Haartman Institute, Helsinki University, [2]Coagulation disorders Unit, Division of Haematology, Department of Internal Medicine, Helsinki University Central Hospital, Helsinki, Finland, [3]Department of Cardiovascular Science, University of Sheffield, and [4]Sheffield Haemophilia and Thrombosis Centre, Royal Hallamshire Hospital, Sheffield, UK

Summary

The introduction of dual viral inactivation of clotting factor concentrates has practically eliminated infections by viruses associated with significant pathogenicity over the last 20 years. Despite this, theoretical concerns about transmission of infection have remained, as it is known that currently available viral inactivation methods are unable to eliminate parvovirus B19 or prions from these products. Recently, concern has been raised following the identification of the new parvoviruses, human parvovirus 4 (PARV4) and new genotypes of parvovirus B19, in blood products. Parvoviruses do not cause chronic pathogenicity similar to human immunodeficiency virus or hepatitis C virus, but nevertheless may cause clinical manifestations, especially in immunosuppressed patients. Manufacturers should institute measures, such as minipool polymerase chain reaction testing, to ensure that their products contain no known viruses. So far, human bocavirus, another new genus of parvovirus, has not been detected in fractionated blood products, and unless their presence can be demonstrated, routine testing during manufacture is not essential. Continued surveillance of the patients and of the safety of blood products remains an important ongoing issue.

Keywords: haemophilia, coagulation, parvovirus, parvovirus 4, clotting factor concentrate.

Patients with inherited bleedings disorders, such as severe haemophilia A, B, von Willebrand disease (VWD), as well as other rare bleeding disorders suffer recurrent spontaneous and traumatic bleeds and are treated with intravenous infusions of the missing clotting factor, so-called replacement therapy.

The treatment of bleeding disorders has developed enormously from the use of fresh frozen plasma in the 1940s, cryoprecipitate in the 1960s, and clotting factor concentrates since the early 1970s. Over the last 20 years recombinant technology, aimed at avoidance of all animal- or human-derived proteins during the manufacture or final formulation of the coagulation factor concentrates, has been developed. Until recently, the major drive in concentrate development has been the reduction in infective risk. Today, the development of allo-antibodies to factor VIII (FVIII) (inhibitors) has taken over as the main problem in haemophilia management.

Plasma-derived clotting factor concentrates are prepared by fractionation of up to 30 000 pooled plasma units. Until viral inactivation was introduced in 1985, viral infections present in the donors could easily be transmitted to the recipient. The result was that virtually all recipients of concentrates prior to 1985 were infected with hepatitis C virus (HCV) and many were also infected with human immunodeficiency virus (HIV) and hepatitis B virus (HBV) (Makris *et al*, 1996). The viral elimination processes are based on destruction of the viruses with dry or wet heat treatment (sometimes under pressure), chemical treatment with combination of solvent and detergent (S/D), and nanofiltration (Mannucci & Tuddenham, 2001).

Viral elimination processes proved to be highly successful in virtually abolishing the risk of infection with HBV, HCV and HIV. However, in 1992, a number of outbreaks of hepatitis A transmission by concentrates were reported (Richardson & Evatt, 2000). This occurred due to the fact that hepatitis A, which does not have a lipid envelope, was resistant to the viral elimination by the S/D method used during manufacture. Subsequently, new regulations require that all plasma-derived clotting factor concentrates undergo two different viral elimination procedures before release.

Despite the success of the currently used viral elimination techniques, two infectious agent problems have remained, parvovirus B19 (B19) and prion transmission. As B19 is relatively resistant to all the currently available elimination methods, manufacturers introduced screening of mini-pools by the polymerase chain reaction (PCR). Positive minipools are not used in fractionation of blood products, but despite this, recent evidence suggests that the risk of B19 transmission is

Correspondence: Dr Mike Makris, Department of Cardiovascular Science, University of Sheffield, Royal Hallamshire Hospital, Sheffield S10 2JF, UK. E-mail: m.makris@sheffield.ac.uk

British Journal of Haematology, 2012, 159, 385–393

First published online 1 October 2012
doi:10.1111/bjh.12060

real (Soucie *et al*, 2011). Variant Creutzfeld Jacob Disease (vCJD), a prion disease, is the human form of the Bovine Spongiform Encephalopathy (BSE) that appeared in the UK cattle population in the 1980s. Transmission by blood products and clotting factor concentrates has been demonstrated (Peden *et al*, 2010). Prions are highly resistant to all currently used elimination techniques but because of geographical restriction of vCJD, some countries have chosen to avoid using plasma collected in those countries, e.g., the UK (Millar *et al*, 2010).

Further viral newcomers in this field include human parvovirus 4 (PARV4), which has been linked with intravenous drug administration, both for therapeutic use, such as for bleeding disorders, and recreational abuse. In recognition of the difficulty of eliminating infective agents from clotting factor concentrates, some countries (e.g., UK, Canada and Ireland) decided to use exclusively recombinant clotting factors, when these became available. This can be envisioned as protectionism in the countries where this kind of national transitions have not been undertaken. The current plasma-derived products have proven safe, at least so far.

Our objective here is to illustrate that, in association with intravenous repetitive coagulation factor replacement therapies – despite the current precautions – the risk of viral transmittance cannot be completely excluded. The surveillance of both old and new patients and concentrates remains our continued task.

We live in a world where new disease entities and viral epidemics continue to emerge in various locations and, with the current air travel frequency, these diseases can spread quite rapidly. The recently identified west Nile virus and swine influenza are examples of suddenly emerging pathogenic viruses that have a relatively strong penetrance and cause local and even worldwide epidemics. Additionally, prions are an example of pathogens, which are very hard to detect and the success of their elimination remains unclear for many years, with potentially serious consequences.

Parvovirus taxonomy and basic features

The name parvovirus originates from the Latin word *parvum*, which means small; parvoviruses are among the smallest known viruses with a virion diameter of 18–26 nm. Parvoviruses infect a wide range of vertebrates and insects causing systemic infections. The family *Parvoviridae* is divided into two sub-families: Parvovirinae and Densovirinae (http://ictvonline.org/virusTaxonomy.asp?version=2009). Viruses from the sub-family Parvovirinae infect vertebrate cells and are divided further into five genera (Table I): *Parvovirus, Dependovirus, Erythrovirus, Bocavirus,* and *Amdovirus*. Of these, *Erythrovirus, Dependovirus* and *Bocavirus* genera contain viruses infecting humans. In addition, a sixth genus, *Partetravirus,* containing human PARV4 and human PARV4-like viruses, has been proposed. The sub-family of Densovirinae contains viruses of invertebrates.

Table I. The taxonomy of parvoviruses.

Subfamily	Genus	Example virus, abbreviation
Parvovirinae	*Parvovirus*	*Minute virus of Mice, MVM*
		Canine parvovirus, CPV
	Dependovirus	*Adeno-assosiated virus AAV*
		Goose parvovirus
	Erythrovirus	*Human parvovirus, B19*
		Simian parvovirus, SPV
	Bocavirus	*Bovine parvovirus, BPV*
		Human bocaviruses 1-4, HBoV1-4
	Amdovirus	*Aleutian mink disease virus, AMVD*
	Partetravirus (proposed)	*Human partetravirus, PARV4*
		Porcine hokovirus, PHoV/PPV3
Densovirinae	*Densovirus*	*Junonia coenia densovirus*
	Brevidensovirus	*Aedes aegypti densovirus*
	Iteravirus	*Bombyx mori densovirus*
	Pefudensovirus	*Periplanta fuliginosa densovirus*

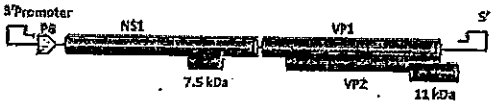


Fig 1. Genome structure and protein encoding reading frames of B19.

The structure of parvoviruses is simple; the icosahedral virion consists of only proteins and linear single-stranded-DNA genome with hairpin structures at both ends. The hairpins are palindromic and the 3'-end can fold and function as a primer during viral replication (Fig 1). The length of the DNA genome is approximately 5–6 kb. In the parvovirus infection cycle, the virus attaches to its receptor, e.g. globoside (P-antigen) in case of human B19, on the surface of host cells (Brown *et al*, 1993) and is transported into the cell by endocytosis. Inside the host cell, the virion is transported to the nucleus where parvovirus replication takes place. Parvoviruses do not encode their own DNA-polymerase, indicating that all parvoviruses are dependent on (i) host cell polymerase and (ii) S-phase of dividing cells. In the case of dependoviruses, co-infection with another virus is needed for efficient DNA replication (Atchison *et al*, 1965).

General aspects and epidemiology of human and porcine parvoviruses

Parvovirus B19

Human parvovirus B19 (B19) is the type species of the *Erythrovirus* and representative member of parvoviruses. B19 was discovered when a serum sample from an asymptomatic blood donor gave a false-positive result in an immunoelectrophoresis assay for HBV (Cossart *et al*, 1975). The virus was detected in panel B and was coded 19, from which its name originates. The most common transmission route of B19 is respiratory, but it can also transmit via

plasma-derived medical products and from mother to fetus. B19 infections are prevalent worldwide, and seroprevalence studies based on B19 IgG have shown that in Europe 60–80% of adults have been infected with this virus during their lifetime (Mossong *et al*, 2008). In Asia the B19 seroprevalence in blood donors was found to be 25–40% (Kishore *et al*, 2010, Ke *et al*, 2011).

B19 DNA prevalence in blood donors has been reported by a number of studies. The rates of positivity were 0·88% in the USA (Kleinman *et al*, 2007), 0·2% in the Netherlands (Koppelman *et al*, 2011) and 0·55–1·3% in the UK and Africa (Candotti *et al*, 2004).

B19 is erythrotropic and replicates in erythroid progenitor cells in human bone marrow. After short viraemia the virus is eliminated from the blood circulation by neutralizing antibodies produced by the host. However, B19 genomic DNA remains detectable in solid tissues of seropositive individuals. Initially, B19 DNA was detected in the synovium of patients with rheumatoid arthritis but, in 1997, the viral DNA was also detected in 48% of synovia collected from healthy controls (Saal *et al*, 1992; Soderlund *et al*, 1997). Later, B19 was shown to persist with full-length coding capacity in several tissue types of both symptomatic and asymptomatic persons, most probably for a lifetime (Soderlund-Venermo *et al*, 2002; Norja *et al*, 2006; Manning *et al*, 2007).

B19 strains have been divided into three divergent genotypes according to their genomic sequence. Genotype 1 is the prototypic virus and is nowadays globally the most predominant circulating virus (Hubschen *et al*, 2009). The genotype 2 virus was first identified in human skin and in the serum of an Italian HIV-positive patient with chronic anaemia (Hokynar *et al*, 2002; Nguyen *et al*, 2002). Genotype 2 has since been found in human solid tissues but only sporadically in blood and seems to have disappeared from wide circulation after the 1970s (Blumel *et al*, 2005; Norja *et al*, 2006; Manning *et al*, 2007; Grabarczyk *et al*, 2011, Koppelman *et al*, 2011). The genotype 3 virus was found in France in the serum and bone marrow of a child with transient aplastic anaemia (Nguyen *et al*, 1999). Following its discovery, genotype 3 has been reported to be endemic in Ghana and Brazil (Candotti *et al*, 2004; Sanabani *et al*, 2006; Freitas *et al*, 2008; Keller *et al*, 2009). Since identification of the genotypes 2 and 3, many commercial and in-house PCR-methods have been shown to detect these B19 genotypes with lower sensitivity or fail to detect either or both of these genotypes (Hokynar *et al*, 2004; Baylis, 2008).

Bocavirus

Human bocavirus 1 (HBoV1) was first identified in 2005 by random molecular screening and large-scale sequencing. HBoV1 was discovered in Sweden, in nasopharyngeal aspirates of children with respiratory tract infections (Allander *et al*, 2005). It belongs to the genus *Bocavirus* and its closest relatives are the bovine parvovirus (BPV) and the minute virus of canines (MVC). The receptors and target cells of HBoV1 are unknown and, to date, HBoV1 has been cultured only in pseudo-stratified human airway epithelium cell culture system (Dijkman *et al*, 2009). Using similar methods of random amplification, three additional human bocaviruses were identified in faecal samples in 2009 and 2010 (Arthur *et al*, 2009; Kapoor *et al*, 2009, 2010). These new HBoVs were named HBoV2, HBoV3 and HBoV4. Of these HBoV2 seems to be the most prevalent and circulates globally (Arthur *et al*, 2009; Kapoor *et al*, 2009, 2010; Chow *et al*, 2010; Kantola *et al*, 2010).

The seroprevalence of HBoV1 has been reported to be more than 90% in adults. However, the HBoV1-4 viral-like particles used in the enzyme-linked immunosorbent assay (ELISA) have been shown to cross-react, which might affect the serological assays. Norja *et al* (2012) detected an HBoV1 seroprevalence of 94·9%, but after removing cross-reacting antibodies the rate was 68·4%. Similar results were obtained by Kantola *et al* (2011), who observed that adult HBoV1 seroprevalence decreased, from 96 to 59%, after removing the cross-reacting antibodies. The Kantola study reported HBoV2–4 seroprevalences among adults of 34% for HBoV2, 15% for HBoV3, and 2% for HBoV4 (Kantola *et al*, 2011). As far as we are aware, human bocavirus DNA has not been detected in blood donations.

Parvovirus 4

PARV4 was identified in 2005 in a HBV-positive intravenous drug abuser with various viral infection-related symptoms by similar methods to the HBoVs (Jones *et al*, 2005). During the following year, a related virus variant (PARV5) was identified in plasma pools used in the manufacture of plasma-derived medicinal products (Fryer *et al*, 2006). Subsequently, the name PARV5 was changed to PARV4 genotype 2 (Fryer *et al*, 2007a). In 2008, a third genotype of PARV4 was identified in HIV-infected African patients (Simmonds *et al*, 2008). DNAs for PARV4 genotype-1 and -2 have been found in bone marrow, lymphoid tissue, and liver of subjects with a history of intravenous drug use, or HIV, or HCV infection (Manning *et al*, 2007; Simmonds *et al*, 2007; Longhi *et al*, 2007; Schneider *et al*, 2008a). In addition, several studies described PARV4 DNA in donor blood samples and coagulation factor concentrates (Fryer *et al*, 2006, 2007a,b; Lurcharchaiwong *et al*, 2008; Schneider *et al*, 2008b). Initially the parenteral transmission route was proposed for PARV4, but the genotype 3 of PARV4 has also been found in subjects without a risk of parenteral exposure (Simmonds *et al*, 2008; Panning *et al*, 2010).

Porcine parvovirus

Porcine parvovirus (PPV1) was first isolated in Germany and the USA in 1965 and today it is found worldwide

(Csagola *et al*, 2012). PPV1 belongs to the genus *Parvovirus*. PPV1 is transmitted oronasally among seronegative dams (female parents) and the virus is then further transmitted through the placenta to fetus, causing reproductive failure. During the last decade, several new parvoviruses have been identified in pigs, including porcine hokovirus (PHoV/PPV3), which is related to PARV4 (Csagola *et al*, 2012).

Human diseases caused by parvoviruses

Until the discovery of HBoVs and PARV4, human B19 was considered to be the only pathogenic parvovirus to humans. The adeno-associate viruses of *Dependovirus* genus are non-pathogenic and are studied as vectors for gene-therapy. Although B19 is associated with various clinical manifestations, subclinical infection is a common finding among both children and adults. In healthy, immunocompetent subjects, B19 infection is usually mild and transient, requiring no treatment.

The most common B19 manifestation among children is a rash causing the disease *Erythema infectiosum* (EI), fifth disease or 'slapped cheek', and arthritis among adults (Anderson *et al*, 1984). In EI, the rash typically appears first on the cheeks, spreading to the neck, trunk, and limbs. In addition, the patient may have headache, fever, nausea, and diarrhoea. Among adults, arthritis can be the only manifestation of B19 infection, affecting 45–80% of infected subjects (Anderson *et al*, 1985; Reid *et al*, 1985; White *et al*, 1985). Joint symptoms are symmetrical and affect fingers, wrists, ankles, and knees. Arthritis is usually transient but in some cases it may be prolonged and fulfill the criteria of rheumatoid arthritis (Naides *et al*, 1990).

Women without B19-specific antibodies are at risk of primary B19 infection and trans-placental transmission. During maternal infection, the risk of vertical transmission is approximately 30% (Brown, 2010). Intrauterine B19 infection has been associated with fetal anaemia, hydrops, miscarriage, and fetal death (Enders *et al*, 2006, 2008).

In subjects with shortened red-cell survival, such as sickle cell disease, B19-infection may lead to aplastic crisis (Pattison *et al*, 1981). Among immunosuppressed subjects with decreased ability to produce antibodies, including patients with leukaemia or lymphoma (Kurtzman *et al*, 1988, 1989) or in the HIV/HCV-infected, the B19 infection may become persistent causing chronic anaemia.

Following the HBoV1 discovery, a large number of studies of the prevalence of HBoV1 have been undertaken in respiratory secretions of young children. According to recent studies, primary infections of HBoV1 are significantly associated with respiratory illnesses, including wheezing, pneumonia, and otitis media (Soderlund-Venermo *et al*, 2009; Don *et al*, 2010; Meriluoto *et al*, 2012). HBoV1 has also been detected in faeces from children with symptoms of gastroenteritis. However, the significance of HBoV1 as an enteric virus is questionable, because in many subjects another enteric virus was detected simultaneously with HBoV1, and there is a lack of evidence of replication of HBoV1 in the enteric tract (Albuquerque *et al*, 2007; Yu *et al*, 2008, Szomor *et al*, 2009). HBoV2 instead may cause gastroenteritis in young children (Kapoor *et al*, 2009; Chow *et al*, 2010; Kantola *et al*, 2010).

So far, no disease associations have been confirmed for PARV4 (Lahtinen *et al*, 2011). The virus has been linked to encephalitis (Benjamin *et al*, 2011), and detected in the blood of three mothers bearing newborns with hydrops (Chen *et al*, 2011). Among patients with haemophilia, clinical presentations concurrent with PARV4 seroconversion were rash and unexplained hepatitis (Sharp *et al*, 2012). The individual in whom PARV4 was first identified presented with fatigue, vomiting, arthralgia, neck stiffness, night sweats, and diarrhoea, but this patient was lost to follow up, and it is not known if the described symptoms were associated with the PARV4 infection (Jones *et al*, 2005).

There are no antiviral drugs or vaccines against human parvovirus infection. However, among immunocompetent patients, treatment is unnecessary and infections are self cleared. Immunodeficient patients with chronic B19 infection, and patients with transient aplastic anaemia and B19, can be managed with intravenous immunoglobulin or erythrocyte transfusions (Frickhofen *et al*, 1990; Koduri *et al*, 1999).

Parvoviruses in blood products

Parvovirus B19

The B19 titre in blood is at its highest, up to 10^{13} genome equivalents/ml blood, during the first days of acute infection. Infected subjects are usually asymptomatic when the viral titres are at their highest. This creates a risk of contaminating blood products by blood donors with asymptomatic B19 infection. Siegl and Cassinotti (1998) reported B19 DNA in 50–80% and in 30–50% of non-virally inactivated VIII concentrates and S/D-inactivated coagulation factor IX (FIX) concentrates, respectively. A more recent German study detected B19 DNA in 26% of coagulation factor concentrates of different types, collected between 2007 and 2008 (Modrow *et al*, 2011). The highest viral loads were observed in the intermediate purity FVIII /VWF concentrates. Because of its small and non-enveloped structure, B19 is relatively resistant to most viral inactivation procedures used in the manufacturing of medical blood-derived products (Willkommen *et al*, 1999; Koenigbauer *et al*, 2000; Schmidt *et al*, 2001) and B19 is only partially removable with small pore size nanofiltration (Burnouf-Radosevich *et al*, 1994).

In Europe, in an attempt to reduce the risk of B19 transmission by blood products, the blood derived products manufactured after 2004 are not allowed to contain B19 DNA of more than 10^4 iu/ml, and nucleic acid testing for B19 is

obligatory for S/D-treated human plasma products (European Pharmacopoeia Commission, Council of Europe European Directorate for the Quality of Medicines, 2011). Similar instructions are given by United States Food and Drug Administration (http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/default.htm). B19 DNA concentrations below the 10^4 iu/ml limit are not considered to lead to seroconversion (Brown *et al*, 2001). However, Soucie *et al* (2011) reported 1·7 times higher B19 IgG seroconversion rates among children who received plasma-derived factor concentrates screened for B19 levels than among children receiving recombinant products. The infectivity of B19 in the blood products is affected by both the level B19-specific IgG in the products and the immune status of the recipient.

In order to quantify the B19 positive units, the quantitative DNA amplification method is used, and primers designed to detect all three genotypes are required. Many in-house PCR methods and one commercial PCR method, designed before the identification of B19 genotypes, are prone to miss B19 genotypes 2 and 3 (Hokynar *et al*, 2004; Baylis *et al*, 2004; Koppelman *et al*, 2004, 2007). Consequently, some plasma pools have remained contaminated with an excessive level of B19. All three genotypes of B19 have been reported in clotting factor concentrates, however, with a reduced frequency for genotypes 2 and 3 (Schneider *et al*, 2004; Modrow *et al*, 2011). To obtain an accurate diagnosis and safety of blood products, methods with capability to detect all B19 genotypes should be used. A reference panel for B19 DNA genotypes by the World Health Organization Expert Committee on Biological Standardization (ECBS) was established at the end of 2009, and it is available for validation of B19 PCR-based detection assays for all three genotypes (Baylis *et al*, 2012). Furthermore, the most recent study of B19 levels in plasma donations described a commercial method for simultaneous B19 and HAV screening (Koppelman *et al*, 2012).

Human bocaviruses

Three studies have analysed whether HBoV1 occurs in blood donor plasma and plasma-derived coagulation factor concentrates (Fryer *et al*, 2007b; Eis-Hubinger *et al*, 2010; Modrow *et al*, 2011). None of the studies reported positive cases of HBoV1. The absence of detectable HBoV1 DNA in blood or plasma donors, however, may be due to the fact that HBoV1 infections are most common among young children (Soderlund-Venermo *et al*, 2009; Meriluoto *et al*, 2012) and less frequent among the blood donor population. Negative results could also be explained by low HBoV1 titres in donors, as possible low-level viraemia could remain undetectable in manufactured plasma pools containing hundreds or thousands of donations.

PARV4 DNA in blood-derived medical products

The first study of PARV4 in blood products was published soon after the virus was discovered and reported PARV4 DNA with prevalence of 5·1% in manufacturing plasma pools (Fryer *et al*, 2006). In this study both PARV4 genotype 1 and 2 DNAs were amplified, and the virus titres ranged between < 500 copies to 10^6 copies/ml. A year later, the same authors reported PARV4 genotype 1 and 2 DNAs in 4% of recently sourced plasma pools collected in Europe and the USA, in 21% of the older plasma pools collected between 1990 and 1993, in 2% of the blood collected from healthy blood donors and in 6% of febrile patients (Fryer *et al*, 2007b). Schneider *et al* (2008a) reported PARV4 in 1–33% of randomly selected plasma-derived concentrate pools. The higher frequency of the PARV4 was detected in the older concentrates manufactured 10 years earlier, but smaller amounts of PARV4 were also detectable in the currently used concentrates. Recently, a study from China reported PARV4 DNA in the blood of 16–22% healthy subjects, in 33% of HBV-infected subjects and in 41% of HCV-infected subjects (Yu *et al*, 2012). In addition, PARV4 DNA has been detected in 26% of blood donor plasma pools collected between 2007 and 2010 in China (Ma *et al*, 2012). Table II summarizes PARV4 DNA detected in plasma- and blood-derived medical products. Even if the disease associations of PARV4 are not currently known, the prevalences of PARV4, especially those detected most recently in France and China, raise a question of whether blood donor minipools should be tested by screening for PARV4 DNA similar to B19.

In contrast to the studies described in Table II, three studies performed in France and Germany analysed high numbers of blood donor plasma donations, minipools or coagulation factor concentrates and failed to detect any

Table II. PARV4 DNA findings in blood donor samples and coagulation factor concentrates.

References	Blood product	PARV4 DNA prevalence (%)
Fryer *et al* (2006)	Plasma pools	5·1
Fryer *et al* (2007a,b)	Plasma pools,	8·7
	individual plasma	4
Fryer *et al* (2007c)	FVIII concentrates	16
Lurcharchaiwong *et al* (2008)	Blood donor sera	3·95
Schneider *et al* (2008b)	Coagulation FFII, FVIII, FIX, activated prothrombin complex concentrates	21
Vallerini *et al* (2008)	Blood donor sera	1
Touinssi *et al* (2010)	Blood donor plasma	24·6
Ma *et al* (2012)	Plasma pools	26

PARV4 DNA positive samples (Servant-Delmas *et al*, 2009; Eis-Hubinger *et al*, 2010; Modrow *et al*, 2011). Whether these negative results are due to seasonal or geographical reasons need further studies. The unanswered question also is, whether the higher frequency of PARV4 in older blood products (Fryer *et al*, 2007a,b; Schneider *et al*, 2008b) represents a timely population-based hazard or whether the manufacturing processes, i.e., nanofiltration, have improved the elimination of the viruses more comprehensively. On the other hand, demonstration of virus genome in plasma-derived products does not translate to infectivity.

PARV4 and haemophilia

In a study of 35 persons with haemophilia from the UK and USA receiving replacement therapy, 15/35 (43%) were positive for the PARV4 IgG whereas only 1/35 (3%) of untreated family members were positive (Sharp *et al*, 2009). The concentrates involved in treatment were non-virally inactivated clotting factors issued from the late 1970s to the early 1980s. The methods of detection were serological, ELISA-type assays, detecting both anti-PARV4 IgG and IgM, developed by the group of Simmonds.

In a 5-year follow-up of a cohort of 194 haemophilia patients who were born between 1972 and 1982, 1–7% of patients/year seroconverted for PARV4 (Sharp *et al*, 2012). They were followed between 1989 and 1994 by 6 monthly blood sampling. At cohort enrolment, almost all patients were HCV-positive and 43% of patients were PARV4 IgG-positive. Among PARV4 seropositive subjects, 46% were HIV-positive and 38% HIV-negative, thus PARV4 exposure did not significantly associate with HIV infection. The active disease forms related to PARV4 detection were rash and exacerbation of hepatitis. PARV4 IgM became positive during acute infections. The concentrates involved were plasma-derived and had undergone S/D treatment and dry or wet heating processes. Overall, the seroprevalence and the risk of seroconversion are significantly higher in patients having replacement therapy than the background population or sibling controls.

Transmission of porcine parvovirus by the old Hyate C porcine concentrate

A serious complication of therapy of persons with haemophilia is the development of antibodies (inhibitors) against the clotting factor, which renders the concentrates ineffective in controlling bleeding. Porcine FVIII concentrate, Hyate C, has been used as a treatment of patients with congenital haemophilia and inhibitory antibodies. Hyate C was developed in 1980 and was manufactured by Ipsen Ltd (Slough, UK) from pig plasma. During the manufacturing process Hyate C underwent a number of purification steps, and cell culture was used to confirm the absence of viruses but, in contrast to human coagulation factor concentrates, it did not undergo viral inactivation. In 1996, PPV1 was found in several Hyate C products and its supply was suspended. The knowledge that many recipients of Hyate C were already infected by HIV and were immunosuppressed led to concerns that PPV1 could in some cases infect humans. Soucie *et al* (2000) detected PPV1 DNA in 95% of porcine FVIII concentrates and confirmed that PPV1 is a common, low level contaminant in Hyate C. However, none of the 98 recipients of Hyate C tested positive for PPV1 antibodies. Most pigs naturally have antibodies to PPV1, but there is no evidence of transmission to humans from physical contact between pigs and humans. In addition to PPV1, porcine hokovirus, closely related to PARV4, has recently been found in porcine plasma and FVIII preparations (Szelei *et al*, 2010). The theoretical risk that porcine parvoviruses could infect humans remains a concern, but, if employed, PCR screening and discarding the porcine parvovirus DNA positive samples could eliminate the risk of transmission. Porcine plasma-derived FVIII concentrates are no longer available and to our knowledge are not being developed but a recombinant porcine FVIII concentrate has recently started clinical trials.

Implications

New viruses and other disease-inducing agents will always continue to evolve. In the first 10 months of 2010 there were 2350 reports of outbreaks of infectious diseases in humans, plants and animals (www.promedmail.org). It must be appreciated that the identification of new infections internationally is the norm rather than the exception. The studies of PARV4 and haemophilia have shown that the virus can be transmitted via blood donations and plasma products, at least when the viral inactivation steps include the methods of S/D and heating. We do not know the infectivity frequency following nanofiltration, but it may eliminate the majority of the viral load (Schneider *et al*, 2008b). To date, the parvoviruses have not been proven to cause significant chronic pathogenesis in patients with a healthy immunological system. However, in patients already infected with HIV or otherwise immunocompromised, B19 has pathogenic consequences. Overall, the current data imply that viruses are able to escape the current plasma fractionation and purification steps.

For infections that are potentially transmissible by clotting factor concentrates, blood donors should be screened serologically and mini-pools of plasma should be genotyped with the virus load measured with PCR (EMA, 2010). The safety of the plasma-derived concentrates demands continuous watchful strategies and surveillance. The regulatory studies required for registration occur early in the introduction of the products onto the market and are not optimal at detecting infection transmission by agents other than hepatitis A/B/C, HIV and Parvovirus where acute seroconversion detection is possible. Continuous vigilance by the haemophilia community is required to identify infective problems

early. Adverse event reporting studies, such as the European Haemophilia Safety Surveillance (EUHASS) system, or national spontaneous reporting schemes have the potential to identify problems, but alertness to new or unusual problems is required for unexpected events (Makris *et al*, 2011). In this way any unexpected clinically significant transmission of infection by plasma products can be traced and eliminated as rapidly as possible.

References

Albuquerque, M.C., Rocha, L.N., Benati, F.J., Soares, C.C., Maranhao, A.G., Ramirez, M.L., Erdman, D. & Santos, N. (2007) Human bocavirus infection in children with gastroenteritis, Brazil. *Emerging Infectious Diseases*, 13, 1756–1758.

Allander, T., Tammi, M.T., Eriksson, M., Bjerkner, A., Tiveljung-Lindell, A. & Andersson, B. (2005) Cloning of a human parvovirus by molecular screening of respiratory tract samples. *Proceedings of the National Academy of Sciences of the United States of America*, 102, 12891–12896.

Anderson, M.J., Lewis, E., Kidd, I.M., Hall, S.M. & Cohen, B.J. (1984) An outbreak of erythema infectiosum associated with human parvovirus infection. *The Journal of Hygiene*, 93, 85–93.

Anderson, M.J., Higgins, P.G., Davis, L.R., Willman, J.S., Jones, S.E., Kidd, I.M., Pattison, J.R. & Tyrrell, D.A. (1985) Experimental parvoviral infection in humans. *The Journal of Infectious Diseases*, 152, 257–265.

Arthur, J.L., Higgins, G.D., Davidson, G.P., Givney, R.C. & Ratcliff, R.M. (2009) A novel bocavirus associated with acute gastroenteritis in Australian children. *PLoS Pathogens*, 5, e1000391.

Atchison, R.W., Casto, B.C. & Hammon, W.M. (1965) Adenovirus-associated defective virus particles. *Science*, 149, 754–756.

Baylis, S.A. (2008) Standardization of nucleic acid amplification technique (NAT)-based assays for different genotypes of parvovirus B19: a meeting summary. *Vox Sanguinis*, 94, 74–80.

Baylis, S.A., Shah, N. & Minor, P.D. (2004) Evaluation of different assays for the detection of parvovirus B19 DNA in human plasma. *Journal of Virological Methods*, 121, 7–16.

Baylis, S.A., Ma, L., Padley, D.J., Heath, A.B., Yu, M.W.; Collaborative Study Group. (2012) Collaborative study to establish a World Health Organization International genotype panel for parvovirus B19 DNA nucleic acid amplification technology (NAT)-based assays. *Vox Sanguinis*, 102, 204–211.

Benjamin, L.A., Lewthwaite, P., Vasanthapuram, R., Zhao, G., Sharp, C., Simmonds, P., Wang, D. & Solomon, T. (2011) Human parvovirus 4 as potential cause of encephalitis in children, India. *Emerging Infectious Diseases*, 17, 1484–1487.

Blumel, J., Eis-Hubinger, A.M., Stuhler, A., Bonsch, C., Gessner, M. & Lower, J. (2005) Characterization of parvovirus B19 genotype 2 in KU812Ep6 cells. *Journal of Virology*, 79, 14197–14206.

Brown, K.E. (2010) The expanding range of parvoviruses which infect humans. *Reviews in Medical Virology*, 20, 231–244.

Brown, K.E., Anderson, S.M. & Young, N.S. (1993) Erythrocyte P antigen: cellular receptor for B19 parvovirus. *Science*(New York, N.Y.), 262, 114–117.

Brown, K.E., Young, N.S., Alving, B.M. & Barbosa, L.H. (2001) Parvovirus B19: implications for transfusion medicine. Summary of a workshop. *Transfusion*, 41, 130–135.

Burnouf-Radosevich, M., Appourchaux, P., Huart, J.J. & Burnouf, T. (1994) Nanofiltration, a new specific virus elimination method applied to high-purity factor IX and factor XI concentrates. *Vox Sanguinis*, 67, 132–138.

Candotti, D., Etiz, N., Parsyan, A. & Allain, J.P. (2004) Identification and characterization of persistent human erythrovirus infection in blood donor samples. *Journal of Virology*, 78, 12169–12178.

Chen, M.Y., Yang, S.J. & Hung, C.C. (2011) Placental transmission of human parvovirus 4 in newborns with hydrops, Taiwan. *Emerging Infectious Diseases*, 17, 1954–1956.

Chow, B.D., Ou, Z. & Esper, F.P. (2010) Newly recognized bocaviruses (HBoV, HBoV2) in children and adults with gastrointestinal illness in the united states. *Journal of Clinical Virology: The Official Publication of the Pan American Society for Clinical Virology*, 47, 143–147.

Cossart, Y.E., Field, A.M., Cant, B. & Widdows, D. (1975) Parvovirus-like particles in human sera. *Lancet*, 1, 72–73.

Csagola, A., Lorincz, M., Cadar, D., Tombacz, K., Biksi, I. & Tuboly, T. (2012) Detection, prevalence and analysis of emerging porcine parvovirus infections. *Archives of Virology*, 157, 1003–1010.

Dijkman, R., Koekkoek, S.M., Molenkamp, R., Schildgen, O. & van der Hoek, L. (2009) Human bocavirus can be cultured in differentiated human airway epithelial cells. *Journal of Virology*, 83, 7739–7748.

Don, M., Soderlund-Venermo, M., Valent, F., Lahtinen, A., Hedman, L., Canciani, M., Hedman, K. & Korppi, M. (2010) Serologically verified human bocavirus pneumonia in children. *Pediatric Pulmonology*, 45, 120–126.

Eis-Hubinger, A.M., Drexler, J.F., Reber, U., Panning, M., Drosten, C., Weber-Schehl, M., Hedges, D., Baylis, S.A. & Roth, W.K. (2010) Absence of detection of novel human parvoviruses in german plasma donations. *Transfusion*, 50, 266–267.

EMA (2010). Guideline on plasma-derived medicinal products. Document reference: EMA/CHMP/BWP/706271/2010. http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2011/07/WC500109627.pdf

Enders, M., Schalasta, G., Baisch, C., Weidner, A., Pukkila, L., Kaikkonen, L., Lankinen, H., Hedman, L., Soderlund-Venermo, M. & Hedman, K. (2006) Human parvovirus B19 infection during pregnancy – value of modern molecular and serological diagnostics. *Journal of Clinical Virology: the Official Publication of the Pan American Society for Clinical Virology*, 35, 400–406.

Enders, M., Weidner, A., Rosenthal, T., Baisch, C., Hedman, L., Soderlund-Venermo, M. & Hedman, K. (2008) Improved diagnosis of gestational parvovirus B19 infection at the time of nonimmune fetal hydrops. *The Journal of Infectious Diseases*, 197, 58–62.

European Pharmacopoeia Commission, Council of Europe European Directorate for the Quality of Medicines (2011) Human plasma (pooled and treated for virus inactication), monograph 1646 (01/2011). In: European Pharmacopeia, 7th edn. Council of Europe, Strasbourg.

Freitas, R.B., Melo, F.L., Oliveira, D.S., Romano, C.M., Freitas, M.R., Macedo, O., Linhares, A.C., de A Zanotto, P.M. & Durigon, E.L. (2008) Molecular characterization of human erythrovirus B19 strains obtained from patients with several clinical presentations in the Amazon region of Brazil. *Journal of Clinical Virology: the Official Publication of the Pan American Society for Clinical Virology*, 43, 60–65.

Frickhofen, N., Abkowitz, J.L., Safford, M., Berry, J.M., Antunez-de-Mayolo, J., Astrow, A., Cohen, R., Halperin, I., King, L. & Mintzer, D. (1990) Persistent B19 parvovirus infection in patients infected with human immunodeficiency virus type 1 (HIV-1): a treatable cause of anemia in AIDS. *Annals of Internal Medicine*, 113, 926–933.

Fryer, J.F., Kapoor, A., Minor, P.D., Delwart, E. & Baylis, S.A. (2006) Novel parvovirus and related variant in human plasma. *Emerging Infectious Diseases*, 12, 151–154.

Fryer, J.F., Delwart, E., Bernardin, F., Tuke, P.W., Lukashov, V.V. & Baylis, S.A. (2007a) Analysis of two human parvovirus PARV4 genotypes identified in human plasma for fractionation. *The Journal of General Virology*, 88, 2162–2167.

Fryer, J.F., Delwart, E., Hecht, F.M., Bernardin, F., Jones, M.S., Shah, N. & Baylis, S.A. (2007b) Frequent detection of the parvoviruses, PARV4 and PARV5, in plasma from blood donors and symptomatic individuals. *Transfusion*, 47, 1054–1061.

Fryer, J.F., Hubbard, A.R. & Baylis, S.A. (2007c) Human parvovirus PARV4 in clotting factor VIII concentrates. *Vox Sanguinis*, 93, 341–347.

Grabarczyk, P., Kalinska, A., Kara, M., Wieczorek, R., Ejduk, A., Sulkowska, E., Golebiowska-Staroszczyk, S., Matysiak, M., Baylis, S.A. & Brojer, E. (2011) Identification and characterization of acute infection with parvovirus B19 genotype 2 in immunocompromised patients in Poland. *Journal of Medical Virology*, 83, 142–149.

Hokynar, K., Soderlund-Venermo, M., Pesonen, M., Ranki, A., Kiviluoto, O., Partio, E.K. & Hedman, K. (2002) A new parvovirus genotype persistent in human skin. *Virology*, 302, 224–228.

Hokynar, K., Norja, P., Laitinen, H., Palomaki, P., Garbarg-Chenon, A., Ranki, A., Hedman, K. & Soderlund-Venermo, M. (2004) Detection and differentiation of human parvovirus variants by commercial quantitative real-time PCR tests. *Journal of Clinical Microbiology*, 42, 2013–2019.

Hubschen, J.M., Mihneva, Z., Mentis, A.F., Schneider, F., Aboudy, Y., Grossman, Z., Rudich, H., Kasymbekova, K., Sarv, I., Nedeljkovic, J., Tahita, M.C., Tarnagda, Z., Ouedraogo, J.B., Gerasimova, A.G., Moskaleva, T.N., Tikhonova, N.T., Chitadze, N., Forbi, J.C., Fancye, A.O., Otegbayo, J.A., Charpentier, E. & Muller, C.P. (2009) Phylogenetic analysis of human parvovirus b19 sequences from eleven different countries confirms the predominance of genotype 1 and suggests the spread of genotype 3b. *Journal of Clinical Microbiology*, 47, 3735–3738.

Jones, M.S., Kapoor, A., Lukashov, V.V., Simmonds, P., Hecht, F. & Delwart, E. (2005) New DNA viruses identified in patients with acute viral infection syndrome. *Journal of Virology*, 79, 8230–8236.

Kantola, K., Sadeghi, M., Antikainen, J., Kirveskari, J., Delwart, E., Hedman, K. & Soderlund-Venermo, M. (2010) Real-time quantitative PCR detection of four human bocaviruses. *Journal of Clinical Microbiology*, 48, 4044–4050.

Kantola, K., Hedman, L., Arthur, J., Alibeto, A., Delwart, E., Jartti, T., Ruuskanen, O., Hedman, K. & Söderlund-Venermo, M. (2011) Seroepidemiology of human bocaviruses 1–4. *Journal of Infectious Diseases*, 9, 1403–1412.

Kapoor, A., Slikas, E., Simmonds, P., Chieochansin, T., Naeem, A., Shaukat, S., Alam, M.M., Sharif, S., Angez, M., Zaidi, S. & Delwart, E. (2009) A newly identified bocavirus species in human stool. *The Journal of Infectious Diseases*, 199, 196–200.

Kapoor, A., Simmonds, P., Slikas, E., Li, L., Bodhidatta, L., Sethabutr, O., Triki, H., Bahri, O., Oderinde, B.S., Baba, M.M., Bukbuk, D.N., Besser, J., Bartkus, J. & Delwart, E. (2010) Human bocaviruses are highly diverse, dispersed, recombination prone, and prevalent in enteric infections. *The Journal of Infectious Diseases*, 201, 1633–1643.

Ke, L., He, M., Li, C., Liu, Y., Gao, L., Yao, F., Li, J., Bi, X., Lv, Y., Wang, J., Hirsch, M.L. & Li, W. (2011) The prevalence of human parvovirus B19 DNA and antibodies in blood donors from four Chinese blood centers. *Transfusion*, 51, 1909–1918.

Keller, L.W., Barbosa, M.L., Melo, F.L., Pereira, L. M., David-Neto, E., Lanhez, L.E. & Durigon, E. L. (2009) Phylogenetic analysis of a near-full-length sequence of an erythrovirus genotype 3 strain isolated in Brazil. *Archives of Virology*, 154, 1685–1687.

Kishore, J., Srivastava, M. & Choudhary, N. (2010) Standardization of B19 IgG ELISA to study the seroepidemiology of parvovirus B19 in North Indian voluntary blood donors. *Asian Journal of Transfusion Science*, 2, 86–90.

Kleinman, S.H., Glynn, S.A., Lee, T.H., Tobler, L., Montalvo, L., Todd, D., Kiss, J.E., Shyamala, V. & Busch, M.P.National Heart, Lung, Blood Institute Retrovirus Epidemiology Donor Study (REDS-II) (2007) Prevalence and quantitation of parvovirus B19 DNA levels in blood donors with a sensitive polymerase chain reaction screening assay. *Transfusion*, 10, 1756–1764.

Koduri, P.R., Kumapley, R., Valladares, J. & Teter, C. (1999) Chronic pure red cell aplasia caused by parvovirus B19 in AIDS: use of intravenous immunoglobulin – a report of eight patients. *American Journal of Hematology*, 61, 16–20.

Koenigbauer, U.P., Eastlund, T. & Day, J.W. (2000) Clinical illness due to parvovirus B19 infection after infusion of solvent/detergent-treated pooled plasma. *Transfusion*, 40, 1203–1206.

Koppelman, M.H., Cuypers, H.T., Emrich, T. & Zaaijer, H.L. (2004) Quantitative real-time detection of parvovirus B19 DNA in plasma. *Transfusion*, 44, 97–103.

Koppelman, M.H., Rood, I.G., Fryer, J.F., Baylis, S. A. & Cuypers, H.T. (2007) Parvovirus B19 genotypes 1 and 2 detection with real-time polymerase chain reaction assays. *Vox Sanguinis*, 93, 208–215.

Koppelman, M.H., van Swieten, P. & Cuijpers, H. T. (2011) Real-time polymerase chain reaction detection of parvovirus B19 DNA in blood donations using a commercial and an in-house assay. *Transfusion*, 51, 1346–1354.

Koppelman, M.H., Cuijpers, H.T., Wessberg, S., Valkeajarvi, A., Pichl, L., Schottstedt, V. & Saldanha, J. (2012) Multicenter evaluation of a commercial multiplex polymerase chain reaction test for screening plasma donations for parvovirus B19 DNA and hepatitis A virus RNA. *Transfusion*, 7, 1498–1508.

Kurtzman, G.J., Cohen, B., Meyers, P., Amunullah, A. & Young, N.S. (1988) Persistent B19 parvovirus infection as a cause of severe chronic anaemia in children with acute lymphocytic leukaemia. *Lancet*, 2, 1159–1162.

Kurtzman, G., Frickhofen, N., Kimball, J., Jenkins, D.W., Nienhuis, A.W. & Young, N.S. (1989) Pure red-cell aplasia of 10 years' duration due to persistent parvovirus B19 infection and its cure with immunoglobulin therapy. *New England Journal of Medicine*, 321, 519–523.

Lahtinen, A., Kivela, P., Hedman, L., Kumar, A., Kantele, A., Lappalainen, M., Liitsola, K., Ristola, M., Delwart, E., Sharp, C., Simmonds, P., Soderlund-Venermo, M. & Hedman, K. (2011) Serodiagnosis of primary infections with human parvovirus 4, Finland. *Emerging Infectious Diseases*, 17, 79–82.

Longhi, E., Bestetti, G., Acquaviva, V., Foschi, A., Piolini, R., Meroni, L., Magni, C., Antinori, S., Parravicini, C. & Corbellino, M.(2007) Human parvovirus 4 in the bone marrow of italian patients with AIDS. *AIDS* (London, England), 21, 1481–1483.

Lurcharchaiwong, W., Chieochansin, T., Payungporn, S., Theamboonlers, A. & Poovorawan, Y. (2008) Parvovirus 4 (PARV4) in serum of intravenous drug users and blood donors. *Infection*, 36, 488–491.

Ma, Y.Y., Guo, Y., Zhao, X., Wang, Z., Lv, M.M., Yan, Q.P. & Zhang, J.G. (2012) Human parvovirus PARV4 in plasma pools of Chinese origin. *Vox Sanguinis*, doi: 10.1111/j.1423-0410.2012.01605.x. [Epub ahead of print].

Makris, M., Preston, F.E., Rosendaal, F.R., Underwood, J.C., Rice, K.M. & Triger, D.R. (1996) The natural history of chronic hepatitis C in haemophiliacs. *British Journal of Haematology*, 94, 746–752.

Makris, M., Calizzani, G., Fischer, K., Gilman, E. A., Hay, C.R.M., Lambert, T., Lassila, R., Ludlam, C.A. & Mannucci, P.M. (2011) EUHASS: The European Haemophilia Safety Surveillance system. *Thrombosis Research*, 127, 22–25.

Manning, A., Willey, S.J., Bell, J.E. & Simmonds, P. (2007) Comparison of tissue distribution, persistence, and molecular epidemiology of parvovirus B19 and novel human parvoviruses PARV4 and human bocavirus. *The Journal of Infectious Diseases*, 195, 1345–1352.

Mannucci, P.M. & Tuddenham, E.G. (2001) The hemophilias – from royal genes to gene therapy. *The New England Journal of Medicine*, 344, 1773–1779.

Meriluoto, M., Hedman, L., Tanner, L., Simell, V., Makinen, M., Simell, S., Mykkanen, J., Korpelainen, J., Ruuskanen, O., Ilonen, J., Knip, M., Simell, O., Hedman, K. & Soderlund-Venermo, M. (2012) Association of human bocavirus 1 infection with respiratory disease in childhood follow-up study, Finland. *Emerging Infectious Diseases*, 18, 264–271.

Millar, C.M., Connor, N., Dolan, G., Lee, C.A., Makris, M., Wilde, J., Winter, M., Ironside, J.W., Gill, N. & Hill, F.G. (2010) Risk reduction strategies for variant creutzfeldt-jakob disease transmission by UK plasma products and their impact on patients with inherited bleeding disorders. *Haemophilia: The Official Journal of the World Federation of Hemophilia*, 16, 305–315.

Modrow, S., Wenzel, J.J., Schimanski, S., Schwarzbeck, J., Rothe, U., Oldenburg, J., Jilg, W. & Eis-Hubinger, A.M. (2011) Prevalence of nucleic acid sequences specific for human parvoviruses, hepatitis A and hepatitis E viruses in coagulation factor concentrates. *Vox Sanguinis*, 100, 351–358.

Mossong, J., Hens, N., Friederichs, V., Davidkin, I., Broman, M., Litwinska, B., Siennicka, J., Trzcinska, A., VAN Damme, P., Beutels, P., Vyse, A., Shkedy, Z., Aerts, M., Massari, M. & Gabutti, G. (2008) Parvovirus B19 infection in five European countries: seroepidemiology, force of infection and maternal risk of infection. *Epidemiology and Infection*, 136, 1059–1068.

Naides, S.J., Scharosch, L.L., Foto, F. & Howard, E.J. (1990) Rheumatologic manifestations of

human parvovirus B19 infection in adults. initial two-year clinical experience. *Arthritis and Rheumatism*, 33, 1297–1309.

Nguyen, Q.T., Sifer, C., Schneider, V., Allaume, X., Servant, A., Bernaudin, F., Auguste, V. & Garbarg-Chenon, A. (1999) Novel human erythrovirus associated with transient aplastic anemia. *Journal of Clinical Microbiology*, 37, 2483–2487.

Nguyen, Q.T., Wong, S., Heegaard, E.D. & Brown, K.E. (2002) Identification and characterization of a second novel human erythrovirus variant, A6. *Virology*, 301, 374–380.

Norja, P., Hokynar, K., Aaltonen, L.M., Chen, R., Ranki, A., Partio, E.K., Kiviluoto, O., Davidkin, I., Leivo, T., Eis-Hubinger, A.M., Schneider, B., Fischer, H.P., Tolba, R., Vapalahti, O., Vaheri, A., Soderlund-Venermo, M. & Hedman, K. (2006) Bioportfolio: lifelong persistence of variant and prototypic erythrovirus DNA genomes in human tissue. *Proceedings of the National Academy of Sciences of the United States of America*, 103, 7450–7453.

Norja, P., Hedman, L., Kantola, K., Kemppainen, K., Suvilehto, J., Pitkäranta, A., Aaltonen, L.M., Seppänen, M., Hedman, K. & Söderlund-Venermo, M. (2012) Occurrence of human bocaviruses and parvovirus 4 in solid tissues. *Journal of Medical Virology*, 8, 1267–1273.

Panning, M., Kobbe, R., Vollbach, S., Drexler, J.F., Adjei, S., Adjei, O., Drosten, C., May, J. & Eis-Hubinger, A.M. (2010) Novel human parvovirus 4 genotype 3 in infants, Ghana. *Emerging Infectious Diseases*, 16, 1143–1146.

Pattison, J.R., Jones, S.E., Hodgson, J., Davis, L.R., White, J.M., Stroud, C.E. & Murtaza, L. (1981) Parvovirus infections and hypoplastic crisis in sickle-cell anaemia. *Lancet*, 1, 664–665.

Peden, A., McCardle, L., Head, M.W., Love, S., Ward, H.J., Cousens, S.N., Keeling, D.M., Millar, C.M., Hill, F.G. & Ironside, J.W. (2010) Variant CJD infection in the spleen of a neurologically asymptomatic UK adult patient with haemophilia. *Haemophilia: The Official Journal of the World Federation of Hemophilia*, 16, 296–304.

Reid, D.M., Reid, T.M., Brown, T., Rennie, J.A. & Eastmond, C.J. (1985) Human parvovirus-associated arthritis: a clinical and laboratory description. *Lancet*, 1, 422–425.

Richardson, L.C. & Evatt, B.L. (2000) Risk of hepatitis A virus infection in persons with hemophilia receiving plasma-derived products. *Transfusion Medicine Reviews*, 14, 64–73.

Saal, J.G., Steidle, M., Einsele, H., Muller, C.A., Fritz, P. & Zacher, J. (1992) Persistence of B19 parvovirus in synovial membranes of patients with rheumatoid arthritis. *Rheumatology International*, 12, 147–151.

Sanabani, S., Neto, W.K., Pereira, J. & Sabino, E.C. (2006) Sequence variability of human erythroviruses present in bone marrow of brazilian patients with various parvovirus B19-related hematological symptoms. *Journal of Clinical Microbiology*, 44, 604–606.

Schmidt, I., Blumel, J., Seitz, H., Willkommen, H. & Lower, J. (2001) Parvovirus B19 DNA in plasma pools and plasma derivatives. *Vox Sanguinis*, 81, 228–235.

Schneider, B., Becker, M., Brackmann, H.H. & Eis-Hubinger, A.M. (2004) Contamination of coagulation factor concentrates with human parvovirus B19 genotype 1 and 2. *Thrombosis and Haemostasis*, 92, 838–845.

Schneider, B., Fryer, J.F., Reber, U., Fischer, H.P., Tolba, R.H., Baylis, S.A. & Eis-Hubinger, A.M. (2008a) Persistence of novel human parvovirus PARV4 in liver tissue of adults. *Journal of Medical Virology*, 80, 345–351.

Schneider, B., Fryer, J.F., Oldenburg, J., Brackmann, H.H., Baylis, S.A. & Eis-Hubinger, A.M. (2008b) Frequency of contamination of coagulation factor concentrates with novel human parvovirus PARV4. *Haemophilia: The Official Journal of the World Federation of Hemophilia*, 14, 978–986.

Servant-Delmas, A., Laperche, S., Mercier, M., Eighouzzi, M.H., Lionnet, F., Stankovic, K., Girot, R. & Lefrere, J.J. (2009) Human parvovirus 4 in recipients of cellular products and in blood donors: epidemiologic similarity with B19 parvovirus. *Transfusion*, 49, 1771–1773.

Sharp, C.P., Lail, A., Donfield, S., Simmons, R., Leen, C., Klenerman, P., Delwart, E., Gomperts, E.D. & Simmonds, P. (2009) High frequencies of exposure to the novel human parvovirus PARV4 in hemophiliacs and injection drug users, as detected by a serological assay for PARV4 antibodies. *The Journal of Infectious Diseases*, 200, 1119–1125.

Sharp, C.P., Lail, A., Donfield, S., Gomperts, E.D. & Simmonds, P. (2012) Virologic and clinical features of primary infection with human parvovirus 4 in subjects with hemophilia: frequent transmission by virally inactivated clotting factor concentrates. *Transfusion*, 52, 1482–1489.

Siegl, G. & Cassinotti, P. (1998) Presence and significance of parvovirus B19 in blood and blood products. *Biologicals: Journal of the International Association of Biological Standardization*, 26, 89–94.

Simmonds, P., Manning, A., Kennell, R., Carnie, F.W. & Bell, J.E. (2007) Parenteral transmission of the novel human parvovirus PARV4. *Emerging Infectious Diseases*, 13, 1386–1388.

Simmonds, P., Douglas, J., Bestetti, G., Longhi, E., Antinori, S., Parravicini, C. & Corbellino, M. (2008) A third genotype of the human parvovirus PARV4 in sub-saharan africa. *The Journal of General Virology*, 89, 2299–2302.

Soderlund, M., von Essen, R., Haapasaari, J., Kiistala, U., Kiviluoto, O. & Hedman, K. (1997) Persistence of parvovirus B19 DNA in synovial membranes of young patients with and without chronic arthropathy. *Lancet*, 349, 1063–1065.

Soderlund-Venermo, M., Hokynar, K., Nieminen, J., Rautakorpi, H. & Hedman, K. (2002) Persistence of human parvovirus B19 in human tissues. *Pathologie-Biologie*, 50, 307–316.

Soderlund-Venermo, M., Lahtinen, A., Jartti, T., Hedman, L., Kemppainen, K., Lehtinen, P., Allander, T., Ruuskanen, O. & Hedman, K. (2009) Clinical assessment and improved diagnosis of bocavirus-induced wheezing in children, Finland. *Emerging Infectious Diseases*, 15, 1423–1430.

Soucie, J.M., Erdman, D.D., Evatt, B.L., Anderson, L.J., Torok, T.J., El-Jamil, M., Barnhart, E., Tepper, M., Burrill, H.N., Pickett, A.M. & Mengeling, W.L. (2000) Investigation of porcine parvovirus among persons with hemophilia receiving hyate: C porcine factor VIII concentrate. *Transfusion*, 40, 708–711.

Soucie, J.M., De Staercke, C., Chitlu, R.M.B., Gruppo, R.A., Hooper, W.C., Kessler, C.M., Roshni Kulkarni, R., Manco-Johnson, M.J., Monahan, P.E., Oakley, Meredith., Powell, J., Pyle, M., Recht, M., Riske, B., Sabio, H. & Trimble, S. (2011) Continued Transmission of Parvovirus B19 in Plasma-Derived Factor Concentrates After the Implementation of B19 Nucleic Acid Plasma Minipool Screening. *Blood (ASH Annual Meeting Abstracts)*, 118, 3378.

Szelei, J., Liu, K., Li, Y., Fernandes, S. & Tijssen, P. (2010) Parvovirus 4-like virus in blood products. *Emerging Infectious Diseases*, 16, 561–564.

Szomor, K.N., Kapusinszky, B., Rigo, Z., Kis, Z., Rozsa, M., Farkas, A., Szilagyi, A., Berencsi, G. & Takacs, M. (2009) Detection of human bocavirus from fecal samples of Hungarian children with acute gastroenteritis. *Intervirology*, 52, 17–21.

Touinssi, M., Brisbarre, N., Picard, C., Frassati, C., Dussol, B., Uch, R., Gallian, P., Cantaloube, J.F., Micco, P. & Biagini, P. (2010) Parvovirus 4 in blood donors, France. *Emerging Infectious Diseases*, 16, 165–166.

Vallerini, D., Barozzi, P., Quadrelli, C., Bosco, R., Potenza, L., Riva, G., Gregorini, G., Sandrini, S., Tironi, A., Montagnani, G., De Palma, M., Torelli, G., Delwart, E. & Luppi, M. (2008) Parvoviruses in blood donors and transplant patients, Italy. *Emerging Infectious Diseases*, 14, 185–186.

White, D.G., Woolf, A.D., Mortimer, P.P., Cohen, B.J., Blake, D.R. & Bacon, P.A. (1985) Human parvovirus arthropathy. *Lancet*, 1, 419–421.

Willkommen, H., Schmidt, I. & Lower, J. (1999) Safety issues for plasma derivatives and benefit from NAT testing. *Biologicals: Journal of the International Association of Biological Standardization*, 27, 325–331.

Yu, J.M., Li, D.D., Xu, Z.Q., Cheng, W.X., Zhang, Q., Li, H.Y., Cui, S.X., Miao-Jin, ?., Yang, S.H., Fang, Z.Y. & Duan, Z.J. (2008) Human bocavirus infection in children hospitalized with acute gastroenteritis in China. *Journal of Clinical Virology: The Official Publication of the Pan American Society for Clinical Virology*, 42, 280–285.

Yu, X., Zhang, J., Hong, L., Wang, J., Yuan, Z., Zhang, X. & Ghildyal, R. (2012) High prevalence of human parvovirus 4 infection in HBV and HCV infected individuals in shanghai. *PLoS ONE*, 7, e29474.

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
				2012. 10. 20	該当なし	

一般的名称	新鮮凍結人血漿	研究報告の公表状況	Emerging Infectious Disease Journal, Vol.18 No.10; Available from: http://wwwnc.cdc.gov/eid/article/18/10/11-1373_article.htm	公表国 ドイツ
販売名(企業名)	新鮮凍結血漿-LR「日赤」(日本赤十字社) 新鮮凍結血漿-LR「日赤」成分採血(日本赤十字社) 新鮮凍結血漿-LR「日赤」120(日本赤十字社) 新鮮凍結血漿-LR「日赤」240(日本赤十字社) 新鮮凍結血漿-LR「日赤」480(日本赤十字社)			

研究報告の概要

○ガーナの小児の鼻腔及び糞便検体から分離されたヒトパルボウイルス4

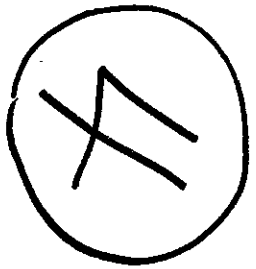
サハラ以南のアフリカ諸国では、経口伝播がヒトパルボウイルス4(PARV4)の感染に関与している可能性がある。ガーナにおいて、気道症状を呈する15歳未満の小児1,904人から鼻腔検体または糞便検体を採取し検査を行った結果、鼻腔検体961例中8例(0.83%)及び糞便検体943例中5例(0.53%)からPARV4 DNAが検出された。ウイルス濃度は、鼻腔検体では1.3×10^{3}～1.8×10^{7}コピー/mL(中央値1.0×10^{4}コピー/mL)、糞便検体では2.3×10^{3}～4.6×10^{6}コピー/mL(中央値6.8×10^{4}コピー/mL)であり、全てPARV4ジェノタイプ3と分類された。ウイルス濃度が約6－7$\log_{10}$コピー/mLの場合もあり、PARV4感染経路として気道あるいは糞口経路が示唆される。

報告企業の意見	今後の対応
ガーナにおいて気道症状を呈する小児の鼻腔及び糞便検体からPARV4が検出され、PARV4感染経路として経口経路が示唆されるとの報告である。	ヒトパルボウイルス4の病原性について未だ詳細は不明であるが、今後も本ウイルスについての情報収集に努める。

使用上の注意記載状況・その他参考事項等

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

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク



Human Parvovirus 4 in Nasal and Fecal Specimens from Children, Ghana

Jan Felix Drexler, Ulrike Reber, Doreen Muth, Petra Herzog, Augustina Annan, Fabian Ebach, Nimarko Sarpong, Samuel Acquah, Julia Adlkofer, Yaw Adu-Sarkodie, Marcus Panning, Egbert Tannich, Jürgen May, Christian Drosten, and Anna Maria Eis-Hübinger

Nonparenteral transmission might contribute to human parvovirus 4 (PARV4) infections in sub-Saharan Africa. PARV4 DNA was detected in 8 (0.83%) of 961 nasal samples and 5 (0.53%) of 943 fecal samples from 1,904 children in Ghana. Virus concentrations ≈6–7 $\log_{10}$ copies/mL suggest respiratory or fecal–oral modes of PARV4 transmission.

Human parvovirus 4 (PARV4; human partetravirus) is a single-stranded DNA virus discovered in 2005 (*1*). PARV4 has been detected in persons at risk for parenteral infections, suggesting blood-borne transmission (*2,3*) although other transmission routes have not been ruled out. Studies in northern Europe demonstrated a high prevalence of antibodies against PARV4 in injection drug users, persons co-infected with HIV and hepatitis C virus, and persons with hemophilia who were exposed to nonvirally inactivated clotting factors; however, antibodies were not detected in the general population (*4,5*).

In contrast, PARV4 seroprevalence was 25%–37% in adults in the Democratic Republic of Congo, Cameroon, and Burkina Faso who were not infected with HIV and hepatitis C virus. (*6*). PARV4 DNA was detected in blood of 8.6% of children 15 or 24 months of age in Ghana (*7*). There was no history of exposure to multiple-use needles or blood transfusion in any of these children. These data suggested alternative modes of PARV4 transmission in countries in Africa. Nonparenteral modes of transmission of PARV4 have also been suggested in South Africa (*6*), Taiwan (*8*), India, (*9*), China (*10*), and Thailand (*11*).

PARV4 has been classified into 3 genotypes. Genotypes 1 and 2 are found in North America, Europe, and Asia (*1–3,9–11*), and genotype 3 is found in in sub-Saharan Africa (*7,12*). To investigate whether PARV4 is found in the respiratory or intestinal tract, we analyzed previously collected specimens from 1,904 children in Ghana.

The Study

Ethical approval for this study was provided by the Committee on Human Research Publication and Ethics, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana. Informed consent was obtained from parents or guardians of all children.

A total of 1,904 anonymous nasal and fecal specimens were obtained during a study on molecular diagnostics for respiratory and enteric tract infections in symptomatic children <15 years of age at the Presbyterian Hospital in Agogo, Ghana. Nasal swab specimens were obtained from children with upper or lower respiratory tract symptoms. Fecal samples were obtained from 504 children with gastrointestinal symptoms (53.4% of sampled children; 294 [58.3%] of symptomatic children with vomiting, 190 [37.7%] with diarrhea, and 144 [28.6%] with acute malnutrition; 9 [1.8%] with incomplete clinical data) and 439 (46.6%) children without gastrointestinal symptoms.

A total of 961 nasal swabs were obtained during February–November 2008 from 520 boys and 441 girls (median age 19 months, range 0–162 months, interquartile range 8–38 months). Nasal swabs were placed in 1.5 mL of RNAlater (QIAGEN, Hilden, Germany). A total of 943 fecal samples were obtained during May–October 2009 from 500 boys and 443 girls (median age 36 months, range 0–165 months, interquartile range 17–78 months). Fecal samples were prepared as 10% suspensions in phosphate-buffered saline. No paired nasal and fecal specimens were available from individual patients.

Viral DNA was purified from 140 µL of nasal swab suspension or 200 µL of fecal suspension by using QIAamp Viral RNA and DNA Stool Mini Kits (QIAGEN), respectively. Two real-time PCRs were performed. One primer/probe set was designed to detect PARV4 genotypes 1 or 2 viruses (*13*), and a second primer set was designed to detect PARV4 genotype 3 viruses (*7*). The sensitivity of both protocols was 1–2 genome copies/reaction. Absolute quantification of PARV4 genome copy numbers relied on photometrically quantified genotype 3 plasmid standards, as described (*7*).

To exclude bias from DNA purification methods, PARV4-negative nasal and fecal specimens were spiked with quantified plasmid standards. Subsequent

Author affiliations: University of Bonn Medical Centre, Bonn, Germany (J.F. Drexler, U. Reber, D. Muth, A. Annan, F. Ebach, C. Drosten, A.M. Eis-Hübinger); Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany (P. Herzog, J. Adlkofer, E. Tannich, J. May); Kumasi Centre for Collaborative Research in Tropical Medicine, Kumasi, Ghana (A. Annan, N. Sarpong, S. Acquah); Kwame Nkrumah University of Science and Technology, Kumasi (Y. Adu-Sarkodie); and Freiburg University Medical Center, Freiburg, Germany (M. Panning)

DOI: http://dx.doi.org/10.3201/eid1810.111373

Table. Nucleotide sequence divergence of parvovirus 4 strains from nasal swab and fecal samples from children, Ghana, from genotype 1, 2, and 3 prototype strains*

Specimen type and no.	Nucleotide position according to GenBank accession no. EU874248	Nucleotide sequence divergence from parvovirus 4 reference strains, %		
		Genotype 1 (GenBank AY622943)	Genotype 2 BR10627–5 (GenBank DQ873390)	Genotype 3 NG-OR (GenBank EU874248)
Nasal swab				
N1	1700–4660	6.56	7.39	0.92
N2	299–4660	7.51	8.07	0.88
N3	50–4660	7.37	8.38†	0.83
N4	1962–2056‡	9.16	6.73	2.14
N4	2117–3413	4.97	5.31	0.93
N5	1962–2056	9.16	6.73	2.14
N5	2117–4183	5.50	6.34	0.98
N6	299–4660	7.51	8.10	0.90
N7	1962–2056	9.16	6.73	2.14
N7	2431–2914	6.24	7.01	1.25
N7	3068–3246	4.61	5.19	1.12
N8	624–3246	7.36	7.84	0.84
Feces				
F1	1700–4183	6.20	6.82	0.89
F2	1700–4460	6.56	7.39	0.92
F3	1700–3716	6.08	6.52	0.85
F4	1700–4183	6.02	6.78	0.89
F5	1700–4183	6.93	6.73	1.04

*Pairwise nucleotide divergence was calculated by using the DNA distance matrix in BioEdit (www.mbio.ncsu.edu/BioEdit/bioedit.html).
†Because the homologs of the first 92 nt of strain N3 are not given in the prototype strain BR10627–5, calculation of divergence started at N3 nt position 93.
‡Nucleotide sequence of the PCR product (primer sequences trimmed) was amplified by using screening PCR designed for detection of PARV4 genotype 3 as described (7).

quantification was equivalent between techniques and specimens, and differences between specimen types in several experiments were <0.5 $\log_{10}$ copies/mL. Standard procedures were used to prevent PCR contamination. Determination of PARV4 genotypes was conducted by nucleotide sequencing of several genomic target regions (Table).

Eight (0.83%) of 961 nasal swabs and 5 (0.53%) of 943 fecal samples tested were positive for PARV4 DNA. Virus concentrations ranged from 1.3×10^3 to 1.8×10^7 copies/mL (median 1.0×10^4 copies/mL) in nasal swab suspensions and from 2.3×10^3 to 4.6×10^6 copies/mL (median 6.8×10^4 copies/mL) in fecal suspensions (Figure 1). The difference in virus concentrations between the 2 groups was not significant ($p = 0.056$, by Mann-Whitney U test).

Nucleotide sequencing of amplicons generated by screening PCRs and sequencing of additional genomic regions classified all viruses as PARV4 genotype 3 (Table) (GenBank accession numbers JN183920–JN183932). This result was confirmed by phylogenetic analysis of a 483-nt fragment of the capsid-encoding open reading frame 2 (Figure 2).

Ages of the 8 children with PARV4-positive nasal swab specimens ranged from 9 to 58 months (median 32 months). Ages of the 5 children with PARV4-positive fecal samples were 1, 36, 43, 57, and 124 months. Nasal swab specimens with the highest viral loads were from a 9-month-old boy and a 29-month-old girl. Fecal samples with the highest viral loads were from 2 boys 43 and 57 months of age.

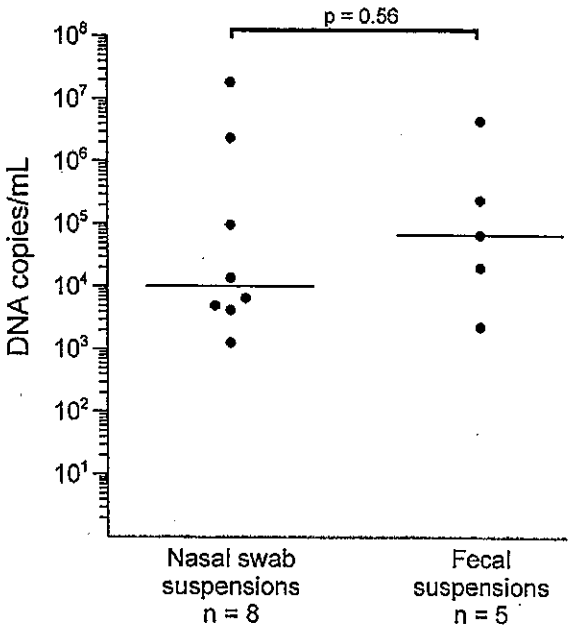


Figure 1. Parvovirus 4 DNA loads in virus-positive nasal and fecal specimens from children, Ghana. Virus concentrations are given on a log scale on the y-axis. Each dot represents 1 specimen. Horizontal lines represent median values for each sample type. For calculation of statistical significance of the difference in viral quantities between sample types, the Mann-Whitney U test was used. Virus quantities in nasal swabs and feces are given for sample suspensions (nasal swabs in 1.5 mL of stabilizing reagent and feces in a 10% suspension in phosphate-buffered saline).

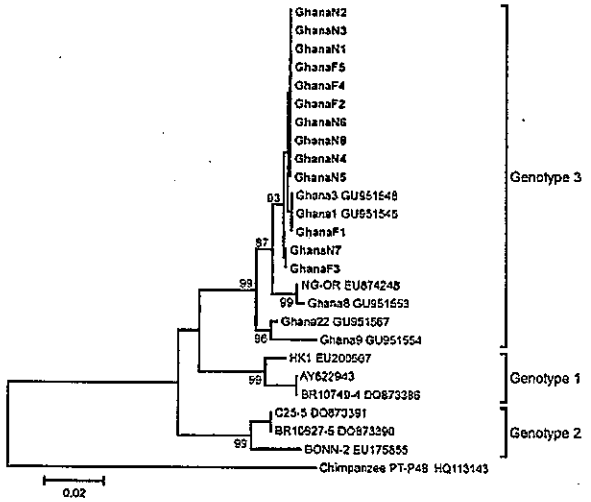


Figure 2. Phylogenetic analysis of a 483-nt fragment of the parvovirus 4 (PARV4) capsid-encoding open reading frame (ORF) 2 for PARV4 strains identified in children, Ghana. Neighbor-joining phylogeny was conducted in MEGA5.05 (www.megasoftware.net) by using a gap-free ORF2 fragment corresponding to positions 2,432–2,914 in the PARV4 genotype 3 prototype strain NG-OR (GenBank accession no. EU874248) with a nucleotide percentage distance substitution model and 1,000 bootstrap replicates. Scale bar indicates percentage uncorrected nucleotide distance. Previously published PARV4 sequences are given with strain names (if available) and GenBank accession numbers. Viruses newly identified are in **boldface**. The source of PARV4 strains identified in the study is indicated by capital letters (N, nasal specimen; F, fecal specimen). PARV4 genotypes are given to the right of taxa. A chimpanzee partetravirus was used as the outgroup.

Conclusions

We found PARV4 in 0.8% of nasal swab specimens and 0.5% of fecal specimens from 2 groups of children in Ghana symptomatic for respiratory illness and with or without diarrheal illness, respectively. Our results provide evidence to suggest that the higher prevalence of PARV4 reported among adults in countries in western Africa (*6*) might be caused by transmission by the respiratory or fecal–oral route.

However, demonstration of PARV4 in the respiratory tract and feces does not identify a transmission route. PARV4 in the respiratory tract could be caused by high viremia, which was recently reported in a child in India with a genotype 2 infection (*9*) and in 2 patients with hemophilia in the United Kingdom, 1 with a genotype 1 infection and 1 with a genotype 2 infection (*14*).

It is unclear to what extent the putative nonparenteral transmission routes of PARV4 genotype 3 in western Africa apply to other areas. Markedly lower PARV4 antibody prevalences observed in Europe (*4,5*) argue against PARV4 spread by nonparenteral routes, e.g., from infected injection drug users to the general population. Likewise, the higher prevalence of PARV4 antibodies in HIV-infected blood donors in South Africa compared with uninfected donors (*6*) appears incompatible with PARV4 transmission primarily by the respiratory route. Therefore, our results do not contradict those of a study conducted in Scotland, which showed no PARV4 in respiratory specimens (*15*).

Because of the small number of children with PARV4 DNA in nasal or fecal specimens, correlation of infection with age groups was not possible. A limitation of our study was the lack of blood specimens from children with current respiratory or fecal PARV4 shedding, and serologic studies are needed to evaluate susceptibility of different age groups to PARV4 infection. Furthermore, detection of PARV4 in patients with respiratory disease does not indicate that PARV4 was the cause of the disease. In 5 of 8 PARV4-positive nasal swabs, typical respiratory viruses (parainfluenza virus, influenza A virus, rhinovirus) were also detected and the pattern of symptoms in PARV4-positive children did not differ from symptoms in PARV4-negative children. Similarly, 3 of 5 children with PARV4-positive feces did not have gastrointestinal symptoms at the time of fecal sampling. One child had vomiting and another child had vomiting and diarrhea. Moreover, in 3 of these 5 children, in addition to PARV4, *Giardia lamblia*, a potential cause of diarrhea, was also detected.

Although data for exposure and risk factors and paired samples were not available, suggested transmission routes might explain the high infection rates in western Africa. Further studies are needed to assess the effect of PARV4 excretion on virus epidemiology and the chronology of PARV4 infection.

Acknowledgments

We thank the children and their parents for participating in the study and Carmen Poster for excellent technical assistance.

This study was supported by grants from the Union Bank of Switzerland Optimus Foundation to E.T., J.M., C.D. and Y.A.; European Union project European Management Platform for Emerging and Re-emerging Infectious Disease Entities (grant 223498); the German Academic Exchange Service to J.A.; the German Research Foundation (grant DR 772/3-1); and BONFOR to A.M.E.-H. (grant O-151.0021).

Dr Drexler is a physician and clinical virologist at the University of Bonn. His research interest is characterization of novel human and zoonotic viruses.

References

1. Jones MS, Kapoor A, Lukashov VV, Simmonds P, Hecht F, Delwart E. New DNA viruses identified in patients with acute viral infection syndrome. J Virol. 2005;79:8230–6. http://dx.doi.org/10.1128/JVI.79.13.8230-8236.2005

2. Fryer JF, Lucas SB, Padley D, Baylis SA. Parvoviruses PARV4/5 in hepatitis C virus–infected patient. Emerg Infect Dis. 2007;13:175–6. http://dx.doi.org/10.3201/eid1301.060856
3. Simmonds P, Manning A, Kenneil R, Carnie FW, Bell JE. Parenteral transmission of the novel human parvovirus PARV4. Emerg Infect Dis. 2007;13:1386–8. http://dx.doi.org/10.3201/eid1309.070428
4. Sharp CP, Lail A, Donfield S, Simmons R, Leen C, Klenerman P, et al. High frequencies of exposure to the novel human parvovirus PARV4 in hemophiliacs and injection drug users, as detected by a serological assay for PARV4 antibodies. J Infect Dis. 2009;200:1119–25. http://dx.doi.org/10.1086/605646
5. Lahtinen A, Kivela P, Hedman L, Kumar A, Kantele A, Lappalainen M, et al. Serodiagnosis of primary infections with human parvovirus 4, Finland. Emerg Infect Dis. 2011;17:79–82. http://dx.doi.org/10.3201/eid1701.100750
6. Sharp CP, Vermeulen M, Nebie Y, Djoko CF, LeBreton M, Tamoufe U, et al. Epidemiology of human parvovirus 4 infection in sub-Saharan Africa. Emerg Infect Dis. 2010;16:1605–7. http://dx.doi.org/10.3201/eid1701.100750
7. Panning M, Kobbe R, Vollbach S, Drexler JF, Adjei S, Adjei O, et al. Novel human parvovirus 4 genotype 3 in infants, Ghana. Emerg Infect Dis. 2010;16:1143–6. http://dx.doi.org/10.3201/eid1607.100025
8. Yang SJ, Hung CC, Chang SY, Lee KL, Chen MY. Immunoglobulin G and M antibodies to human parvovirus 4 (PARV4) are frequently detected in patients with HIV-1 infection. J Clin Virol. 2011;51:64–7. http://dx.doi.org/10.1016/j.jcv.2011.01.017
9. Benjamin LA, Lewthwaite P, Vasanthapuram R, Zhao G, Sharp C, Simmonds P, et al. Human parvovirus 4 as potential cause of encephalitis in children, India. Emerg Infect Dis. 2011;17:1484–7. http://dx.doi.org/10.3201/eid1708.110165
10. Yu X, Zhang J, Hong L, Wang L, Yuan Z, Zhang X, et al. High prevalence of human parvovirus 4 infection in HBV and HCV infected individuals in Shanghai. PLoS ONE. 2012;7:e29474. http://dx.doi.org/10.1371/journal.pone.0029474
11. Lurcharchaiwong W, Chieochansin T, Payungporn S, Theamboonlers A, Poovorawan Y. Parvovirus 4 (PARV4) in serum of intravenous drug users and blood donors. Infection. 2008;36:488–91. http://dx.doi.org/10.1007/s15010-008-7336-4
12. Simmonds P, Douglas J, Bestetti G, Longhi E, Antinori S, Parravicini C, et al. A third genotype of the human parvovirus PARV4 in sub-Saharan Africa. J Gen Virol. 2008;89:2299–302. http://dx.doi.org/10.1099/vir.0.2008/001180-0
13. Fryer JF, Delwart E, Hecht FM, Bernardin F, Jones MS, Shah N, et al. Frequent detection of the parvoviruses, PARV4 and PARV5, in plasma from blood donors and symptomatic individuals. Transfusion. 2007;47:1054–61. http://dx.doi.org/10.1111/j.1537-2995.2007.01235.x
14. Sharp CP, Lail A, Donfield S, Gomperts ED, Simmonds P. Virologic and clinical features of primary infection with human parvovirus 4 in subjects with hemophilia: frequent transmission by virally inactivated clotting factor concentrates. Transfusion. 2012;52:1482–9. http://dx.doi.org/10.1111/j.1537-2995.2011.03420.x
15. Manning A, Russell V, Eastick K, Leadbetter GH, Hallam N, Templeton K, et al. Epidemiological profile and clinical associations of human bocavirus and other human parvoviruses. J Infect Dis. 2006;194:1283–90. http://dx.doi.org/10.1086/508219

Address for correspondence: Anna Maria Eis-Hübinger, Institute of Virology, University of Bonn Medical Centre, Sigmund-Freud-Str. 25, D-53127 Bonn, Germany; email: anna-maria.eis-huebinger@ukb.uni-bonn.de

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
				2012. 10. 20	該当なし	

一般的名称	新鮮凍結人血漿	研究報告の公表状況		公表国
販売名(企業名)	新鮮凍結血漿-LR「日赤」(日本赤十字社) 新鮮凍結血漿-LR「日赤」成分採血(日本赤十字社) 新鮮凍結血漿-LR「日赤」120(日本赤十字社) 新鮮凍結血漿-LR「日赤」240(日本赤十字社) 新鮮凍結血漿-LR「日赤」480(日本赤十字社)		E P Notari, J Brodsky, W R Steele, R Y Dodd, S L Stramer. AABB Annual Meeting&CTTXPO 2012; October 6-9, 2012, BOSTON.	米国

研究報告の概要

○大規模かつ様々な地域の供血者サンプルにおける2009-2011年のHTLV-I/IIの抗体陽性率

背景/症例報告: 近年、米国ではHTLV-I/IIの検査結果が陽性となる供血者数が減少しているが、感染は今も確認されており、性別と地域によって異なっている。今回、複数回供血者においてHTLV単回検査が実施可能か評価を行った。

研究デザイン/方法: 2009～11年、米国赤十字社で確認されたHTLV-I/II陽性供血者のデータを検索した。HTLV-I/II抗体検査に繰り返し反応を示した供血者は、EIAで確認し、IFA、ウェスタンブロット(WB)及びRIPAの併用で最終確認した。抗体陽性率は米国国勢調査の性別と地域分布に基づいて計算した。北東部(NE)、南部(S)、中西部(MW)、及び西部(W)の地域について、カイ二乗検定とボンフェローニ補正による多重比較を行った。罹患密度(ID)は、過去の供血歴が1095日(3年)を超えない供血者人年(PY)に対する抗体陽転者の人数とした。

結果/所見: 3年間で、HTLV陽性の443人を含む7,098,612人の同種血供血者からの1,900万を超える供血を調査した。HTLV供血の総抗体陽性率は10万人当たり2.3人であった。うち女性供血者は72%(443人中319人)、男性は28%で、抗体陽性率はそれぞれ3.6/10万人、1.2/10万人であった(p<0.0001、OR= 2.9、95%CI 2.4及び3.6)。全体として、地域及び供血者10万人当たりのHTLV 陽性供血者数は、NE対W(p=0.013)、MW対NE(p=0.002)、MW対W(p=0.0001)、MW対S(p=0.0001)で有意差があった;SとNE、SとW間では差は認められず、NWは抗体陽性率が最も低かった。複数回供血者のうち、36人がHTLV陽性であり、22人は3年を超えた供血、14人は3年以内の供血が陰性であった。同14人の総IDは0.18/10万人年(95%CI、0.10、0.30)であり、女性供血者(13人)のIDが0.34/10万人年(95%CI、0.18、0.58)、男性供血者(1人)が0.03/10万人年であった(95%CI、0.001、0.141)。14人中7人がHTLV-II、5人がHTLV-I/II、2人がHTLV-Iであった。14人中11人がIFA陽性(エンドポイント 1:64－2:1024)であり、残りの3人がWB/RIPAで確認された。

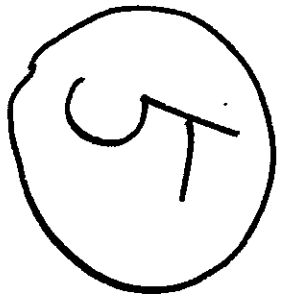
結論: HTLVの抗体陽性率と性別に有意な差が観察された。3年以内の新規感染14例が確認されたことから、HTLV単回抗体検査のみで供血者スクリーニングを行うことは有効とは言えない。

使用上の注意記載状況・その他参考事項等

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

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
大規模かつ様々な地域の供血者においてHTLV-I/IIの抗体陽性率を評価したところ、抗体陽性率と性別に有意な差が確認され、また複数回供血者におけるHTLV単回検査は、供血者スクリーニングとしては有効ではないことが明らかとなったとの報告である。	日本赤十字社では、献血時のスクリーニング法として、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い、確認検査としてウエスタンブロット法による検査を行っている。今後も引き続き情報の収集に努める。



Plenary

P1-030A

Transfused RBCs Can Be Immunogenic in Splenectomized Mice: of Inflammation, Adjuvants, and Anamnestic Responses

J E Hendrickson[1] (jeanne.hendrickson@emory.edu), S R Stowell[2], N H Smith[3], K Girard-Pierce[2], K Hudson[4], J C Zimring[5]. *[1]Pediatrics and Pathology, Emory University, Atlanta, GA, United States; [2]Pathology, Emory University, Atlanta, GA, United States; [3]Pediatrics, Emory University, Atlanta, GA, United States; [4]Scripps Research Institute, San Diego, CA, United States; [5]Puget Sound Blood Center, Seattle, WA, United States*

Background/Case Studies: Patients with thalassemia major may have higher rates of RBC alloimmunization than the general transfused population, and splenectomy has been reported to be associated with alloimmunization in this patient population. Previous murine studies with RBCs expressing the mHEL model antigen have shown that splenectomy largely abrogates alloimmunization. However, many factors (donor as well as recipient) may influence RBC alloimmunization, and we hypothesize that some RBC antigens under some conditions have the capacity to be immunogenic upon initial exposure in splenectomized animals. **Study Design/Methods:** Blood from donor mice expressing the HOD (lysozyme, ovalbumin, and human Duffy b), the hGPA (human glycophorin A), or the KEL2 (human Cellano) antigen on their RBCs was transfused into C57BL/6 or FVB recipients who had been surgically splenectomized; control animals were sham splenectomized. A subset of recipients were pretreated with poly (I:C) or CFA prior to transfusion, and a subset of recipients received multiple KEL2 transfusions. Two weeks after transfusion, alloimmunization (IgG, IgM) was assessed by flow cytometric crossmatch, utilizing transfused RBCs and antigen negative RBCs as targets. **Results/Findings:** No splenectomized mice transfused with HOD or KEL2 RBCs in the absence of induced inflammation made a detectable IgM or IgG alloantibody response (n = 3 experiments, 60 animals total). However, 100% of control, sham splenectomized animals made detectable alloantibodies, and control animals multiply transfused with KEL2 RBCs demonstrated a boostable response. In contrast to findings in the mHEL system, HOD, hGPA , and KEL2 expressing RBCs led to detectable alloantibodies following transfusion into recipients pretreated with CFA (HOD) or poly (I:C) (hGPA, KEL2) (n = 7 independent experiments, 70 animals total). **Conclusion:** Although a spleen plays a critical role in primary RBC alloimmune responses in mice transfused in their baseline state, a spleen is not essential for responses to 3 different RBC antigens when transfusion occurs in the presence of adjuvants or recipient inflammation. Under no studied condition, however, did splenectomized animals make higher levels of RBC alloantibodies than their control counterparts. It has been suggested that splenectomized thalassemia patients may have elevated IL-6 levels, and the presumed alteration in immune function in such patients may be involved in the reported findings; other factors to consider in this patient population include RBC transfusion burden, timing of initial RBC antigen exposure (pre vs post-splenectomy), and transfused RBC life span. A better understanding of the potential impact of splenectomy (be it surgical or autosplenectomy) on RBC alloimmunization may benefit thalassemia major and sickle cell anemia patients alike.

Disclosure of Commercial Conflict of Interest

K. Girard-Pierce: Nothing to disclose; J. E. Hendrickson: Nothing to disclose; K. Hudson: Nothing to disclose; N. H. Smith: Nothing to disclose; S. R. Stowell: No Answer; J. C. Zimring: Nothing to disclose

Disclosure of Grants Conflict of Interest

K. Girard-Pierce: Nothing to disclose; J. E. Hendrickson: Immucor, Grants or Research Support; K. Hudson: Nothing to disclose; N. H. Smith: Nothing to disclose; S. R. Stowell: No Answer; J. C. Zimring: Immucor, Grants or Research Support

P2-030A

HTLV-I/II Prevalence and Incidence from 2009-2011 in a Large, Geographically Diverse Sample of US Blood Donors

E P Notari[1] (ed.notari@redcross.org), J Brodsky[2], W R Steele[1], R Y Dodd[1], S L Stramer[2]. *[1]Transmissible Diseases, American Red Cross, Rockville, MD, United States; [2]Scientific Support Office, American Red Cross, Gaithersburg, MD, United States; [3]Quality Analytics Inc., Riverwood, IL, United States*

Background/Case Studies: The number of US blood donors testing positive (pos) for HTLV-I/-II infection has decreased in recent years, but prevalent and incident infections are still detected and differ by gender and geographic distribution. The feasibility of one-time testing for HTLV was assessed by evaluating HTLV incidence in repeat donors. **Study Design/Methods:** Data for HTLV-I/II pos donors identified by the American Red Cross (ARC) from 2009-2011 were retrieved. Antibody in donors testing anti-HTLV-I/II repeat reactive (Abbott PRISM) were confirmed by a 2nd EIA followed by a combination of IFA, western blot (WB) and RIPA (CA Viral & Rickettsial Diseases Lab). Prevalence rates were calculated by gender and geographic distribution based on US Census Regions. Rates for the Northeast (NE), South (S), Midwest (MW) and West (W) were compared using multiple pairwise chi-square comparisons with Bonferroni adjustment. Incidence density (ID) was calculated as the number of seroconverters over total donor person years (PY) with prior donation histories not to exceed 1095 days (3 years). **Results/Findings:** For the 3-year period, >19 million donations were tested from 7,098,612 allogeneic donors including 443 HTLV pos. Overall HTLV donation prevalence was 2.3 per 100,000 (PHT). Female donors accounted for 72% (319/443) and males 28% with prevalence rates of 3.6 PHT and 1.2 PHT, respectively ($p < 0.0001$, OR = 2.9, 95% CI 2.4, 3.6). Overall; the number of HTLV pos donors by region and prevalence rates of PHT donors differed significantly; between the NE vs. W ($p = 0.013$), MW vs. NE ($p = 0.002$), MW vs. W ($p < 0.0001$) and MW vs. S ($p < 0.0001$); no differences were noted between the S and the NE or the S and the W; the MW had the lowest rates. Of repeat donors, 36 were HTLV pos; 22 had nonreactive donations >3 yrs prior and 14 within 3 yrs. For these 14, an overall ID of 0.18 PHT PY was calculated (95% CI, 0.10, 0.30). Female donors accounted for nearly all of the calculable incident donors; the 13 female donors had an ID of 0.34 PHT PY (95% CI, 0.18, 0.58) with 0.03 PHT PY (95% CI, 0.001, 0.141) for the 1 male. Of the 14, 7 were typed as HTLV-II, 5 HTLV-I/II and 2 HTLV-I; 11/14 were IFA pos (1:64-1:1024 endpoint) with the remaining 3 confirmed by WB/RIPA. **Conclusion:** Significant differences in HTLV prevalence rates and gender were observed. With 14 incident infections identified, it is not feasible to screen donors only once for anti-HTLV. These data are consistent with those of earlier time periods.

Disclosure of Commercial Conflict of Interest

J. Brodsky: Nothing to disclose; R. Y. Dodd: Abbott Laboratories, Stocks or Bonds; Abbott Laboratories, Grants or Research Support; Novartis, Travel Support or Honorarium; Ortho Diagnostics, Travel Support or Honorarium; E. P. Notari: Nothing to disclose; W. R. Steele: Nothing to disclose; S. L. Stramer: Nothing to disclose

Disclosure of Grants Conflict of Interest

J. Brodsky: Nothing to disclose; R. Y. Dodd: Abbott Laboratories, Grants or Research Support; E. P. Notari: Nothing to disclose; W. R. Steele: Nothing to disclose; S. L. Stramer: Nothing to disclose

Donor Based anti-HTLV Prevalence Rates per 100,000 (PHT)

US Census Region	Total Number of Donors	Number of anti-HTLV Positive	Prevalence Rate	Lower 95% CI	Upper 95% CI
Northeast	1,662,757	98	6.3	5.1	7.6
Midwest	2,123,544	77	3.6	2.9	4.5
South	2,298,417	161	7.0	6.0	8.2
West	1,113,894	107	9.6	7.9	11.6

P3-030A

Age of Red Blood Cells in Premature Infants (ARIPI)

D.A Fergusson[1,8] (dafergusson@ohri.ca), P C Hebert[1,8], L LeBel[1], N G Rouvinez-Bouali[2,8], J A Smyth[3,12], K Sankaran[4,8], A T Tinmouth[1,8], M A Blajchman[5], L Kovacs[6,10], C Lachance[7,11]. *[1]Clinical Epidemiology, Ottawa Hospital Research Institute, Ottawa, ON, Canada; [2]Division of Neonatology, The Ottawa Hospital, Ottawa, ON, Canada; [3]Division of Neonatology, Children's and Women's Health Centre of British Columbia, Vancouver, BC, Canada; [4]Division of Neonatology, Royal University Hospital, Saskatoon, SK, Canada; [5]Faculty of Health Sciences, McMaster University, Hamilton, ON, Canada; [6]Division of Neonatology, Jewish General Hospital, Montreal, QC, Canada; [7]Division of Neonatology, Ste Justine Hospital, Montreal, QC, Canada; [8]Faculty of Medicine, University of Ottawa, Ottawa, ON, Canada; [9]College of Medicine, University of Saskatchewan, Saskatoon, SK, Canada; [10]Faculty of Medicine, McGill University, Montreal, QC, Canada; [11]Faculty of Medicine, University of Montreal, Montreal, QC, Canada; [12]Faculty of Medicine, University of British Columbia, Vancouver, BC, Canada*

Background/Case Studies: Despite recent trends in decreasing transfusion thresholds and the development of technologies designed to avoid allogeneic exposure, allogeneic red blood cell (RBC) transfusions remain an important supportive and life-saving measure for neonatal intensive care patients experiencing illness and anemia of prematurity. However, a number

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
				2013. 1. 17	該当なし	

一般的名称	新鮮凍結人血漿	研究報告の公表状況		公表国
販売名（企業名）	新鮮凍結血漿-LR「日赤」（日本赤十字社） 新鮮凍結血漿-LR「日赤」成分採血（日本赤十字社） 新鮮凍結血漿-LR「日赤」120（日本赤十字社） 新鮮凍結血漿-LR「日赤」240（日本赤十字社） 新鮮凍結血漿-LR「日赤」480（日本赤十字社）		Francis RO, Strauss D, Williams JD, Whaley S, Shaz BH. Transfusion. 2012 Dec;52(12):2664-70. doi: 10.1111/j.1537-2995.2012.03639.x. Epub 2012 Apr 9.	米国

研究報告の概要

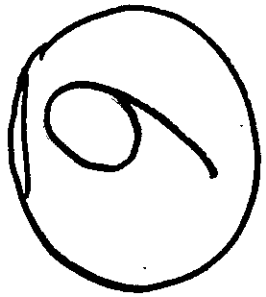
○2010年季節的流行期間中の、ニューヨークにおける供血者のウエストナイルウイルス感染症
背景：供血者のウエストナイルウイルス（WNV）急性感染について、ミニプール（MP）NATから個別（ID）NATへの転換に関する一律の開始戦略は現在存在しない。1999年以降最大の流行となった2010年のWNV季節的流行期間中の、ニューヨーク血液センターにおけるWNVスクリーニングの結果について報告する。
研究デザインと方法：2010年7月1日～10月31日の期間中、MP-NATまたはID-NATを用いて供血者スクリーニングを行い、NAT陽性の血液についてWNV-IgM及びIgG抗体の有無を調べた。
結果：ウイルス血症であるとみられる血液20本（0.0129%、1/7752）が確認された。MP-NATでは検出されなかった可能性がある9本が同定され、これらのうち2本はIgM、IgG抗体がいずれも陰性であり、うち1本はID-NATの開始に2本以上の陽性血液が必要であるという条件下では検出できなかったと考えられる。遡及的なID-NATでは2本の陽性血液が確認された。ニューヨーク州におけるNAT陽性血液の多く（16/19本）は、州の中でもヒトWNV症例が多い郡（ナッソー郡及びサフォーク郡）の居住者から採取されたものであった。
結論：陽性血液が1件検出された後のID-NATの開始、遡及的な検査によって、感染性が想定される血液の出荷が防止できた。しかし、遡及的な検査によりNAT陽性血液が検出されたことは、ID-NATの開始基準の変更が必要であることを示唆している可能性がある。

使用上の注意記載状況・その他参考事項等

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

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
2010年のウエストナイルウイルス（WNV）季節的流行期間中、ニューヨーク血液センターで行ったNATスクリーニングの結果である。MP-NATとID-NATを適切に切り替えることにより、WNVの感染性を有する血液の出荷を効果的に防ぐ事ができたが、さらに改善の余地があるとの報告である。	日本赤十字社では、輸血感染症対策として受付時に海外滞在歴の有無を確認し、帰国（入国）後4週間は献血不適としている。また、ウエストナイルウイルス感染の国内発生に備え、平成17年10月25日付血液対策課発事務連絡に基づく緊急対応（献血制限、NAT検査）のほか、厚生労働科学研究「血液製剤の安全性確保と安定供給のための新興・再興感染症の研究」班と共同して対応について検討している。今後も引き続き情報の収集に努める。



West Nile virus infection in blood donors in the New York City area during the 2010 seasonal epidemic

Richard O. Francis, Donna Strauss, Joan Dunn Williams, Shavonne Whaley, and Beth H. Shaz

BACKGROUND: A uniform threshold strategy for converting from minipool (MP)-nucleic acid testing (NAT) to individual donation (ID)-NAT screening for acute West Nile virus (WNV) infection among blood donors is lacking. We report on WNV screening at the New York Blood Center during the 2010 seasonal WNV epidemic, the most severe epidemic in that state since the original outbreak in 1999.

STUDY DESIGN AND METHODS: Between July 1 and October 31, 2010, blood donations were screened by MP-NAT or ID-NAT and the presence of anti-WNV immunoglobulin (Ig)M and IgG was evaluated among NAT-positive donations.

RESULTS: Twenty presumed viremic donations were identified for a frequency of 0.0129% (1 in 7752 donations). Nine donations that could have been missed by MP-NAT were identified. Two of these donations were both IgM and IgG negative, one of which would have been missed if more than one positive donation was required for initiating ID-NAT. Retrospective ID-NAT revealed two positive donations. The majority of the NAT-positive donations in New York (16/19) were from donors who lived in counties that had the highest incidence of human WNV cases in the state.

CONCLUSION: Our data details the identification of WNV NAT-positive blood donations during a severe seasonal epidemic in the New York area. By initiating ID-NAT after one positive donation, using retrospective testing, and triggering ID-NAT regionally, we were able to prevent the release of presumably infectious donations. The detection of NAT-positive donations with retrospective testing, however, may indicate the need for changes in our trigger criteria.

West Nile virus (WNV) is a single-stranded RNA virus that is transmitted by the *Culex* mosquito and usually infects birds. Mammals such as humans and horses are incidental hosts and several human outbreaks have been reported around the world in Romania, Russia, Israel, and most recently in the United States. Approximately 20% of WNV infections lead to a febrile illness, West Nile fever, and less than 1% of infected individuals have neurologic disease (meningoencephalitis).[1] The large proportion of asymptomatic infections, 80%, poses the threat that acutely infected persons may present for blood donation without symptoms of illness. As such, the risk of transfusion-transmitted WNV (TT-WNV) infection was predicted.[2,3] In addition, it has been shown that the virus is stable in stored blood for 42 days under refrigerated conditions.[4]

Twenty-three cases of TT-WNV infection from the 2002 season were retrospectively confirmed in 2003.[5] The outcomes of these cases included asymptomatic infection, febrile illness, meningoencephalitis, and death. Since viral nucleic acid can be detected before the generation of IgM and IgG antibodies against WNV,[6] the Food and Drug Administration, private industry, and blood collection agencies partnered to begin nucleic acid testing

ABBREVIATIONS: ID = individual donation; MP(s) = minipool(s); PVD(s) = presumed viremic donation(s); TT-WNV = transfusion-transmitted West Nile virus; WNV = West Nile virus.

From the New York Blood Center, New York, New York; the Department of Pathology and Cell Biology, Columbia University Medical Center–New York Presbyterian Hospital, New York, New York; Creative Testing Solutions, Tempe, Arizona; and the Department of Pathology and Laboratory Medicine, Emory University, Atlanta, Georgia.

Address reprint requests to: Beth H. Shaz, New York Blood Center, 310 East 67 Street, New York, NY 10065, e-mail: BShaz@NYBloodCenter.org.

Received for publication July 20, 2011; revision received February 5, 2012, and accepted February 7, 2012.

doi: 10.1111/j.1537-2995.2012.03639.x

TRANSFUSION 2012;52:2664-2670.

(NAT) of blood donations in minipools (MPs) to detect presumed viremic donations (PVDs). MP-NAT for WNV was implemented across the United States in July 2003. It was soon apparent, however, that MP-NAT was not sufficiently sensitive to detect all PVDs as additional cases of TT-WNV infection occurred in 2003.[7]

Because of the prohibitive cost of individual-donation (ID)-NAT for all donations, several strategies were developed for determining when it would be appropriate to transition from MP- to ID-NAT to detect and remove as many PVDs as possible from the blood supply.[8,9] Taken into account in these screening strategies are the number of positive donations after which ID-NAT should be initiated, the size of the geographic area for which the threshold criteria applies, the use of retrospective testing after a PVD is found, and the appropriate interval during which no positive donations are identified to revert to MP-NAT. The effectiveness of these strategies in detecting PVDs as well as the cost associated with them have been evaluated by several authors.[10-12]

The 2010 WNV season in New York had the greatest number of clinical cases of WNV infection since the original outbreak in 1999 in that state.[13] The collection area of our blood center includes New York City, neighboring counties in New York State, and portions of central New Jersey. We report on the detection of PVDs during the 2010 WNV seasonal epidemic in the New York City area using our regional triggering strategy.

MATERIALS AND METHODS

NAT

Blood donations were screened during all months of the year by NAT using the Procleix WNV transcription-mediated amplification assay (Gen-Probe, San Diego, CA). A signal-to-cutoff ratio (S/CO) of 1 or greater defined a positive result. MP-NAT was performed in pools of 16 samples and positive MPs were resolved by testing the individual samples to identify the positive donation(s). ID-NAT was performed on all hematopoietic cellular therapy products. To determine if a sample initially detected by ID-NAT would have been detected in a MP, NAT-positive samples were diluted 1:16 in WNV-negative plasma and NAT was performed. A PVD was defined as an initially reactive donation that repeated as reactive on the original sample from the donation or one that had a signal-to-cutoff ratio of 17 or greater.[14]

Detection of anti-WNV

Anti-WNV were detected in a sample from the index NAT-positive donation. An IgM capture enzyme-linked-immunosorbent assay (MAC-ELISA) and IgG ELISA were performed by a reference laboratory (Sonora Quest Laboratories, Phoenix, AZ). For the IgM MAC-ELISA, an index value of less than 0.90 was negative, an index value of 0.90 to 1.10 was equivocal, and an index value of greater than 1.10 was positive. For the IgG ELISA, an index value of less than 1.30 was negative, an index value of 1.30 to 1.49 was equivocal, and an index value of 1.50 or greater was positive.

Criteria for conversion between MP-NAT and ID-NAT

Triggering to ID-NAT for the collection area was from July 1, 2010 to October 31, 2010. The trigger to ID-NAT was one NAT-positive donation. The algorithm for conversion between MP- and ID-NAT is shown in Fig. 1. Upon identification of a NAT-positive donation the zip code and county of residence of the donor were obtained. ID-NAT was then initiated in the county of residence of the donor from whom the positive donation originated. Retrospec-

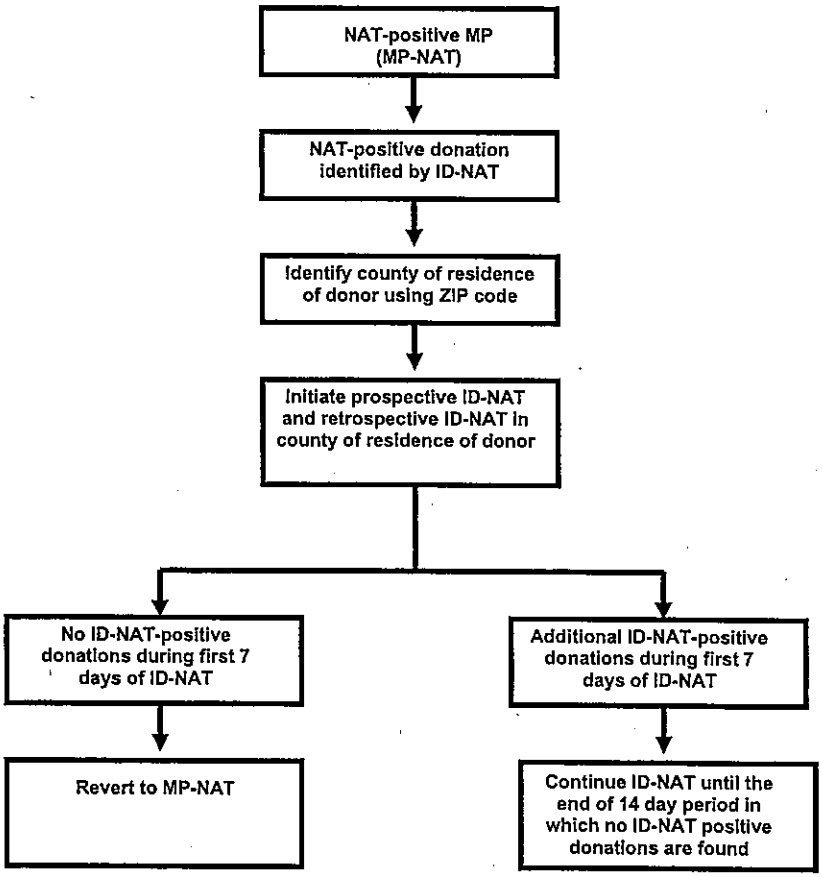


Fig. 1. Algorithm for conversion between MP and ID-NAT.

tive ID-NAT of donations from the affected county was performed beginning on the day the positive donation was identified back to the date of collection of the positive donation. Reversion to MP-NAT in the affected county occurred after 7 days if no other positives were detected or after 14 days from the last date with a NAT-positive donation, if additional positive samples were found. Conversion between MP- and ID-NAT testing for the adjacent counties of Long Island (Nassau and Suffolk Counties) was coordinated such that MP- and ID-NAT were used in both counties at the same time.

Collection of public health data

Public health data for WNV cases and PVDs were obtained from the websites for the various governmental health agencies. Information about WNV cases and PVDs in the 50 states were from the Centers for Disease Control and Prevention at http://www.cdc.gov/ncidod/dvbid/westnile/surv&control_archive.htm. Data for case counts by week for New York and New Jersey were obtained from the United States Geological Survey at http://diseasemaps.usgs.gov/2010/wnv_us_human.html. Case counts and distribution of WNV throughout counties in New York State were found at http://www.health.state.ny.us/nysdoh/westnile/update/update.htm.

RESULTS

The collection area for the New York Blood Center is shown in Fig. 2 and encompasses the five boroughs of New York City (Bronx, Kings, Manhattan, Queens, and Richmond Counties), Long Island (Nassau and Suffolk Counties), the Hudson Valley region of New York (Dutchess, Orange, Putnam, Rockland, Ulster, and Westchester Counties), and central New Jersey (Hunterdon, Middlesex,

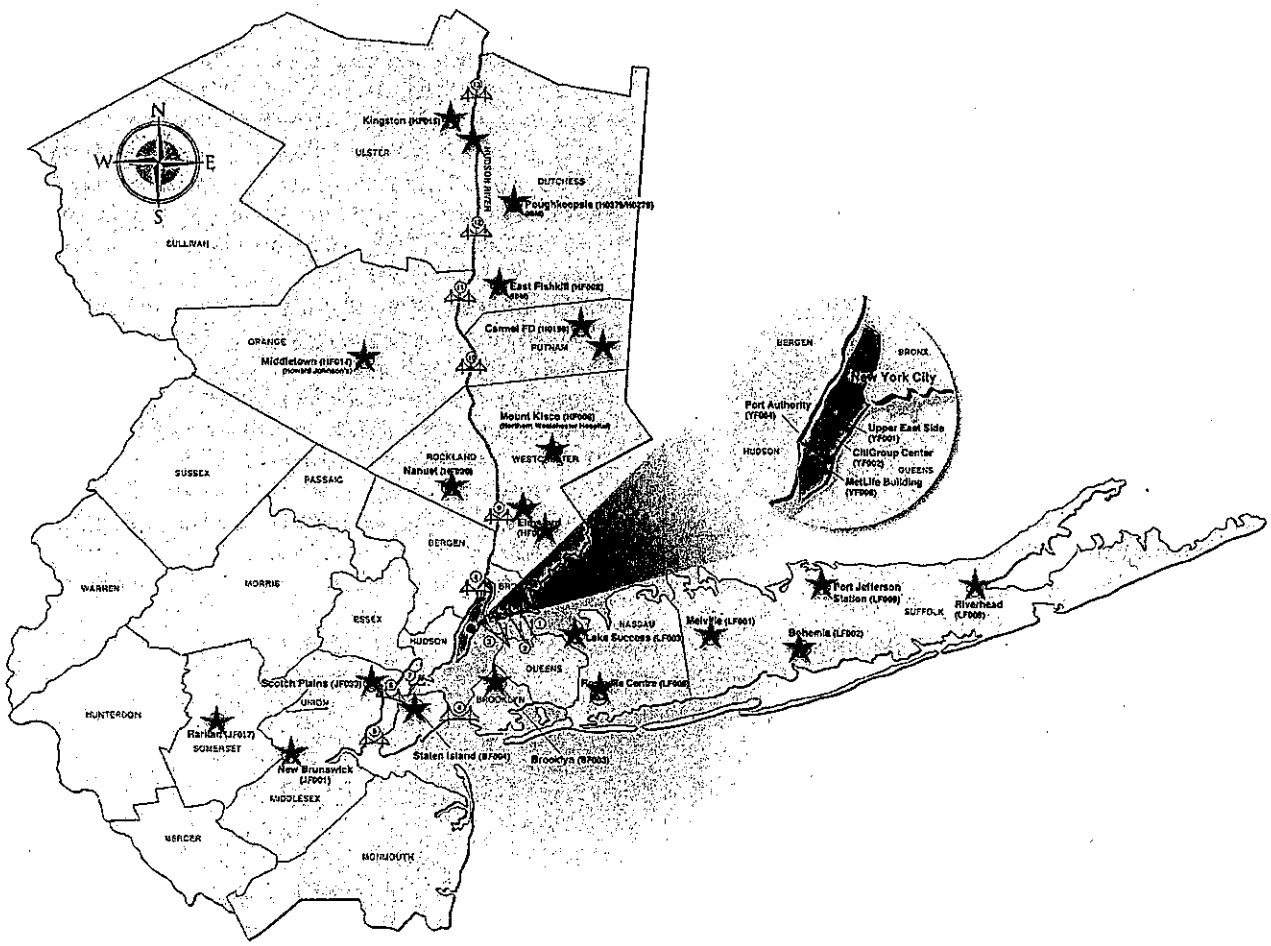


Fig. 2. Blood center collection area. The collection area for the New York Blood Center includes areas of New York and New Jersey. (★) Fixed donation site. Counties in New York City: Bronx, Kings (Brooklyn), Manhattan, Queens, and Richmond (Staten Island). Counties in the Hudson Valley region of New York: Dutchess, Orange, Putnam, Rockland, Ulster, and Westchester. Counties in Long Island: Nassau and Suffolk. Counties in New Jersey: Hunterdon, Middlesex, Somerset, and Union.

Somerset, and Union Counties). Between July 1, 2010, and October 31, 2010, a total of 155,280 donations were screened by NAT with 133,306 (86%) donations tested in MPs. The remaining 21,974 (14%) donations were screened by ID-NAT either due to the ID-NAT trigger being activated (21,129/21,974) or because they were hematopoietic cellular therapy products (845/21,974).

Twenty PVDs were detected for a frequency of 0.0129% or 1 in 7752 donations. As shown in Table 1, 19 of 20 PVDs were from New York and one was from New Jersey. Eight PVDs (40%) were detected by MP-NAT and 12 (60%) were detected by ID-NAT due to the ID-NAT trigger being activated. All reactive MPs were resolved by ID-NAT. The two PVDs from August 13 were detected retrospectively after conversion to ID-NAT in response to the two positive donations collected on August 12. In all, 1636 donations were tested retrospectively, for a frequency of 0.12% or 1 in 833 PVDs detected among retrospectively tested donations. In addition, there was one false-positive sample that was reactive on initial testing, but was nonreactive when repeated and was negative for anti-WNV IgM and IgG. There were no reports of TT-WNV infection.

TABLE 1. WNV-PVDs by collection date, collection county, and original method of NAT detection (MP vs. ID)

Collection date	County (state)	NAT detection method
July 14	Nassau (NY)	MP
July 22	Suffolk (NY)	MP
July 23	Nassau (NY)	MP
July 26	Suffolk (NY)	ID
July 28	Suffolk (NY)	ID
August 6	Bronx (NY)	MP
August 12	Suffolk (NY)	MP
August 12	Suffolk (NY)	MP
August 13*	Nassau (NY)	ID
August 13*	Nassau (NY)	ID
August 14	Kings (NY)	MP
August 16	Suffolk (NY)	ID
August 17	Nassau (NY)	ID
August 19	Kings (NY)	ID
August 24	Suffolk (NY)	ID
August 25	Suffolk (NY)	ID
August 30	Suffolk (NY)	ID
September 5	Suffolk (NY)	ID
September 7	Suffolk (NY)	ID
September 8	Middlesex (NJ)	MP

* Donations detected with retrospective testing.

Figure 3 depicts the number of PVDs at our blood center by week, during the WNV season as well as the incidence of reported human WNV clinical cases in New York and New Jersey. The greatest number of PVDs detected in a single week, five, occurred during the week of August 8 to August 14. This week of peak detection of PVDs was also the week during which the greatest number of reported clinical cases in New York occurred. In addition, the PVD collected in New Jersey on September 8 occurred during 1 of 2 weeks (September 5–September 11) in which six WNV cases were reported in New Jersey, the highest during the season for that state. Therefore, the peak detection of PVDs correlated with the period of peak incidence of reported WNV cases in both New York and New Jersey.

Plasma samples from the 20 NAT-positive donations were tested in replicates of eight at a dilution of 1:16 to simulate MP-NAT to determine the likelihood of detecting WNV in these specimens in MPs. The results of this testing are shown in Table 2. Eight of these donations were positive in only none of eight (n = 4), one of eight (n = 3), and two of eight (n = 1) replicates and would be expected to be detected in ID-NAT and not MP-NAT (yield cases). These eight yield cases were all originally detected by ID-NAT. A donation that was originally detected by ID-NAT was positive in four of eight replicates, suggesting that there was only a 50% chance of detection by MP-NAT. The remaining 11 donations were positive in eight of eight replicates and therefore would be detected by MP-NAT.

The plasma samples from the 20 PVDs were tested for the presence of anti-WNV IgM and IgG to assess the WNV immunity status of the donors. The antibody testing results were not used for making decisions about convert-

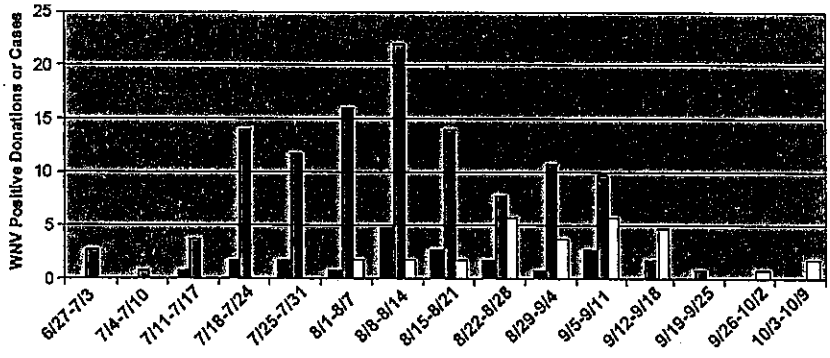


Fig. 3. WNV-positive donations and WNV cases in New York and New Jersey by week during the 2010 season. Data for WNV cases for New York and New Jersey are from the United States Geological Survey website at: http://diseasemaps.usgs.gov/2010/wnv_us_human.html. (■) WNV-PVDs; (▣) clinical WNV cases in New York; (□) clinical WNV cases in New Jersey.

TABLE 2. WNV-PVDs: dilution studies and detection of anti-WNV

Collection date	1:16 dilution testing	Expect to detect by MP or ID	IgM	IgG
July 14	8/8 positive	MP	Negative	Negative
July 22	8/8 positive	MP	Negative	Positive
July 23	8/8 positive	MP	Negative	Negative
July 26	8/8 positive	MP	Negative	Negative
July 28	8/8 positive	MP	Negative	Negative
August 6	8/8 positive	MP	Negative	Negative
August 12	8/8 positive	MP	Negative	Negative
August 12	8/8 positive	MP	Negative	Negative
August 13*	1/8 positive	ID	Positive	Negative
August 13*	2/8 positive	ID	Positive	Positive
August 14	8/8 positive	MP	Negative	Equivocal
August 16	0/8 positive	ID	Positive	Positive
August 17	0/8 positive	ID	Positive	Positive
August 19†	1/8 positive	ID	Negative	Negative
August 24	0/8 positive	ID	Positive	Positive
August 25	1/8 positive	ID	Positive	Negative
August 30	0/8 positive	ID	Positive	Positive
September 5†	4/8 positive	MP/ID	Negative	Negative
September 7	8/8 positive	MP	Negative	Negative
September 8	8/8 positive	MP	Negative	Negative

* Donations detected with retrospective testing.
† Donations possibly not detected by MP-NAT and IgM and IgG negative.

ing between MP- and ID-NAT. PVDs detected in the earlier part of the season from July 14 to August 12 tended to be detectable by MP-NAT and IgM negative (none of eight IgM-positive, one of eight IgG-positive) while NAT-positive donations in the middle to latter part of the season from August 13 to August 30 typically required detection by ID-NAT and were IgM positive (7/12 IgM positive, 5/12 IgG positive). Considering the eight yield cases that would not be detectable by MP-NAT, five were IgM and IgG positive, two were IgM positive and IgG negative, and one was IgM and IgG negative. In addition, the donation that was positive on four of eight replicates in dilution testing was IgM and IgG negative. Therefore, 2 of the 20 PVDs were WNV antibody negative and may not have been detected by MP-NAT.

The majority of the 19 PVDs from New York, 16, were collected from residents of Nassau and Suffolk Counties (Long Island). The remaining three PVDs were from New York City residents (one in Bronx County and two in Kings County). The greater proportion of PVDs collected in Long Island correlated with the majority of clinical cases of WNV being reported in residents of Long Island (82 cases) compared to residents of New York City (42 cases). These results demonstrate that regions of our collection area that had the highest incidence of WNV cases also had the highest incidence of PVDs.

DISCUSSION

We report on the incidence of WNV PVDs in the New York City area during the most active WNV season in that state since the original outbreak in 1999. In the New York Blood Center's collection area that included New York City, Long Island, the Hudson Valley region, and central New Jersey, the frequency of PVDs was 0.0129% (1 in 7752 donations). Of the 20 PVDs that were collected, eight (40%) were yield cases that would not have been detected by MP-NAT and one donation would have had a 50% chance of being detected by MP-NAT. Two PVDs were identified upon retrospective testing in Long Island, the portion of our collection area that had the greatest proportion of PVDs and clinical cases in New York. In addition, two donations that may have been missed by MP-NAT were detected by ID-NAT due to activation of the ID-NAT trigger and were both anti-WNV IgM and IgG negative. Historically, PVDs that were negative by MP-NAT and anti-WNV IgM negative have been associated with TT-WNV infection.[7,15,16] The frequency of 0.0129% for WNV NAT-positive blood donations is comparable to what has been reported in other areas of the United States in which seasonal WNV-epidemics occur.[17-19]

In this study conversion from MP-NAT to ID-NAT within a county occurred after detecting one PVD from a resident of that county. Investigations of triggering strategies have demonstrated that switching from MP-NAT to ID-NAT after detecting one PVD, without a rate requirement, is the most sensitive method for detecting PVDs.[10,20] Among the PVDs in this study, the donation collected on August 19 in Kings County would have only been detected by ID-NAT as demonstrated by dilution testing, was IgM and IgG negative, and was initially tested with ID-NAT because of one prior MP-NAT–positive sample that was detected in the same county on August 14. Therefore, by initiating ID-NAT on one instead of two positive donations, the release of this presumably infectious blood product was prevented.

Reverting to MP-NAT is typically done after either 7 or 14 days of not detecting additional PVDs during ID-NAT. It has been demonstrated that continuing ID testing for 14 instead of 7 days increases the number of low-viremic donations that are detected,[10] albeit at the cost of prolonging ID-NAT. AABB recommends considering continuing ID-NAT for 14 days in areas with ongoing WNV activity.[14] Our strategy entailed ID-NAT for 7 days if no other positive donations were found or 14 days if any additional positive samples were encountered.

Retrospective ID-NAT was performed when converting from MP-NAT to ID-NAT by testing donations from the day of reporting of a NAT-positive donation back to the day of collection of that donation. The frequency of detecting a NAT-positive donation among retrospectively tested donations was almost 10 times that of detecting positive donations among the general donor population (0.12% vs. 0.0129%). While these results demonstrate the utility of retrospective testing for identifying PVDs during periods of high WNV activity, they also indicate that ID-NAT perhaps should have been used for all donations during this epidemic period.

Conversion between MP- and ID-NAT was done for individual counties, except for Nassau and Suffolk Counties in Long Island, which were converted together. As demonstrated by data from Table 1 and Fig. 3, this strategy resulted in MP-NAT being used during peak periods of clinical cases and detection of PVDs. For example, a PVD from Bronx County was collected on August 6, detected by MP-NAT. ID-NAT was initiated only in that county while within the next 7 days four additional PVDs were collected in Suffolk and Nassau Counties. Of these four PVDs, two were collected on August 12, detected by MP-NAT, and two were collected on August 13, detected by retrospective ID-NAT. If conversion to ID-NAT was done for the entire New York City area on August 6, retrospective testing would not have been necessary to detect the August 13 donations. These data suggest that perhaps a wider geographic area than individual counties should be considered for conversion between MP- and ID-NAT in our collection region.

This study has several limitations. First, data about the donors are not available to investigate relationships between the presence of symptoms before, at the time of, and after donation and viremia. Viral loads were not determined, an additional NAT method was not used to confirm transcription-mediated amplification results, and donor follow-up was not performed. Second, our estimate of the frequency of viremic blood donors may be an underestimate because we did not perform ID-NAT throughout the entire season and therefore may have missed cases with levels of viremia that were below the level of detection of MP-NAT during a time that ID-NAT was not triggered. In addition, donors with low levels of viremia that could not be detected by ID-NAT would also not be represented. Third, we are unable to estimate the frequency of WNV infection among blood donors in our collection area because prospective donors who were symptomatic may have not gone to donate or may have been deferred from donation because they reported not feeling well when they presented. Fourth, county of residence of the blood donors from our study may not, in all cases, reflect the location where they became infected. It is expected that a person's exposure to mosquitoes most likely occurs during the evening or early morning hours (when mosquitoes are most active) when the individual is at home. This may not hold true, however, for a person who works an evening or night shift in a different county, outdoors, where he or she could come in contact with mosquitoes. Thus, the expectation that the incidence of PVDs will correlate with the same areas that have high disease activity may not always be the case. Finally, our study does not address the question of whether WNV testing should be performed at all during the parts of the year in which there is no mosquito activity and no WNV cases are being reported.

In conclusion, our results demonstrate the importance of weighing the many variables involved in selecting a strategy for conversion between MP- and ID-NAT for detecting WNV among blood donors. Using our current strategy we were able to prevent 20 PVDs from being released, nine of which may not have been detectable by MP-NAT and two of which may have led to TT-WNV infection. The high rate of detection of PVDs among retrospectively tested samples, however, indicates that improvements in our triggering strategy may be warranted. Initiation of ID-NAT in a single county based on detection of one PVD proved advantageous for detecting subsequent PVDs that would have been missed by MP-NAT. The ability to detect PVDs may be increased even more by considering a larger geographic area for conversion between MP- and ID-NAT, as well as increasing the minimum period of ID-NAT to 14 days as suggested by some.[10] By considering these factors as well as continuing to evaluate seasonal WNV activity as information becomes available, we will improve our ability to protect our blood supply while managing the increased costs of increased use of ID-NAT.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest relevant to the manuscript submitted to TRANSFUSION.

REFERENCES

1. Petersen LR, Marfin AA. West Nile virus: a primer for the clinician. Ann Intern Med 2002;137:173-9.
2. Biggerstaff BJ, Petersen LR. Estimated risk of West Nile virus transmission through blood transfusion during an epidemic in Queens, New York City. Transfusion 2002;42: 1019-26.

3. Biggerstaff BJ, Petersen LR. Estimated risk of transmission of the West Nile virus through blood transfusion in the US, 2002. Transfusion 2003;43:1007-17.
4. Mather T, Takeda T, Tassello J, Ohagen A, Serebryanik D, Kramer E, Brown F, Tesh R, Alford B, Chapman J, Lazo A. West Nile virus in blood: stability, distribution, and susceptibility to PEN110 inactivation. Transfusion 2003;43:1029-37.
5. Pealer LN, Marfin AA, Petersen LR, Lanciotti RS, Page PL, Stramer SL, Stobierski MG, Signs K, Newman B, Kapoor H, Goodman JL, Chamberland ME. Transmission of West Nile virus through blood transfusion in the United States in 2002. N Engl J Med 2003;349:1236-45.
6. Busch MP, Kleinman SH, Tobler LH, Kamel HT, Norris PJ, Walsh I, Matud JL, Prince HE, Lanciotti RS, Wright DJ, Linnen JM, Caglioti S. Virus and antibody dynamics in acute West Nile virus infection. J Infect Dis 2008;198:984-93.
7. Montgomery SP, Brown JA, Kuehnert M, Smith TL, Crall N, Lanciotti RS, Macedo de Oliveira A, Boo T, Marfin AA. Transfusion-associated transmission of West Nile virus, United States 2003 through 2005. Transfusion 2006;46: 2038-46.
8. Custer B, Tomasulo PA, Murphy EL, Caglioti S, Harpool D, McEvoy P, Busch MP. Triggers for switching from minipool testing by nucleic acid technology to individual-donation nucleic acid testing for West Nile virus: analysis of 2003 data to inform 2004 decision making. Transfusion 2004;44: 1547-54.
9. Kleinman SH, Williams JD, Robertson G, Caglioti S, Williams RC, Spizman R, Morgan L, Tomasulo P, Busch MP. West Nile virus testing experience in 2007: evaluation of different criteria for triggering individual-donation nucleic acid testing. Transfusion 2009;49:1160-70.
10. Biggerstaff BJ, Petersen LR. A modeling framework for evaluation and comparison of trigger strategies for switching from minipool to individual-donation testing for West Nile virus. Transfusion 2009;49:1151-9.
11. Custer B, Busch MP, Marfin AA, Petersen LR. The cost-effectiveness of screening the U.S. blood supply for West Nile virus. Ann Intern Med 2005;143:486-92.
12. Korves CT, Goldie SJ, Murray MB. Cost-effectiveness of alternative blood-screening strategies for West Nile virus in the United States. PLoS Med 2006;3:e21.
13. New York State Department of Health. West Nile virus update [monograph on the internet]. 2011. [cited 2011 Jun 30]. Available from: URL: http://www.health.state.ny.us/nysdoh/westnile/update/update.htm
14. Connor DJ, Lipton KS. West-Nile virus—revised recommendations for triggering individual donation nucleic acid testing and use of communication plans. Association Bulletin No. 08-03. Bethesda (MD): American Association of Blood Banks; 2008.
15. Harrington T, Kuehnert MJ, Kamel H, Lanciotti RS, Hand S, Currier M, Chamberland ME, Petersen LR, Marfin AA. West Nile virus infection transmitted by blood transfusion. Transfusion 2003;43:1018-22.
16. Macedo de Oliveira A, Beecham BD, Montgomery SP, Lanciotti RS, Linnen JM, Giachetti C, Pietrelli LA, Stramer SL, Safranek TJ. West Nile virus blood transfusion-related infection despite nucleic acid testing. Transfusion 2004;44: 1695-9.
17. Busch MP, Caglioti S, Robertson EF, McAuley JD, Tobler LH, Kamel H, Linnen JM, Shyamala V, Tomasulo P, Kleinman SH. Screening the blood supply for West Nile virus RNA by nucleic acid amplification testing. N Engl J Med 2005;353:460-7.
18. Kleinman S, Glynn SA, Busch M, Todd D, Powell L, Pietrelli L, Nemo G, Schreiber G, Bianco C, Katz L. The 2003 West Nile virus United States epidemic: the America's Blood Centers experience. Transfusion 2005;45:469-79.
19. Stramer SL, Fang CT, Foster GA, Wagner AG, Brodsky JP, Dodd RY. West Nile virus among blood donors in the United States, 2003 and 2004. N Engl J Med 2005;353: 451-9.
20. Stramer SL, Foster G, Krysztof D, Dodd R. Evaluation of triggering strategies for single unit NAT West Nile virus. Transfusion 2008;48:23A.

別紙様式第2-1

No. 17

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
				2012. 10. 20	該当なし	

一般的名称	新鮮凍結人血漿	研究報告の公表状況		公表国
販売名(企業名)	新鮮凍結血漿-LR「日赤」(日本赤十字社) 新鮮凍結血漿-LR「日赤」成分採血(日本赤十字社) 新鮮凍結血漿-LR「日赤」120(日本赤十字社) 新鮮凍結血漿-LR「日赤」240(日本赤十字社) 新鮮凍結血漿-LR「日赤」480(日本赤十字社)		ProMED 20121007.1328469	カンボジア ほか

研究報告の概要

○デング熱／デング出血熱 更新情報(抜粋)
カンボジア
今年(2012年)9月第1週までに少なくとも34,483人のデング熱症例が報告され、昨年の同時期の12,972人と比較して2.66倍に増加した。また、今年9月までに146人の子どもがデング熱で死亡し、昨年の同期間の死亡者数59人と比較して2.47倍に増加した。デング熱により1週間に3～5人の子どもが死亡し続けていると言う。保護者が患児をまず最初に個人医院に連れて行き、治療が無効で疾患がより重篤になってから公立病院を訪れるため、その時には既に手遅れとなっているので死亡者数が増加したと当局の専門官は述べた。

使用上の注意記載状況・その他参考事項等

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

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
カンボジアでは2012年9月第1週までのデング熱報告数が前年の同時期と比べて2.66倍に増加したとの報告である。	日本赤十字社では、輸血感染症対策として受付時に海外滞在歴の有無を確認し、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。

JRC2012T-041

Published Date: 2012-10-07 11:56:41
Subject: PRO> Dengue/DHF update 2012 (49): Asia
Archive Number: 20121007.1328469

DENGUE/DHF UPDATE 2012 (49) ASIA
****
A ProMED-mail post
http://www.promedmail.org
ProMED-mail is a program of the
International Society for Infectious Diseases
http://www.isid.org

In this update:
Cases in various countries:
[1] Cambodia
[2] Cases in various countries
Thailand, Maha Sarakham province
India, Delhi area
India, Chandigarh, Harayana/Punjab states
Pakistan, Karachi, Sindh province

******
[1] Cambodia
Date: Wed 3 Oct 2012
Source: Xinhua News Agency [edited]
http://news.xinhuanet.com/english/health/2012-10/03/c_131886401.htm

At least 34 483 dengue fever cases were reported in Cambodia in the 1st 9 months of this year [2012], a 166 percent increase compared with 12 972 cases in the same period last year [2011], a report of the National Center for Parasitology, Entomology and Malaria Control showed Wednesday [3 Oct 2012].

From January to September this year [2012], the disease had killed 146 Cambodian children, up 147 percent compared with 59 deaths during the same period last year [2011]. "The disease continues to kill between 3 and 5 children a week," said Dr Char Meng Chuor, director of the center.

He explained that there were more deaths this year [2012] because parents had sent their ill children to private clinics 1st, and when the treatment was ineffective and the disease became more severe, they would send them to public hospitals, but it was too late for them to be cured.

Dengue fever is a viral disease transmitted by _Aedes_ mosquitoes. The disease causes an acute illness of sudden onset that usually follows symptoms such as headache, fever, exhaustion, severe muscle and joint pain, swollen glands, vomiting, and rash.

In Cambodia, outbreaks of dengue fever usually begin at the onset of the rainy season in May and last until October.

Char Meng Chuor said that to prevent outbreaks, the center has distributed some 270 tones of Abate (a chemical substance used to kill larvae in water pots) to households this year [2012].

Last year [2011], the country reported 15 980 dengue fever cases, and 73 children died.

-- Communicated by: PRO/MBDS <promed-mbds@promedmail.org>

[According to the newswire above, a total of 34 483 cases and 146 fatalities due to dengue infection were reported in Cambodia during the 1st 9 months of 2012. According to the WHO Western Pacific Regional Office (WPRO) report on the dengue situation, dated 20 Sep 2012, a total of 31 061 cases and 127 deaths due to dengue infection, with a CFR of 0.4 percent, were reported in Cambodia during the 1st 8 months of 2012.

The trend of dengue activity in Cambodia is declining. However, the activity remains above the historic seasonal baseline; 11 017 cases and 48 deaths, with a CFR of 0.4 percent for the same period in 2011 (see http://www.wpro.who.int/emerging_diseases/Dengue.Biweekly.20Sep2012.pdf).

For a map of Cambodia with provinces, see http://ephotopix.com/image/asia/cambodia_province_map.gif. For the interactive HealthMap/ProMED-mail map with links to other recent PRO/MBDS and ProMED-mail postings on Cambodia and neighboring countries, see http://healthmap.org/r/1iGB. - Mod.SCM]

******

[2]
Cases in various countries:

- Thailand, Maha Sarakham province. 5 Oct 2012. Dengue 713 cases; Deaths 1; Increasing.
http://www.pattayadailynews.com/en/2012/10/06/dengue-fever-outbreak-kills-isan-teen-girl/

[A map showing the location of Maha Sarakham province can be accessed at
http://www.lib.utexas.edu/maps/middle_east_and_asia/thailand_admin_2005.jpg. - Mod.TY]

India, Delhi area. 3 Oct 2012. Dengue 98 cases; Deaths 2; Increasing. http://www.hindustantimes.com/India-news/NewDelhi/10-new-dengue-cases-in-city/Article1-939488.aspx

[Maps of India can be seen at http://www.mapsofindia.com/maps/india/india-political-map.htm and
http://healthmap.org/r/1pSH. - Mod.TY]

- India, Chandigarh, Harayana/Punjab states. 3 Oct 2012. Dengue, September 2012 only (conf.) 105 cases; Deaths 1. http://timesofindia.indiatimes.com/city/chandigarh/105-dengue-cases-in-Chandigarh/articleshow/16648380.cms

- Pakistan, Karachi, Sindh province. 2 Oct 2012. Dengue for 1-2 Oct 2012 (conf.) 16 cases, (susp.) 243 cases; Increasing. http://www.brecorder.com/pakistan/general-news/83134-16-confirmed-cases-of-dengue-fever-reported-from-different-hospitals-.html

[A HealthMap/ProMED-mail interactive map showing the location of Karachi in Sindh province can be accessed at
http://healthmap.org/r/3DHW. - Mod.TY]

See Also

Dengue/DHF update 2012 (47): Asia 20120930.1316993
Chikungunya & dengue - Cambodia (04): comment 20120925.1308762
Dengue/DHF update 2012 (43): Asia 20120917.1297396
.....sb/dk/ty/msp

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
				2013. 1. 17	該当なし	

一般的名称	新鮮凍結人血漿	研究報告の公表状況	Trottier H, Buteau C, Robitaille N, Duval M, Tucci M, Lacroix J, Alfieri C. Transfusion. 2012 Dec;52(12):2653-63. doi: 10.1111/j.1537-2995.2012.03611.x. Epub 2012 Mar 15.	公表国
販売名(企業名)	新鮮凍結血漿-LR「日赤」(日本赤十字社) 新鮮凍結血漿-LR「日赤」成分採血(日本赤十字社) 新鮮凍結血漿-LR「日赤」120(日本赤十字社) 新鮮凍結血漿-LR「日赤」240(日本赤十字社) 新鮮凍結血漿-LR「日赤」480(日本赤十字社)			カナダ

研究報告の概要

○幹細胞移植レシピエントにおける、輸血関連エプスタイン・バーウイルス感染:小児後方視的コホート研究
背景: 血液の安全性において、現在スクリーニング検査が行われていない病原体の輸血感染は懸念事項である。このような未検査の病原体のひとつが、リンパ増殖性疾患と関連するエプスタイン・バーウイルス(EBV)である。本研究で、造血幹細胞(HSC)移植を受ける小児における移植後のEBV感染率及び血液製剤の投与と感染との関連性についての分析を行った。
研究デザインと方法: HSC移植小児レシピエントの移植前の血清及びHSCドナーの血清中のEBV抗体の存在について評価し、移植後のEBV感染率、患者の輸血歴について調査した。EBVの移植後累積感染率は、移植前の血清学的データに従い、カプラン・マイヤー法で推定した。血液製剤とEBV感染の関連性についてはコックス回帰モデルを用いて評価した。
結果: 移植前のEBV抗体陽性率は、レシピエントで77.9%、ドナーで61.8%であった。レシピエントの全員が移植前後の期間に血液製剤の投与を受けていた。抗体陰性患者における30日及び60日のEBVの移植後累積感染率は、それぞれ4.6%(95%CI、1.2−17.3%)、13.4%(95%CI、5.8−29.4%)であった。分析を臍帯血移植を受けた抗体陰性患者のみに限定した場合、60日の累積感染率は8.3%(95%CI、2.2−29.4%)であった。重要なことに、EBV感染と輸血量の関連性を肯定する傾向が明確にみられた。
結論: 本研究は、HSC移植レシピエントにおける輸血と移植後EBV感染の関連性を示唆している。

報告企業の意見	今後の対応
造血幹細胞(HSC)移植を受ける小児における血液製剤の投与と移植後エプスタイン・バーウイルス(EBV)感染率の関連性について調査したところ、HSC移植レシピエントにおける輸血と移植後EBV感染の関連性が示唆されたとの報告である。	今後も引き続き情報の収集に努める。

使用上の注意記載状況・その他参考事項等

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

血液を介するウイルス、
細菌、原虫等の感染
vCJD等の伝播のリスク

Transfusion-related Epstein-Barr virus infection among stem cell transplant recipients: a retrospective cohort study in children

Helen Trottier, Chantal Buteau, Nancy Robitaille, Michel Duval, Marisa Tucci, Jacques Lacroix, and Caroline Alfieri

BACKGROUND: Blood safety warrants strict screening measures to minimize the risk of transmitting blood-borne pathogens. However, transfusion-transmitted infections for which testing is not currently performed continue to be a concern. Among these untested agents is Epstein-Barr virus (EBV) which, in the transplant setting, is associated with lymphoproliferative disease, a potentially fatal cancer. The aim of this study was to analyze the incidence of posttransplant EBV infection and its association with administration of blood products in children receiving a hematopoietic stem cell (HSC) graft.

STUDY DESIGN AND METHODS: This retrospective cohort study sought to review charts of pediatric recipients of HSC grafts to collect information on the presence of EBV antibodies in the recipients' pretransplant sera and in HSC donor sera, incidence of posttransplant EBV infection, and patients' transfusion history. Cumulative incidence of posttransplant EBV infection was estimated by Kaplan-Meier methods according to pretransplant serology. The association between blood products and EBV infection was measured by Cox regression models.

RESULTS: The pretransplant EBV seroprevalence was 77.9% for recipients and 61.8% for graft donors. Virtually, all recipients received blood products during the peritransplant period. Among seronegative recipients, the 30- and 60-day cumulative incidences of posttransplant EBV infection were 4.6 (95% confidence interval [CI], 1.2-17.3) and 13.4% (95% CI, 5.8%-29.4%), respectively. The 60-day cumulative incidence was 8.3% (95% CI, 2.2%-29.4%) when restricting the analysis to seronegative recipients of cord blood grafts. Importantly, there was a clear positive trend associating EBV infection to transfusion volume.

CONCLUSION: This study suggests an association between transfusions and posttransplant EBV infection in HSC transplant recipients.

In many countries, numerous steps are taken to minimize the risk of infection from transfused blood products. Typically, blood banking organizations will screen for an array of infectious pathogens as part of their quality control protocol. These include hepatitis B and C viruses, human immunodeficiency virus, human T-cell leukemia virus, syphilis, West Nile virus, Chagas disease (*Trypanosoma cruzi*), and on selected units, cytomegalovirus (CMV/human herpesvirus-5).[1-3] Thus, while transmission of these infections via transfusion has become exceedingly rare, the risk of transfusion-transmitted infections for which testing is not currently performed continues to be a concern.[4-8] Among these untested infectious agents is Epstein-Barr virus (EBV, also known as human herpesvirus-4), which in

ABBREVIATIONS: CSA = cyclosporine A; EBNA = Epstein-Barr nuclear antigen; HSC = hematopoietic stem cell; HSCT = hematopoietic stem cell transplantation; IQR = interquartile range; PTLD = posttransplant lymphoproliferative disease; RR(s) = relative risk(s); VCA = virus capsid antigen.

From the Department of Social and Preventive Medicine; the Department of Pediatrics, Division of Infectious Diseases; the Department of Pediatrics, Division of Hematology-Oncology; the Department of Pediatrics, Division of Pediatric Intensive Care Medicine; and the Department of Microbiology and Immunology, University of Montreal, Sainte-Justine Hospital Research Centre, Montreal, Canada.

Address reprint requests to: Helen Trottier, Sainte-Justine Hospital Research Center, Department of Social and Preventive Medicine, University of Montreal, 3175 Côte Ste-Catherine, Room A-830, Montreal, QC, Canada, H3T 1C5; e-mail: helen.trottier@umontreal.ca.

This study was supported by a grant from the Fonds de la Recherche en Santé du Québec (FRSQ; Grant 13904) held by JL and MT.

Received for publication November 14, 2011; revision received January 26, 2012, and accepted January 26, 2012.

doi: 10.1111/j.1537-2995.2012.03611.x

TRANSFUSION 2012;52:2653-2663.

immunocompromised patients, can induce lymphoproliferative disease, a potentially fatal cancer.[9-12]

In countries with stringent hygiene practices, EBV seroprevalence tends to increase gradually with age, typically showing two seroconversion peaks: at 2 to 4 years and at 14 to 18 years.[13,14] Hence the mean seroprevalence in children is approximately 50%, which increases steadily to values of 90% to 99% in adults.[11,14] Healthy EBV-seropositive individuals harbor approximately 0.1 to 5 infected B lymphocytes per 10^6 peripheral blood mononuclear cells (MNCs).[15,16] This explains the transmissibility of EBV via the white blood cell (WBC) component of blood. In the United States the prevalence of EBV, detected by polymerase chain reaction (PCR) testing in blood from 100 randomly selected blood donors, has been reported to be 72%.[17] While leukoreduction can significantly reduce the number of EBV genomes in red blood cells (RBCs), it does not completely eliminate EBV-carrying cells.[18]

Nonetheless, with only a handful of documented cases, transfusion-transmitted EBV infection is apparently a relatively rare event. The first report was published by Gerber and colleagues,[19] who showed seroconversion in four of five EBV-seronegative patients undergoing open heart surgery. This was confirmed by another early study that reported that six of 18 EBV-seronegative patients acquired EBV infection following transfusions received after open heart surgery.[20] The risk of primary infection via transfusion in patients without existing antibodies to EBV has been estimated to be 33% to 46% or higher.[20] Several more case reports of EBV-induced postperfusion syndrome can be found in the literature.[21-25] Early studies estimated that EBV infection could be detected 2.5 to 9 weeks after transfusion.[26,27] Other more recent examples of EBV transfusion-transmitted infections have been published. One such case was that of a 19-year-old immune-competent man presenting with infectious mononucleosis 13 days after transfusion with blood that had not been leukoreduced.[28] In the transplant setting, Alfieri and colleagues[29] described the occurrence of a transfusion-related EBV transmission event from a blood donor to a 16-year-old liver transplant recipient who developed protracted infectious mononucleosis 6 weeks after liver transplant surgery. Another situation that provides evidence for the transmissibility of EBV via transfusion is the high prevalence of multiple EBV strains in hemophiliacs.[30]

Because EBV infection is linked to posttransplant lymphoproliferative disease (PTLD), a life-threatening complication occurring after hematopoietic stem cell transplantation (HSCT) and solid organ transplantation,[31] it is important to eliminate the source of infection when possible. For pediatric HSCT patients receiving an EBV-negative graft, transfused EBV-positive blood products may represent an important source of infection. This study was initiated, therefore, to document the risk of acquiring posttransplant EBV infection after blood product transfusion in a pediatric cohort of HSCT patients. Within this cohort we focused more specifically on the umbilical cord blood transplant group as these patients were most likely to have received EBV-negative grafts and EBV-positive blood products. The level of transfusion-related risk for this particular patient group to acquire EBV infection has, to our knowledge, not been reported, and is the focus of this study.

MATERIALS AND METHODS

Study design

A retrospective cohort study was initiated through chart review of all pediatric patients who received hematopoietic stem cell (HSC) grafts at Sainte-Justine Hospital from 1993 to 2009. Existing conditions for HSC transplant in these patients included acute myeloid leukemia, acute lymphoblastic leukemia, juvenile myelomonocytic leukemia, chronic myeloid leukemia, familial erythrophagocytic lymphohistiocytosis, Fanconi anemia, metachromatic leukodystrophy, and lymphoma. Patient charts were reviewed to retrieve information on: 1) the presence of EBV antibodies in pretransplant sera from recipients and HSC donors, 2) the incidence of posttransplant EBV infection in recipients until 1 year posttransplantation, 3) the transfusion history of recipients, and 4) the general characteristics of recipients. This study was approved by the research ethics committee of Sainte-Justine Hospital.

HSCT procedures

HSCT procedures were performed as previously reported.[32] Briefly, children with leukemia were treated with total body irradiation (12 Gy in eight fractions), 120 mg/kg cyclophosphamide over 2 days, and 40 mg/kg etoposide. Alternatively, intravenous (IV) busulfan, adjusted by therapeutic dosing to target a steady-state concentration of 1200 ng/mL (roughly equivalent to 0.8 mg/kg/dose), was given in 16 fractions over 4 days and with 200 mg/kg cyclophosphamide given over 4 days. After transplant, all acute leukemia patients received four to 12 monthly intrathecal methotrexate treatments followed by oral leucovorin rescue. Variations of this protocol for patients with Fanconi anemia, hemophagocytic syndrome, and other congenital or inherited conditions were described previously.[32] Graft-versus-host disease prophylaxis in all patients consisted of 2 mg/kg/day rabbit antithymocyte globulin (Thymoglobulin, Genzyme Corp., Cambridge, MA) administered on Days −2, −1, +1, and +2, along with cyclosporine A (CSA) given IV from Day −3 to Day +21 and orally thereafter. Dose adjustments were made to obtain CSA levels of 250 to 400 ng/mL. In the

absence of graft-versus-host disease, CSA was tapered off weekly by 5% starting at Day +100. In addition, cord blood recipients were treated with IV methylprednisolone, followed by 2 mg/kg/day oral prednisolone (tapered by 10% weekly, starting on Day +30). Supportive care was performed as previously reported.[32] This included weekly IV immunoglobulin (500 mg/kg) administered from transplant to Day +100, followed by monthly administration for 6 months. In addition, until 2006, during the months of October to April, monthly treatments of RSV hyperimmune globulin (400 mg/kg Respigam, Medimmune, Boston, MA) were given. Weekly monitoring for EBV and CMV was performed for at least the first 100 days posttransplant. Patients received irradiated, CMV-negative, and leukoreduced blood products to maintain platelet (PLT) counts higher than 20×10^9/L and hemoglobin levels above 70 g/L. Appropriate anti-CMV treatment was provided if two consecutive clinical samples were positive at the required threshold or if there was evidence of clinical disease. Our institution's protocol for diagnosis and treatment of EBV lymphoproliferative disease in allogeneic graft recipients specifies that PCR monitoring for EBV viral load be performed at regular intervals of at least 2 weeks or sooner posttransplant for approximately 4 months or as long as the patient remains immune suppressed. During their hospital stay, patients were isolated to prevent infection and were worked up for PTLD if the EBV PCR test attained the high-risk threshold. Patients were discharged approximately 6 weeks after transplantation.

Chart review for EBV serology, EBV viral load, and transfusion history

Pretransplant sera from recipients and HSC donors were tested for IgG antibodies to the EBV capsid antigen (VCA) using a standard indirect immune fluorescence assay and for antibodies to the Epstein-Barr nuclear antigen (EBNA) by anticomplement immune fluorescence assay. IgG antibody titers to EBV early antigen were also determined by immune fluorescence assay. Donors and recipients were classified according to their pretransplant serologic status as: 1) having past infection (VCA and EBNA IgG titers > 10), 2) having recent infection or being immune suppressed (VCA IgG titers > 10 and EBNA titers < 10), 3) having reactivated infection (VCA, EBNA, and early antigen IgG titers > 10), or 4) being seronegative or naïve (no serologic sign of prior infection). The incidence of posttransplant EBV infection was measured during the first year posttransplantation by semi- and quantitative PCR testing on samples of the recipient's blood taken regularly after transplantation (see above-mentioned protocol). The PCR test was scored as positive if the viral load surpassed the minimum threshold value. All blood products (measured in milliliters) received by the recipients were documented.

Statistical analysis

Descriptive statistics and Kaplan-Meier curves were used to analyze the cumulative incidence (and 95% confidence intervals [CIs]) of infection until 1 year of follow-up according to each recipient's pretransplant serologic status. This was also done for the group of recipients receiving only cord-blood (EBV-negative) grafts. Time zero was defined as the date of transplantation. Patients contributed to follow-up time until documentation of a positive EBV PCR test or until the last recorded visit date up to 1 year posttransplantation for censored observations. Cox regression was used to measure the association (relative risks [RRs] and 95% CI) between posttransplant EBV infection and 1) transfusion of blood products and 2) volume of blood products transfused. Tertile or quartile was used for the categorization of the variable "volume of transfusion." For the analysis regarding the risk conferred by the volume of transfusion, we also tested for trend by fitting models using the volume variable treated as ordinal based on the median value for each quartile or tertile of volume transfused. Types of blood products analyzed were those with potential for viral transmission, such as RBCs, plasma, and PLT concentrates (labile blood products).[33,34] Blood products manufactured with pasteurization (heat inactivation) and solvent/detergent viral inactivation procedures, such as albumin, were not considered in this analysis. Confusion was controlled for using the 5% change in estimate method considering variables such as type of transplantation (autologous, allogeneic cord blood, allogeneic other, or haploidentical), age (linear), sex (male or female), and year of diagnosis (before or after 2000, seeing that universal leukoreduction was instituted in Canada in 1999). All analyses were performed with computer software (Stata 11.1, StataCorp, College Station, TX).

RESULTS

EBV seroprevalence and infection in the cohort

A total of 487 charts were reviewed for HSC grafts performed on 422 pediatric patients between 1993 and 2009. All 422 pediatric recipients were included in this analysis, but only the first transplant was considered for patients receiving more than one graft. The majority of HSC grafts (317 of 422 [75%]) were performed after the implementation of universal leukoreduction. The mean and median ages at transplantation were 8.9 (standard deviation [SD], 5.2) and 8.5 (interquartile range [IQR], 3.6-14.1) years, respectively. There were 177 (42%) females and 245 (58%) males. Grafts were subdivided into three categories, namely 150 autologous (36%), 111 allogeneic cord blood

(26%), and 161 allogeneic other (38%; Table 1). The pretransplant EBV seroprevalence was 77.9% in this recipient cohort; thus 22.1% of our pediatric patients were EBV seronegative before transplantation. EBV seroprevalence data in HSC donors were calculated after excluding autologous and cord blood grafts and were available for 68% of the allogeneic HSC donors (110 of 161). EBV seropositivity among these donors was 61.8%, as determined by the presence of antibodies to VCA IgG. The median time between pretransplant serologic testing and the date of transplantation was 28 days (IQR, 17-54 days) for both recipients and HSC donors.

Table 2 and Fig. 1 show, for HSC recipients with EBV PCR testing, the cumulative incidence of posttransplant EBV infection at different time points stratified according to their pretransplant EBV serostatus. Only patients with EBV PCR results have been included in these analyses (238 patients). Patients with missing PCR results (most of whom are autologous transplant recipients) have been excluded. Among seronegative patients (EBV seronegative before transplantation), the 1-year cumulative incidence of EBV infection was 28.5% (95% CI, 14.2%-51.9%). A total of eight seronegative recipients developed EBV DNAemia within 1 year posttransplant. By 30 and 60 days, 4.6 (95% CI, 1.2-17.3) and 13.4% (95% CI, 5.8%-29.4%), respectively, had evidence of EBV DNAemia as revealed by positive PCR testing in blood. Among the group of seronegative recipients with posttransplant EBV DNAemia, one probable PTLD case was diagnosed and was fatal (data not shown).

TABLE 1. Graft category in recipient population*

Type of transplantation	Number* (%)
Autologous	
Peripheral blood stem cells	132 (31.3)
Marrow	18 (4.3)
Allogeneic	
Related marrow	115 (27.3)
Unrelated marrow	42 (10.0)
Related peripheral blood stem cells	3 (0.6)
Unrelated peripheral blood stem cells	1 (0.2)
Unrelated cord blood	109 (25.8)
Related cord blood	2 (0.5)
Total	422 (100)

* Includes information for first transplantation only in the case of patients who received more than one graft.

This deceased patient had been transfused with 6825 mL of RBCs and 9790 mL of PLTs, respectively, during the peritransplant period and had received a graft from a partially mismatched related donor who was EBV seropositive. The graft was not T depleted. The first positive EBV PCR test occurred on Day 48 posttransplant and progressed with unexplained fever, pleural effusion, digestive symptoms, increasing EBV DNAemia, and high EBV DNA viral load on biopsy specimen (antrum, duodenum, and sygmoid colon) on Day 68. Rituximab was administered, but the patient died on Day 88 posttransplant. This patient had not received immunosuppressive therapy before transplantation, apart from the conditioning regimen, which was begun after serologic testing.

We also noted a case of hemophagocytic syndrome probably related to EBV. This patient was seronegative before transplantation. However, due to a diagnosis of immune deficiency (Griscelli disease), the patient was classified among the group with unknown pretransplant serostatus (even though the serology result was probably valid). This recipient received 657 mL of RBCs and 1430 mL of PLTs during the peritransplant period and had received a marrow graft from a seropositive donor. The patient was first positive by EBV PCR testing at Day 98 posttransplant and did not receive immunosuppressive therapy before transplantation, apart from the conditioning therapy that was begun after serologic testing.

EBV seroprevalence and infection in the cord blood recipient subgroup

With rare exceptions, umbilical cord blood is typically negative for EBV.[35] This allowed us to consider the subgroup of EBV-negative recipients of cord blood who become EBV positive posttransplant as the ideal population to examine to resolve the question of whether EBV might infect HSC transplant patients through EBV-positive transfused blood products. Table 3 and Fig. 2 show the incidence of posttransplant EBV infection in the subgroup of children who received cord blood grafts. Interestingly, the 30-day cumulative incidence of EBV was 8.3% (95% CI, 2.2%-29.4%; two recipients of 24 had EBV DNAemia), whereas the 1-year cumulative incidence was 27.1% (95% CI, 10.1%-60.8%). All of these recipients received RBC and PLT transfusions during the peritrans-

TABLE 2. Cumulative incidence of posttransplant EBV infection* after HSCT according to recipient pretransplant EBV serostatus†

Cumulative incidence % (95% CI)	At 30 days	At 60 days	At 100 days	At 200 days	At 1 year
Seronegative recipients (n = 42)	4.6 (1.2-17.3)	13.4 (5.8-29.4)	16.4 (7.7-33.1)	16.4 (7.7-33.1)	28.5 (14.2-51.9)
Recipients with prior EBV antibodies (n = 185)	7.2 (4.2-12.1)	24.3 (18.6-31.5)	33.1 (26.4-40.9)	38.5 (31.2-46.9)	48.6 (39.5-58.5)
Unknown pretransplant serostatus (n = 11)	19.9 (5.2-57.7)	29.3 (10.5-66.3)	39.4 (16.9-74.2)	67.8 (36.2-94.1)	67.8 (36.2-94.1)

* EBV infection measured by PCR testing in blood. n = number of subjects (excludes patients who did not have EBV-PCR testing [missing EBV PCR] most of whom are autologous transplant recipients).
† Data are reported as percentage (95% CI).

plant period (mean of 805 mL RBCs [SD, 531 mL] and 1178 mL PLTs [SD, 697 mL]).

Classification and volume of blood products received by recipients and RR calculation

Table 4 provides a description of the total volume of blood products received by the recipients during the peritransplant period. The proportion of recipients who received at least one RBC transfusion was 93.3%. Nearly all recipients (99%) received PLTs. Thus only a few recipients (less than 1%) were free of transfused products.

Table 5 provides the adjusted RR for the association between transfusion of blood products (as well as for volume of transfusion) and posttransplant EBV infection. The adjusted RRs between posttransplant EBV infection and transfusion of RBCs and fresh-frozen plasma (FFP) were 2.36 (95% CI, 0.58-9.70) and 1.34 (95% CI, 0.62-2.93), respectively. It was not possible to study the association between EBV infection and the reception of PLTs because 99% of stem cell transplant recipients received PLTs. It was also not possible to analyze the association with cryoprecipitate and granulocyte transfusions because less than 2% of recipients had been transfused with these blood products. However, it was possible to analyze the association between the volume of blood products transfused and the posttransplant EBV infection. For all these labile blood products, a clear positive trend was shown. The risk of EBV infection increased with the augmentation of the volume transfused (most of the RR in the highest quartile or tertile as well as the p values for trend were significant). Also, as it is impossible to analyze the risk for RBCs independently from PLTs (seeing that virtually all recipients received PLTs), we analyzed the risk for RBCs restricted to the group of patients who received the lowest volume (less than 2000 mL) of PLTs (data not shown) and found similar results with a significant p value for trend ($p = 0.035$). We also ran the analysis after exclusion of recipients who received a graft before year 2000 (thus eliminating patients grafted before implementation of the universal leukoreduction system) and we obtained similar results (data not shown). For example, even with the use of leukoreduced products, the adjusted RR for patients who received over 200 mL of FFP was 3.80 (95% CI, 1.13-12.80) compared to those who did not receive FFP and the adjusted RR for patients receiving more than 2530 mL of PLTs was 2.54 (95% CI, 1.32-4.87) compared to those who received less than 1260 mL.

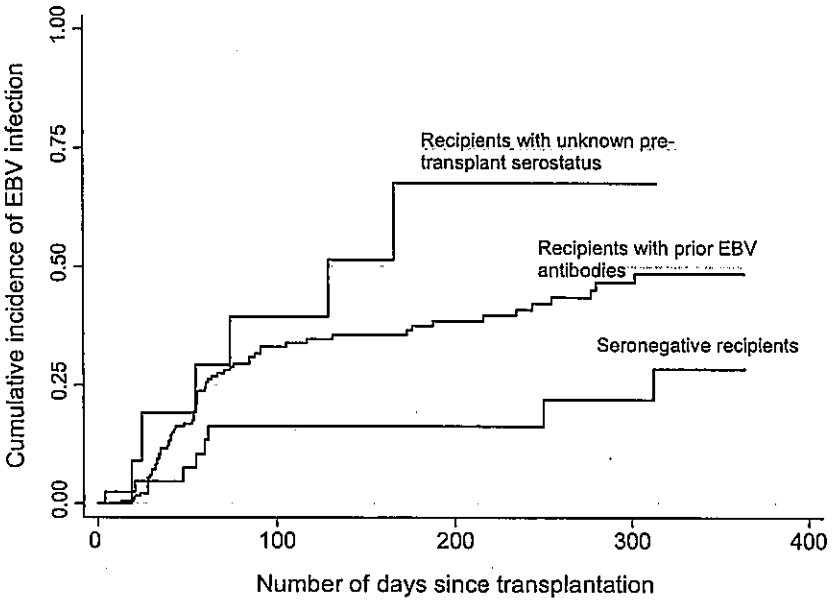


Fig. 1. Kaplan-Meier curve for the cumulative incidence of posttransplant EBV infection among all HSCT recipients according to their pretransplant EBV serostatus. EBV infection measured by PCR testing in blood. The difference between survival curves was not significant (p value = 0.08 by log-rank test).

DISCUSSION

Globally, the results of this study suggest that transfused leukoreduced blood is a vehicle for EBV transmission in immunosuppressed HSCT patients. In support of this we noted a significant and clear positive trend associating EBV infection to transfusion volume. Also, two cases of EBV DNAemia occurred in seronegative recipients of cord blood grafts within 30 days posttransplant;

TABLE 3. Posttransplant EBV infection* in recipients of cord blood grafts†

Cumulative incidence % (95% CI)	At 30 days	At 60 days	At 100 days	At 200 days	At 1 year
Seronegative recipients (n = 24)	8.3 (2.2-29.4)	8.3 (2.2-29.4)	8.3 (2.2-29.4)	8.3 (2.1-29.4)	27.1 (10.1-60.8)
Recipients with prior EBV antibodies (n = 70)	1.4 (0.2-9.7)	10.5 (5.2-20.8)	15.9 (8.8-27.7)	18.6 (8.8-27.7)	32.1 (19.7-49.4)
Unknown pretransplant serostatus (n = 6)	0	20.0 (3.1-79.6)	20.0 (3.1-79.6)	46.7 (13.7-93.2)	46.7 (13.7-93.2)

* EBV infection measured by PCR testing in blood. n = number of subjects (excludes patients who did not have EBV PCR testing [missing EBV PCR] most of whom are autologous transplant recipients).
† Data are reported as percentage (95% CI).

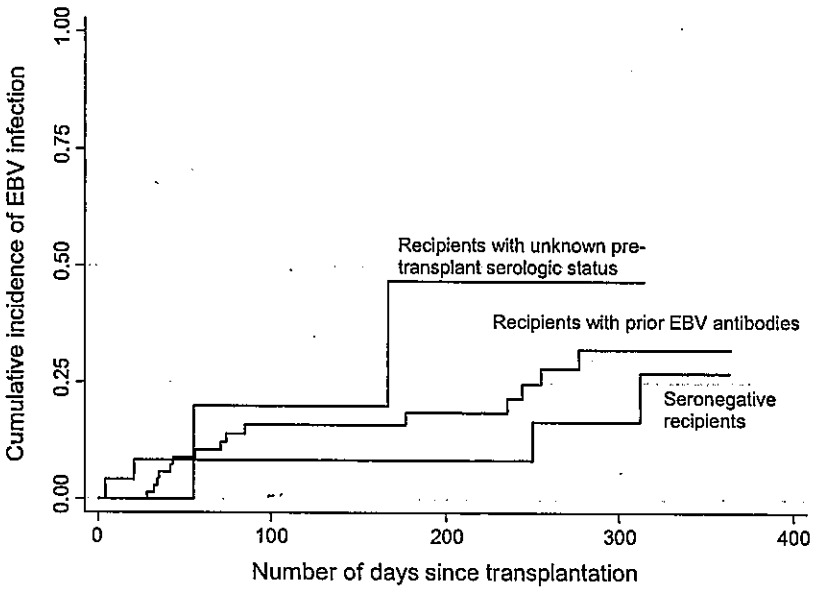


Fig. 2. Kaplan-Meier curve for the cumulative incidence of posttransplant EBV infection* among cord blood transplant recipients. *EBV infection measured by PCR testing in blood. The difference between survival curves was not significant (p value = 0.61 by log-rank test).

TABLE 4. Type of transfused blood product and quantity received by recipients

Type	Number of patients* (%)	Total volume (mL)	
		Median (IQR)	Mean (SD)
RBCs			
No	28 (6.7)		
Yes	389 (93.3)	1050 (600-2150)	1806 (2342)
FFP or frozen plasma			
No	383 (91.9)		
Yes	34 (8.1)	300 (200-1065)	2056 (6044)
PLT concentrates			
No	4 (1.0)		
Yes	413 (99.0)	1806 (825-3677)	3373 (5289)
Cryoprecipitates			
No	413 (99.0)		
Yes	4 (1.0)	90 (23-188)	105 (100)
Albumin			
5%			
No	369 (88.5)		
Yes	48 (11.5)	250 (250-500)	427 (383)
25%			
No	375 (89.9)		
Yes	42 (10.1)	250 (200-518)	500 (717)
Granulocytes			
No	414 (98.1)		
Yes	8 (1.9)	1682 (1568-3434)	2707 (2156)
Any product			
No	5 (1)		
Yes	417 (99)		

* Total frequencies may differ slightly from the total number of recipients because missing data are not listed. Includes data related to the first transplantation only.

these cannot be attributed to a reactivation event of endogenous recipient virus nor can they implicate the graft as a source of infection.

EBV pretransplant seroprevalence for our pediatric recipient cohort was 77.9%, a proportion substantially higher than the 50% documented in the solid organ pediatric transplant population.[36,37] It is possible that the higher seroprevalence in our cohort was due to passive antibody transfer given that children with leukemia and other malignant hematologic disorders are more likely to receive blood products before transplantation. On the other hand, we might be less confident about serology for the detection of a recent infection in patients receiving cancer chemotherapy. While we cannot completely rule out the possibility that EBV infection might have been missed in these patients before transplant, the patient charts show that our cohort had a higher prevalence of EBV antibodies before transplant than the expected EBV seroprevalence rate in children. Although our protocol does not include pretransplant PCR testing, serologic testing at our institution always includes both VCA IgG and EBNA antibody testing. We are therefore confident that the EBV serologic data for patients with a seronegative profile are accurate. Seroprevalence among HSC donors was 61.8%, which is within the range usually found in children. This was not surprising considering that donors are often the patients' siblings.

Our data indicate that 13.4% (95% CI, 5.8%-29.4%) of seronegative recipients developed EBV DNAemia within 60 days posttransplant. Moreover, among the group of eight seronegative recipients who developed EBV DNAemia, one case of probable PTLD was diagnosed and was fatal. Interestingly, this patient had received substantial amounts of RBCs (6825 mL) and PLTs (9790 mL) during the peritransplant period, a transfusion volume much greater than the average for this group of recipients (Table 4). Furthermore, it is noteworthy that this case of probable PTLD in seronegative patients, as well as most of

TABLE 5. Adjusted RR* for the association between transfusion of blood products and incidence of posttransplant EBV infection

Type of blood product†	Adjusted RR (95% CI)	p value for trend‡
RBC		
Transfusion		
No	1.00 (reference)	
Yes	2.37 (0.58-9.70)	
Volume of transfusion (mL)		
0	1.00 (reference)	0.047§
<850.0	1.99 (0.47-8.44)	
850.0-1890.0	2.40 (0.56-10.24)	
>1890.0	2.86 (0.68-12.11)	
FFP		
Transfusion		
No	1.00 (reference)	
Yes	1.34 (0.62-2.93)	
Volume of transfusion (mL)		
0	1.00 (reference)	0.079
<200.0	0.70 (0.22-2.25)	
>200.0	3.16 (1.00-11.17)§	
PLTs		
Transfusion		
No		
Yes		
Volume of transfusion (mL)		
< 1260	1.00 (reference)	0.012§
1260-2530	1.65 (0.86-3.18)	
>2530	2.19 (1.21-3.97)§	

* RRs are hazard ratio estimated with Cox regression and adjusted for empirical confounders using 5% change in estimate method (for variables such as type of transplantation [autologous, allogeneic cord blood, allogeneic other, or haploidentical], age [linear], year of diagnosis [before or after 2000], and sex [male or female]).

† It was not possible to analyze the risk related to the reception of PLTs because virtually all recipients received PLT concentrates. However, it was possible to analyze the risk associated with different volumes of PLTs transfused. Also, because of too little data, it was not possible to analyze the risk related to cryoprecipitate and granulocyte transfusions. Because albumin has no potential for viral transmission it was not considered in our analysis.

‡ We tested for trend by fitting models using the volume variable treated as ordinal based on the median value for each quartile or tertile of volume transfused.

§ Results significant.

the incidences of EBV posttransplant infection in our seronegative patients, occurred after the year 2000, and were subsequent to the implementation of universal prestorage leukoreduction in Canada.

Transmission of EBV in seronegative recipients may have occurred through virus contained either in the donor graft or in the transfused blood products. If EBV DNAemia were to occur in seronegative patients receiving an EBV-negative graft, then blood products could be suspected as the vector for transmission. Numerous cases of EBV DNAemia occurred in recipients for which the pretransplant serostatus of the donor was unknown (missing data). However, many children were transplanted with cord blood, which is normally negative for EBV.[35] Interestingly, within 30 days, EBV infection had occurred in 8.3% (95% CI, 2.1%-29.4%) of seronegative recipients after cord blood transplantation. Barring natural infection, which is possible but unlikely in such a short time period—more so because recipients were isolated in hospital in a HEPA air-filtered room—this strongly points to blood products as the vehicle for transmission. It is noteworthy that EBV seroconversion also occurred after 3 months, but these "late" cases are difficult to attribute to transfusion as most transfusions would have been expected in the first 3 months posttransplant. EBV is a ubiquitous virus transmitted by saliva; therefore, we cannot rule out the possibility that patients were exposed naturally to the virus after the isolation period. Natural infection may explain the cases of DNAemia especially those that occurred long after the transplant. One might also argue on the validity of the pretransplant serology of children with leukemia receiving immunosuppressive therapy. However, this cannot explain the seronegative status of our EBV-negative recipients of cord blood who developed EBV DNAemia, as none of these patients (except for one case which occurred within 30 days posttransplant) received pretransplant immunosuppressive therapy (apart from the pretransplant conditioning therapy, which always begins after testing for EBV serology).

Finally, it is not possible to completely rule out the possibility that EBV originated from the cord blood graft. Although such an event would be exceedingly rare, such unusual cases of EBV-infected cord blood have been documented. For example, Weinberg and colleagues[35] reported no case of positive EBV PCR among 362 cord blood samples. However, in 1973, Chang and Blakenship[38] showed that one of the 696 cord blood samples tested was EBV positive. One such case has also been documented by Haut and coworkers.[39]

The results of this study indicate that recipients who received RBC transfusions were 2.37 times more at risk of developing EBV DNAemia than those who did not receive RBCs (although this was not significant). We also showed a clear positive association between the volume of RBCs, plasma, and PLTs transfused and the incidence of posttransplant EBV infection. The RRs for the highest quartile or tertile of volume transfused was 2.86 (95% CI, 0.68-12.11) for RBCs, 3.16 (95% CI, 1.00-11.17) for plasma, and 2.19 (95% CI, 1.21-3.97) for PLTs and the p value for trend was significant for the volume of RBCs and PLTs transfused. This shows a clear association between transfusion and EBV infection.

In an effort to prevent transfusion reactions and transfusion-transmitted infectious diseases, Canadian Blood Services and Héma-Québec implemented systematic prestorage RBC unit leukoreduction in the summer of 1999; in addition, prestorage leukoreduction of PLTs had been available since February 1998. Leukoreduction is a process in which WBCs, ordinarily present in collected blood components, are intentionally reduced in number. Typically, the number of WBCs in a RBC unit is decreased from 5×10^9 to less than 5×10^6 WBCs per unit by prestorage leukoreduction.[40] Through this process the number of viral copies associated with WBCs would be expected to be reduced accordingly. It would have been very interesting to perform our analysis by comparing data from specimens taken before with those taken after the implementation of leukoreduction to measure the impact of leukoreduction on the risk of EBV transmission. Unfortunately, too few data were available before year 2000 to allow this stratification. However, our results indicate significant RRs even when the analysis was restricted to patients who received a graft after the implementation of universal leukoreduction. It is important to point out that leukoreduction does not reduce to zero the risk of transmitting certain viruses. For example, it has been shown that CMV-seronegative units may provide greater protection than leukoreduced products in some at-risk population groups such as transplant recipients and immunosuppressed patients.[41,42] This might also be the case for EBV.

Transmission of EBV infection by transfusion is thought to be relatively infrequent for the following reasons: 1) most adult recipients of blood and blood products are already immune to EBV; 2) whole blood and serum from seropositive donors contain EBV-neutralizing antibodies, which may protect the recipient from infection; 3) the viability of B lymphocytes carrying the EBV genome may decline during blood storage; 4) viral load in blood from healthy seropositive donors is normally low ($5/10^6$-$1/10^7$ peripheral blood MNCs); and 5) the risk of EBV transmission from RBC and/or PLT transfusions is significantly reduced by leukoreduction. Thus, in most instances, EBV genomes contained in blood products should not cause severe disease when the transfused recipient is immune competent. In fact, with regard to EBV, blood products are safe for the general adult population since over 90% of adults have immunity to EBV. Occurrence of infectious mononucleosis in EBV-negative recipients receiving EBV-positive blood products has been documented, but is rare.[28]

While immune-competent individuals can control the infection, those with congenital or acquired immunodeficiency are highly vulnerable to developing EBV-associated lymphoproliferative disease.[43-47] The overall incidence of PTLD among allogeneic HSCT adult recipients has been estimated to be approximately 1% (approx. 3% for pediatric HSCT).[12,48,49] This risk increases to more than 8% with the presence of risk factors such as T-cell depletion of the donor marrow.[10,49,50] The occurrence of PTLD is higher during the early posttransplant period due to the ablated state of the immune system. Lack of a robust immune response may lead to high EBV viral load which is a risk factor and prognostic indicator for PTLD.[10] Among allogeneic stem cell recipients who develop PTLD, approximately 25% will die and 25% will incur graft failure.[9] The mortality incidence after PTLD may reach 82%.[12] It stands to reason, therefore, that transfusion of EBV-positive blood products to immune-suppressed stem cell transplant pediatric patients may prove detrimental during the early posttransplant period.

Despite the limitations of this study, which include its retrospective design, missing chart data, and inclusion of only one center, there are nonetheless numerous strengths. One of these is the study's appreciable sample size. Further, the population is diverse and thus representative of a typical transplant population sampling from a large North American city. The results are clinically significant and suggest an association between EBV infection and transfusion of leukoreduced blood product units. The number of patients was too small to draw conclusions on any potential association between blood product transfusion and PTLD, but large enough to yield interesting RRs and to consider designing a prospective study in the pediatric transplant population. Unfortunately, typing of donor-recipient strains is not possible. Legal and ethical norms pertinent to blood donation require anonymity, thus impeding any tracing of donor units for EBV isolation and typing postdonation.

Our patient population included EBV-seronegative patients who showed a surprisingly high rate of EBV infection acquired within a time frame unlikely to be compatible with acquisition through an infected contact. Indeed, our data suggest that transmission of EBV infection occurred through the transfusion of blood products. To our knowledge, this is the first report to document the level of transfusion-related risk of acquiring EBV infection in an immunosuppressed population. Pretransplant EBV-seropositive recipients also showed evidence of EBV DNAemia at various time points posttransplant. For the latter, the source of the DNAemia may be reactivation of their own virus or new infection or reinfection by virus originating from the graft or from transfused blood products. Moreover, because cord blood progenitor cells are increasingly used for transplantation in children and because EBV is not normally found in cord blood,[35] the probability of EBV infection via the donor graft is essentially eliminated. This points to the potential importance of EBV in blood products as a source of infection among the pediatric transplant population. It also suggests a need to consider instituting EBV screening of blood products destined to immunosuppressed pediatric patients and

developing appropriate EBV prophylactic measures (vaccine, antibody therapy) for use in such patients. Instituting EBV screening of blood products may not be easy to achieve given the high prevalence of EBV seropositivity in adults, but it would be theoretically possible taking into account that approximately 1% to 10% of blood donors might be called upon to give blood for such a small subgroup of patients.

ACKNOWLEDGMENTS

HT holds a salary award from the Fonds de la Recherche en Santé du Québec (FRSQ). The authors are grateful to Nicole Poitras, Anne-Marie Fontaine, Aniela Pruteanu, Hugues Charron, Mariana Dumitrascu, and Louise Laporte for the extraction of data and chart review. HT designed the study, provided oversight for all aspects of the study including data extraction from patient charts, performed the statistical analyses, and was the principal author of the manuscript. CB reviewed the PTLD charts and provided essential clinical information with regard to patient outcome. MD contributed his cohort of HSC transplant patients. NR lent her expertise in blood banking procedures and transfusion medicine. MT and JL contributed their knowledge of blood safety and assisted with study design and data interpretation. CA provided expert advice on interpretation of EBV analyses and assisted with writing the manuscript. All authors have read and commented on the various versions of this paper.

CONFLICT OF INTEREST

The authors declare no competing financial interests. The authors were not restricted in experimental design, in data collection and analysis, nor in public disclosure of the findings contained in this manuscript.

REFERENCES

1. Hema-Québec. Safety measures in place. [cited 2011 May 24]. Available from: URL: http://www.hema-quebec.qc.ca/comprendre/mesures-de-securite/securite-en-place.en.html
2. Canadian Blood Services. Safety and testing. [cited 2011 May 3]. Available from: URL: http://www.bloodservices.ca/CentreApps/Internet/UW_V502_MainEngine.nsf/page/Testing?OpenDocument
3. Macdonald N. Transfusion and risk of infection in Canada: update 2005. Can J Infect Dis Med Microbiol 2005; 16:161-5.
4. Chamberland ME, Alter HJ, Busch MP, Nemo G, Ricketts M. Emerging infectious disease issues in blood safety. Emerg Infect Dis 2001;7 Suppl:552-3.
5. Strong DM, Katz L. Blood-bank testing for infectious diseases: how safe is blood transfusion? Trends Mol Med 2002;8:355-8.
6. Vamvakas EC. Is human herpesvirus-8 transmitted by transfusion? Transfus Med Rev 2010;24:1-14.
7. Karimi G, Samiei S, Hatami H, Gharehbaghian A, Vafaiyan V, Namini MT. Detection of Chlamydia pneumoniae in peripheral blood mononuclear cells of healthy blood donors in Tehran Regional Educational Blood Transfusion Centre. Transfus Med 2010;20:237-43.
8. Trimble SR, Parker CS, Grant AM, Soucie JM, Reyes N. Assessing emerging infectious threats to blood safety for the blood disorders community. Am J Prev Med 2010;38 Suppl:S468-74.
9. Smith JM, Corey L, Healey PJ, Davis CL, McDonald RA. Adolescents are more likely to develop posttransplant lymphoproliferative disorder after primary Epstein-Barr virus infection than younger renal transplant recipients. Transplantation 2007;83:1423-8.
10. Aalto SM, Juvonen E, Tarkkanen J, Volin L, Haario H, Ruutu T, Hedman K. Epstein-Barr viral load and disease prediction in a large cohort of allogeneic stem cell transplant recipients. Clin Infect Dis 2007;45:1305-9.
11. Allen U, Alfieri C, Preiksaitis J, Humar A, Moore D, Tapiero B, Tellier R, Green M, Davies D, Hébert D, Weitzman S, Petric M, Jacobson K; Canadian PTLD Workshop Group— 1999. Epstein-Barr virus infection in transplant recipients: summary of a workshop on surveillance, prevention and treatment. Can J Infect Dis 2002;3:89-99.
12. Ocheni S, Kroeger N, Zabelina T, Sobottka I, Ayuk F, Wolschke C, Muth A, Lellek H, Petersen L, Erttmann R, Kabisch H, Zander AR, Bacher U. EBV reactivation and post transplant lymphoproliferative disorders following allogeneic SCT. Bone Marrow Transplant 2008;42: 181-6.
13. Higgins CD, Swerdlow AJ, Macsween KF, Harrison N, Williams H, McAulay K, Thomas R, Reid S, Conacher M, Britton K, Crawford DH. A study of risk factors for acquisition of Epstein-Barr virus and its subtypes. J Infect Dis 2007;195:474-82.
14. Pariente M, Bartolome J, Lorente S, Crespo MD. Age distribution of serological profiles of Epstein-Barr virus infection: review of results from a diagnostic laboratory. Enferm Infecc Microbiol Clin 2007;25:108-10.
15. Rocchi G, de Felici A, Ragona G, Heinz A. Quantitative evaluation of Epstein-Barr virus-infected mononuclear peripheral blood leukocytes in infectious mononucleosis. N Engl J Med 1977;296:132-4.
16. Riddler SA, Breinig MC, McKnight JL. Increased levels of circulating Epstein-Barr virus (EBV)-infected lymphocytes and decreased EBV nuclear antigen antibody responses are associated with the development of posttransplant lymphoproliferative disease in solid organ transplant recipients. Blood 1994;84:972-84.
17. Hudnall SD, Chen T, Allison P, Tyring SK, Heath A. Herpesvirus prevalence and viral load in healthy blood donors by quantitative real-time polymerase chain reaction. Transfusion 2008;48:1180-7.

18. Wagner HJ, Kluter H, Kruse A, Bucsky P, Hornef M, Kirchner H. Determination of the number of Epstein-Barr virus genomes in whole blood and red cell concentrates. Transfus Med 1995;5:297-302.
19. Gerber P, Walsh JH, Rosenblum EN, Purcell RH. Association of EB-virus infection with the post-perfusion syndrome. Lancet 1969;1:593-5.
20. Henle W, Henle G, Scriba M, Joyner CR, Harrison FS Jr, Von Essen R, Paloheimo J, Klemola E. Antibody responses to the Epstein-Barr virus and cytomegaloviruses after open-heart and other surgery. N Engl J Med 1970;282: 1068-74.
21. Carter JW, Edson RS, Kennedy CC. Infectious mononucleosis in the older patient. Mayo Clin Proc 1978;53:146-50.
22. McMonigal K, Horwitz CA, Henle W, Henle G, Lawton J, Polesky H, Peterson L. Post-perfusion syndrome due to Epstein-Barr virus. Report of two cases and review of the literature. Transfusion 1983;23:331-5.
23. Turner AR, McDonald RN, Cooper BA. Transmission of infectious mononucleosis by transfusion of pre-illness plasma. Ann Int Med 1972;77:751-3.
24. Blacklow NR, Watson BK, Miller G, Jacobson BM. Mononucleosis with heterophil antibodies and EB virus infection. Acquisition by an elderly patient in hospital. Am J Med 1971;51:549-52.
25. Henle W, Henle G. Epstein-Barr virus and blood transfusion. In: Dodd RY, Barker LF, editors. Infection, immunity and blood transfusion. Proceedings of the XVth Annual Scientific Sympoium of the American Red Cross. New York: Liss; 1985. p. 201.
26. Solem JH, Jörgensen W. Accidentally transmitted infectious mononucleosis. Report of a case. Acta Med Scand 1969; 186:433-7.
27. Kapsenberg JG, Langenhuysen MM, Nieweg HO, Deiss H. Posttransfusion mononucleosis with heterophil antibodies. Simultaneous infection with cytomegalovirus and EB virus. Acta Med Scand 1970;187:79-82.
28. Tattevin P, Crémieux AC, Descamps D, Carbon C. Transfusion-related infectious mononucleosis. Scand J Infect Dis 2002;34:777-8.
29. Alfieri C, Tanner J, Carpentier L, Perpête C, Savoie A, Paradis K, Delage G, Joncas J. Epstein-Barr virus transmission from a blood donor to an organ transplant recipient with recovery of the same virus strain from the recipient's blood and oropharynx. Blood 1996;87:812-7.
30. Yao QY, Croom-Carter DS, Tierney RJ, Habeshaw G, Wilde JT, Hill FG, Conlon C, Rickinson AB. Epidemiology of infection with Epstein-Barr virus types 1 and 2: lessons from the study of a T-cell-immunocompromised hemophilic cohort. J Virol 1998;72:4352-63.
31. Murdock J, Morris TC, Alexander HD, McNamee P. Red cell aplasia due to erythrovirus parvovirus B19 infection in a Jehovah's witness with post-transplant EBV-induced aggressive lymphoma: management considerations. Leuk Lymphoma 2002;43:211-3.
32. Dalle J, Duval M, Moghrabi A, Wagner E, Vachon MF, Barrette S, Bernstein M, Champagne J, David M, Demers J, Rousseau P, Winikoff R, Champagne MA. Results of an unrelated transplant search strategy using partially HLA-mismatched cord-blood as an immediate alternative to HLA-matched bone marrow. Bone Marrow Transplant 2004;33:605-11.
33. Tabor E. The epidemiology of virus transmission by plasma derivatives: clinical studies verifying the lack of transmission of hepatitis B and C viruses and HIV type 1. Transfusion 1999;39:1160-8.
34. Hillyer CD, Silberstein LE, Ness PM, Anderson KC, Roback JD, editors. Blood banking and transfusion medicine—basic principles and practice. 2nd ed. Philadelphia (PA): Churchill Livingstone; 2007.
35. Weinberg A, Enomoto L, Li S, Shen D, Coll J, Shpall EJ. Risk of transmission of herpesviruses through cord blood transplantation. Biol Blood Marrow Transplant 2005;11:35-8.
36. Breinig MK, Zitelli B, Starzl TE, Ho M. Epstein-Barr virus, cytomegalovirus, and other viral infections in children after liver transplantation. J Infect Dis 1987;156:273-9.
37. Ho M, Jaffe R, Miller G, Breinig MK, Dummer JS, Makowka L, Atchison RW, Karrer F, Nalesnik MA, Starzl TE. The frequency of Epstein-Barr virus infection and associated lymphoproliferative syndrome after transplantation and its manifestation in children. Transplantation 1988;45:719-27.
38. Chang RS, Blakenship W. Spontaneous in vitro transformation of leukocytes from a neonate. Proc Soc Exp Biol Med 1973;144:337-9.
39. Haut PR, Kovarik P, Shaw PH, Walterhouse D, Jenson HB, Kletzel M. Detection of EBV DNA in the cord blood donor for a patient developing Epstein-Barr virus-associated lymphoproliferative disorder following mismatched unrelated umbilical cord blood transplantation. Bone Marrow Transplant 2001;27:761-5.
40. Gibson BE, Todd A, Roberts I, Pamphilon D, Rodeck C, Bolton-Maggs P, Burbin G, Duguid J, Boulton F, Cohen H, Smith N, McClelland DB, Rowley M, Turner G; British Commitee for Standards in Haematology Transfusion Task Force: Writing group. Transfusion guidelines for neonates and older children. Br J Haematol 2004;124:433-53.
41. Nichols WG, Price TH, Gooley T, Corey L, Boeckh M. Transfusion-transmitted cytomegalovirus infection after receipt of leukoreduced blood products. Blood 2003;101: 4195-200.
42. Hillyer CD, Strauss RG, Luban NL. Handbook of pediatric transfusion medicine. San Diago (CA): Academic; 2004.
43. Joncas JH, Russo P, Brochu P, Simard P, Brisebois J, Dubé J, Marton D, Leclerc JM, Hume H, Rivard GE. Epstein-Barr virus polymorphic B-cell lymphoma associated with leukemia and with congenital immunodeficiencies. J Clin Oncol 1990;8:378-84.
44. Young L, Alfieri C, Hennessy K, Evans H, O'Hara C, Anderson KC, Ritz J, Shapiro RS, Rickinson A, Kieff E, Cohen JI. Expression of Epstein-Barr virus transformation-associated

genes in tissues of patients with EBV lymphoproliferative disease. N Engl J Med 1989;321:1080-5.
45. Hanto DW, Gajl-Peczalska KJ, Frizzera G, Arthur DC, Balfour HH Jr, McClain K, Simmons RL, Najarian JS. Epstein-Barr virus (EBV) induced polyclonal and monoclonal B-cell lymphoproliferative diseases occurring after renal transplantation. Clinical, pathologic, and virologic findings and implications for therapy. Ann Surg 1983;198: 356-69.
46. Zutter MM, Martin PJ, Sale GE, Shulman HM, Fisher L, Thomas ED, Durnam DM. Epstein-Barr virus lymphoproliferation after bone marrow transplantation. Blood 1988; 72:520-9.
47. Cohen JI. Epstein-Barr virus lymphoproliferative disease associated with acquired immunodeficiency. Medicine (Baltimore) 1991;70:137-60.
48. Curtis RE, Travis LB, Rowlings PA, Socié G, Kingma DW, Banks PM, Jaffe ES, Sale GE, Horowitz MM, Witherspoon RP, Shriner DA, Weisdorf DJ, Kolb HJ, Sullivan KM, Sobocinski KA, Gale RP, Hoover RN, Fraumeni JF Jr, Deeg HJ. Risk of lymphoproliferative disorders after bone marrow transplantation: a multi-institutional study. Blood 1999;94: 2208-16.
49. Landgren O, Gilbert ES, Rizzo JD, Socié G, Banks PM, Sobocinski KA, Horowitz MM, Jaffe ES, Kingma DW, Travis LB, Flowers ME, Martin PJ, Deeg HJ, Curtis RE. Risk factors for lymphoproliferative disorders after allogeneic hematopoietic cell transplantation. Blood 2009;113:4992-5001.
50. Cesaro S, Pegoraro A, Tridello G, Calore E, Pillon M, Varotto S, Abate D, Barzon L, Mengoli C, Carli M, Messina C. A prospective study on modulation of immunosuppression for Epstein-Barr virus reactivation in pediatric patients who underwent unrelated hematopoietic stem-cell transplantation. Transplantation 2010;89:1533-40.

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
				2012. 11. 19	該当なし	

一般的名称	新鮮凍結人血漿	研究報告の公表状況	Gobbini F, Owusu-Ofori S, Marcelin AG, Candotti D, Allain JP. Transfusion. 2012 Nov;52(11):2294-9. doi: 10.1111/j.1537-2995.2012.03607.x. Epub 2012 Mar 15.	公表国 英国
販売名（企業名）	新鮮凍結血漿-LR「日赤」（日本赤十字社） 新鮮凍結血漿-LR「日赤」成分採血（日本赤十字社） 新鮮凍結血漿-LR「日赤」120（日本赤十字社） 新鮮凍結血漿-LR「日赤」240（日本赤十字社） 新鮮凍結血漿-LR「日赤」480（日本赤十字社）			

研究報告の概要

○西アフリカの流行地域であるガーナにおける、ヒトヘルペスウイルス8の輸血感染
ヒトヘルペスウイルス8（HHV-8）の抗体陽性率は、欧州や北米では5%未満、サハラ以南アフリカでは50～70%と地域によって開きがある。HHV-8の輸血感染の証拠は間接的なものに留まっている。今回、供血者と全血輸血を受けた免疫正常受血者のペア252組から得た検体に対して血清学的検査及び分子生物学的検査を行った。その結果、受血者28人（11%）及び供血者16人（6%）がHHV-8抗体陽性であり、抗体陽性の血液を輸血された抗体陰性受血者12人中1人（8.3%　信頼区間0～23%）に感染の疑いが高いことが確認された。当該供血者の血液にはHHV-8 DNAが含まれており、当該供血者を含む5人のHHV-8 DNAの配列は、ブートストラップ値97%で既知のジェノタイプとは異なるクラスターを形成していた。そのため、新しいジェノタイプ（HHV-8-G）を命名法に加えることを提案する。
今回のHHV-8伝播は、受血者の多くが免疫正常者であったため、臨床的影響はなかった。しかし、サハラ以南アフリカでは、免疫抑制剤の使用の増加に伴い、臨床的なリスクは増大するだろう。

使用上の注意記載状況・その他参考事項等

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

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
ヒトヘルペスウイルス8（HHV-8）の流行地域であるガーナにおいて、血液供血者と受血者のペア252組についてHHV-8の検査を行ったところ、抗体陽性血液を輸血した抗体陰性受血者12人中1人に感染の疑いが高いことが示され、また当該供血者を含む5人のHHV-8 DNA配列は既知のジェノタイプとは異なるクラスターを形成していることが分かったとの報告である。	今後も情報の収集に努める。

10

Human herpesvirus 8 transfusion transmission in Ghana, an endemic region of West Africa

Francesca Gobbini, Shirley Owusu-Ofori, Anne-Geneviève Marcelin, Daniel Candotti, and Jean-Pierre Allain

BACKGROUND: Human herpesvirus 8 (HHV-8) seroprevalence ranges between less than 5% in Europe and North America and 50% to 70% in sub-Saharan Africa. Evidence of HHV-8 transfusion transmission is only indirect. We conducted a serologic (anti-HHV-8) and molecular (HHV-8 DNA) study of samples from paired donor-immunocompetent recipients transfused with whole blood.

STUDY DESIGN AND METHODS: Samples from 252 donor-recipient pairs were tested. Immunoglobulin G to HHV-8 was detected with enzyme immunoassays and confirmed with an in-house immunofluorescence assay. The cellular fraction from seroreactive donors and their recipients was tested for HHV-8 DNA.

RESULTS: Anti-HHV-8 was positive (reactive in two or more assays) in 28 (11%) patients and 16 (6%) donors. Of 12 seronegative recipients (at risk of transmission) receiving seropositive blood, one very likely transmission was identified (8.3% confidence interval, 0% 23%). The donor blood contained HHV-8 DNA and his and four other donors' sequences clustered separately from recorded genotypes with a 97% bootstrap constituting a distinct genotype.

CONCLUSIONS: HHV-8 is transmitted in Ghana but does not carry clinical consequences since most patients are immunocompetent. The clinical risk will increase with the availability of immunosuppressive drugs in sub-Saharan Africa. We propose that a new genotype (HHV-8-G for Ghana) be added to the current nomenclature.

Human herpesvirus 8 (HHV-8), also known as Kaposi sarcoma–associated herpes virus, was identified as the etiologic agent of Kaposi's sarcoma and was associated with two B-cell lymphoproliferative disorders: primary effusion lymphoma and multicentric Castelman's disease.[1]

HHV-8 seroprevalence varies geographically. In Africa, up to 50% of the population is seropositive,[2] while in northern Europe and America the seroprevalence is less than 5%, increasing to 10% to 15% in Mediterranean regions.[3] Eight genotypes have been identified so far. Genotypes A/C, J, and K are prevalent in Europe, the United States, North of Asia, and the Middle East; in South Asia and Polynesia Genotype D/F has been found while B, Q, R, and N have been identified in sub-Saharan Africa.[4]

In sub-Saharan Africa, routes of HHV-8 transmission include saliva contact within family members and infection occurs mostly during childhood.[2,5,6] In low-prevalence developed countries, sexual transmission between men appears more frequent than in heterosexual relationships.[3] HHV-8 transmission after transplantation

ABBREVIATIONS: HHV-8 = human herpesvirus 8; IFA = immunofluorescence assay; qPCR = quantitative polymerase chain reaction; S/CO = sample-to-cutoff ratio; SNP(s) = single-polymorphism nucleotide(s).

From the Division of Transfusion Medicine, Department of Haematology, University of Cambridge, Cambridge, UK; the Transfusion Medicine Unit, Komfo Anokye Teaching Hospital, Kumasi, Ghana; the Virology Laboratory Hospital Pitié-Salpêtrière, Paris, France; and the National Blood Service, Cambridge Blood Centre, Cambridge, UK.

Address reprint requests to: J.-P. Allain, Department of Haematology, Cambridge Blood Centre, Long Road, Cambridge CB2 2PT, UK; e-mail: jpa1000@cam.ac.uk.

The BOTIA repository was supported by a grant from the FP6 EC (SP23-CT-2006-006487).

Received for publication October 12, 2011; revision received January 10, 2012, and accepted January 10, 2012.

doi: 10.1111/j.1537-2995.2012.03607.x

TRANSFUSION 2012;52:2294-2299.

of organs (mostly kidney) from HHV-8–seropositive donors was reported as well as viral reactivation in seropositive patients receiving immunosuppressive drugs.[1]

Transmission through blood products remains unclear but is considered low risk.[7] In 2009, Cannon and colleagues[8] conducted a large study including linked donor-recipient samples and found no transmission of HHV-8 through blood transfusion. In Uganda, a high-prevalence country, Hladik and colleagues[9] concluded on the basis of serologic indirect evidence to an HHV-8 transfusion transmission risk of 2.8% in seronegative recipients receiving blood from a seropositive donor.

To estimate the risk of transfusion-transmitted HHV-8 infection in a different sub-Saharan African endemic country (Ghana), we conducted a serologic and molecular study on paired donor-recipient blood samples from immunocompetent recipients transfused with whole blood.

MATERIALS AND METHODS

Samples collection

Whole blood samples from patients transfused at the Komfo Anokye Teaching Hospital (Kumasi, Ghana, West Africa) were obtained from the Blood and Organ Transmissible Infectious Agent repository.[10] None of the units transfused were leukoreduced nor washed. A total of 252 sets of donor (<2 weeks of storage before transfusion) and before and 3-months-posttransfusion recipient samples were tested. Whole blood was collected in 10 mL K3 EDTA tubes, separated by decantation, into plasma and cellular fraction, and both were stored at –40°C or below.

HHV-8 serology

Immunoglobulin (Ig)G antibodies to HHV-8 lytic antigens were tested using a bipeptide enzyme immunoassay (EIA; Biotrin International GmbH, Heidelberg, Germany) following the manufacturer's instructions. Samples with a sample-to-cutoff ratio (S/CO) value of less than 0.8 were considered nonreactive; S/CO values of more than 1.2 were reactive and S/CO values between 0.8 and 1.2 were considered undetermined (gray zone). The manufacturer discontinued distribution of this kit halfway through the study so the commercial Advanced Biotechnologies (Columbia, MD) EIA was used to test the rest of the samples. Sixty-nine samples were tested with both commercial kits and only 43 showed concordant results. Because of the high frequency of discrepancy between the two assays, we decided to use as confirmation method for positive samples an in-house immunofluorescence assay in Prof. Agut's Laboratory at Hospital Pitié-Salpêtrière, Paris, France.[11]

HHV-8 DNA extraction, PCR, and sequencing

Viral DNA was isolated from 200 µL of cell fraction by using a viral nucleic acid kit (High Pure, Roche, Lewes, UK) according to the manufacturer's instructions. HHV-8 DNA was detected by a real-time quantitative PCR (qPCR) assay as previously described.[12] Tenfold dilutions of an in-house plasmid pKS2471 containing the HHV-8 ORF26 were used to construct the standard curve and estimate the viral load. To confirm qPCR results, a 1251-nucleotide region including the minor capsid protein coded by ORF26 was amplified by a more sensitive seminested PCR. The forward primers used were KS26/D[4] in the first round of amplification and Orf26Fwd1[13] in the second round. LGH2574[4] was used as reverse primer in both rounds. The two amplification reactions were performed in identical conditions. Briefly, 5 µL of extracted DNA was amplified in a 50-µL mixture containing 1× HiFi Buffer 3 (Roche), 1.5 mmol/L $MgCl_2$, 0.8 mmol/L dNTPs, 0.6 µmol/L of each primer, 2.6 units of enzyme blend (Expand High-Fidelity, Roche), and 26.25 µL of DNase-free water. After an initial denaturation at 94°C for 5 minutes, a touchdown amplification was carried out as follows: 94°C for 1 minute, 60°C for 1 minute, and 72°C for 2 minutes. The 1-minute annealing temperature was 60°C for five cycles, 58°C for five cycles, 57°C for five cycles, and then 55°C for the last 15 cycles. Nested PCR products were purified from gel and sequenced at the University of Cambridge Biochemistry Department with a DNA analyzer (3730xl, Applied Biosystems) using the primers utilized in the PCR. All the ORF26 sequences obtained were submitted to GenBank under the Accession Numbers JN662003 to JN662017.

RESULTS

HHV-8 seroprevalence and transfusion transmission

The Blood and Organ Transmissible Infectious Agent repository including paired pretransfusion and 3-months-posttransfusion samples from Ghanaian immunocompetent whole blood recipients and corresponding donors were tested to investigate the largely unknown transfusion transmission of HHV-8 in a context of reported high endemicity.

Posttransfusion samples from 252 Ghanaian patients and 248 blood donors were tested for HHV-8 IgG antibodies. Results are summarized in Table 1. After a first enzyme-linked immunosorbent assay (ELISA) screening, 72 (29%) of the 252 patients and 56 (23%) of the 248 donors tested positive for HHV-8 antibodies. However, only 29 (11%) patients and 16 (6%) donors were confirmed reactive. Recipients of 16 transfusions with seropositive blood and donors of 29 seroreactive recipients after transfusion were retrospectively investigated.

TABLE 1. Serologic and molecular results of Ghanaian donor and recipient samples

	Anti-HHV-8 (S/CO)		
	Reactive	Nonreactive	HHV-8 DNA
Whole blood donations	16	232	2 positive
Posttransfusion recipients	29	223	2 positive
Pretransfusion recipients of seropositive blood	2	12	0
Posttransfusion exposed* susceptible recipients	1	11	0
Donor 2027	1 (4.7)		Positive
Recipient 2027			
Before transfusion		1	Negative
After transfusion	1 (9.5)		Negative
Donor 2003	1 (3.0)		Positive
Recipient 2003			
Before transfusion		1	Negative
After transfusion		1	Negative

* Seronegative before transfusion.

Before transfusion, 12 patients exposed to seroreactive blood were anti-HHV-8 seronegative and considered susceptible (at risk) to whole blood transfusion–related infection. One of them was seropositive after transfusion and 11 remained seronegative (Table 1). Donor 2027 and the paired susceptible recipient after transfusion were both anti-HHV-8 reactive with 4.7 and 9.5 S/CO, respectively. Anti-HHV-8 in donation 2027 was confirmed by immunofluorescence. Plasma and cellular fraction samples from this pair were tested for HHV-8 DNA. Donor 2027 blood contained HHV-8 DNA but not the recipient's sample collected 3 months after transfusion. In contrast, the recipient of donation 2003 that contained both antibodies and HHV-8 DNA remained seronegative after transfusion (Table 1). The other 10 susceptible recipients exposed to seropositive but DNA negative whole blood did not show serologic or molecular evidence of HHV-8 infection.

HHV-8 genomic sequence and new subtype

To confirm the qPCR screening results, HHV-8 DNA from two viremic patients and two donors was amplified by nested PCR targeting ORF26. Phylogenetic analysis of the sequences suggested that they formed a cluster different from previously reported African strains. To investigate further this genetic variability, 34 Ghanaian HIV-infected samples identified as anti-HHV-8 positive in a previous study were tested for HHV-8 DNA. The ORF26 region was successfully amplified and sequenced in four samples. In parallel, 84 random blood donors' samples from Guinea, another West African country, were screened for HHV-8 DNA and seven sequences were obtained. The Guinean samples clustered with the Q genotype but five of eight Ghanaian sequences clustered separately from the other GenBank references with a bootstrap value of 97% over 1000 replicates (Fig. 1) constituting a separate and new genotype. The other three Ghanaian sequences (Gh1623, 2003D, 2027D) clustered with other genotypes (two R and one Q; Fig. 1).

DISCUSSION

Transmission of HHV-8 through blood transfusion remains a controversial topic. Despite the seroprevalence in the general population and in blood donors in North America and North Europe ranging between 3.5 and 7.3%, no cases of transfusion transmission were reported.[3] HHV-8 DNA has been found in blood donors and healthy individuals in low- and higher-prevalence endemic areas such as Italy[14] and Africa.[4,12,13] These findings, in addition to the reports of HHV-8 infection associated with injection drug use,[7] raised the concern of HHV-8 transmission through blood products. Two studies conducted in Uganda by Hladik and coworkers[9] and Mbulaiteye and coworkers[15] found a risk of HHV-8 transmission through blood product of 2.8 and 2.6%, respectively. However, the conclusions of these two reports were purely based on serological testing and did not include DNA detection.

In this study the risk of HHV-8 transmission through blood transfusion in Ghana was investigated. A seroprevalence of 6% in blood donors and 11% in immunocompetent patients who received blood transfusion was found (Table 1). The relatively low seroprevalence compared to the study of Ablashi and colleagues[5] might be explained by the fact that we used a very specific immunofluorescence assay (IFA) to confirm initial results obtained by a less laborious, more sensitive, but less specific ELISA kit. Admittedly, however, IFA may present its own problems. Our results are more in line with the lower seroprevalence found in Zimbawe and South Africa.[6] One likely case of HHV-8 transmission was identified. Recipient 2027, negative for IgG to HHV-8 before transfusion, seroconverted after receiving an antibody and DNA positive whole blood transfusion. As demonstrated by Fowlkes and coworkers,[16] passive antibodies are detected immediately after transfusion with a relatively high titer and become undetectable in approximately 3 weeks. Immune response becomes detectable 4 to 10 weeks posttransfusion and is high titer. Our case clearly falls in the second scenario.

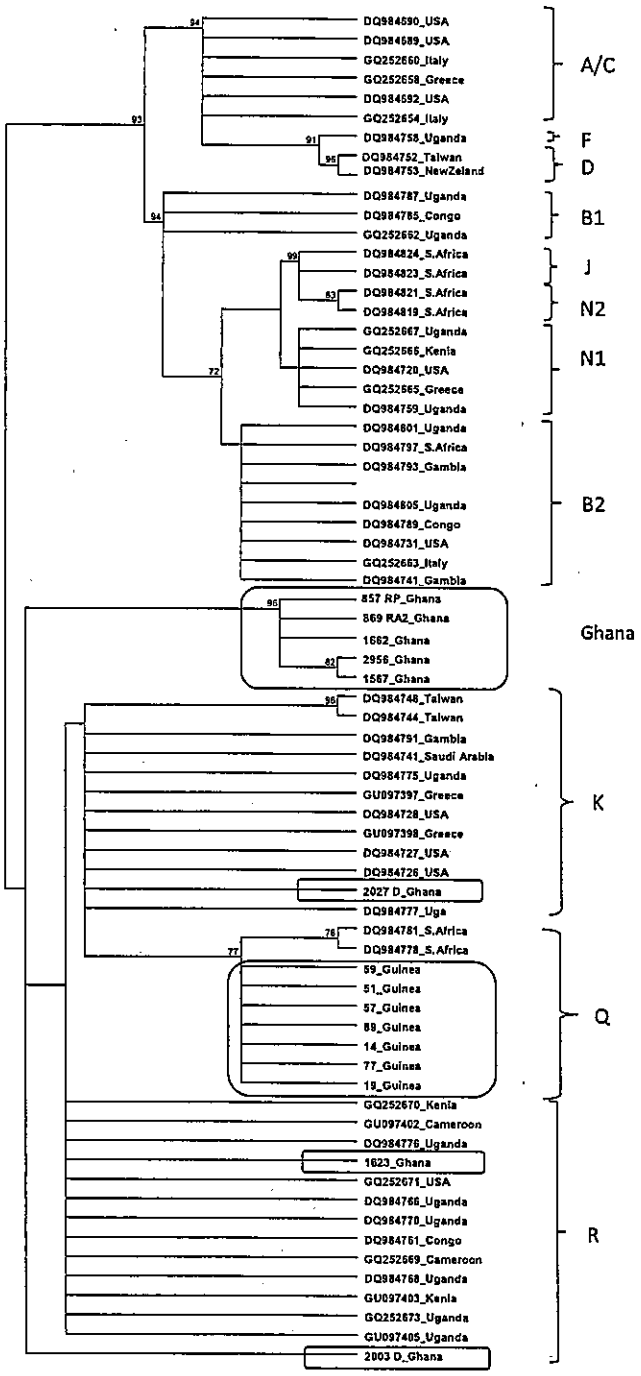


Fig. 1. Neighbor-joining phylogenetic tree of HHV-8 sequences found in Ghana and Guinea. The eight HHV-8 genotypes and their subgenotypes are indicated on the right and highlighted in orange. The red boxes indicate strains from Ghana while the blue box indicates strains from Guinea. Bootstrap values of more than 70% over 1000 replicates are considered significant and are indicated. Five Ghanaian sequences form a subcluster (98% bootstrap value) inside the larger group constituted of K, Q, and R genotypes. The six Guinean samples are part of the Q genotype with a bootstrap of 77%. The last sequence from Ghana (Gh-1623) is clustering with R genotype references.

Indeed, we detected high-titer antibodies (S/CO 9.5 compared to S/CO 4.5 in the donor) 3 months after transfusion, excluding passive transmission (Table 1). Such exclusion was further supported by data from five recipients transfused with seropositive donor blood with S/CO values of at least 9 who were seronegative in the posttransfusion samples. The likelihood of finding viral DNA 3 months postinfection is very low. Therefore, it is not surprising that we did not detect any HHV-8 DNA in the posttransfusion sample. The presence of HHV-8 DNA in the transfused whole blood, the confirmation of antibody to HHV-8 in the posttransfusion sample by an alternative method (immunofluorescence), and the high antibody level strongly suggest transfusion transmission. However, the absence of DNA in the recipient makes impossible to have a direct link with the donor strain. Therefore, community infection cannot be totally ruled out. A second recipient receiving blood from DNA-positive Donor 2003 showed no evidence of seroconversion 3 months after transfusion, a period of time that would be sufficient for the development of an immune response (Table 1). It was assumed that blood donations with detectable DNA contained a higher load of HHV-8 and were presumed more infectious than seropositive DNA-negative blood. Eleven susceptible patients exposed to HHV-8–seropositive blood did not seroconvert (Table 1). This might be related to either seropositive DNA-negative blood containing virus below the infectious dose or to false-positive antibody testing. The latter appears unlikely since both screening EIAs and IFAs were reactive in all 10 HHV-8 DNA–negative donations. Altogether, among 12 patients susceptible to infection and exposed (seronegative individuals receiving seropositive blood) one of two HHV-8 DNA–positive blood transmitted but none of 10 seropositive, HHV-8 DNA–negative blood did. This corresponds to an estimated transmission risk of 8% (95% confidence interval, 0%-23.3%), which is consistent with the 2.8% excess risk reported by

Hladick and colleagues.[9] These data also suggest low infectivity of HHV-8 DNA–negative blood being below the threshold of infectivity in immunocompetent recipients. The lack of HHV-8 transmission found by previous studies might be related to low viral load or to false-positive serologic results due to a lack of serologic confirmation.

HHV-8 may not represent a major issue for blood safety in developed areas but still remains a concern in countries with high seroprevalence among blood donors such as Ghana. Despite the fact that HHV-8 in immunocompetent individuals is usually associated with mild or no symptoms, infections in immunodeficient patients can lead to severe complications and, in some cases, to fatal outcomes. A relatively high incidence of HHV-8 infection has been described in solid organ transplantation patients such as liver and kidney transplant recipients caused by both transmission through the graft and reactivation of a previous infection.[17] Thus, HHV-8 infection in high endemic areas is a growing concern with the emergence of a population of immunodeficient blood recipients in sub-Saharan Africa related to the increasing availability of cancer chemotherapy and immunosuppressive drugs.

New HHV-8 subtype in Ghana

Despite the limited variability of HHV-8 genome, eight genotypes (or clusters) have been identified based on single-polymorphism nucleotides (SNPs) in the ORF26 (minor capsid protein) extended sequence.[4] In this study, we successfully amplified the ORF26 of eight Ghanaian individuals (six patients and two donors) and of seven Guinean blood donors. Five Ghanaian sequences clustered separately from the references with high bootstrap values while three other Ghanaian and seven Guinean strains appeared to belong to Genotype Q (Fig. 1). HHV-8 genotypes were defined by SNPs in an approximately 1-kb region including ORF26 and only three SNPs were necessary to distinguish between the two related Genotypes K and R. These are also the closest genotypes to the Ghanaian sequences. However, six SNPs differentiate five Ghanaian samples from K and R genotypes. For this reason, we propose that a new genotype (G for Ghana) be added. However, this new subgroup is found only in Ghana and does not seem to extend to Guinea, west of Ghana. Indeed, the samples from Guinea have different SNPs and cluster with Q and K whose subclassification into separate subtypes is unconvincing. This study contributes to the understanding of HHV-8 genome variation and distribution that can be used for further studies linking HHV-8 virulence and Kaposi sarcoma incidence.

ACKNOWLEDGMENTS

The authors thank Prof. Henri Agut from the Laboratory Hospital Pitié-Salpêtrière (Paris, France) for agreeing to perform the immunofluorescence tests; Dr Andre Loua Director of the National Blood Transfusion Centre, Conakry (Guinea); and Mr Francis Sarkodie who supervised the samples collection in Ghana.

CONFLICT OF INTEREST

None.

REFERENCES

1. Laurent C, Meggetto F, Brousset P. Human herpesvirus 8 infections in patients with immunodeficiencies. Hum Pathol 2008;39:983-93.
2. Butler LM, Were WA, Balinandi S, Downing R, Dollard S, Neilands TB, Gupta S, Rutherford GW, Mermin J. Human herpesvirus 8 infection in children and adults in a population-based study in rural Uganda. J Infect Dis 2011; 203:625-34.
3. Soulier J, Grollet L, Oksenhendler E, Cacoub P, Cazals-Hatem D, Babinet P, d'Agay MF, Clauvel JP, Raphael M, Degos L, Sigaux F. Kaposi's sarcoma-associated herpesvirus-like DNA sequences in multicentric Castleman's disease. Blood 1995;86:1276-80.
4. Tornesello ML, Biryahwaho B, Downing R, Hatzakis A, Alessi E, Cusini M, Ruocco V, Katongole-Mbidde E, Loquercio G, Buonaguro L, Buonaguro FM. Human herpesvirus type 8 variants circulating in Europe, Africa and North America in classic, endemic and epidemic Kaposi's sarcoma lesions during pre-AIDS and AIDS era. Virology 2010;398:280-9.
5. Ablashi D, Chatlynne L, Cooper H, Thomas D, Yadav M, Norhanom AW, Chandana AK, Churdboonchart V, Kulpradist SA, Patnaik M, Liegmann K, Masood R, Reitz M, Cleghorn F, Manns A, Levine PH, Rabkin C, Biggar R, Jensen F, Gill P, Jack N, Edwards J, Whitman J, Boshoff C. Seroprevalence of human herpesvirus-8 (HHV-8) in countries of Southeast Asia compared to the USA, the Caribbean and Africa. Br J Cancer 1999;81:893-7.
6. Dollard SC, Butler LM, Jones AM, Mermin JH, Chidzonga M, Chipato T, Shiboski CH, Brander C, Mosam A, Kiepiela P, Hladik W, Martin JN. Substantial regional differences in human herpesvirus 8 seroprevalence in sub-Saharan Africa: insights on the origin of the "Kaposi's sarcoma belt." Int J Cancer 2010;127:2395-401.
7. Vamvakas EC. Is human herpesvirus-8 transmitted by transfusion? Transfus Med Rev 2010;24:1-14.
8. Cannon MJ, Operskalski EA, Mosley JW, Radford K, Dollard SC. Lack of evidence for human herpesvirus-8 transmission via blood transfusion in a historical US cohort. J Infect Dis 2009;199:1592-8.
9. Hladik W, Dollard SC, Mermin J, Fowlkes AL, Downing R, Amin MM, Banage F, Nzaro E, Kataaha P, Dondero TJ, Pellett PE, Lackritz EM. Transmission of human herpesvirus 8 by blood transfusion. New Engl J Med 2006;355:1331-8.
10. Lefrère JJ. [The BOTIA project ("Blood and Organ Trans-

missible Infectious Agents"): a European collection of blood samples and an observatory of agents transmitted by blood transfusion or organ transplantation]. Transfus Clin Biol 2005;12:93-4.
11. Simpson GR, Schulz TF, Whitby D, Cook PM, Boshoff C, Rainbow L, Howard MR, Gao SJ, Bohenzky RA, Simmonds P, Lee C, de Ruiter A, Hatzakis A, Tedder RS, Weller IV, Weiss RA, Moore PS. Prevalence of Kaposi's sarcoma associated herpesvirus infection measured by antibodies to recombinant capsid protein and latent immunofluorescence antigen. Lancet 1996;348:1133-8.
12. Compston LI, Sarkobie F, Li C, Candotti D, Opare-Sem O, Allain JP. Multiplex real-time PCR for the detection and quantification of latent and persistent viral genomes in cellular or plasma blood fractions. J Virol Methods 2008; 151:47-54.
13. Endo T, Miura T, Koibuchi T, Nakamura H, Takahashi T, Odawara T, Goto M, Ajisawa A, Iwamoto A, Nakamura T. Molecular analysis of human herpesvirus 8 by using single nucleotide polymorphisms in open reading frame 26. J Clin Microbiol 2003;41:2492-7.
14. Viviano E, Vitale F, Ajello F, Perna AM, Villafrate MR, Bonura F, Aricò M, Mazzola G, Romano N. Human herpesvirus type 8 DNA sequences in biological samples of HIV-positive and negative individuals in Sicily. AIDS 1997;11: 607-12.
15. Mbulaiteye SM, Biggar RJ, Bakaki PM, Pfeiffer RM, Whitby D, Owor AM, Katongole-Mbidde E, Goedert JJ, Ndugwa CM, Engels EA. Human herpesvirus 8 infection and transfusion history in children with sickle-cell disease in Uganda. J Natl Cancer Inst 2003;95:1330-5.
16. Fowlkes AL, Brown C, Amin MM, Roback JD, Downing R, Nzaro E, Mermin J, Hladik W, Dollard SC. Quantitation of human herpesvirus 8 (HHV-8) antibody in patients transfused with HHV-8-seropositive blood. Transfusion 2009;49: 2208-13.
17. Andreoni M, Goletti D, Pezzotti P, Pozzetto A, Monini P, Sarmati L, Farchi F, Tisone G, Piazza A, Pisani F, Angelico M, Leone P, Citterio F, Ensoli B, Rezza G. Prevalence, incidence and correlates of HHV-8/KSHV infection and Kaposi's sarcoma in renal and liver transplant recipients. J Infect 2001;43:195-9.

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
				2012. 11. 19	該当なし	

一般的名称	新鮮凍結人血漿	研究報告の公表状況	Hladik W, Pellett PE, Hancock J, Downing R, Gao H, Packel L, Mimbe D, Nzaro E, Mermin J. J Infect Dis. 2012 Nov;206(10):1497-503. doi: 10.1093/infdis/jis543. Epub 2012 Sep 4.	公表国 ウガンダ 共和国
販売名(企業名)	新鮮凍結血漿-LR「日赤」(日本赤十字社) 新鮮凍結血漿-LR「日赤」成分採血(日本赤十字社) 新鮮凍結血漿-LR「日赤」120(日本赤十字社) 新鮮凍結血漿-LR「日赤」240(日本赤十字社) 新鮮凍結血漿-LR「日赤」480(日本赤十字社)			

研究報告の概要

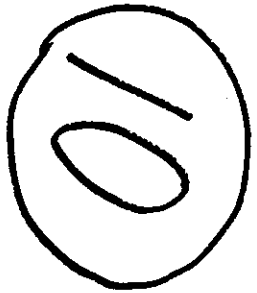
○ヒトヘルペスウイルス8抗体陽性血液の輸血とその後の死亡率の関連
背景: ヒトヘルペスウイルス8(HHV-8)はウガンダで流行しており、血液により伝播する。今回、HHV-8抗体陽性血液の輸血後の死亡率を調査した。
方法: 6カ月の追跡期間を設けた病院ベースの前向きコホート研究で、少なくとも7日間生存した輸血患者にHHV-8抗体陽性血液が及ぼした影響について調べた。
結果: 1092人の受血者中471人(43.1%)にHHV-8抗体陽性血液が輸血された。年齢中央値は1.8歳(0.1～78歳)で、111人(10.2%)が追跡期間中に死亡した。交絡因子(加齢、HIV感染、マラリア以外の疾患、複数回輸血)の調整後、短期(4日以内)保管したHHV-8抗体陽性血液の受血者はHHV-8抗体陰性血液受血者と比較して死亡率が高かった(補正ハザード比[AHR]、1.92;95%CI、1.21～3.05;P=0.01)。一方、長期(5日以上)保管HHV-8陽性血液の輸血は死亡率の増加と有意な関連がなかった(P=0.58)。
結論: 短期保管HHV-8抗体陽性血液は、死亡リスクの増加と関連していた。急性HHV-8感染と若年死亡率の間に観察された関連性を検証するために更なる研究が必要である。

使用上の注意記載状況・その他参考事項等

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

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
ヒトヘルペスウイルス8(HHV-8)抗体陽性血液の輸血後の死亡率について調査したところ、短期(4日以内)保管したHHV-8抗体陽性血液の受血者は、HHV-8抗体陰性血液の受血者と比較して死亡率が高かったとの報告である。	今後も情報の収集に努める。



MAJOR ARTICLE

Association Between Transfusion With Human Herpesvirus 8 Antibody–Positive Blood and Subsequent Mortality

Wolfgang Hladik,[1,3] Philip E. Pellett,[4] John Hancock,[1] Robert Downing,[1] Hongjiang Gao,[5] Laura Packel,[7] Derrick Mimbe,[1] Esau Nzaro,[2] and Jonathan Mermin[6]

[1]Division of Global HIV/AIDS, Center for Global Health, Centers for Disease Control and Prevention (CDC), Entebbe, and [2]Department of Haematology, Mulago Hospital, Kampala, Uganda; [3]Department of Clinical Epidemiology, Academic Medical Center, University of Amsterdam, The Netherlands; [4]Department of Immunology and Microbiology, Wayne State University, Detroit, Michigan; [5]Division of Global HIV/AIDS, Center for Global Health, and [6]Division of HIV/AIDS Prevention, National Center for Hepatitis, HIV, STD, and Tuberculosis Prevention, Centers for Disease Control and Prevention (CDC), Atlanta, Georgia; and [7]Global Health Sciences, University of California, San Francisco

(See the editorial commentary by Operskalski, on pages 1485–7.)

Background. Human herpesvirus 8 (HHV-8) is endemic in Uganda and transmissible by blood. We evaluated mortality following transfusion of HHV-8 antibody–positive blood.

Methods. In a hospital-based, observational, prospective cohort study with a 6-month follow-up, we examined the effect of HHV-8 antibody–positive blood on transfusion recipients surviving at least 7 days.

Results. Of 1092 recipients, 471 (43.1%) were transfused with HHV-8 antibody–positive blood. Median age was 1.8 years (range, 0.1–78); 111 (10.2%) died during follow-up. After adjusting for confounders (increasing age, human immunodeficiency virus infection, illness other than malaria, receipt of multiple transfusions), recipients of HHV-8 antibody–positive blood stored ≤4 days ("short-stored") were more likely to die than recipients of HHV-8 antibody–negative blood (adjusted hazards ratio [AHR], 1.92; 95% confidence interval [CI], 1.21–3.05; $P = .01$). The AHR of the effect of each additional short-stored HHV-8 antibody–positive transfusion was 1.79 (95% CI, 1.33–2.41; $P = .001$).

Conclusions. Transfusion with short-stored HHV-8 antibody–positive blood was associated with an increased risk of death. Further research is warranted to determine if a causal pathway exists and to verify the observed association between acute HHV-8 infection and premature mortality.

Human herpesvirus 8 (HHV-8 or Kaposi's sarcoma–associated herpes virus) causes Kaposi's sarcoma, multicentric Castleman's disease, and primary effusion lymphoma [1]. In Uganda and other sub-Saharan African countries, Kaposi's sarcoma is frequent [2] and causes substantial morbidity and mortality. However, there is a paucity of literature describing any adverse outcomes following acute HHV-8 infection.

In sub-Saharan Africa, adult HHV-8 seroprevalence can exceed 50%, [1] with similarly high seroprevalence in healthy blood donors. The possibility of HHV-8 infection through blood transfusion has been suggested [3–5] and was demonstrated in a study in Uganda [6]. We analyzed data from the same prospective, observational cohort study to compare the risk of death within 6 months following transfusion of blood that was positive for HHV-8 antibodies with that following transfusion of blood that was negative for HHV-8 antibodies.

METHODS

Transfusion Recipients and Blood Donations

As previously described [6], between December 2000 and July 2001, written informed consent (and assent, as appropriate) was obtained from transfusion recipients or their parents or guardians if participants were

Received 20 February 2012; accepted 4 June 2012; electronically published 4 September 2012.

Presented in part: Conference on Retroviruses and Opportunistic Infections, Denver, Colorado, 15–18 February 2006.

Correspondence: Wolfgang Hladik, MD, Uganda Virus Research Institute, PO Box 49, Entebbe, Uganda (wfh3@cdc.gov).

The Journal of Infectious Diseases 2012;206:1497–503
Published by Oxford University Press on behalf of the Infectious Diseases Society of America 2012.
DOI: 10.1093/infdis/jis543

aged <18 years at Mulago Hospital, Kampala, Uganda. Transfusion recipients were eligible for enrollment if their pretransfusion specimen after blood typing and cross-matching was available, identifying information for the transfused blood was known, and no other transfusions had taken place in the previous 6 months. Follow-up visits were scheduled at 1, 2, and 4 weeks post-transfusion, then monthly for 5 additional months. Participants were also seen at the study clinic for unscheduled acute care visits free of charge. At enrollment and each follow-up visit, blood was drawn, and a questionnaire was administered to collect information on patient demographics, health, and repeat transfusions. Participants who did not return for scheduled visits were followed up at home, and any deaths were recorded (Figure 1).

From November 2000 to September 2001, all blood donors in central Uganda were offered study participation, and blood specimens from consenting donors were stored for HHV-8 serologic testing. Donations were screened at the Uganda Blood Transfusion Services for human immunodeficiency virus (HIV), hepatitis B surface antigen, and *Treponema pallidum* and stored at 4°–8° C according to routine procedures. Most blood was divided into plasma and several smaller packed red blood cell units for use in young children; some blood units were left undivided for use in adults, although such units were sometimes split at the hospital into smaller units for use in children. Leukoreduction filters were not used; the buffy coat was partially removed from packed cell units.

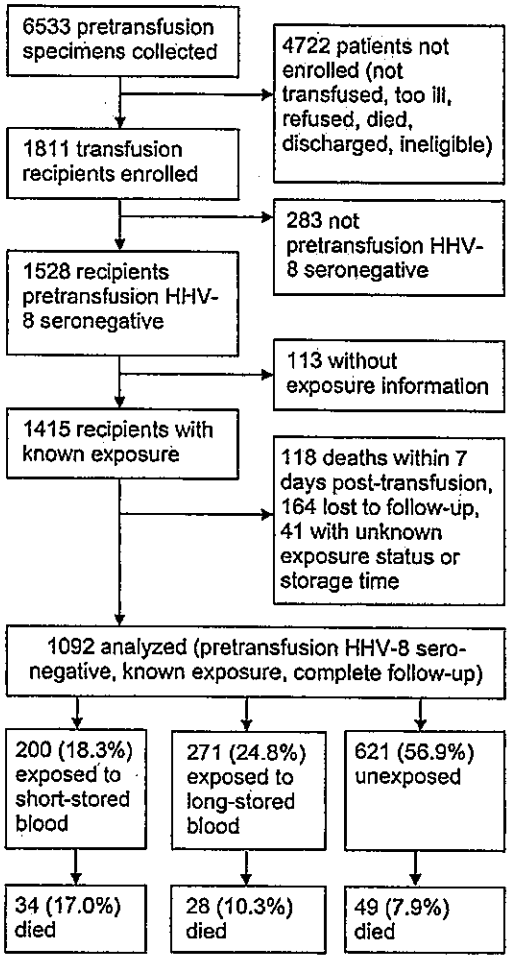


Figure 1. Trial profile of transfusion recipients.

Laboratory Procedures

Recipient plasma collected pretransfusion was tested for hemoglobin levels and HIV antibodies. HIV reactivity was confirmed by polymerase chain reaction if recipients were aged ≤24 months. Pretransfusion recipient blood and linked blood donor specimens were tested for HHV-8 antibodies at the Centers for Disease Control and Prevention (CDC) laboratory in Atlanta, as previously described [6].

Exposure Classification and Transfusion Events

Each transfusion was treated as a discrete event and was counted separately. Each transfusion could comprise ≥1 blood units (depending on patient body weight and degree of anemia as well as blood unit size and availability). Most recipients who received multiple transfusions did so within the first 7 days of their hospital stay. For the purpose of this analysis, an "exposed" person received ≥1 transfusions with HHV-8 antibody–positive blood products whether or not exposure to or infection with the virus occurred. Laboratory testing for antibodies against HHV-8 took place only after completion of follow-up. Recipients transfused with any HHV-8 antibody–positive blood units in the first 7 days were classified as "exposed," whereas recipients transfused exclusively with HHV-8 antibody–negative blood were classified as "unexposed." Because previous analysis of data from the same study found that HHV-8 antibody–positive blood stored ≤4 days was likely responsible for most transfusion-associated HHV-8 infections [6], recipients were grouped into risk categories from high to low as follows: (1) exposed to (any) HHV-8 antibody–positive blood stored ≤4 days (short-stored); (2) exposed to HHV-8 antibody–positive blood stored >4 days (long-stored); or (3) unexposed. Transfusions of blood products with any HHV-8 antibody status occurring after 7 days of the first transfusion (usually following readmission to the hospital) were regarded as "repeat" transfusions.

Data Management and Analysis

Data were entered in duplicate using Epi Info 6.04 (CDC) and analyzed using SAS software (SAS Institute). We excluded participants who were positive for HHV-8 antibodies pretransfusion or who were lost to follow-up. Recipients who received blood of unknown or equivocal HHV-8 serostatus and were not

also transfused with short-stored HHV-8 antibody–positive blood were also excluded. Participants were censored for the first 7 days following their initial transfusion and the first 7 days following the first subsequent transfusion that changed their HHV-8 exposure status to exposed. Transfusion recipients who died within 7 days of their initial transfusion or their first HHV-8 antibody–positive transfusion were removed from analysis, assuming that any effect of receipt of HHV-8 antibody–positive blood on mortality would take longer than 1 week to materialize. Using SAS Tphreg, we performed Cox proportional hazards analysis to estimate the hazard of death for potential risk factors (age, HIV serostatus, pretransfusion anemia, reason for transfusion, number of transfusions, and exposure to HHV-8 antibody–positive blood). We then estimated the adjusted hazard ratio (AHR) for receipt of short-stored HHV-8 antibody–positive blood by controlling simultaneously for these confounders. We repeated the main multivariate analysis with the reference group ("unexposed") restricted to recipients of short-stored HHV-8 antibody–negative blood only. No data anomalies or interactions were noted when we examined multiple-variate results in conjunction with individual-variate results.

Using SAS Proc Logistics, we adjusted for the same confounders and estimated the adjusted odds ratio for death among recipients of short-stored HHV-8 antibody–positive blood within the first 60 days of follow-up. We also estimated the adjusted population-attributable fraction of death due to receipt of short-stored HHV-8 antibody–positive blood as a function of follow-up time under the assumptions of the proportional hazard model and that censoring time is independent of event time [7].

The study was approved by the institutional review boards of the Uganda Virus Research Institute, the Uganda National Council for Science and Technology, and the CDC.

Enrollment and Follow-Up

Pretransfusion blood specimens for 6533 potential transfusion recipients were sent to the hospital's blood bank for typing and cross-matching (Figure 1). Of these, 1811 participants were enrolled; the remaining were not transfused (31%), were ineligible (28%), were too ill to consent (5%), refused to consent (13%), died prior to enrollment (2%), or were discharged prior to enrollment (22%). Of the 1811 enrolled recipients, 1528 (84%) were negative for HHV-8 antibodies pretransfusion. Of these, 436 (29%) were excluded from analysis because of unknown exposure status (10%), early death within 7 days of transfusion (8%), or loss to follow-up (11%).

RESULTS

We included 1092 pretransfusion HHV-8 antibody–negative recipients in the analysis (Table 1). These patients were transfused a total of 1328 times (median, 1; range, 1–8) with 2416 blood units (median, 1; range, 1–16) from 1498 blood donations. Most blood units transfused were packed red blood cells (78%), followed by whole blood (14%), blood of unknown product type (8%), and plasma and/or platelet products (<1%). Most recipients were aged <5 years (median age, 1.8 years; range, 0.1–78 years) and had malaria as a baseline diagnosis. Recipients transfused for malaria were younger than recipients transfused for other reasons (median age, 1.3 vs 17.0 years).

Median follow-up was 167 days (interquartile range [IQR], 116–169 days) and was similar among exposed and unexposed recipients. Among blood donations linked to study participants, HHV-8 antibody positivity was 36.5%. Among study participants, 471 (43.1%) were exposed, and 621 (56.9%) were unexposed. Among the exposed recipients, most (69%) were transfused with a single HHV-8 antibody–positive unit; the remainder received 2 (17%) or >2 (14%) units. Among those exposed to short-stored HHV-8 antibody–positive blood, 67% received 1 such blood product, 19% received 2, and 14% received ≥3. Recipients across the different exposure groups had similar HIV prevalence, pretransfusion anemia status, and reason for transfusion, but they differed by sex, age, and number of transfusions or blood units received (Table 1).

One hundred eleven (10.2%) recipients died during follow-up, with a median time from transfusion to death of 43 days (IQR, 19–73 days). Of the 621 unexposed recipients, 49 (7.9%) died, and of the 271 recipients of long-stored HHV-8 antibody–positive blood, 28 (10.3%) died, compared with 34 (17.0%) of the 200 recipients of short-stored HHV-8 antibody–positive blood. Using person-time as the denominator, unadjusted mortality per 100 person-years was 20.1 for recipients transfused with HHV-8 antibody–negative blood, 26.0 for recipients transfused with long-stored HHV-8 antibody–positive blood, and 44.2 for recipients transfused with short-stored HHV-8 antibody–positive blood.

In bivariate analysis, significant risk factors for death included age, HIV infection, illness other than malaria, receipt of multiple transfusions, and receipt of short-stored HHV-8 antibody–positive blood (Table 2). In multivariate analysis, transfusion with short-stored HHV-8 antibody–positive blood remained significantly associated with mortality during follow-up (AHR, 1.92; $P = .01$) (Table 2). When we restricted the multivariate analysis to the first 60 days of follow-up, the risk of death remained significant (adjusted odds ratio 2.29; 95% confidence interval [CI], 1.29–4.09, $P = .005$). Receipt of long-stored HHV-8 antibody–positive blood was not significantly associated with an excess risk of death ($P = .58$). When the reference group for the multivariate analysis was restricted to recipients of short-stored HHV-8 antibody–negative blood, the AHR due to receipt of short-stored HHV-8 antibody–positive blood remained statistically significant (AHR, 2.39; $P = .005$) and no significant risk of death was associated with

Table 1. Characteristics of Study Participants by Human Herpesvirus 8 (HHV-8) Antibody Exposure Status

Characteristic	Exposure Status (transfusion with HHV-8 antibody–positive blood, by storage time)				*P* Value
	All (N = 1092)	Stored >4 days (n = 271)	Stored ≤4 days (n = 200)	Unexposed (n = 621)	
Age, years					
Median (range)	1.80 (0.1–78)	1.50 (0.2–59)	1.85 (0.1–78)	1.50 (0.1–78)	.03[a]
Sex, female	575 (52.7)	140 (51.7)	123 (61.5)	312 (50.2)	.02
HIV status					
Negative	948 (86.8)	233 (86.0)	177 (88.5)	538 (86.6)	.24
Positive	112 (10.3)	25 (9.2)	18 (9.0)	69 (11.1)	
Missing	32 (2.9)	13 (4.8)	5 (2.5)	14 (2.3)	
Pretransfusion anemia status					
Anemic	791 (72.4)	197 (72.7)	140 (70.0)	454 (73.1)	.67
Not anemic	17 (1.6)	6 (2.2)	4 (2.0)	7 (1.1)	
Unknown	284 (26.0)	68 (25.1)	56 (28.0)	160 (25.8)	
No. transfusions received					
1	937 (85.8)	210 (77.5)	147 (73.5)	580 (93.4)	<.0001
≥2	155 (14.2)	61 (22.5)	53 (26.5)	41 (6.6)	
No. blood units received					
1	868 (79.5)	191 (70.5)	134 (67.0)	543 (87.4)	<.0001
2	135 (12.4)	43 (15.9)	38 (19.0)	54 (8.7)	
≥3	89 (8.1)	37 (13.6)	28 (14.0)	24 (3.9)	
Reason for transfusion					
Malaria	912 (83.5)	220 (81.2)	163 (81.5)	529 (85.2)	.23
Other/unknown	180 (16.5)	51 (18.8)	37 (18.5)	92 (14.8)	
Survival status					
Alive	981 (89.8)	243 (89.7)	166 (83.0)	572 (92.1)	.001
Dead	111 (10.2)	28 (10.3)	34 (17.0)	49 (7.9)	
Time to death, days					
Median	43	50	35	37	<.0001[a]

Data are no. (%) unless otherwise noted.

Abbreviation: HIV, human immunodeficiency virus.

[a] *P* value based on difference in mean values.

transfusion of either long-stored HHV-8 antibody–positive or long-stored HHV-8 antibody–negative positive blood (Table 3).

With the multivariate model restricted to recipients of a single transfusion (n = 937), the hazard for death due to receipt of short-stored HHV-8 antibody–positive blood remained (AHR, 1.95; 95% CI, 1.10–3.45; $P = .02$). With the model restricted to recipients of a single blood unit (n = 868), the hazard for death upon receipt of short-stored HHV-8 antibody–positive blood was similar but not statistically significant (AHR, 1.70; 95% CI, .94–3.09; $P = .08$).

In a separate analysis, we restricted the risk set to recipients of a single blood unit and kept the reference group defined as recipients of a single short-stored HHV-8 antibody–negative blood unit. In this model, recipients of a single short-stored HHV-8 antibody–positive blood unit had a significantly higher mortality than reference group recipients (AHR, 2.19; 95% CI, 1.06–4.53; $P = .03$), whereas there was no excess risk of death among recipients of a single long-stored HHV-8 antibody–negative or HHV-8 antibody–positive blood unit. We also analyzed the data in a separate multivariate model similar to that shown in Table 2 except that exposure to HHV-8 antibody–positive blood was expressed as the continuous number of short- or long-stored HHV-8 antibody–positive or HHV-8 antibody–negative blood units. In this model, additional short-stored HHV-8 antibody–positive blood units transfused provided no survival benefit (AHR, 0.94; 95% CI, .61–1.43); whereas for all other blood units, each additional transfused unit had a protective effect on survival (long-stored HHV-8 antibody–positive: AHR, 0.67; 95% CI, .49–.93; short-stored HHV-8 antibody–negative: AHR, 0.53, 95% CI, .33–.83; compared with transfusion with long-stored HHV-8 antibody–negative units).

We also altered the main model (as shown in Table 2) such that the number of transfusions (by HHV-8 serostatus and storage time) replaced the categorical exposure variables and

Table 2. Risk Factors for Post-Transfusion Mortality, N = 1092 (human herpesvirus 8 [HHV-8] reference group: transfused with short- or long-stored HHV-8 antibody–negative blood)

	Recipients			Unadjusted Hazard Ratio			Adjusted Hazard Ratio		
Risk Factor	Person-time	No. of Recipients	Mortality	Point Estimate	95% CI	*P* Value	Point Estimate	95% CI	*P* Value
Age, years, continuous	428.0	1092	25.9	1.02	1.01–1.03	.04	1.00	.98–1.02	.78
HIV uninfected	377.3	948	17.5	Ref	...	...	Ref	...	...
HIV infected	37.4	112	109.5	5.96	4.03–8.80	.01	6.50	4.33–9.76	<.0001
HIV unknown	13.3	32	30.8	1.80	.67–4.94	.25	2.13	.77–5.91	.14
Not pretransfusion anemic	6.6	17	30.1	Ref	...	...	Ref	...	...
Pretransfusion anemic	304.3	791	28.0	0.89	.22–3.60	.87	1.43	.33–6.16	.63
Unknown anemia status	117.1	284	20.5	0.65	.16–2.81	.58	1.17	.26–5.22	.84
Transfused for malaria	359.3	912	22.5	Ref	...	.01	Ref	...	.06
Transfused for other reasons	68.8	180	43.6	1.92	1.26–2.97		1.64	.97–2.78	
Number of transfusions (continuous)	428.0	1328[a]	25.9	1.55	1.35–1.79	.01	1.60	1.36–1.88	<.0001
Transfused with									
HHV-8-seronegative blood	243.6	621	20.1	Ref	...	...	Ref	...	...
HHV-8-seropositive blood stored >4 days	107.2	271	26.0	1.30	.82–2.69	.27	1.15	.71–1.86	.58
HHV-8-seropositive blood stored ≤4 days	77.6	200	44.2	2.18	1.41–3.37	.01	1.92	1.21–3.05	.01

Person-time in years. Mortality expressed as number of deaths per 100 person-years, Hazard ratios: the hazard of death among patients by differing characteristic. *P* values apply to differences in the hazards observed.

Abbreviations: CI, confidence interval; HIV, human immunodeficiency virus.

[a] Refers to number of transfusions (rather than recipients).

the overall number of transfusions. In this model, too, short-stored HHV-8 antibody–positive blood was associated with an increased risk for death (AHR for each additional transfusion, 1.79; 95% CI, 1.33–2.41; $P = .0001$), whereas the AHR for each additional long-stored HHV-8 antibody–positive transfusion (1.23), short-stored HHV-8 antibody–negative transfusion (1.11), and long-stored HHV-8 antibody–negative transfusion (1.18) was nonsignificant. When replacing the number of transfusions with the number of blood units transfused in the model shown in Table 2, the AHR for exposure to short-stored HHV-8 antibody–positive blood remained significant (AHR, 2.25; 95% CI, 1.44–3.53; $P < .001$), and each additional blood unit transfused carried a significant risk for death (AHR, 1.19; 95% CI, 1.06–1.34; $P = .004$), similar to the number of transfusions. When including both the number of blood units and the number of transfusions in the same main model, HIV infection, additional transfusions during follow-up (AHR, 2.76), and exposure to short-stored HHV-8 antibody–positive blood (AHR, 1.76) remained significant predictors for death, whereas each additional blood unit (of any HHV-8 antibody status) transfused was associated with a decreased risk of death (AHR, 0.67; 95% CI, .48–.92; $P = .014$).

Stratifying the analysis by the major reason for transfusion did not alter the point estimate of association but led to wide confidence intervals for the AHR in each strata (data not shown). We detected no significant effect related to age, illness leading to transfusion, or blood product type transfused on the association between exposure and death. We estimated the median adjusted population attributable fraction of mortality due to short-stored HHV-8 antibody–positive blood to be 13.7% (95% CI, 2.9%–23.4%), which decreased from 16.9% at the beginning of follow-up to 11.0% at the end of follow-up.

We excluded deaths occurring within the first week following transfusion. During this time period, a total of 104 deaths occurred, with a median time to death of 2 days (IQR, 1–4 days). Transfusion of HHV-8 antibody–positive blood was not associated with an increased risk of death within these 7 days (overall: AHR, 0.95; $P = .83$; for short-stored HHV-8 antibody–positive blood: AHR, 0.61; $P = .23$; and for long-stored HHV-8 antibody–positive blood: AHR, 1.14; $P = .61$). Confounding by passive antibody transfer made it difficult to identify active HHV-8 seroconversions among the deceased. Three active HHV-8 seroconvertors were identified (2 recipients of short-stored HHV-8 antibody–positive blood, 1 recipient of long-stored HHV-8 antibody–positive blood, none in

Table 3. Risk Factors for Post-Transfusion Mortality, N = 1074 (human herpesvirus 8 [HHV-8] reference group: transfused with short-stored HHV-8 antibody–negative blood)

	Adjusted Hazard Ratio		
Risk Factor	Point Estimate	95% CI	*P* Value
Age, years, continuous	1.00	.98–1.02	.90
HIV uninfected	Ref	...	...
HIV infected	6.61	4.40–9.93	<.0001
HIV unknown	2.29	.83–6.35	.11
Not pretransfusion anemic	Ref	...	...
Pretransfusion anemic	1.55	.36–6.71	.56
Unknown anemia status	1.31	.29–5.89	.72
Transfused for malaria	Ref	...	.06
Transfused for other reasons	1.67	.98–2.83	
Number of transfusions (continuous)	1.57	1.33–1.86	<.0001
Transfused with			
HHV-8 Ab–negative blood stored ≤4 d	Ref	...	...
HHV-8 Ab–negative blood stored >4 d	1.51	.83–2.75	.18
HHV-8 Ab–positive blood stored >4 d	1.45	.78–2.72	.24
HHV-8 Ab–positive blood stored ≤4 d	2.39	1.30–4.42	.005

Hazard ratios: the hazard of death among patients by differing characteristic. *P* values apply to differences in the hazards observed.

Abbreviations: Ab, antibody; CI, confidence interval; HIV, human immunodeficiency virus.

the unexposed group), which was insufficient for further analysis.

DISCUSSION

In this study, recipients of HHV-8 antibody–positive blood stored ≤4 days had a 1.9-fold greater risk of death than recipients of HHV-8 antibody–negative blood. The risk of death increased with each additional unit of short-stored HHV-8 antibody–positive blood transfused; in contrast, unexposed recipients experienced no additional risk from receipt of additional HHV-8 antibody–negative units regardless of their storage time.

We note several study limitations. We were unable to collect extensive information on the causes of death. Due to the observational study design, study participants were not truly randomized to the different exposure categories. However, this was unlikely to have biased our results because we adjusted for the number of transfusions received throughout the observation period. Also, the mortality risk remained when we restricted analysis to recipients without repeat transfusions during follow-up, and it remained when we right-censored both exposed and unexposed in the same fashion (ie, upon receipt of an HHV-8 antibody–positive transfusion during follow-up).

Our adjusted analysis accounted for several confounders, some of which remained significant in our model. However, several observations support the hypothesis of an exposure-related risk of death. First, the mortality risk was significant only for transfusion with short-stored blood. This is consistent with our earlier finding that most transfusion-associated HHV-8 infections were likely due to short-stored HHV-8 antibody–positive blood [6] and a similar infection risk differential is known for other infectious agents (eg, cytomegalovirus) [8, 9]. Further, the increased mortality risk for each additional short-stored HHV-8 antibody–positive blood unit transfused suggests a dose-response relationship between exposure and subsequent death that was not observed for HHV-8 antibody–negative units and remained after controlling for the total number of transfusions. Lastly, the absence of an exposure-related risk of death during the first 7 days following transfusion indirectly supports our hypothesis because a causal association between transfusion of HHV-8 antibody–positive blood and post-transfusion death would likely take time to manifest itself and suggests that at the time of the baseline transfusion recipients of HHV-8 antibody–positive blood were not more acutely ill than others.

The adjusted estimated attributable risk of death due to transfusion with short-stored HHV-8 antibody-positive blood implies that approximately 5 (95% CI, 1.0–8.0) of the 34 deaths among recipients of short-stored HHV-8-antibody-positive blood or 4.2% of all 111 deaths may have been due to transfusion of short-stored HHV-8 antibody-positive blood. The association with mortality could be due to transfusion-associated HHV-8 being rapidly and highly pathogenic in some patients or to a different infectious agent or other hazard associated with HHV-8 seropositivity. We previously estimated the excess HHV-8 infection risk due to transfusion of short-stored HHV-8-antibody-positive blood alone as 4.2% (95% CI, .1–8.3) [6], or approximately 13 excess HHV-8 infections in this cohort. Among exposed patients who completed >4 weeks of follow-up before dying, there was no serological evidence of HHV-8 infection. However, some individuals may have died of acute illness before seroconversion would have been detected in the context of our sampling intervals. Acute disease has been associated with HHV-8 infection in both immunocompetent [10, 11] and immunocompromised persons, including well-documented severe disease in HIV-infected patients and organ transplant recipients [12–15]. All of our study participants were sufficiently ill to require transfusion; their immune status may have been further compromised by the immunosuppressive effects of transfused blood [16], especially if it

contained allogeneic leukocytes [17, 18]. Thus, it is plausible that HHV-8 itself directly contributed to the observed mortality. Additional research that considers cause of death, HHV-8 DNA in donors and recipients, or the effect of leukoreduction or irradiation on the outcome of transfused short-stored HHV-8 antibody–positive blood in transfusion recipients may clarify the association of HHV-8 with mortality among transfusion recipients.

In conclusion, transfusion of short-stored HHV-8 antibody–positive blood was associated with increased risk of death during the 2–28 weeks following transfusion. If this association is confirmed, blood transfusion systems in HHV-8 endemic areas will face a dilemma. Donated blood is a scarce resource in most countries, particularly in sub–Saharan Africa; removal of HHV-8 antibody–positive blood would further exacerbate existing shortages. The benefits of transfused blood will need to be weighed against its known and potential adverse effects.

Notes

Acknowledgments. All authors substantially contributed to the study's design and conduct or to data analysis and interpretation. All approved the final version for publication.

W. H. had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy the data analysis. P. E. P., W. H., R. D., and J. M. were responsible for the study concept and design. D. M., W. H., and E. N. were responsible for the acquisition of data. L. P., J. H., H. G., D. M., and W. H. analysed and interpreted the data. W. H., J. M., and P. E. P. drafted the manuscript. W. H., J. M., P. E. P., E. N., R. D., and J. H. critically revised the manuscript for important intellectual content. J. H., L. P., and H. G. provided statistical analysis. W. H. obtained funding. J. M. and W. H. provided administrative, technical, or material support.

We thank all investigators for their efforts and all reviewers for their comments and advice. We especially thank the study participants and field staff from the study clinic, Mulago Hospital, Nakasero Blood Bank, and the Uganda Virus Research Institute.

Disclaimer. The findings and conclusions in this report are those of the authors and do not necessarily represent the views of CDC or the U.S. Department of Health and Human Services.

Financial support. This work was funded by the CDC. CDC staff were involved in the design and conduct of the study; in the collection, management, analysis, and interpretation of the data; and in the preparation, review, and approval of the manuscript.

Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Henke-Gendo C, Schulz TF. Transmission and disease association of Kaposi's sarcoma–associated herpesvirus: recent developments. Curr Opin Infect Dis 2004; 17:53–7.
2. Dedicoat M, Newton R. Review of the distribution of Kaposi's sarcoma–associated herpesvirus (KSHV) in Africa in relation to the incidence of Kaposi's sarcoma. Br J Cancer 2003; 88:1–3.
3. Blackbourn DJ, Ambroziak J, Lennette E, Adams M, Ramachandran BLevy JA. Infectious human herpesvirus 8 in a healthy North American blood donor. Lancet 1997; 349:609–11.
4. Dollard SC, Nelson KE, Ness PM, et al. Possible transmission of human herpesvirus-8 by blood transfusion in a historical United States cohort. Transfusion 2005; 45:500–3.
5. Enbom M, Urassa W, Massambu C, Thorstensson R, Mhalu F, Linde A. Detection of human herpesvirus 8 DNA in serum from blood donors with HHV-8 antibodies indicates possible bloodborne virus transmission. J Med Virol 2002; 68:264–7.
6. Hladik W, Dollard SC, Mermin J, et al. Transmission of human herpesvirus 8 by blood transfusion. N Engl J Med 2006; 355:1331–8.
7. Chen L, Lin DY, Zeng D. Attributable fraction functions for censored event times. Biometrika 2010; 97:713–26.
8. Roberts TC, Buller RS, Gaudreault-Keener M, et al. Effects of storage temperature and time on qualitative and quantitative detection of cytomegalovirus in blood specimens by shell vial culture and PCR. J Clin Microbiol 1997; 35:2224–8.
9. Roback JD. CMV and blood transfusions. Rev Med Virol 2002; 12:211–9.
10. Andreoni M, Sarmati L, Nicastri E, et al. Primary human herpesvirus 8 infection in immunocompetent children. JAMA 2002; 287:1295–300.
11. Wang QJ, Jenkins FJ, Jacobson LP, et al. Primary human herpesvirus 8 infection generates a broadly specific CD8(+) T-cell response to viral lytic cycle proteins. Blood 2001; 97:2366–73.
12. Oksenhendler E, Cazals-Hatem D, Schulz TF, et al. Transient angiolymphoid hyperplasia and Kaposi's sarcoma after primary infection with human herpesvirus 8 in a patient with human immunodeficiency virus infection. N Engl J Med 1998; 338:1585–90.
13. Thaunat O, Mamzer-Bruneel MF, Agbalika F, et al. Severe human herpesvirus-8 primary infection in a renal transplant patient successfully treated with anti-CD20 monoclonal antibody. Blood 2006; 107:3009–10.
14. Luppi M, Barozzi P, Schulz TF, et al. Bone marrow failure associated with human herpesvirus 8 infection after transplantation. N Engl J Med 2000; 343:1378–85.
15. Pietrosi G, Vizzini G, Pipitone L, et al. Primary and reactivated HHV8 infection and disease after liver transplantation: a prospective study. Am J Transplant 2011; 11:2715–23.
16. Vamvakas EC, Blajchman MA. Deleterious clinical effects of transfusion-associated immunomodulation: fact or fiction? Blood 2001; 97:1180–95.
17. Blajchman MA. Immunomodulation and blood transfusion. Am J Ther 2002; 9:389–95.
18. Kao KJ. Mechanisms and new approaches for the allogeneic blood transfusion-induced immunomodulatory effects. Transfus Med Rev 2000; 14:12–22.

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
				2012. 10. 20	該当なし	

一般的名称	新鮮凍結人血漿	研究報告の公表状況		公表国
販売名（企業名）	新鮮凍結血漿-LR「日赤」（日本赤十字社） 新鮮凍結血漿-LR「日赤」成分採血（日本赤十字社） 新鮮凍結血漿-LR「日赤」120（日本赤十字社） 新鮮凍結血漿-LR「日赤」240（日本赤十字社） 新鮮凍結血漿-LR「日赤」480（日本赤十字社）		H Alhumaidan, B Westley, C Esteva, V Berardi, C Young, J Sweeney. AABB Annual Meeting& CTTXPO 2012; October 6-9, 2012, BOSTON.	米国

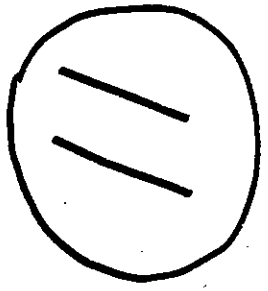
研究報告の概要

○白血球除去赤血球による*Anaplasma phagocytophilum*伝播
背景：ヒト顆粒球アナプラズマ症（HGA）はマダニの一種によって伝播されるリケッチア感染症である。輸血伝播によるアナプラズマ症（TTA）は過去に4例が報告されているのみで、全例が米国中西部で発生している。
症例・所見：64歳男性患者は、3日間続く倦怠感、労作時呼吸困難、下血により入院し、慢性閉塞性肺疾患の既往歴及び鉄欠乏性貧血を伴う再発性胃炎を有していた。保存前白血球除去赤血球製剤5ユニットが輸血され、その後容態は安定し退院した。その2日後、頭痛、発熱、悪寒により再入院した。末梢血スメアによりHGAと一致する桑実胚を持つ多形核白血球が確認された。受血者及び全5ユニットの白血球除去赤血球製剤の供血者セグメント検体について*Anaplasma phagocytophilum*の検査を行ったところ、1名の供血者に感染が確認された。当該供血者は媒介ダニIxodesの多発地帯であるロードアイランド州在住の81歳健常男性で、屋外活動は行うがダニ刺咬歴はなかった。
結論：白血球除去はHGA伝播の予防とはならず、赤血球製剤がHGA多発地帯から非多発地帯へ供給されて輸血される例は多いため、受血者が予期せず発熱した場合は地域に関係なくTTAの可能性を考慮すべきである。

使用上の注意記載状況・その他参考事項等

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

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク



報告企業の意見	今後の対応
白血球除去赤血球の輸血により*Anaplasma phagocytophilum*が伝播したとの報告である。	今後も引き続き、新興・再興感染症の発生状況等に関する情報の収集に努める。

compared with donors without Chagas cardiomyopathy (178/340 [52%]; mean 7.1 parasites/20 mL). In the univariate model PCR positivity was associated with pro-BNP level ($p < 0.001$), left ventricular ejection fraction ($p = 0.024$), Troponin ($p < 0.001$) and CKMB ($p = 0.043$). **Conclusion:** *T. cruzi* PCR positivity correlates with presence of cardiomyopathy and with markers of severity of disease indicating a direct role of parasite persistence in disease pathogenesis. These findings support guidelines for treatment of chronically infected patients and potential utility of *T. cruzi* PCR for prognosis and therapeutic monitoring.

Disclosure of Commercial Conflict of Interest

M. P. Busch: NIH, Grants or Research Support; Novartis/Gen-Probe, Grants or Research Support; Terumo/Caridian, Grants or Research Support; Gen-Probe, Consulting or Board of Director Fees; Johnson & Johnson/Merck, Ortho, Consulting or Board of Director Fees; Abbott, Travel Support or Honorarium; Novartis, Travel Support or Honorarium; D. M. Carrick: Nothing to disclose; B. Custer: Nothing to disclose; X. Deng: Nothing to disclose; K. Kavounis: No Answer; S. M. Keating: Nothing to disclose; T. Lee: Nothing to disclose; C. D. Oliveira: Nothing to disclose; A. L. Ribeiro: No Answer; E. C. Sabino: Nothing to disclose

Disclosure of Grants Conflict of Interest

M. P. Busch: Novartis, Grants or Research Support; Ortho, Grants or Research Support; Terumo/Caridian, Grants or Research Support; D. M. Carrick: Nothing to disclose; B. Custer: Nothing to disclose; X. Deng: Nothing to disclose; K. Kavounis: No Answer; S. M. Keating: Nothing to disclose; T. Lee: Nothing to disclose; C. D. Oliveira: Nothing to disclose; A. L. Ribeiro: No Answer; E. C. Sabino: Nothing to disclose

S64-030J

Increasing Rate of Babesiosis in Transfused Patients at a New York City Hospital

C Goss[1] (chg9006@med.cornell.edu), P Giardina[2], M S Simon[3], D A Kessler[4], B H Shaz[4], M Cushing[1]. *[1]Transfusion Medicine and Cellular Therapy, Weill Cornell Medical College, New York, NY, United States; [2]Pediatrics, Weill Cornell Medical College, New York, NY, United States; [3]Internal Medicine, Weill Cornell Medical College, New York, NY, United States; [4]New York Blood Center, New York, NY, United States*

Background/Case Studies: Babesiosis, a tick-borne infection primarily due to the intra-erythrocytic protozoa, *Babesia microti*, results in a wide-spectrum of clinical illness that ranges from asymptomatic to fatal. Asymptomatic donors in endemic areas are difficult to identify, and transfusion-transmitted babesiosis (TTB) has been increasingly identified in certain regions particularly the Northeastern U.S. We describe a 10-month experience at our institution, during which 7 cases of suspected TTB were reported to the transfusion service (TS). **Study Design/Methods:** All suspected cases of TTB are reported to the TS by clinicians, microbiologists, or epidemiology. TS physicians investigate the possibility of TTB, and if RBC or granulocyte units were transfused within three months prior to the babesiosis diagnosis, suspected units are reported to the blood supplier and New York State Department of Health. The patient's chart is reviewed for demographics, comorbidities, specific risk factors for babesiosis, and the clinical course. **Results/Findings:** Seven cases of suspected TTB were reported from 8/2011 to 5/2012 (1 reported case for 2857 RBC units transfused). In the prior 6 years, 8 cases had been reported, 3 of which occurred in 2010. Four patients (57%) had thalassemia, 3 (43%) had hematological malignancies, and (57%) were asplenic. The non-thalassemic patients were all older than 60 years of age. The parasitemia at diagnosis ranged from 0.3% to 7.9%. Five of 7 experienced laboratory evidence of hemolysis. Two patients required hospitalization and a third required transfer to the ICU for management of babesiosis. Four were treated as outpatients with a prolonged course of antibiotics. Eight of 15 (53%) diagnosed with babesiosis in the last 8 years had warm RBC autoantibodies and in 5 cases the autoantibody was identified within 1 month of the babesiosis diagnosis. For 2 patients, review of pre-transfusion specimens revealed evidence of babesia infection prior to transfusion. Three patients were considered presumed to have TTB (1 in 6668 RBC transfused) with donor titers ranging from 64 to 256. The two remaining patients received blood solely at outside hospitals and remain unconfirmed for TTB. No fatalities due to TTB occurred during this time; 1 patient died within 42 days of diagnosis from complications of their underlying condition. **Conclusion:** In the past year, our institution experienced an alarming increase in frequency of TTB associated with significant morbidity and financial cost. These results are consistent with recent publications documenting the parasite's expanding geographic range in New York State and highlight the urgent need for donor screening assays. Until such a test is licensed, clinicians must be aware of this risk in transfused patients particularly in vulnerable populations such as the elderly and neonates, and patients with asplenia, hemoglobinopathies or hematologic malignancies.

Disclosure of Commercial Conflict of Interest

M. Cushing: Nothing to disclose; P. Giardina: Nothing to disclose; C. Goss: Nothing to disclose; D. A. Kessler: Immunetics, Other; B. H. Shaz: Immunetics, Other; M. S. Simon: Nothing to disclose

Disclosure of Grants Conflict of Interest

M. Cushing: Nothing to disclose; P. Giardina: Nothing to disclose; C. Goss: Nothing to disclose; D. A. Kessler: Nothing to disclose; B. H. Shaz: Nothing to disclose; M. S. Simon: Nothing to disclose

S65-030J

Anaplasma phagocytophilum Transmission by Leukoreduced Red Blood Cells

H Alhumaidan[1] (alhumaidan@me.com), B Westley[2], C Esteva[1], V Berardi[4], C Young[3], J Sweeney[1]. *[1]Blood Bank and Transfusion Medicine, Miriam Hospital, Providence, RI, United States; [2]Division of Infectious Disease, Miriam Hospital, Providence, RI, United States; [3]Rhode Island Blood Center, Providence, RI, United States; [4]Imugen Inc, Norwood, MA, United States*

Background/Case Studies: Human Granulocytic Anaplasmosis (HGA) is a tickborne rickettsial infectious disease caused by gram-negative, obligate intracellular bacteria infecting neutrophils and transmitted to humans by species of the Ixodes tick. *Ixodes scapularis* is endemic in the Northeast and upper Midwest regions of the US. Only four previous cases of transfusion transmitted anaplasmosis (TTA) have been described, all from the Midwest and only one was proven to be from a leukoreduced red blood cell (RBC). **Study Design/Methods:** A 64 year old male patient was admitted to hospital with a 3 day history of fatigue, dyspnea on exertion and melena for several days. The Patient had a history of chronic obstructive pulmonary disease (COPD) and was on oral prednisone. There was a history of recurrent gastritis with iron deficiency anemia. His hemoglobin on admission was 6.2 g/dL. He received five units of prestorage leukoreduced RBCs, subsequently stabilized and was discharged. Two days after discharge, he developed headache, fever and chills and was readmitted. He was started on antibiotics and IV methylprednisolone for exacerbation of his COPD. The patient's symptoms did not improve and on day 5 of his second admission, the temperature was 101.4 F. and the WBC decreased to 2.3 x10^9/L. Polymorphonuclear leukocytes containing morulae consistent with HGA were reported in the peripheral smear. Samples from the recipient and donor segments from all 5 leukoreduced RBC units were retrieved and tested for *Anaplasma phagocytophilum*. **Results/Findings:** All five donor segments were evaluated by polymerase chain reaction (PCR). One donor tested PCR positive for HGA. This unit had been stored for 12 days prior to transfusion. The donor was a healthy 81 year old male from an Ixodes endemic area in RI with outdoor activities who reported no tick bites. Laboratory data are shown in the Table. **Conclusion:** Leukoreduction does not interdict the transmission of HGA. TTA requires consideration in recipients of red cell transfusion with unexplained fever, regardless of the geographic location of the transfusion since red cells are commonly exported from HGA endemic to HGA nonendemic areas.

Disclosure of Commercial Conflict of Interest

H. Alhumaidan: PALL Medical, Grants or Research Support; V. Berardi: IMUGEN Inc, Ownership or Partnership; C. Esteva: No Answer; J. Sweeney: Nothing to disclose; B. Westley: No Answer; C. Young: No Answer

Disclosure of Grants Conflict of Interest

H. Alhumaidan: PALL Medical, Grants or Research Support; V. Berardi: IMUGEN Inc, Grants or Research Support; C. Esteva: No Answer; J. Sweeney: Nothing to disclose; B. Westley: No Answer; C. Young: No Answer

Anaplasma phagocytophilum		
Donor testing (segment)	Serology PCR	ELISA positive IgM > 17.4 IgG > 12.1 Positive
Recipient testing (WB and serum)	Serology PCR	ELISA IgG negative IgM not reported Positive

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
				2012. 10. 20	該当なし	

一般的名称	新鮮凍結人血漿	研究報告の公表状況	Jimenez-Marco T, Fisa R, Riera C, Girona-Llobera E, Sedeño M, Saura A, Iniesta L, Guillen C, Muncunill J. Vox Sang. 2012 Nov;103(4):356-8. doi: 10.1111/j.1423-0410.2012.01622.x. Epub 2012 Jun 19.	公表国
販売名(企業名)	新鮮凍結血漿-LR「日赤」(日本赤十字社) 新鮮凍結血漿-LR「日赤」成分採血(日本赤十字社) 新鮮凍結血漿-LR「日赤」120(日本赤十字社) 新鮮凍結血漿-LR「日赤」240(日本赤十字社) 新鮮凍結血漿-LR「日赤」480(日本赤十字社)			スペイン

研究報告の概要

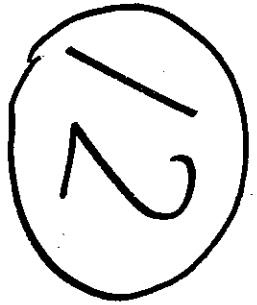
○*Leishmania infantum*の無症候性キャリア由来の血液成分に適用される病原体不活化技術
無症候性リーシュマニア感染症は、流行地域における輸血感染の主な原因となっている。バレアレス諸島では、供血者の無症候性*L.infantum*の感染率は極めて高い(調査対象供血者の5.9%)。現在、血液銀行の基準を満たす供血者のためのリーシュマニアスクリーニング検査は存在しないため、血液製剤中のリーシュマニアの除去のために数種類の方法が用いられている。今回、アモトサレンとUVA照射(INTERCEPT)を用いて無症候性*L.infantum*感染供血者から採取した血液製剤を用いて病原体除去技術の能力を調査した。病原体不活化処理実施前の血小板製剤6例中5例で、RT-PCR結果が陽性であった。INTERCEPTでの不活化後、これらの血小板製剤はRT-PCRで陰性となり、in vitro培養において6カ月後も全て陰性であった。これは供血者の血液成分から原虫を除去する目的でINTERCEPTが用いられた初の報告である。この所見を確認するためには更なる研究が必要である。

報告企業の意見	今後の対応
無症候性*Leishmania infantum*感染供血者からの血小板製剤中の原虫を除去するためにINTERCEPTが用いられ、有効であったとの報告である。	日本赤十字社では、輸血感染症対策として問診時に海外滞在歴の有無を確認し、帰国(入国)後4週間は献血不適としている。今後も引き続き、新興・再興感染症の発生状況等に関する情報の収集に努める。

使用上の注意記載状況・その他参考事項等

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

血液を介するウイルス、
細菌、原虫等の感染
vCJD等の伝播のリスク



VoxSanguinis
The International Journal of Transfusion Medicine
ISBT International Society of Blood Transfusion
Vox Sanguinis (2012) 103, 356–358

CASE REPORT

DOI: 10.1111/j.1423-0410.2012.01622.x

Pathogen inactivation technology applied to a blood component collected from an asymptomatic carrier of Leishmania infantum: a case report

T. Jimenez-Marco,[1,*] R. Fisa,[2,*] C. Riera,[2] E. Girona-Llobera,[1] M. Sedeño,[1] A. Saura,[2] L. Iniesta,[2] C. Guillen[2] & J. Muncunill[1]
[1]*Fundació Banc de Sang i Teixits de les Illes Balears, Balearic Islands, Mallorca, Spain*
[2]*Laboratori de Parasitologia, Facultat de Farmàcia, Universitat de Barcelona, Barcelona, Spain*

Vox Sanguinis

Received: 19 December 2011
revised 11 May 2012
accepted 12 May 2012,
published online 19 June 2012

Asymptomatic *Leishmania* infections have been the main cause of transfusion transmission in endemic areas. Polymerase chain reaction has been used to detect *L. infantum* DNA in the peripheral blood of asymptomatic *Leishmania* carriers. In our region, the prevalence of asymptomatic *L. infantum* infection in donors is markedly high (5·9% of donors studied). We investigated the ability of pathogen inactivation technology, using amotosalen and UVA illumination, to eliminate *L. infantum* in a blood component collected from an asymptomatic *L. infantum* infected donor. This is the first report of the INTERCEPT system being used to eliminate a parasite from a component collected from a donor.

Key words: donors, malaria and protozoal infections, pathogen inactivation.

Introduction

Visceral leishmaniasis caused by *Leishmania infantum* is endemic in the Mediterranean basin. Most *L. infantum* infections are asymptomatic and resolve spontaneously in immunocompetent individuals. A minority progress to classic visceral leishmaniasis [1]. *Leishmania* infection is naturally transmitted through the bite of phlebotomine sand flies, but transmission of *Leishmania* by transfusion has also been reported [2]. The existence of asymptomatic *L. infantum* carriers, associated with intermittent low-density circulation of the parasite [1,3], has been proposed as the main cause of transmission by blood.

In the Balearic Islands, the prevalence of asymptomatic *L. infantum* infection in blood donors is substantially high (*L. infantum* DNA in blood was detected in 5·9% of blood donors studied) [4], which is consistent with other findings regarding asymptomatic carriers from the Mediterranean region [1,5].

Although some research studies have investigated *Leishmania* infection in blood donors [1,3–5], at present there are no *Leishmania* donor screening tests that are capable of meeting Blood Bank criteria with respect to speed, standardization and automation.

As no suitable donor screening tests are currently available, several methods have been used to eliminate *Leishmania* in blood products [6,7]. As an approach to reducing the risk of transfusion transmission in our area, we investigated the ability of a pathogen inactivation technology using amotosalen HCl and ultraviolet-A (320–400 nm) light [INTERCEPT Blood System for Platelets; Cerus, BV, Amersfoot, the Netherlands] to eliminate *L. infantum* in apheresis platelet units obtained from an asymptomatic infected blood donor.

Materials and methods

This study was conducted under a protocol approved by the Balearic Island Ethic Committee after written informed consent was obtained from the participating donor. A 53-year-old male donor, previously known to be asymptomatically infected with *L. infantum* by detecting *L. infantum* DNA in his peripheral blood, was enrolled in this study. He gave a platelet apheresis donation using the Amicus device (Fenwal, Lake Zurich, IL, USA). According to the Spanish specifications for platelet products, the targeted platelet content of each unit suitable for transfusion had to be $\geq 3{\cdot}0 \times 10^{11}$/component. Apheresis products were

Correspondence: Teresa Jimenez-Marco, Fundació Banc de Sang i Teixits de les Illes Balears, C/Rosselló i Caçador, 20 07004, Balearic Islands, Palma de Mallorca, Spain
E-mail: matejimenez@hotmail.com; tjimenez@fbstib.org
*Both authors contributed equally to this work.

suspended in approximately 35% plasma and 65% platelet additive solution (Intersol, Fenwal) and treated according to the INTERCEPT manufacturer's instructions for use. Western blot (WB) and real-time polymerase chain reaction (RT-PCR) analyses were carried out on peripheral blood samples on the day of the donation. RT-PCR was also carried out on the platelet unit both before and after inactivation with the INTERCEPT.

Anti-*Leishmania* antibodies were tested by WB using a whole *L. infantum* antigen (MHOM/FR/78/LEM75) as previously described [8]. We considered a serum positive when immunoreactivity against the 14 and/or 16 kDa *L. infantum* antigen fraction was observed.

The presence of *Leishmania* DNA was analysed by amplification of kinetoplast DNA sequence by RT-PCR as previously described [9]. DNA was extracted in duplicate, and each amplification was performed in triplicate using the ABI Prism 7700 system. In total, six DNA amplifications were performed for each platelet unit. RT-PCR was considered positive for *Leishmania* spp. when the threshold cycle (tC) was <45. The tC for a given sample is the first cycle of the PCR reaction where fluorescence is detected above the baseline. '*In vitro*' culture was performed as previously described [4]. All samples were cultured regardless of the RT-PCR results.

Results

The *Leishmania*-specific antibodies were revealed by WB in the sera of this donor and showed the characteristic 16-kDa band. Both WB and RT-PCR analyses on peripheral blood samples were positive on the day of the donation. The preinactivation platelet units were detected positive by RT-PCR at 0·01 parasites/ml in five of six DNA amplifications performed. After inactivation, the platelet unit was RT-PCRs negative. All '*in vitro*' cultures were negative after 6-month follow-up.

Discussion

Asymptomatic carriers of *L. infantum* are a major cause of transfusion transmission in endemic areas [2]. There is a reasonable possibility that blood products from infected persons, if parasitemic at the time of blood donation, may transmit leishmaniasis to the recipient. Several methods based on pathogen inactivation technology have been used to prevent transfusion-acquired leishmaniasis [6,7], including the INTERCEPT Blood System [7]. Peripheral blood PCR is a good, noninvasive alternative to traditional diagnosis methods, such as microscopic examination and/or bone marrow and spleen aspirate cultures, for detecting *L. infantum* asymptomatic carriers [1,3,4]. PCR testing can be considered as a true direct method for detecting parasite presence as DNA is rapidly degraded following parasite death [10]. Culture methods, in reality, have a low sensitivity compared with the high sensitivity of PCR when these methods are used for the detection of asymptomatic carriers [3,4]. This may be due to the low level of circulating parasites in asymptomatic individuals, ranging from 0·001 parasites/ml to 1 parasite/ml [11], which it is sufficient to render a positive PCR but not a positive culture result [12].

Therefore, culture techniques do not seem to be the best method to detect asymptomatic carriers due to the low level of circulating parasites found in these individuals.

We studied a blood donor with detectable *Leishmania* DNA in peripheral blood but who was otherwise a healthy individual. WB and RT-PCR analyses performed on the donor's peripheral blood samples taken on the day of the donation were positive. The preinactivation platelet unit RT-PCR was positive at 0·01 parasites/ml. However, the '*in vitro*' culture results were negative. This result is not surprising given the small size of the 'inoculum' (0·01 parasites/ml), which is far below the dose that is considered necessary to produce cell growth (over 10^4 parasites/ml) [12]. However, RT-PCRs were negative after platelet unit inactivation, thereby guaranteeing the absence of viable parasites since *Leishmania* nucleic acids are rapidly degraded following parasite death [10].

Until now, studies into pathogen inactivation technologies applied to the reduction in *Leishmania* risks have been based on '*in vitro*' studies. Basically, *Leishmania*-infected monocytes and/or promastigotes were deliberately added at high doses to blood components collected from healthy, noninfected donors. The presence of viable postinactivation parasites in these studies was evaluated by culture methods [6,7]. In reality, it is more likely that the levels of parasites in blood donations from asymptomatic individuals, and which need to be inactivated, are much lower than those used for '*in vitro*' spiking studies. Essentially, if pathogen inactivation technology is able to inactivate the high doses used for '*in vitro*' spiking studies, this gives more weight to the indication that it will be able to inactivate the low doses presented in asymptomatic blood donors. The application of this technology should, therefore, provide a wide margin of safety.

The INTERCEPT Blood System may represent an interesting approach to prevent transfusion-transmitted leishmaniasis. However, these findings need to be confirmed through additional studies.

Acknowledgements

We thank the blood donor for his willingness to participate in the study. The authors also wish to thank Martin Hadley-Adams for assisting with the English language and preparation of the manuscript. This work was supported by the National Plan of I+D+I 2008-2011 and ISC III-Subdirección

General de Evaluación y Fomento de la Investigación (PI 10/00533).

Conflict of interest

The authors declare that there are no conflicts of interest.

References

1 le Fichoux Y, Quaranta JF, Aufeuvre JP, *et al.* Ocurrence of *Leishmania infantum* parasitemia in asymptomatic blood donors living in an area of endemicity in southern France. *J Clin Microbiol* 1999; 37: 153–157

2 Singh S. Transfusion transmitted leishmaniasis: a case report and review of literature. *Indian J of Med Microbiol* 2006; 24: 165–170

3 Riera C, Fisa R, Udina M, *et al.* Detection of *Leishmania infantum* cryptic infection in asymptomatic blood donors living in an endemic area (Eivissa, Balearic Islands, Spain) by different diagnostic methods. *Trans R Soc Trop Med Hyg* 2004; 98: 102–110

4 Riera C, Fisa R, López-Chejade P, *et al.* Asymptomatic infection by *Leishmania infantum* in blood donors from the Balearic Islands (Spain). *Transfusion* 2008; 48: 1383–1389

5 Biglio A, Bola C, Concialdi E, *et al.* Asymptomatic *Leishmania infantum* infection in an area of north-western Italy (Piedmont region) where such infections are traditionally endemic. *J Clin Microbiol* 2010; 48: 131–136

6 Cardo LJ, Rentas FJ, Ketchum L, *et al.* Pathogen inactivation of Leishmania donovani infantum in plasma and platelet concentrate using riboflavin and ultraviolet light. *Vox Sang* 2006; 90: 85–91

7 Eastman RT, Barrett LK, Dupuis K, *et al.* Leishmania inactivation in human apheresis platelets by a psoralen (amotosalen HCL) and long-wavelength ultraviolet irradiation. *Transfusion* 2005; 45: 1459–1463

8 Riera C, Valladares JE, Gállego M, *et al.* Serological and parasitological follow-up in dogs experimentally infected with *Leishmania infantum* and treated with meglumine antimoniate. *Vet Parasitol* 1999; 2: 33–47

9 Martin-Ezquerra G, Fisa R, Riera C, *et al.* Role of Leishmania spp. infestation in nondiagnostic cutaneous granulomatous lesions: report of a series of patients from a Western Mediterranean area. *Br J Dermatol* 2009; 161: 320–325

10 Prina E, Roux E, Mattei D, *et al.* *Leishmania* DNA is rapidly degraded following parasite death: an analysis by microscopy and real-time PCR. *Microbes Infect* 2007; 9: 1307–1315

11 Mary C, Faraut F, Drogoul MP, *et al.* Reference values for *Leishmania infatum* parasitemia in different clinical presentations: quantitative polymerase chain reaction for therapeutic monitoring and patient follow-up. *Am J Trop Med Hyg* 2006; 75: 858–863

12 Lemesre JL, Darcy F, Kweider M, *et al.* Requirements of defined cultivation conditions for standard growth of *Leishmania* promastigotes in vitro. *Acta Trop* 1988; 45: 99–108

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
				2013. 2. 20	該当なし	

一般的名称	新鮮凍結人血漿	研究報告の公表状況		公表国
販売名（企業名）	新鮮凍結血漿-LR「日赤」（日本赤十字社） 新鮮凍結血漿-LR「日赤」成分採血（日本赤十字社） 新鮮凍結血漿-LR「日赤」120（日本赤十字社） 新鮮凍結血漿-LR「日赤」240（日本赤十字社） 新鮮凍結血漿-LR「日赤」480（日本赤十字社）		ProMED 20130217.1546451	スペイン

研究報告の概要

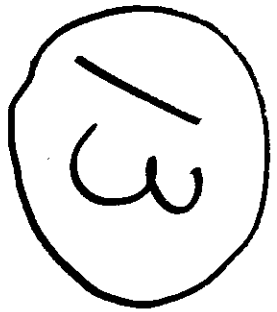
○リーシュマニア症－スペイン、マドリッド
スペイン、マドリッド南部の市町村（Bosquesurの緑地帯周辺のFuenlabrada、Leganes、Getafe、Humanes等）で発生しているリーシュマニア症のアウトブレイクは、2012年の150症例を含めて過去3年間に500症例が報告されており、まだ継続しているとみられる。
リーシュマニア症は感染動物を刺咬したサシチョウバエに刺咬されることにより伝播し、内臓リーシュマニア症を発症した場合、治療を行わなければ死亡することもある。Bosquesurで増加しているウサギが保有宿主となっている可能性がある。2012年、Fuenlabradaは緊急事態地域と宣言され、事態を制御するためにウサギの駆除が許可された。厚生大臣は、2012年末までに報告された内臓リーシュマニア症は3例で、2011年の47例と比べて改善したと述べたが、2013年の現時点で既に3例の新規症例が報告されており、アウトブレイクはまだ終了していないとみられる。

報告企業の意見	今後の対応
スペイン、マドリッド南部の市町村におけるリーシュマニア症のアウトブレイクは過去3年間で500症例が報告され、未だ終息していないとの報告である。	日本赤十字社では、輸血感染症対策として問診時に海外滞在歴の有無を確認し、帰国（入国）後4週間は献血不適としている。今後も引き続き、新興・再興感染症の発生状況等に関する情報の収集に努める。

使用上の注意記載状況・その他参考事項等

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

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク



Published Date: 2013-02-17 11:35:32
Subject: PRO/AH/EDR> Leishmaniasis - Spain: Madrid
Archive Number: 20130217.1546451

LEISHMANIASIS - SPAIN: MADRID
****

A ProMED-mail post
http://www.promedmail.org
ProMED-mail is a program of the
International Society for Infectious Diseases
http://www.isid.org

Date: Wed 13 Feb 2013
Source: SER Madrid Sur [in Spanish, trans. Corr.SB, edited]
http://www.sermadridsur.com/noticias/el-brote-de-leishmaniasisque-afecta-a-municipios-del-sur-no-remitio-en-2012-con-150-casos_31239/

The outbreak of leishmaniasis that has affected the southern municipalities (of Madrid) has not decreased in 2012, with 150 cases reported.

According to the "Report on the Health Status of the Population of Madrid, 2012," the outbreak of leishmaniasis is not over, as evidenced by the 150 cases detected last year [2012], including nearly 500 cases which have emerged in the last 3 years. These cases have occurred in southern municipalities of the region, such as Fuenlabrada, Leganes, Getafe or Humanes, near the green zones of Bosquesur.

Leishmaniasis is spread by the bite of an insect (a _Phlebotominae_ female sand fly that has previously bitten an infected animal) and can lead to death if the visceral disease occurs and if it is left untreated.

The strong increase occurred from 2009, with 471 cases, compared to 15 or 25 that had been reported in previous years. The cases have been found in municipalities in southern Madrid, such as Fuenlabrada with 322 cases, Leganes with 37, Getafe with 20 affected, and Humanes, which had 5 patients. All of them were near the green area of Bosquesur, which officials believe may be the focus of leishmaniasis.

This disease is transmitted by a sandfly that has previously bitten an infected animal. It is believed that hares and rabbits, which proliferate in Bosquesur, can act as reservoirs or carriers of the parasite.

Leishmaniasis causes 2 types of disease: cutaneous, and visceral, which can affect organs such as the spleen and liver and is fatal if not treated properly.

Last year [2012], the community declared Fuenlabrada an "Emergency Area," allowing the free hunting of rabbits to try to control the extent of the problem. The Ministry of Health said that by the end of 2012, there were 3 cases of visceral leishmaniasis, while in 2011, there had been 47, which was good progress. Still, the outbreak is not considered to be over. So far this year [2013], there have been 3 new cases.

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

[Dogs are considered the main reservoir of Leishmania in Spain (M.G. et al. Current situation of _Leishmania infantum_ infection in shelter dogs in northern Spain. Parasit. Vectors. 2012;5:60). It has been hypothesized that rabbits, hares, and squirrels may also be hosts, but this remains to be demonstrated. - Mod.EP

A HealthMap/ProMED-mail map can be accessed at: http://healthmap.org/r/1zJm.]

See Also

2012

Leishmaniasis - Spain: Madrid: 20120504.1123085
Leishmaniasis, human, canine - Spain (02): background 20120329.1084736
Leishmaniasis, human, canine - Spain: (MD) 20120328.1083656
Leishmaniasis, canine - Singapore ex Spain: OIE 20120125.1022003
2010

Leishmaniasis, human - Spain 20100612.1969
2004

Leishmaniasis, dog reservoir - Spain 20040524.1388
2000

Leishmaniasis - Germany ex Spain (02) 20000729.1254
Leishmaniasis, Germany ex Spain: background 20000727.1248
Leishmaniasis - Germany ex Spain 20000725.1236
.....sb/ep/msp/dk

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
一般的名称	－		研究報告の公表状況	Transfusion. 2012 Nov;52(11):2285-93.	公表国 アイルランド	
販売名（企業名）	－					

研究報告の概要

変異型クロイツフェルトヤコブ病(vCJD)は、まれで進行性の致命的な非炎症性の神経変性疾患である。アイルランドは輸血を通してのvCJD伝播率が英国(176/6200万)に次いで、世界中で2番目に高い(2/4500万)。
vCJD伝播の危険を減らすためプリオンを取り除くフィルター(the P-Capt filter)が開発された。
この研究は、アイルランド共和国で赤血球のプリオンろ過を実行することについての費用効果を評価することを目的とした。
費用対効果モデルは、受血者が感染した赤血球を輸血された結果、臨床的変異型CJDを発症する可能性をシミュレートするために開発された。
モデル変数は公表文献や専門家の意見を収集し、費用はプリオンろ過を実装するために必要な処理の変更に基づいて推定した。
プリオン濾過を行わなければ、10年間に2人が赤血球輸注によるvCJD発症すると推定され、失われる寿命は18.5年となるがプリオン濾過を行えば、寿命は失われない。
普遍的なプリオン濾過の費用は68.2百万ユーロで、寿命1年当たりのコストは3.7百万ユーロである。
シミュレーションの25.3%においては、プリオン濾過の有無にかかわらず、感染血液の輸血を介したvCJD感染の死亡例はない。
そのため、プリオン濾過導入の費用対効果は高くないと考えられた。
多くの非費用対効果の高い血液の安全戦略が過去に実施されてきたが、輸血医療における有限な資源の最も効率的な使用を考慮するべきである。

報告企業の意見	今後の対応
赤血球製剤に対するプリオンろ過フィルターの費用対効果に関する情報である。 現時点まで血友病以外で血漿分画製剤からvCJD伝播が疑われた報告はなく、血漿分画製剤の製造工程でプリオンが除去できるとの情報もある。	今後ともvCJDに関する安全性情報等に留意していく。

使用上の注意記載状況・その他参考事項等

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

Cost-effectiveness of prion filtration of red blood cells to reduce the risk of transfusion-transmitted variant Creutzfeldt-Jakob disease in the Republic of Ireland

Conor Teljeur, Martin Flattery, Patricia Harrington, Michelle O'Neill, Patrick S. Moran, Linda Murphy, and Máirín Ryan

BACKGROUND: Variant Creutzfeldt-Jakob disease (vCJD) is a rare, progressive fatal noninflammatory neurodegenerative disease. Ireland has the second-highest rate of vCJD in the world with an ongoing risk of vCJD transmission through blood transfusion. Prion-removing filters have been developed to reduce the risk of vCJD transmission. This study aimed to evaluate the cost-effectiveness of implementing a policy of prion filtration of red blood cells (RBCs) in the Republic of Ireland.

STUDY DESIGN AND METHODS: A cost-effectiveness model was developed to simulate the likelihood of RBC recipients developing clinical vCJD as a result of being transfused with infected RBCs. Model variables were collected from published literature and expert opinion. Costs were estimated based on the processing changes required to implement prion filtration.

RESULTS: In the absence of prion filtration, it is estimated that two individuals will develop clinical vCJD arising from RBC transfusions over a 10-year time horizon. The discounted life-years lost will be 18.5 years. With prion filtration, there will be no deaths or life-years lost. The discounted cost of universal prion filtration is €68.2 million over 10 years with a corresponding incremental cost-effectiveness ratio of €3.7 million per life-year gained. In 25.3% of simulations there were no deaths from vCJD infection through infected blood transfusions, irrespective of prion filtration.

CONCLUSION: Prion filtration is considered not cost-effective by traditional measures. Although numerous non–cost-effective blood safety strategies have been implemented in the past, consideration should be given to the most efficient use of finite resources in transfusion medicine.

Variant Creutzfeldt-Jakob disease (vCJD) is one of a group of rare, progressive fatal noninflammatory neurodegenerative diseases known as transmissible spongiform encephalopathies (TSEs).[1] CJD is the most common TSE affecting humans; a new variant form, termed vCJD, was first described in 1996. This variant form is characterized by having a different neuropathologic profile, a younger age of onset, different clinical findings, and the absence of abnormal electroencephalogram findings typical of other CJD forms.[2] The origin of vCJD is linked to the consumption of beef from cattle infected with a bovine form of the disease, bovine spongiform encephalitis (BSE)[3] which was prevalent in the United Kingdom and elsewhere in the 1980s and 1990s. Of the 224 cases of vCJD worldwide to date, 175 have occurred in the United Kingdom,[4] where the incidence peaked in 2000, declining since. Ireland has the second highest rate of vCJD in the world behind the United Kingdom, with four cases reported to date in a population of 4.5 million.[4]

There is an ongoing risk of vCJD transmission from transfusion of blood or blood products originating from subclinical carriers of the disease. Measures have been taken by transfusion services worldwide in accordance

ABBREVIATIONS: BSE = bovine spongiform encephalitis; IBTS = Irish Blood Transfusion Service; ICER = incremental cost-effectiveness ratio; QALY = quality-adjusted life-year; TSE(s) = transmissible spongiform encephalopathy (-ies); VAT = value-added tax; vCJD = variant Creutzfeldt-Jakob disease.

From the Health Information and Quality Authority, Dublin, Ireland.

Address correspondence to: Conor Teljeur, Health Information and Quality Authority (HIQA), George's Court, George's Lane, Dublin 7, Ireland; e-mail: cteljeur@hiqa.ie.

Received for publication November 2, 2011; revision received January 11, 2012, and accepted February 7, 2012.

doi: 10.1111/j.1537-2995.2012.03637.x

TRANSFUSION 2012;52:2285-2293.

with best available evidence to minimize this risk. These measures include donor deferral policies, importation of plasma from countries with a low incidence of vCJD and BSE, and universal leukoreduction of red blood cells (RBCs). Worldwide there have been four documented cases of vCJD infection arising from transfusion of RBCs, resulting in three deaths from clinical vCJD.[5] All of these cases occurred in the United Kingdom before the introduction of universal leukoreduction of RBCs. The high incidence of vCJD in Ireland has raised concerns that there may be a significant risk of secondary transmission through infected blood products.[6]

Prototype blood tests have been developed for detection of vCJD in symptomatic individuals, but as yet there are no tests sufficiently sensitive to screen blood from individuals who are asymptomatic carriers of the infectious agent.[7,8] Prion-removing filters have been developed to further reduce the risk of vCJD transmission from transfusion of RBCs by, it is claimed, substantially reducing any residual prion protein present in donated blood.[9] The use of such filters would complement existing measures adopted to contain the risk of transmission. Although prion-removing filters may alter the composition of RBCs that are passed through them, the resulting RBCs are considered safe for transfusion.[6,10] The aim of this study was to evaluate the cost-effectiveness of implementing a policy of prion filtration of RBCs in the Republic of Ireland.

MATERIALS AND METHODS

Literature review on efficacy and safety of prion filters

Published literature was obtained by searching MEDLINE, CINAHL, the Cochrane Library, Database of Abstracts of Reviews of Effects, NHS Economic Evaluation Database, and EBSCO Psychology and Behavioural Sciences Collection and Health Business on the PubMed and EBSCO systems. Regular alerts were established on PubMed and EBSCO and relevant information retrieved via alerts was current to December 16, 2010. Given the limited data available on the topic, a broad search was used to capture as many potentially relevant results as possible. No date restrictions or other filters were applied to limit the retrieval to specific study designs or document types.

Economic model

A cost-utility analysis is the preferred type of economic evaluation for assessing health care interventions in Ireland.[11] As no published quality-of-life data were found for vCJD, or for TSEs generally, a cost-effectiveness analysis was performed as an alternative in this study. Prion filtration of all RBCs was compared to a policy of no filtration. The perspective was that of the publicly funded health and social care system in Ireland with only direct costs to the Irish Blood Transfusion Service (IBTS) and Health Service Executive included. The target population included all individuals receiving a transfusion of RBCs. It was assumed that no additional transfusion-related adverse events would arise due to filter-related changes in the composition of RBCs. Discounting is a technique that allows comparison between costs and benefits that occur at different times. It reflects a societal preference for benefits to be realized in the present and costs to be experienced in the future. Costs and benefits were discounted at the rate of 4% as prescribed in Irish guidelines.[11] A 10-year time horizon was used for evaluating costs. Benefits were determined as the life-years gained based on infections prevented from blood transfusions in the 10-year horizon. The benefits could therefore extend beyond the 10-year horizon as long as the original transfusion took place during the 10-year interval. No utility data associated with vCJD or CJD were available to develop a suitable quality-adjusted life-year (QALY) measure, hence a cost-utility analysis was not possible. Variable values were determined using peer-reviewed literature, with gray literature and expert opinion used in the absence of peer-reviewed evidence.

The model comprised two distinct components: the transmission model and the costs model. The former simulated the transfusion of infected units of RBCs into recipients; the latter estimated the costs associated with prion filtration. The transmission model was adapted from a model originally developed by the UK Department of Health. The UK model was deterministic and estimated cost-effectiveness for a number of best- and worst-case scenarios. This was converted into a fully probabilistic model that allowed the inherent uncertainty around variable estimates to be incorporated.

The transmission model simulated the likelihood of RBC recipients developing vCJD as a result of being transfused with blood donated by individuals who were subclinical carriers of vCJD. There are approximately 96,000 donors and 32,000 recipients of units of RBCs each year in Ireland. The median age of recipients is 69 and on average 41% of recipients are alive 5 years after transfusion.[12] The model relied on a number of key variables (see Table 1): the prevalence of subclinical vCJD in the donor population; the infectivity of blood sourced from an infected donor; the susceptibility of the recipient to developing clinical vCJD; once infected, the incubation period before developing clinical vCJD; and the efficacy of the filter. The prevalence of subclinical vCJD in Ireland was estimated using the results of the UK Hilton study.[13] To account for the difference in observed clinical cases, the prevalence was multiplied by the ratio of indigenous clinical prevalence in Ireland (2 in 4.5 million) to the UK (176 in 62 million). The infectivity of human blood has been inferred from animal studies. All confirmed clinical cases

TABLE 1. Transmission model variable distributions and values

Variable	Median (95% CI)	Distribution
National prevalence of preclinical vCJD*	153 (35-413)†	Beta (3, 12,671) × 0.158[13]
Susceptibility to developing clinical vCJD (%)	9.7 (5.0-16.6)	Beta (10, 90)[14]
Donations per infected donor (units per annum)	1 (1-4)	Sampled[15]
Percentage of collected units used (%)	89 (86-91)	Beta (512, 63)[15]
Infectivity of vCJD infected blood (ID/mL)	9.3 (0.9-35.6)	Gamma (1.57, 0.135)[15]
Infectivity removed by leukoreduction (%)	50.0 (32.4-67.5)	Beta (14.9, 14.9)[9,17]
Residual plasma (mL)		
Top and top (TT)	20.2 (13.7-26.7)	Normal (20.2, 3.3)[16]
Top and bottom (TB)	9.3 (5.2-13.4)	Normal (9.3, 2.1)[18]
Incubation (years)		
MM homozygous	7.6 (5.6-9.6)	Normal (7.60, 1.02)[19]
Non-MM homozygous‡	21.3 (15.9-28.6)	Log normal (3.06, 0.15)
Percentage population MM homozygous	39.2 (34.5-44.0)	Beta (156, 242)[20]
Probability of infectious doses after prion filtration	6.9×10^{-6} (2.5×10^{-7}-3.7×10^{-5})	Beta (1, 9,999)[21]

* A factor of 0.158 is applied to the prevalence to reflect the relative difference in observed indigenous clinical cases between Ireland and the United Kingdom.
† Prevalence has been multiplied by national population of 4.5 million to give number of infected individuals nationally.
‡ As there are no observed cases of clinical vCJD in non–MM-homozygous individuals, this distribution is based on expert opinion alone.
Beta = α and β in parentheses; Gamma = shape and rate in parentheses; normal and log normal = mean and SD in parentheses.

of vCJD to date have been methionine-homozygous (MM) at Codon 129 of the prion protein expressing gene (*PRNP*). However, it cannot be ruled out that clinical vCJD could occur in individuals with other polymorphisms.[22] A Poisson dose–response model was used that entails a high probability of infection even at minimal exposure levels. Under this model, there is a 99% probability of transmission with exposure to only five infectious doses. Susceptibility may also be linked to the age of the recipient, but there are insufficient data to support a parameterization.[23] Values for susceptibility and incubation period in non–MM-homozygous individuals are unknown and were based on published modeling exercises.[21,24] It was assumed that all individuals can be infected by exposure to infected RBCs but that only those who are susceptible may go on to develop clinical vCJD. To generate plausible results, susceptibility and incubation length must be negatively correlated.[24] There is no biologic rationale for a negative correlation and this relationship may simply reflect a shortcoming of the model rather than the characteristics of vCJD transmission. Susceptibility was set at a lower range and a corresponding short incubation period was used for this study. The same values for susceptibility to clinical vCJD were applied to MM and non–MM-homozygous individuals. The efficacy of the filter is derived from a single study that determined that 1 in 10,000 infectious doses would not be retained by the filter.[25] The filters therefore appear to be very efficacious, but no subsequent studies are yet available to either confirm or contradict those findings.

There are two methods for extracting RBCs from whole blood: top and top and top and bottom. The method used impacts on the amount of residual plasma in the processed RBCs and therefore the model takes into account the proportions processed by each method in the IBTS. It is thought that vCJD infectivity in human blood is distributed such that half is in the plasma and the remaining 50% split between the white blood cells (WBCs) and buffy coat; hence the amount of residual plasma in RBCs affects the amount of residual infectivity.[26] Leukoreduction is assumed to remove approximately 50% of infectivity.

The wide distributions around variable values reflect the uncertainty in the underlying scientific evidence (see Table 1). There were no data to support the choice of incubation period in non–MM-homozygous individuals, so expert opinion was used to select a plausible value. The age–sex profile of recipients was sampled from national hospital inpatient data and posttransfusion survival data were based on a UK study.[12]

Costs model

Two models of prion-removing filter were considered in this assessment: the P-Capt filter (MacoPharma Ltd, Mouvaux, France) and the Leukotrap Affinity Plus combined WBC and prion-removing filter system (PRF2BE; Pall Medical, Portsmouth, UK). Unlike the PRF2BE, the P-Capt has been independently validated and could be adopted immediately. Such studies typically take 3 to 4 years to complete and it is therefore assumed the PRF2BE filter system could only be adopted after 4 to 5 years under the assumption that it will be shown to have equivalent efficacy to the P-Capt filter.

Costs related to the introduction of prion filtration of RBCs were identified (see Table 2). The costs inputs for the cost-effectiveness model relate to the incremental cost of prion filtration. Where appropriate, cost savings related to the reduced consumption of existing resources were included. Costs considered in estimating the marginal

TABLE 2. Cost data included in the model (2010 costs)*

Item	Cost (€)
P-Capt filter	55.00 (46.20-63.80)
PRF2BE filter†	43.70 (36.71-50.69)
Processing cost per unit (excluding prion filtration)	248.71 (208.92-288.50)
Wafer	2.73 (2.29-3.17)
Macopharma bag	9.87 (8.29-11.45)
Pall bag	8.75 (7.35-10.15)
Classic bag	6.95 (5.84-8.06)
F IX assay	6.20 (5.21-7.19)
Waste bin (per unit)	0.12 (0.10-0.14)
Incineration (per unit)	0.26 (0.22-0.30)
Mean annual staffing costs‡	295,074.00 (247,862.16-342,285.84)

* Data are reported as median (95% CI).
† Filter price estimated based on quoted price in sterling for purchase of 150,000 units.
‡ Comprises three medical laboratory aides and one senior medical scientist. Salary cost includes social insurance, pension costs, and IBTS overheads.

unit cost for the intervention included the cost of procurement, processing, storage, and distribution of prion-filtered RBCs. Consistent with national guidelines, value-added tax (VAT) was not applied to costs.[11] Filter costs were supplied by the manufacturers. Prices for the PRF2BE filter system were quoted in sterling and converted to Euro using the exchange rate at the time of the analysis (€1.14 to GBP£1.00, October 2010). Variation in the exchange rate is assumed to follow a normal distribution (mean, 1; standard deviation [SD], 0.025) around the previous years' exchange rate. Prices for processing equipment were supplied by the IBTS. Salaries were derived from IBTS pay scales and subsequently adjusted for pay-related costs. All costs were based on 2010 prices. All costs were varied by ±20% according to a beta distribution ($\alpha = 2$, $\beta = 2$).

The costs are based on the total number of units processed rather than transfused, as not all processed units are transfused. Some units are not used within the allowable time or else are brought to the operating room and then not transfused. Approximately 148,000 units are processed per annum with 137,000 used. Prion filtration has been associated with a reduction in the hemoglobin (Hb) content of filtered RBCs. The reduction in Hb content per unit may have clinical consequences for transfusion-dependent patients with a percentage of these patients requiring additional units of RBCs annually. It was assumed that this would equate to an increase of 0.5% in the number of units required nationally per annum.

The P-Capt filter represents an additional processing step after leukoreduction whereas the PRF2BE is an integrated WBC and prion removal filter. The use of the P-Capt filter would require an additional sterile connector device (wafer) and associated waste bin and incineration charges. With use of the PRF2BE filter, no leukoreduced plasma (for issue as fresh-frozen plasma) would be generated. In Ireland, approximately 500 units are generated locally per annum with the remaining units required imported from abroad. The cost of an additional processing step to produce leukoreduced plasma was also included. A Factor (F)IX assay is used as a process control measure to indicate filter exposure. It is assumed that 1% of prion filtered units will be selected for testing using F IX assays.

Two-year supply contracts are expected to apply to the purchasing of prion-removing filters. For the cost-effectiveness model, the cost of filters only changes every second year while all consumables vary from year to year. Every 2 years, at the point of filter costs changing, it is assumed that the IBTS will select from the two filter models based on lowest price. Although the PRF2BE filter is less expensive, applying the price fluctuation of ±20% results in the P-Capt filter being less expensive in some simulations.

The budget impact analysis was also determined for a 5-year time horizon using the same perspective as the economic analysis. The data for the budget impact analysis are the same as those used in the cost-effectiveness analysis with the difference being that prices are inclusive of VAT, and no discounting is applied. All items are subject to VAT at 21% apart from staff and the cost per unit of the processed RBCs, which is classified as VAT exempt.

The model was developed and run in the open-source statistics program R.[27] The model was run for 25,000 simulations. Discounting was applied to the results from each year and the results were then aggregated to generate a simulation-level result. The incremental cost-effectiveness ratio (ICER) was computed as the additional cost of prion filtration divided by the additional benefits of prion filtration, in this case calculated as life-years gained. The median, 2.5th percentile, and 97.5th percentile were computed for each outcome across all simulations. These values represent the point estimate, lower and upper bounds, respectively, for each outcome. A univariate sensitivity analysis was used to determine the impact of setting each individual variable in turn to the upper and lower bounds, respectively, while varying all other variables as per the standard model. The results of the sensitivity analysis are presented using a tornado plot, which ranks the variables by their impact on the results. Although the discount rate was not varied in the main model, it was varied between 0 and 6% in the univariate sensitivity analysis. The same discount rate was applied to costs and benefits.

RESULTS

Over 10 years an estimated 45 donors (95% confidence interval [CI], 2-142) infected with subclinical vCJD will donate a total of 70 units (95% CI, 3-224) of RBCs. In the

absence of prion filtration, the infected units of blood will be transfused to six recipients (95% CI, 0-26) that are susceptible to clinical vCJD. Of those six, two (95% CI, 0-11) will survive to 5 years posttransfusion and two individuals (95% CI, 0-8) will develop clinical vCJD and die from this disease (Table 3). In the absence of prion filtration, the life-years lost will be 18.5 (95% CI, 0-102.5). With prion filtration, there will be no deaths or life-years lost. The discounted cost of universal prion filtration will be €68.2 million over 10 years. The corresponding ICER is €3.7 million (95% CI, €0.7 m-∞) per life-year gained.

TABLE 3. Outcomes with and without prion filtration*

Outcome	Without prion filtration	With prion filtration
Deaths from clinical vCJD	2 (0-8)	0 (0-0)
Discounted life-years lost	18.5 (0-102.5)	0 (0-0)
Discounted life-years gained		18.4 (0-101.3)
Discounted cost (€m)		68.2 (61.7-75.0)
ICER (€m/LYG)		3.7 (0.7-∞)
Five-year budget impact (€m)		51.6 (46.4-57.5)

* Data are reported as median (95% CI).

In 25.3% of simulations there were no deaths from vCJD infection through infected blood transfusions, irrespective of prion filtration. Indeed, this is the single most probable outcome. For simulations where there was at least one predicted death, the introduction of prion filtration was predicted to prevent all deaths in 96.8% of cases. The probability of different outcomes is shown in Fig. 1.

The benefits in terms of life-years gained follows a very skewed distribution (Fig. 2). The clustering of results with a gain of 0 is pronounced with 25.6% of simulations resulting in no life-years gained using prion filtration. Just over 83% of simulations had an incremental benefit of less than 50 life-years gained. Given the shorter life expectancy posttransfusion coupled with the older age profile of transfusion recipients, not all cases of prevented infections will result in life-years gained.

The median 5-year budget impact is €51.6 million. In the first few years, when only the P-Capt filter is available, the annual budget impact is approximately €11 million but this reduces to under €9 million by Year 5 when the PRF2BE filter is available. When the budget impact of prion filtration is distributed across the units transfused, it represents an additional cost of €74 (95% CI, €66-€82) per unit, in other words an additional 30% on the price of a unit of RBCs.

A univariate sensitivity analysis was carried out to assess the influence of different variables on the ICER (Fig. 3). The single most significant variable that impacts on the calculated ICER is the prevalence estimate of .subclinical disease. The upper and lower bounds for prevalence are equivalent to 413 and 35 cases of subclinical disease nationally. If the prevalence of subclinical vCJD nationally is 35 cases, for example, then the ICER will be €13.4 million per life-year gained. If, on the other hand, the prevalence is 413 cases nationally, then the ICER will be €2.4 million per life-year gained. The susceptibility is the next most influential variable with an ICER of €7.8 million per life-year gained when susceptibility is at its lowest value and €2.8 million at its highest value. When the discount rate is at its lowest value the mean ICER is €3.1 million per life-year gained.

The cost-effectiveness acceptability curve for prion filtration is shown in Fig. 4. The cost-effectiveness acceptability curve shows the probability that prion filtration is cost-effective over a range of willingness-to-pay thresholds. The probability of cost-effectiveness is

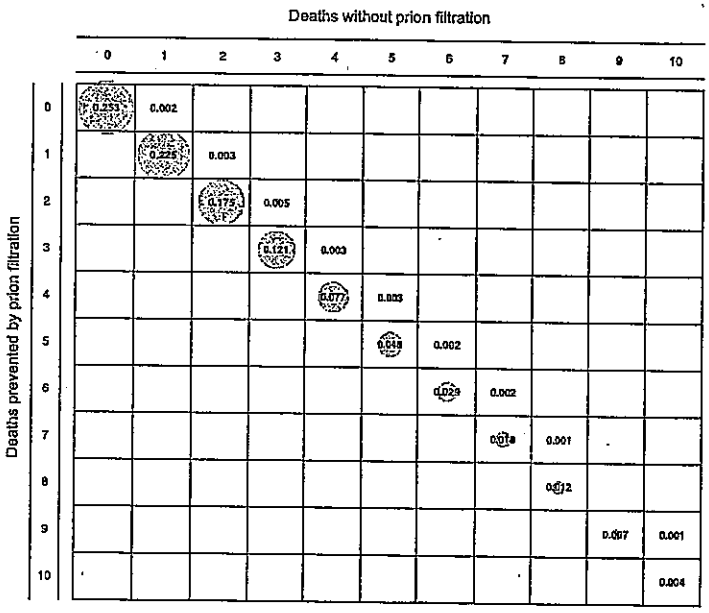


Fig. 1. Balloon plot showing probability of different outcomes with and without prion filtration. Notes: 1) The value in a cell represents the probability of that particular outcome. For example, there is a probability of 0.005 that there will be three deaths without prion filtration where two of the three would be prevented by filtration. Blank cells have zero probability. 2) For clarity the balloon plot only extends to 10 deaths—this excludes 0.8% (n = 194) of simulations with more than 10 deaths. Outcomes with a probability of less than 0.001 are not shown.

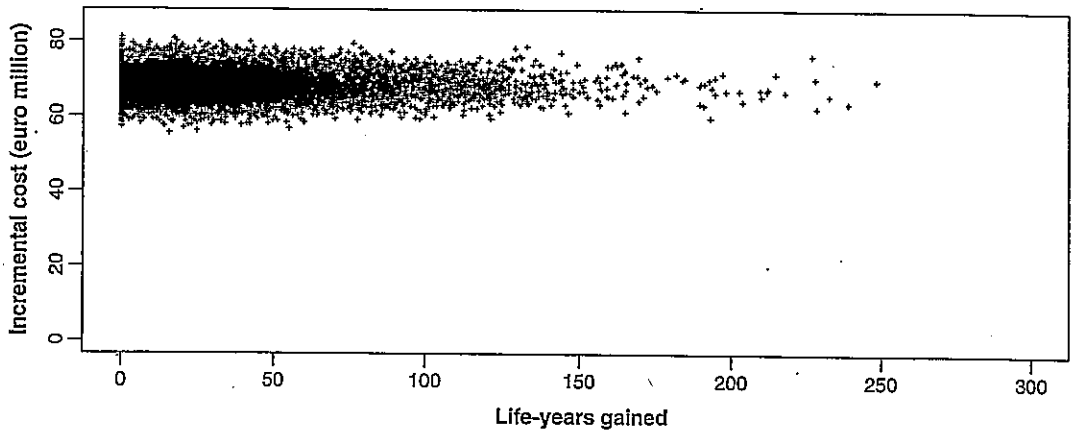


Fig. 2. Plot of cost against health benefit for 25,000 model simulations. Note: In 25.3% (n = 6334) of simulations there were no deaths from vCJD infection through infected blood transfusions, irrespective of prion filtration. Benefits are for the total transfused Irish population.

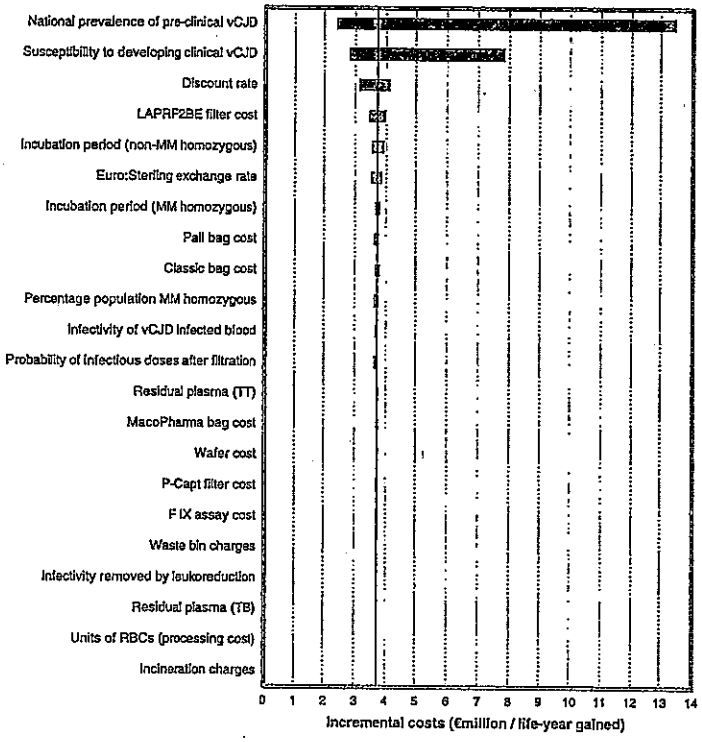


Fig. 3. Tornado plot of univariate sensitivity analysis. Note: base-case and upper and lower values for each variable are derived from Tables 1 and 2. The upper and lower bounds for the discount rate were 5.7 and 1.7%, respectively. Exchange rate variation is approximately ±5% per annum.

zero below a willingness-to-pay threshold of €228,000 per life-year gained. The probability of cost-effectiveness is 0.09 at a willingness-to-pay threshold of €1 million per life-year gained. The probability of cost-effectiveness exceeds 0.5 at a willingness-to-pay threshold of €3.7 million per life-year gained.

DISCUSSION

In the absence of a policy to prion filter RBCs, it was estimated in this study that arising from transfusion of vCJD-infected RBCs over the next 10 years in Ireland there will be two (95% CI, 0-8) deaths from vCJD. This would correspond with 18.5 (95% CI, 0.0-102.5) life-years lost. A policy of universal prion filtration of RBCs is predicted to prevent these two deaths. However, the single most likely outcome is that there will be no deaths arising from vCJD transmission through infected RBCs. Compared to the base-case of no prion filtration, the estimated ICER is €3.7 million (95% CI, €0.7 m-€∞) per life-year gained. The 5-year budget impact of prion filtration would be €51.6 million (95% CI, €46.4 m-€57.5 m), which corresponds to an additional €74 per unit of RBCs transfused.

The ICER of €3.7 million per life-year gained is considered not cost-effective by traditional measures of cost-

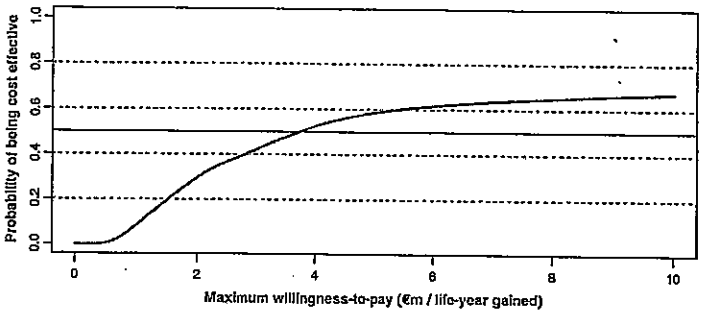


Fig. 4. Cost-effectiveness acceptability curve. Note: as prion filtration offers no benefit in 25.6% (n = 6397) of simulations, the probability of cost-effectiveness never exceeds 0.744.

effectiveness. Although there is no explicit threshold in Ireland, historically an upper limit of €45,000 per QALY has been used, although this has been lowered in recent years, notionally to €20,000 per QALY. Internationally, the cost-effectiveness of blood safety strategies does not compare favorably to that of other health technologies. For example, the addition of nucleic acid testing (NAT) to an existing strategy of viral serologic testing (including human immunodeficiency virus [HIV], hepatitis B, and hepatitis C) was found to be not cost-effective in published European and US studies, with ICERs ranging from €300,000 to €47 million per QALY.[28-32] Despite being not cost-effective, NAT has been adopted by blood transfusion services in most developed countries, including Ireland.[15] The introduction of NAT in Ireland must be viewed in context. From the 1970s to the early 1990s, some patients with hemophilia and von Willebrand disease received contaminated blood products leading to HIV and hepatitis C infections. Contaminated anti-D was also administered resulting in hepatitis C infections. This historical context, coupled with favorable economic conditions at the time, facilitated the introduction of NAT. With no reported Irish cases of vCJD transmission through infected RBCs and less favorable economic circumstances, the precedent of NAT is unlikely to influence a decision on prion filtration. A possible reason for the differing standards of cost-effectiveness of blood safety strategies relates to their purpose—they are aimed at risk reduction rather than improved effectiveness. It is possible that society has a greater preference for risk reduction than health gains.[33] Blood transfusion in Ireland is managed through a State agency so liability claims must also be considered. The negative publicity generated by failing to prevent cases of vCJD infection may also lead to a loss of donors.

The effect of discounting is not inconsiderable in this economic evaluation. Owing to the long incubation period after infection with vCJD, benefits from prion filtration (life-years saved) do not occur for many years. As per the Irish guidelines for the economic evaluation of health care technologies, the same discount rate is applied to both costs and benefits.[11] This practice is not without controversy, particularly when potential benefits do not accrue for a long time (such as in vaccination programs and other preventative public health strategies). Prion filtration is predicted to result in 18.4 (95% CI, 0-101.3) life-years gained with discounting compared to 36.3 (95% CI, 0-228.2) life-years gained without discounting. Differential rates of 3.5 and 1.5% for costs and benefits, respectively, have been used in the United Kingdom. By applying these differential rates, the ICER reduced to €2.3 million (95% CI, €0.5 m-€∞) per life-year gained. While this represents a substantial reduction from the estimate of €3.7 million per life-year gained, prion filtration would still be considered not cost-effective by traditional standards for cost-effectiveness.

The quality of the model was assessed by evaluating the plausibility of the results compared to similar studies. The only study to estimate future cases of vCJD in Ireland predicted that there would be one to two future clinical cases—this is in line with the findings of this study.[34] Until now, cases of vCJD have been driven by primary infection through consumption of BSE-infected beef. Based on the assumption of susceptibility, there is a large cohort who may never develop vCJD, but who are carriers that could infect others. It is therefore possible that a second wave of vCJD may occur in the future due to secondary transmission through infected blood products. In a study published in 2010, the possibility of a second wave of vCJD cases in the United Kingdom was investigated, distinguishing between primary and secondary transmission.[23] It was estimated that over the next 100 years the number of vCJD deaths due to secondary transmission would be approximately 1.7 times the number of vCJD deaths observed to date. If that ratio is applied to Ireland then there would be approximately seven cases in the next 100 years or less than one case per decade through blood transfusion. This would be within the confidence bounds estimated by this study.

The cost-effectiveness model is subject to a number of limitations that may impact on the results or their interpretation. There is substantial uncertainty around both the suitable point estimates and the associated ranges of probable values for many of the key model variables. By using a fully probabilistic model, the uncertainty in the variable values is reflected in the uncertainty in the estimate of cost-effectiveness. The prevalence in the model is assumed to be constant over the 10-year time horizon. This represents a pessimistic view: the risk of primary

transmission through the consumption of infected meat products is believed to have been eliminated; accordingly the number of subclinical donors in the population will decline over time. The cost-effectiveness results reflect the conservative approach adopted—that all genotypes can develop the disease. To date, however, all deaths due to confirmed clinical vCJD have been in MM homozygotes. In assuming, as we have done, that non–MM-homozygotes may develop clinical vCJD, we may be overestimating the number of cases of vCJD arising from blood transfusion. If only MM-homozygous individuals are susceptible to developing clinical disease, then prion filtration would have a true ICER that is substantially higher than €3.7 million per life-year gained. The costs considered in this study were limited to the direct costs to the publicly funded health care system. No costs attached to treatment of individuals with symptomatic vCJD were included. Costs to the individual (for example, out-of-pocket expenditure related to treatment or transport to appointments) or to society (for example, lost productivity in those diagnosed with vCJD) were not considered.

The knowledge of vCJD and its transmission is limited and constantly being updated with new information. While the evidence is sometimes seemingly contradictory or at odds with our understanding of the disease process, it provides an opportunity to refine variable values. The variable values used in this study may be viewed as conservative and reflective of a worst-case scenario. Emerging evidence suggests that some variables, such as infectivity, may be lower than previously thought and hence overstated in this model.[35] However, taking a public health perspective and viewing prion filtration as a means to prevent a civil risk, it is pragmatic to view the potential exposure in a pessimistic rather than optimistic light. Given Ireland's legacy regarding hepatitis C and HIV infection, a conservative approach is more appropriate. As knowledge improves, models can be refined to hopefully produce more accurate and precise estimates of the future course of vCJD.

In conclusion, in the absence of a reliable screening test for donors with subclinical vCJD, it has been proposed that prion filtration of RBCs would complement existing risk reduction strategies and further reduce the risk of transfusion-transmitted vCJD. The introduction of prion filtration for all transfusion recipients was found to be not cost-effective by traditional standards of cost-effectiveness. Although the results of this study may be closer to a worst-case scenario as a result of conservative modeling assumptions, they should not be viewed as improbable. Prion filtration could have a true ICER that is substantially higher than €3.7 million per life-year gained. Although other blood safety interventions regarded as not cost-effective have been implemented, the most effective use of finite resources in transfusion medicine must be taken into consideration.

ACKNOWLEDGMENTS

We would like to thank Mrs Katie Gronow, Dr Maren Daraktchiev, and Dr Peter Bennett of the UK Department of Health who supplied the original model which was adapted to the model used in this study. This project was assisted by an Expert Advisory Group comprising a range of stakeholders and experts in transfusion medicine: Dr David Asher, US Food and Drug Administration; Dr Colette Bonner, Department of Health and Children, Ireland; Dr Francesca Brett, Beaumont Hospital, Dublin; Matthew Collins, Department of Health, Ireland; Dr Patrick Costello, Irish Medicines Board; Dr Robert Cunney, Health Service Executive; Dr Tony Finch, Irish Blood Transfusion Service; Dr Catherine Flynn, St James's Hospital, Dublin; Dr Jenny Kieran, Trinity College, Dublin; Stephen McMahon, Irish Patients' Association; Dr Deirdre Madden, University College Cork; Dr William Murphy, Irish Blood Transfusion Service; Brian O'Mahony, Irish Haemophilia Association; Dr Joan O'Riordan, Irish Blood Transfusion Service; Professor Mark Sculpher, University of York; Dr Lesley Tilson, National Centre for Pharmacoeconomics, Dublin; and Prof. Marc Turner, Scottish National Blood Transfusion Service.

CONFLICT OF INTEREST

MF had previously been employed, until 2008, by Fannin Healthcare, the Irish distributors of the Macopharma range of products. CT, PH, MON, PSM, LM, and MR do not have any conflicts of interest.

REFERENCES

1. US Department of Health and Human Services, Centers for Disease Control and Prevention. Chronic wasting disease [Internet]. CDC; 2011. [cited 2011 Jan 21]. Available from: URL: http://www.cdc.gov/ncidod/dvrd/cwd/
2. Will RG, Ironside JW, Zeidler M, Cousens SN, Estibeiro K, Alperovitch A, Poser S, Pocchiari , M, Hofman A, Smith, PG. A new variant of Creutzfeldt-Jakob disease in the UK. Lancet 1996;347:921-5.
3. Hilton DA. Pathogenesis and prevalence of variant Creutzfeldt-Jakob disease. J Pathol 2006;208:134-41.
4. European Creutzfeldt Jakob Disease Surveillance Network. Current data (August 2011) [Internet]. EUROCJD; 2011. [cited 2011 Aug 31]. Available from: URL: http://www.eurocjd.ed.ac.uk
5. Knight R. The risk of transmitting prion disease by blood or plasma products. Transfus Apher Sci 2010;43:387-91.
6. Murphy CV, Eakins E, Fagan J, Croxon H, Murphy WG. In vitro assessment of red-cell concentrates in SAG-M filtered through the MacoPharma P-CAPT prion-reduction filter. Transfus Med 2009;19:109-16.
7. Orru CD, Wilham JM, Raymond LD, Kuhn F, Schroeder B, Raeber AJ, Caughey B. Prion disease blood test using immunoprecipitation and improved quaking-induced conversion. Mbio 2011;2:e00078-11.

8. Edgeworth JA, Farmer M, Sicilia A, Tavares P, Beck J, Campbell T, Lowe J, Mead S, Rudge P, Collinge J, Jackson GS. Detection of prion infection in variant Creutzfeldt-Jakob disease: a blood-based assay. Lancet 2011;377:487-93.
9. Gregori L, Gurgel PV, Lathrop JT, Edwardson P, Lambert BC, Carbonell RG, Burton SJ, Hammond DJ, Rohwer RG. Reduction in infectivity of endogenous transmissible spongiform encephalopathies present in blood by adsorption to selective affinity resins. Lancet 2006;368:2226-30.
10. Cancelas JA, Rugg N, Pratt PG, Worsham DN, Pehta JC, Banks K, Davenport RD, Judd WJ. Infusion of P-Capt prion-filtered red blood cell products demonstrate acceptable in vivo viability and no evidence of neoantigen formation. Transfusion 2011;51:2228-36.
11. Health Information and Quality Authority. Guidelines for the economic evaluation of health technologies in Ireland. Dublin: HIQA; 2010.
12. Wells AW, Llewelyn CA, Casbard A, Johnson AJ, Amin M, Ballard S, Buck J, Malfroy M, Murphy MF, Williamson LM. The EASTR Study: indications for transfusion and estimates of transfusion recipient numbers in hospitals supplied by the National Blood Service. Transfus Med 2009;19:315-28.
13. Hilton DA, Ghani AC, Conyers L, Edwards P, McCardle L, Ritchie D, Penney M, Hegazy D, Ironside JW. Prevalence of lymphoreticular prion protein accumulation in UK tissue samples. J Pathol 2004;203:733-9.
14. Clarke P, Ghani AC. Projections of the future course of the primary vCJD epidemic in the UK: inclusion of subclinical infection and the possibility of wider genetic susceptibility. J R Soc Interface 2005;2:19-31.
15. Irish Blood Transfusion Service. Annual report 2009. Dublin: Irish Blood Transfusion Service; 2010.
16. Spongiform Encephalopathy Advisory Committee (SEAC) (UK). SEAC position statement on TSE infectivity in blood [Internet]. SEAC Website; 2008. [cited 2010 Apr 26]. Available from: URL: http://webarchive.nationalarchives.gov.uk/20110316162913/http://www.seac.gov.uk/statements/statement0806.htm
17. Gregori L, McCombie N, Palmer D, Birch P, Sowemimo-Coker SO, Giulivi A, Rohwer RG. Effectiveness of leucoreduction for removal of infectivity of transmissible spongiform encephalopathies from blood. Lancet 2004;364:529-31.
18. Wiltshire M, Thomas S, Scott J, Hicks V, Haines M, Cookson P, Garwood M, Cardigan R. Prion reduction of red blood cells: impact on component quality. Transfusion 2009;50:970-9.
19. Zou S, Fang CT, Schonberger LB. Transfusion transmission of human prion diseases. Transfus Med Rev 2008;22:58-69.
20. Alperovitch A, Zerr I, Pocchiari M, Mitrova E, Cuesta JDP, Hegyi I, Collins S, Kretzschmar H, van Duijn C, Will RG. Codon 129 prion protein genotype and sporadic Creutzfeldt-Jakob disease. Lancet 1999;353:1673-4.
21. Ghani AC, Donnelly CA, Ferguson NM, Anderson RM. Updated projections of future vCJD deaths in the UK. BMC Infect Dis 2003;3:4.
22. Turner ML, Ludlam CA. An update on the assessment and management of the risk of transmission of variant Creutzfeldt-Jakob disease by blood and plasma products. Br J Haematol 2009;144:14-23.
23. Boelle PY, Cesbron JY, Valleron AJ. Epidemiological evidence of higher susceptibility to vCJD in the young. BMC Infect Dis 2004;4:26.
24. Garske T, Ghani AC. Uncertainty in the tail of the variant Creutzfeldt-Jakob disease epidemic in the UK. PLoS ONE 2010;5:e15626.
25. Gregori L, Lambert BC, Gurgel PV, Gheorghiu L, Edwardson P, Lathrop JT, Macauley C, Carbonell RG, Burton SJ, Hammond D, Rohwer RG. Reduction of transmissible spongiform encephalopathy infectivity from human red blood cells with prion protein affinity ligands. Transfusion 2006;46:1152-61.
26. Knight R. The diagnosis of prion diseases. Parasitology 1998;117(Suppl):S3-11.
27. R Development Core Team. R: a language and environment for statistical computing [Internet]. Vienna. 2010. [cited 2010 Oct 5]. Available from: URL: http://www.R-project.org
28. Borkent-Raven BA, Janssen MP, van der Poel CL, de Wit GA, Bonsel GJ, van Hout BA. Cost-effectiveness of additional hepatitis B virus nucleic acid testing of individual donations or minipools of six donations in the Netherlands. Transfusion 2009;49:311-9.
29. Jackson BR, Busch MP, Stramer SL, AuBuchon JP. The cost-effectiveness of NAT for HIV, HCV, and HBV in whole-blood donations. Transfusion 2003;43:721-9.
30. Korves CT, Goldie SJ, Murray MB. Cost-effectiveness of alternative blood-screening strategies for West Nile virus in the United States. PLoS Med 2006;3:e21.
31. Marshall DA, Kleinman SH, Wong JB, AuBuchon JP, Grima DT, Kulin NA, Weinstein MC. Cost-effectiveness of nucleic acid test screening of volunteer blood donations for hepatitis B, hepatitis C and human immunodeficiency virus in the United States. Vox Sang 2004;86:28-40.
32. Pereira A. Health and economic impact of posttransfusion hepatitis B and cost-effectiveness analysis of expanded HBV testing protocols of blood donors: a study focused on the European Union. Transfusion 2003;43:192-201.
33. van Hulst M, de Wolf JT, Staginnus U, Ruitenberg EJ, Postma MJ. Pharmaco-economics of blood transfusion safety: review of the available evidence. Vox Sang 2002;83:146-55.
34. Harney MS, Ghani AC, Donnelly CA, Walsh RM, Walsh M, Howley R, Brett F, Farrell M. vCJD risk in the Republic of Ireland. BMC Infect Dis 2003;3:28.
35. Gregori L, Yang H, Anderson S. Estimation of variant Creutzfeldt-Jakob disease infectivity titers in human blood. Transfusion 2011;51:2596-602.

別紙様式第 2-1

別紙 3

医薬品研究報告　調査報告書

識別番号・報告回数		回	報告日 年　月　日	第一報入手日 2012 年 12 月 17 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	BSE, Bovine-Brazil http://www.promedmail.org/direct.php?id=20121208.1443015	公表国 ブラジル	
販売名（企業名）						

研究報告の概要

2010 年 12 月 18 日に、ブラジルの Official Veterinary Services(OVS)は、Sertanopolis 自治体の農業借地所有者から、四肢を硬直させ横臥状態のウシについて報告を受けた。このウシは定期検査の際に発見されたとの報告であった。翌日に OVS がその農業借地を尋ねたところ、当該ウシは死亡したとの情報を入手した。

当該ウシは、肉牛繁殖用で、死亡時の年齢は 13 歳であった。OVS は関連情報を収集すると伴に死亡原因特定のための試料を採取した。この地区では草食動物における狂犬病の発生が認められていたので、国の指示に従い、狂犬病診断および鑑別診断のための検査試料が採取された。

神経疾患が疑われる場合に適応される規制と通常の手続きに従い、試料を用いて狂犬病の検査が施行されたが、結果は陰性であった。成獣であったので、試料は、牛海綿状脳症の監視体制のための分析研究所に送付された。

2011 年 4 月 11 日に、OVS 公認の研究所において、病理組織学的検査により牛海綿状脳症は陰性であるとの結果が得られた。一方、試料は、National Reference Laboratory、National Agricultural Laboratory にも送付され、2012 年 6 月 15 日に、免疫組織化学的検査によって牛海綿状脳症が陽性であるとの結果が得られた。

同試料は、イギリスにある国際獣疫事務局の付託研究施設にも送付された。2012 年 12 月 6 日に、免疫組織化学検査により牛海綿状脳症陽性であることが確認された。今回の牛海綿状脳症はブラジルにおける最初の症例であった。

使用上の注意記載状況・その他参考事項等

BYL-2013-0415

BSE, Bovine-Brazil
http://www.promedmail.org/direct.php?id=20121208.1443015

報告企業の意見	今後の対応
コージネイト FS の製造工程においてアフィニティークロマトグラフィーを用いているが、このリガンドであるマウス IgG モノクローナル抗体産生細胞の培養液にウシインスリンが添加されている。このウシインスリンの一連の製造・精製工程はプリオンを高率に除去できることが確認されている。従って，プリオンがコージネイト FS に混入する可能性は極めて低いと考えられる。	現時点で新たな安全対策上の措置を講じる必要はないと考える。引き続き関連情報の収集に努める。

15

MedDRA version 15.1

BYL-2013-0415

Published Date: 2012-12-08 11:46:03
Subject: PRO/AH/EDR> BSE, bovine - Brazil: (PR)
Archive Number: 20121208.1443015

BSE, BOVINE - BRAZIL: (PARANA)
****
A ProMED-mail post
http://www.promedmail.org
ProMED-mail is a program of the
International Society for Infectious Diseases
http://www.isid.org

Date: 7 Dec 2012

Source: OIE [edited]

http://www.oie.int/wahis_2/temp/reports/en_imm_0000012682_20121207_181754.pdf

Information received on 07 Dec 2012 from Dr Figueiredo Marques Guilherme Henrique, Director, Departamento de Saede Animal, Ministerio da Agricultura, Pecuaria e Abastecimento, Brasilia, Brazil

Summary:

Report type: Immediate notification

Date of start of the event 18 Dec 2012

Date of pre-confirmation of the event 15 Jun 2012

Report date: 07 Dec 2012

Date submitted to OIE: 07 Dec 2012

Reason for notification: 1st occurrence of a listed disease

Manifestation of disease: Sub-clinical infection

Causal agent Prion responsible for bovine spongiform encephalopathy

Nature of diagnosis Laboratory (advanced)

This event pertains to the whole country

New outbreaks

Summary of outbreaks: Total outbreaks: 1

Outbreak Location: Parana (.Sertanopolis)

Total animals affected

Species / Susceptible / Cases / Deaths / Destroyed / Slaughtered

Cattle / 148 / 1 / 1 / 0 / 0

Outbreak statistics:

Species: Cattle

Apparent morbidity rate: 0.68 percent

Apparent mortality rate: 0.68 percent

Apparent case fatality rate: 100 percent

Proportion susceptible animals lost* 0.68 percent *

*Removed from the susceptible population through death, destruction and/or slaughter;

Epidemiology

Source of the outbreak(s) or origin of infection: Unknown or inconclusive

Epidemiological comments: On 18 Dec 2010, the Official Veterinary Services (OVS) were informed by the owner of a holding in the municipality of Sertanopolis (State of Parana) on a recumbent bovine showing limb stiffness which was detected during routine inspection. Next day, when the OVS were going to visit the holding, they were informed by the stockman that the animal was dead.

The OVS went to the holding to collect information and samples for the diagnosis of the cause of the death. As it is an area where rabies is present in herbivores, samples were taken for the diagnosis of this disease and for differential diagnosis, as recommended by the national protocol. The animal was properly buried on site. The animal was a beef breeding cow almost 13 years old at the time of death, according to information obtained during the epidemiological investigations.

According to regulations and routine procedures to be implemented in case of suspected neurological diseases, the sample was tested for rabies and it was negative. As it was an adult animal negative for rabies, the sample was sent for laboratory analysis within the surveillance system for bovine spongiform encephalopathy (BSE).

On 11 Apr 2011, a negative histopathological result for BSE was obtained in a laboratory accredited by the OVS. The sample was sent to the National Reference Laboratory, National Agricultural Laboratory (LANAGRO-PE), Recife, Pernambuco, for BSE diagnosis and it tested positive on 15 Jun 2012 by immunohistochemical test.

The delay between the 2 tests was caused by an incident occurred in one of the laboratories of the accredited network for the diagnosis of BSE. That led to overload the system and to prioritize the diagnosis of samples which met BSE-risk characteristics, as established by the OIE. The sample belonged to the group "fallen stock" and to the age group "over 9 years," according to the Article 11.5.22 of the OIE Terrestrial Animal Health Code. This classification led to consider the sample as showing a low diagnosis priority level, which resulted in a longer than expected delay from histopathological to immunohistochemical tests.

According to the procedure manual on response to the occurrence of a BSE event in Brazil and as it is

the 1st occurrence in the country, the sample was sent for confirmatory diagnosis to the OIE Reference Laboratory for this disease, Animal Health and Veterinary Laboratories Agency (AHVLA), Weybridge, United Kingdom. The sample tested positive in immunohistochemical test on 6 Dec 2012.

The epidemiological investigation shows that the animal's death was not caused by BSE and suggests that it may be an atypical case of the disease occurring in the oldest animals. Information collected during the epidemiological investigation shows also that the animal was reared in an extensive system on grazing.

Note by the OIE: Brazil is still recognized by the OIE as having a negligible BSE risk in accordance with Chapter 11.5. of the OIE Terrestrial Animal Health Code.

Control measures

Measures applied: No vaccination

No treatment of affected animals

Measures to be applied: No other measures

Diagnostic test results

Laboratory name and type: Animal Health Laboratory - IMA (National laboratory)

Tests and results

Species / Test / Test date / Result

Cattle / histological test / 11 Apr 2011 / Negative

Laboratory name and type: National Agricultural Laboratory (LANAGRO-PE) (National laboratory)

Tests and results:

Species / Tests / Test date / Result

Cattle / immunohistochemical tests / 15 Jun 2012 / Positive

Laboratory name and type: Animal Health and Veterinary Laboratories Agency (AHVLA) (OIE's Reference Laboratory)

Tests and results:

Species / Test / Test date / Result

Cattle / immunohistochemical test / 06 Dec 2012 / Positive

--

Communicated by:

ProMED-mail

<promed@promedmail.org>

[This is the 1st report of BSE (Bovine Spongiform Encephalopathy) in Brazil. While the OIE regards Brazil as a negligible risk, there are some countries who may view this differently, especially as they look at meat that may be exported.

The determination of this case as a sporadic case may take some investigation or analysis. Without such an analysis to prove this was a sporadic case, there is a large shadow of doubt that may creep over countries importing product from Brazil.

Other countries and likely the OIE will be looking to see what type of surveillance program Brazil may put into effect. With an eye toward the export markets, Brazil will likely analyze the situation and put into place a surveillance mechanism and a thorough investigation of the situation.

Parana, Brazil, may be found on the interactive Healthmap/ProMED-mail map at:
http://healthmap.org/r/3yzA - Mod.TG]

See Also

BSE, bovine - USA (06): (CA) 20120805.1228663
BSE, bovine - USA (05): (CA) 20120504.1122322
BSE, bovine - USA (04): (CA) 20120501.1119136
BSE, bovine - USA (03): (CA) 20120429.1117352
BSE, bovine - USA (02): (CA) OIE 20120428.1116584
BSE, bovine - USA: (CA) 4th animal confirmed 20120425.1113102
2011
--
BSE - Japan (03): NOT, official statement 20111202.3501
BSE - Japan (02): 37th case, NOT 20111129.3485
BSE - Japan: 37th case, atypical, RFI 20111129.3480
BSE - Switzerland (02): (BE) OIE 20110527.1621
BSE - Switzerland: (SG) OIE 20110504.1381
BSE, bovine - Canada: (MB), Correction (AB) 20110306.0725
BSE, bovine - Canada: (AB) 20110305.0720
BSE, bovine - Canada: (MB) 20110305.0720
BSE - Netherlands: (FR), OIE 20110122.0272
2010
--
BSE - Netherlands (02): (NB), OIE 20101023.3843
BSE - Netherlands: new case 20100904.3176
BSE, bovine - Canada: (AB) 20100311.0792
.....as/tg/ejp/mpp

医薬品　研究報告　調査報告書

<table>
<tr><td>識別番号・報告回数</td><td colspan="2"></td><td>報告日</td><td>第一報入手日
2013年1月31日</td><td>新医薬品等の区分
該当なし。</td><td rowspan="2">総合機構処理欄</td></tr>
<tr><td>一　般　的　名　称</td><td colspan="2">別紙のとおり。</td><td rowspan="2">研究報告の
公表状況</td><td rowspan="2">重症熱性血小板減少症候群に関する報道発表資料（厚生労働省、2013年1月30日）</td><td rowspan="2">公表国
日本</td></tr>
<tr><td>販売名（企業名）</td><td colspan="2">別紙のとおり。</td><td></td></tr>
<tr><td>研究報告の概要</td><td colspan="5">問題点：山口県において、中国で2009頃より発生が報告された、新規なフレボウイルス属ウイルスによる新しいダニ媒介性疾患「重症熱性血小板減少症候群（Severe Fever with Thrombocytopenia Syndrome: SFTS)」の国内最初の症例が確認された。

山口県において、中国で2009頃より発生が報告された、新規なフレボウイルス属ウイルスによる新しいダニ媒介性疾患「重症熱性血小板減少症候群（Severe Fever with Thrombocytopenia Syndrome:SFTS)」の国内最初の症例（患者1名：昨秋に死亡。最近の海外渡航歴なし。）が確認された。本ウイルスはSFTSウイルス（SFTSV）と命名され、クリミア・コンゴ出血熱やリフトバレー熱等の原因ウイルスと同じブニヤウイルス科に属し、ヒトへの感染はダニに咬まれることの他、患者体液との直接接触による感染も報告されている。潜伏期間は6日～2週間で、発熱、頭痛等の他、意識障害等の症状を呈することもあり、致死率は10%を超える。
厚生労働省では、都道府県等へ本疾患に関する情報提供を行うとともに、医療機関に対して、同様の患者を診察した際は情報提供するよう、自治体を通じて協力を要請している。</td><td>使用上の注意記載状況・その他参考事項等

記載なし。</td></tr>
<tr><td colspan="3">報告企業の意見</td><td colspan="3">今後の対応</td><td></td></tr>
<tr><td colspan="3">別紙のとおり。</td><td colspan="3">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。</td><td></td></tr>
</table>

MedDRA/J ver. 15.1

別紙

一般的名称	①人血清アルブミン、②人血清アルブミン、③人血清アルブミン*、④人免役グロブリン、⑤人免役グロブリン、⑥乾燥ペプシン処理人免疫グロブリン、⑦乾燥ペプシン処理人免疫グロブリン、⑧乾燥スルホ化人免疫グロブリン、⑨乾燥スルホ化人免疫グロブリン、⑩乾燥スルホ化人免疫グロブリン、⑪乾燥スルホ化人免疫グロブリン、⑫乾燥スルホ化人免疫グロブリン*、⑬乾燥濃縮人活性化プロテインC、⑭乾燥濃縮人血液凝固第Ⅷ因子、⑮乾燥濃縮人血液凝固第Ⅷ因子、⑯乾燥濃縮人血液凝固第Ⅷ因子、⑰乾燥濃縮人血液凝固第Ⅸ因子*、⑱乾燥濃縮人血液凝固第Ⅸ因子*、⑲乾燥濃縮人血液凝固第Ⅸ因子*、⑳乾燥濃縮人血液凝固第Ⅸ因子、㉑乾燥濃縮人血液凝固第Ⅸ因子、㉒乾燥濃縮人血液凝固第Ⅸ因子、㉓乾燥抗破傷風人免疫グロブリン、㉔抗HBs人免疫グロブリン、㉕トロンビン、㉖フィブリノゲン加第ⅩⅢ因子*、㉗フィブリノゲン加第ⅩⅢ因子、㉘乾燥濃縮人アンチトロンビンⅢ、㉙ヒスタミン加人免疫グロブリン製剤、㉚人血清アルブミン*、㉛人血清アルブミン*、㉜乾燥ペプシン処理人免役グロブリン*、㉝乾燥濃縮人アンチトロンビンⅢ
販売名（企業名）	①献血アルブミン20“化血研”、②献血アルブミン25“化血研”、③人血清アルブミン“化血研”*、④ガンマーグロブリン筋注450mg/3mL「化血研」、⑤ガンマーグロブリン筋注1500mg/10mL「化血研」、⑥献血静注グロブリン“化血研”、⑦献血グロブリン注射用2500mg「化血研」、⑧献血ベニロンーⅠ静注用500mg、⑨献血ベニロンーⅠ静注用1000mg、⑩献血ベニロンーⅠ静注用2500mg、⑪献血ベニロンーⅠ静注用5000mg、⑫ベニロン*、⑬注射用アナクトC2,500単位、⑭コンファクトF注射用250、⑮コンファクトF注射用500、⑯コンファクトF注射用1000、⑰ノバクトM注射用250*、⑱ノバクトM注射用500*、⑲ノバクトM注射用1000*、⑳ノバクトM静注用400単位、㉑ノバクトM静注用800単位、㉒ノバクトM静注用1600単位、㉓テタノセーラ筋注用250単位、㉔ヘパトセーラ筋注200単位/mL、㉕トロンビン“化血研”、㉖ボルヒール*、㉗ボルヒール組織接着用、㉘アンスロビンP500注射用、㉙ヒスタグロビン皮下注用、㉚アルブミン20%化血研*、㉛アルブミン5%化血研*、㉜静注グロブリン*、㉝アンスロビンP1500注射用
報告企業の意見	Sever Fever with Thrombocytopenia syndrome virus（SFTSV）は新規なフレボウイルス属のウイルスで、核酸は一本鎖RNA、エンベロープを有し、中国で2009年頃から報告されている重症熱性血小板減少症候群の原因ウイルスである。 今回の報告は、山口県で重症熱性血小板減少症候群の国内最初の症例が確認され、当該患者は死亡したとの報告である。 上記製剤の製造工程には、冷アルコール分画工程、ウイルス除去膜ろ過工程、加熱工程等の原理の異なるウイルスクリアランス工程が導入されており、各工程のウイルスクリアランス効果は「血漿分画製剤のウイルスに対する安全性確保に関するガイドライン（医薬発第1047号、平成11年8月30日）」に基づく、モデルウイルスを用いたウイルスプロセスバリデーションにより確認されている。今回報告したSFTSVのモデルウイルスには、エンベロープの有無、核酸の種類等から、ウシウイルス性下痢ウイルス（BVDV）が該当すると考えられるが、上記工程のBVDVクリアランス効果については上記バリデーションにより確認されている。また、これまでに上記製剤によるSFTSVへの感染報告例は無い。 以上の点から、上記製剤は新規フレボウイルス属のウイルス感染に対する安全性を確保していると考える。

*：現在製造を行っていない

INF2012-004

厚生労働省
Ministry of Health, Labour and Welfare

平成25年1月30日
【照会先】厚生労働省健康局結核感染症課
感染症情報管理室長　中嶋　建介(内線2389)
課長補佐　難波江　功二(内線2373)
(代表番号)　03(5253)1111
(直通番号)　03(3595)2257

報道関係者　各位

中国で近年報告されている新しいダニ媒介性疾患の患者が国内で確認されました

今般、中国において2009年頃より発生が報告され、2011年に初めて原因ウイルスが特定された新しいダニ媒介性疾患「重症熱性血小板減少症候群(Severe Fever with Thrombocytopenia Syndrome: SFTS)」の症例(患者1名：昨秋に死亡。最近の海外渡航歴なし。)が、山口県において確認されました(別添1)。
これを受けて、厚生労働省では、本疾患に関する資料(別添2、3)を作成し、都道府県等に情報提供を行うとともに、医療機関に対して、同様の患者を診察した際は情報提供するよう、自治体を通じて協力を要請したところです(別添4)。
厚生労働省では、引き続き、本疾患に関する情報収集や調査研究を実施し、適切な対応を行ってまいります。

- (別添1)病原微生物検出情報(IASR)速報　国内で初めて診断された重症熱性血小板減少症候群患者(PDF:KB)
- (別添2)重症熱性血小板減少症候群について(PDF:KB)
- (別添3)重症熱性血小板減少症候群に関するQ&A(PDF:KB)
- (別添4)厚生労働省結核感染症課長通知「重症熱性血小板減少症候群(SFTS)の国内での発生について(情報提供及び協力依頼)」(平成25年1月30日)(PDF:KB)

厚生労働省
Ministry of Health, Labour and Welfare

〒100-8916 東京都千代田区霞が関1-2-2 電話:03-5253-1111(代表)

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
				2013. 1. 30	該当なし	

一般的名称	新鮮凍結人血漿	研究報告の公表状況		公表国
販売名(企業名)	新鮮凍結血漿-LR「日赤」(日本赤十字社) 新鮮凍結血漿-LR「日赤」成分採血(日本赤十字社) 新鮮凍結血漿-LR「日赤」120(日本赤十字社) 新鮮凍結血漿-LR「日赤」240(日本赤十字社) 新鮮凍結血漿-LR「日赤」480(日本赤十字社)		病原微生物検出情報(IASR);Available from: http://www.nih.go.jp/niid/ja/sfts/sfts-iasrs/3142-pr3963.html	日本

研究報告の概要

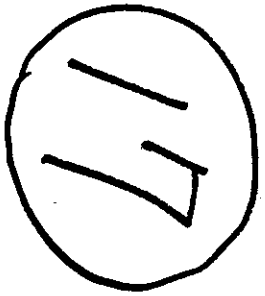
○国内で初めて診断された重症熱性血小板減少症候群患者
国内で初めて、発熱や血小板減少等の症状を呈して亡くなった患者が、ウイルス学的に重症熱性血小板減少症候群ウイルス(SFTSV)による感染症であると診断された。
患者(海外渡航歴なし)は2012年秋、発熱、嘔吐、下痢(黒色便)を呈し入院したが、全身状態が悪化し死亡した。入院中に採取された血液からウイルスが分離され、SFTSVと同定された。また血液中にSFTSV遺伝子が含まれることが確認された。
SFTSVはクリミア・コンゴ出血熱やリフトバレー熱、腎症候性出血熱やハンタウイルス肺症候群の原因ウイルスと同様にブニヤウイルス科に属する。中国からの報告ではマダニからウイルスが分離されており、SFTSVの宿主はダニであると考えられている。ヒトへの感染はSFTSVを有するダニに咬まれることによるが、患者血液や体液との直接接触による感染も報告されている。SFTSVに感染すると6日～2週間の潜伏期を経て、発熱、消化器症状、頭痛、筋肉痛、神経症状、リンパ節腫脹、呼吸器症状、出血症状等の症状が出現し、致死率は10%を超える。
有効性が確認された治療法やワクチンはない。SFTSVに感染しないようにするには、ダニに咬まれないようにすることが重要である。

使用上の注意記載状況・その他参考事項等

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

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
発熱や血小板減少等の症状を呈して亡くなった海外渡航歴のない患者が、国内で初めて、ウイルス学的にブニヤウイルスに属する重症熱性血小板減少症候群ウイルス(SFTSV)による感染症であると診断されたとの報告である。	今後も引き続き情報の収集に努める。



JRC2013T-003

IASR

＜速報＞ 国内で初めて診断された重症熱性血小板減少症候群患者

(掲載日 2013/1/30)

重症熱性血小板減少症候群(severe fever with thrombocytopenia syndrome, SFTS)はブニヤウイルス科フレボウイルス属に分類される新規ウイルス、SFTSウイルス(SFTSV)、によるダニ媒介性感染症である。2011年に中国でSFTSと命名された新規感染性疾患が報告されて以来1)、中国国内の調査から現在7つの省(遼寧省、山東省、江蘇省、安徽省、河南省、河北省、浙江省)で患者発生が確認されている1, 2)。国内で初めて、発熱や血小板減少等の症状を呈し亡くなられた患者が、ウイルス学的にSFTSVによる感染症と診断されたので報告する。

2012年秋、海外渡航歴のない成人患者に、発熱、嘔吐、下痢(黒色便)が出現した。入院時身体所見では、明らかなダニ咬傷はなく、血液検査所見では、白血球数(400/mm^3)と血小板数(8.9×10^4/mm^3)が著明に低下していた。また、AST、ALT、LDH、CKの高値が認められた。血液凝固系の異常、フェリチンの著明な上昇も認められた。尿検査で血尿、蛋白尿が認められた。胸腹部単純CTでは右腋窩リンパ節腫大を認めた。骨髄穿刺検査により、マクロファージによる血球貪食像を伴う低形成髄の所見が認められた。その後に四肢脱力および肉眼的血尿と多量の黒色便を認め、全身状態が不良となり死亡した。入院中に採取された血液からウイルスが分離され、SFTSVと同定された。また血液中にSFTSV遺伝子が含まれることが確認された。血清はELISA、IF法によるSFTSVに対する抗体検査において陰性であった。病理組織においてSFTSVの抗原及び核酸が確認された。

SFTSVは3分節の1本鎖RNAを有するウイルスで、クリミア・コンゴ出血熱やリフトバレー熱、腎症候性出血熱やハンタウイルス肺症候群の原因ウイルスと同様にブニヤウイルス科に属する。中国からの報告では、マダニ[フタトゲチマダニ(*Haemophysalis longicornis*)、オウシマダニ(*Rhipicephalus microplus*)]からウイルスが分離されており1, 3)、SFTSVの宿主はダニであると考えられている。また、ダニに咬まれることの多い哺乳動物からSFTSVに対する抗体が検出されていることから、これらの動物もSFTSVに感染するものと考えられる1)。ヒトへの感染は、SFTSVを有するダニに咬まれることによるが、他に患者血液や体液との直接接触による感染も報告されている4)。ウイルス血症を伴う動物との接触による感染経路もあり得ると考えられる。SFTSVに感染すると6日～2週間の潜伏期を経て、発熱、消化器症状(食欲低下、嘔気、嘔吐、下痢、腹痛)、頭痛、筋肉痛、神経症状(意識障害、けいれん、昏睡)、リンパ節腫脹、呼吸器症状(咳、咽頭痛)、出血症状(紫斑、下血)等の症状が出現し、致死率は10%を超える1, 5)。SFTSはダニ媒介性ウイルス感染症であることから、流行期はダニの活動が活発化する春から秋と考えられる。ダニは日本国内に広く分布する。ただし、詳細はこれからの研究を待たなくてはならない。

確定診断には、血液などからのSFTSVの分離・同定、RT-PCRによるSFTSV遺伝子検出、急性期及び回復期におけるSFTSVに対する血清IgG抗体価、中和抗体価の有意な上昇の確認が必要であり、現在国立感染症研究所ウイルス第一部で検査が可能である。治療に関しては、リバビリン使用の報告があるが2)、その有効性は確認されていない。基本的に対症療法となる。有効なワクチンはない。

医療機関における院内感染予防には、ヒトからヒトに感染する接触感染経路があることから4)、標準予防策の遵守が重要である。また、臨床症状が似た患者を診た場合にはSFTSを鑑別診断に挙げることが重要である。

SFTSVに感染しないようにするには、ダニに咬まれないようにすることが重要である。草むらや藪など、ダニの生息する場所に入る場合には、長袖の服、長ズボン、足を完全に覆う靴を着用し、肌の露出を少なくすることが重要である。

SFTSが疑われる患者を診た場合には、最寄りの保健所、または、国立感染症研究所問い合わせ窓口(info[アットマーク]nih.go.jp)に連絡していただきたい。
＊[アットマーク]は@に置き換えて送信してください。

参考文献

1) Yu XJ, *et al.*, N Engl J Med 364:1523-32, 2011
2) Li S, *et al.*, Biosci Trends 5:273-6, 2011
3) Zhang YZ, *et al.*, J Virol 86:2864-8, 2012

4) Tang X, *et al.*, J Infect Dis ahead of print. 2013
5) Xu B, *et al.*, PLoS Pathog 7:e1002369, 2011

国立感染症研究所ウイルス第一部　西條政幸　下島昌幸
同感染症情報センター　山岸拓也　大石和徳
同獣医科学部　森川　茂
同感染病理部　長谷川秀樹

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
				2013. 2. 15	該当なし	

一般的名称	新鮮凍結人血漿	研究報告の公表状況	Tang X, Wu W, Wang H, Du Y, Liu L, Kang K, Huang X, Ma H, Mu F, Zhang S, Zhao G, Cui N, Zhu BP, You A, Chen H, Liu G, Chen W, Xu B. J Infect Dis. 2013 Mar;207(5):736-9. doi: 10.1093/infdis/jis748. Epub 2012 Dec 6.	公表国
販売名（企業名）	新鮮凍結血漿-LR「日赤」（日本赤十字社） 新鮮凍結血漿-LR「日赤」成分採血（日本赤十字社） 新鮮凍結血漿-LR「日赤」120（日本赤十字社） 新鮮凍結血漿-LR「日赤」240（日本赤十字社） 新鮮凍結血漿-LR「日赤」480（日本赤十字社）			中国

研究報告の概要

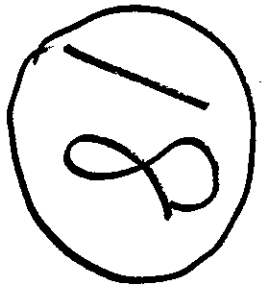
○感染血液との接触による重症熱性血小板減少症候群ブニヤウイルスのヒトからヒトへの伝播
2010年5月～6月に発生した重症熱性血小板減少症候群（SFTS）のアウトブレイクの感染経路を同定するために調査を行った。発端患者は58歳男性で、5月20日に発熱、疲労、筋肉痛、咳、悪心を呈し、5月30日に死亡した。6月6日～8日、発端患者と接触があった31人（医療従事者、家族、親戚と友人、葬儀屋）のうち4人（3人は家族）が二次感染し、SFTSと一致する症状を発症した。これらの二次感染患者には、ダニの咬傷、野生動物との接触、他のSFTS患者との接触がなかった。二次感染した3人の家族は、手袋等の防護をせずに患者の血液に触れており、その後発症した。患者の血液に触れていない家族や親戚、友人は発症しなかった。また、患者を診た医療従事者16人も、保護具を装着せずに診察した医師も含め、発症していない。
調査によると、二次感染には患者血液との接触が有意に関連していたが、呼吸器からの分泌物、尿、便との接触とは有意な関連性はなかった。発端患者の粘膜や皮膚の創傷からの血液への接触及び看護時の保護具の未装着がSFTSのリスクを用量反応的に上昇させた。発端患者及び二次感染した息子の急性期血清からSFTSウイルスが分離され、これら2分離株の全ゲノム配列は99.99%の類似性を示した。二次感染患者4人全員の回復期血清において、IgG抗体が陽性であった。無症状の接触者27人は、ウイルスRNA及びIgG抗体のいずれも陰性であった。
これらのことから、発端患者の血液との接触が二次感染者の感染源と推定できるが、他の感染源がある可能性もある。患者の家族及び医療従事者は、患者の血液や体液に直接触れないよう、防護策をとることを推奨する。

使用上の注意記載状況・その他参考事項等

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

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
2010年5月～6月に発生した重症熱性血小板減少症候群アウトブレイクの感染経路を調査した結果、発端患者の血液への接触が二次感染に有意に関連していたことが分かったとの報告である。	今後も引き続き情報の収集に努める。



BRIEF REPORT

Human-to-Human Transmission of Severe Fever With Thrombocytopenia Syndrome Bunyavirus Through Contact With Infectious Blood

Xiaoyan Tang,[1,a] Weili Wu,[2,a] Haifeng Wang,[1,a] Yanhua Du,[1] Licheng Liu,[3,6] Kai Kang,[1] Xueyong Huang,[1] Hong Ma,[1] Feng Mu,[6] Shiqiang Zhang,[7] Guohua Zhao,[7] Ning Cui,[4] Bao-Ping Zhu,[5] Aiguo You,[1] Haomin Chen,[1] Guohua Liu,[1] Weijun Chen,[2,3,5] and Bianli Xu[1]

[1]Center for Disease Control and Prevention of Henan Province, Zhengzhou; [2]Key Laboratory of Genome Sciences and Information, Beijing Institute of Genomics, Chinese Academy of Sciences, [3]State Key Laboratory of Pathogen and Biosecurity, Institute of Microbiology and Epidemiology, Academy of Military Medical Sciences, and [4]Chinese Filed Epidemiology Training Program, Beijing; [5]Beijing Genomics Institute in Wuhan, Wuhan, [6]Center for Disease Control and Prevention of Xinyang City, and [7]154th Hospital, Xinyang, People's Republic of China

We investigated an outbreak of severe fever with thrombocytopenia syndrome (SFTS) that occurred during May and June 2010, to identify the mode of transmission. Contact with the index patient's blood was significantly associated with development of SFTS ($P = .01$, by the χ^2 test for linear trend); the frequency of contact with the index patient's blood increased the risk of SFTS in a dose-response manner ($P = .03$, by the χ^2 test for linear trend). We concluded that human-to-human transmission caused this cluster of cases.

Keywords. severe fever with thrombocytopenia syndrome virus (SFTSV); human-to-human transmission; blood.

In May 2007, a life-threatening disease characterized by the sudden onset of fever, thrombocytopenia, and leukopenia was first reported in several provinces in central and northeast China [1, 2]. A novel bunyavirus was identified as the causative agent of this disease. The disease is referred to as fever, thrombocytopenia, and leukopenia syndrome (FTLS) or as severe fever with thrombocytopenia syndrome (SFTS), and the virus is designated FTLSV or SFTSV, respectively [1, 2]. Tick bites were presumed to be the mode of transmission, although no definitive evidence associated with this hypothesis has been identified [1, 2].

During May–June 2010, a cluster of 5 suspected cases of SFTS occurred in Henan Province in central China, with 1 death. We investigated this cluster to confirm the diagnosis and identify the mode of transmission.

METHODS

We defined a laboratory-confirmed case of SFTSV infection as the presence of ≥1 of the following findings: a blood culture positive for SFTSV, identification of viral RNA through reverse transcription polymerase chain reaction (RT-PCR), and seroconversion or a 4-fold increase in anti-SFTSV immunoglobulin G (IgG) titers between acute- and convalescent-phase sera.

We collected acute-phase serum from the index patient and paired sera from the ill contacts, with acute-phase sera collected <7 days after onset and convalescent-phase sera collected >6 weeks after onset. Sera were also collected from the asymptomatic contacts of the index patient 6 weeks after exposure. Ticks were collected from the domestic animals (2 cows and 1 dog) kept by the index patient. An immunofluorescence assay was used to detect anti-SFTSV IgG [1], and RT-PCR (QIAamp viral RNA Mini Kit 52904, Qiagen, Hilden, Germany), using a specific RNA-dependent RNA polymerase gene primer set, was performed to detect SFTSV RNA [1]. Virus was isolated by inoculating acute-phase sera into 2 wells of Vero E6 cells.

In a retrospective cohort study, we performed a verbal autopsy of the deceased index patient by questioning his wife, younger son, and daughter; the village clinic doctors; the head of the village; and the doctors and nurses who treated the patient. We also interviewed the ill and asymptomatic contacts of the index patient about their symptoms of SFTS and possible risk factors for infection, including their exposure to the index patient, exposure to wild animals, and history of tick bites. All participants provided verbal informed consent for anonymous use of their specimens and clinical information for research. The institutional review boards of all participating institutions approved this study.

Received 31 July 2012; accepted 19 September 2012; electronically published 6 December 2012.

[a]X. T., W. W., and H. W. contributed equally to the study.

Correspondence: Weijun Chen, PhD, Beijing Genomics Institute in Wuhan, No. 666, Hitech Rd, Wuhan 430075, China (chenwj@genomics.org.cn) and Bianli Xu, Center for Disease Control and Prevention of Henan Province, Zhengzhou 450016, Henan Province, China (xubl@hncdc.com.cn).

The Journal of Infectious Diseases 2013;207:736–9

DOI: 10.1093/infdis/jis748

CASE REPORT

The index patient was a 58-year-old man, who, on 20 May 2010, experienced a sudden onset of fever (39.5°C), fatigue, myalgia, cough, and nausea. He initially received a diagnosis of influenza and was treated for 4 days in the village clinic with cefazolin, Shuanghuanglian (an herbal antiviral and antibiotic [3]), and dexamethasone (for fever reduction). On 25 May, his symptoms worsened, and he developed facial flushing and conjunctivitis and began vomiting, and he was transferred to municipal hospital A. On 26 May, he was transferred to municipal hospital B, where he received a diagnosis of suspected human granulocytic anaplasmosis [4] and was treated with doxycycline. However, his condition continued to deteriorate progressively; he developed nasal and oral bleeding at approximately 6:15 AM on 30 May and died at approximately 12:45 PM. During the verbal autopsy, the index patient's next of kin denied that the index patient had a history of tick bite before onset of illness. The index patient mostly worked in the field around his house during the 15 days prior to the onset of his illness. However, he often took cows to graze in the hills, and ticks were often found on the cows. During our investigation, we found that the index patient had 2 cows and 1 dog. We collected 9 ticks from the 2 cows, but no ticks were found on the family dog; all ticks, however, tested negative for SFTSV RNA by RT-PCR [1].

We identified 31 contacts with the index patient during his illness, including 16 healthcare workers, 10 family members, 4 relatives and friends, and the village funeral director. During 6–8 June, 4 of these individuals (13%) developed secondary SFTSV infection, with clinical signs and symptoms consistent with SFTS (Supplementary Figure 1) [1]. Of these 4 individuals, 3 were members of the index patient's family. Since 2006, one son (son 1) had resided in another city (Ninbo, Zhejiang Province), approximately 1300 km away, in which SFTSV infection has never been reported [1, 2]. Hearing of his father's grave illness, son 1 went directly to the hospital on 29 May and stayed at the bedside for 2 days, until his father's death. Son 1 became ill on 6 June. The index patient's other son (son 2) resided in the same village as the index patient, and he had visited his father every 3–5 days before his father became ill [1]. Son 2 began caring for the index patient on 25 May. The index patient's daughter resided in another county, approximately 20 km away, and went to the hospital to care for her father during 26–30 May. She had not seen her father during the 30-day period before 26 May. Son 2 and the daughter both became ill on 7 June. The only nonfamilial secondary case was the village funeral director, who resided in the same village and had unprotected contact with the index patient's blood from 1:00–4:00 PM on 30 May, after he sustained a cut on his right index finger while washing and clothing the body with his bare hands. He became ill on 8 June. All secondary cases denied tick bites, contact with wild animals, or exposure to other patients with SFTS during the 15-day period before the onset of their illness.

During the index patient's final hours of life, while he bled profusely from his mouth and nose, 5 of 10 family members were at the bedside; none wore rubber gloves or gowns. Three of these family members helped to wipe off the index patient's blood without wearing personal protection, and blood splashed onto their faces. All 3 became ill, showing clinical signs and symptoms consistent with SFTS [1]. The other 2 family members had no contact with the patient's blood but were only present in the ward; neither became ill. Four relatives and friends visited the index patient and talked with him during the early stages of his illness, when there was no bleeding, and none became ill.

The 16 healthcare workers with contact with the index patient consisted of 8 doctors and 8 nurses. Before the index patient was transferred to hospital B, 2 village doctors and 2 doctors in hospital A had unprotected contact with him during physical examinations, including taking his temperature and testing for coated tongue and lymph node enlargement, and intramuscular injection; none wore wear rubber gloves or gowns and none became ill. Following transfer of the index patient to hospital B, 4 healthcare workers had protected contact with the index patient before he developed bleeding, during physical examinations, including taking his temperature and testing for coated tongue and lymph node enlargement, and intravenous injections; none became ill. When he was bleeding on 30 May, 8 healthcare workers provided care, including wiping off his blood and administering intravenous injections, but only 1 did not wear rubber gloves, surgical masks, and gowns. None of these workers became ill.

RESULTS

Overall, contact with the index patient's blood was significantly associated with developing secondary illness ($P = .01$, by the χ^2 test for linear trend), whereas contact with the index patient's respiratory secretions, urine, and feces was not (Table 1). Of the various modes of exposure, contact with the index patient's blood on mucous membranes or skin wounds ($P < .01$, by the χ^2 test for linear trend) and not wearing personal protective equipment while providing care ($P = .01$, by the χ^2 test for linear trend) were significantly associated with disease risk. Additionally, frequency of contact with blood was associated with disease risk in a dose-response fashion ($P = .03$, by the χ^2 test for linear trend; Table 2).

Two isolates of SFTSV were obtained, one from the acute-phase sera of the index patient and the other from son 1. Whole-genome sequencing showed that the 2 isolates (GenBank accession numbers: HN01: HQ642766, HQ642767,

Table 1. Risk Factors for Secondary Severe Fever With Thrombocytopenia Syndrome Among 31 Close Contacts of the Index Patient, Henan Province, China, May–June 2010

Secretion	Close Contacts, No.		Attack Rate, %	P^a	OR (95% CI)[a]
	Overall	Developed Secondary Case			
Blood					
Exposed	12	4	33.33	.01	...
Unexposed	19	0	0		
Respiratory secretion					
Exposed	4	1	25.00	.44	2.67 (.21–34.56)
Unexposed	27	3	11.11		
Urine					
Exposed	5	1	20.00	.52	1.92 (.16–23.35)
Unexposed	26	3	11.54		
Feces					
Exposed	2	0	0	>.99	
Unexposed	29	4	13.79		

Abbreviations: CI, confidence interval; OR, odds ratio.

[a] Calculated using the 2-sided Fisher exact test.

and HQ642768; HN69: JF682776, JF682777, and JF682778) were nearly identical (99.99% similarity). These 2 isolates showed slightly less similarity (99.83% and 99.83%, respectively) with an isolate obtained from a patient in Xinyang City on 23 June 2009 (GenBank accession numbers: HN20: JF682773, JF682774, JF682775).

Table 2. Risk Factors for Secondary Severe Fever With Thrombocytopenia Syndrome Among 12 Close Contacts Exposed to the Index Patient's Blood, Henan Province, China, May–June 2010

Variable	Close Contacts, No.		Attack Rate, %	P
	Overall	Developed Secondary Case		
Exposure route				
Mucosa, mouth, nose, wound	4	4	100.00	<.01[a]
Skin, clothes, shoes	8	0	0	
Personal protective equipment				
Did not use	5	4	80.00	.01[a]
Use	7	0	0	
Exposure frequency, no. of episodes				
≥3	4	3	75.00	.03[b]
2	5	1	20.00	
1	3	0	0	

[a] Calculated using the Fisher exact test.

[b] Calculated using the 2-sided χ^2 test for trend.

The acute-phase sera from all 5 disease-positive patients were positive for SFTSV RNA by RT-PCR and negative for IgG to the virus. The convalescent-phase sera from the 4 secondary patients had IgG to SFTSV. The sera IgG titers were 1:80 (for son 1), 1:160 (for son 2), 1:640 (for the daughter), and 1:160 (for the funeral director). Sera from all 27 asymptomatic contacts tested negative for both viral RNA (by RT-PCR) and IgG to the virus.

Although contact with the index patient's blood was a point source of exposure, other exposures were also possible. We estimated that the incubation period of SFTSV for this mode of transmission was 7–13 days. However, our sample size was small, and this incubation period might not apply to other modes of transmission, such as tick- or mosquito-borne infection.

The index patient was treated with dexamethasone for fever reduction, during the first 4 days after onset of illness. Dexamethasone and other glucocorticoids lower innate immunity and increase the severity of viral infections [5, 6]. Although it was impossible to determine the role of dexamethasone in the severity of the index patient's illness, it is nonetheless advisable that dexamethasone not be used to treat simple fever. Steroids may have increased the number of circulating virions in his blood and excreted into other body fluids. This may have led to human-to-human transmission of SFTSV.

DISCUSSION

In summary, we have documented an outbreak of infection with the recently identified SFTSV and provided strong

epidemiologic and viral genomic evidence that SFTSV can be transmitted between humans through contact with infected blood. This finding underscores the importance of protecting healthcare workers and patients' family members from exposure to blood. Our data also indicated that practicing standard isolation precautions [7] may minimize the risk of virus transmission by blood.

Since the submission of this manuscript, probable human-to-human transmission of SFTSV has been reported in patients who were not treated with steroids [8, 9]. We recommend that healthcare workers and family members caring for patients with suspected SFTS, as well as persons handling the bodies of those who have died of this disease, wear personal protective equipment, including gloves, gowns, eye protection, and masks, and avoid touching patients' blood and other body fluids. Patients with SFTS should be isolated until they no longer have detectable viremia, and all who come in contact with these patients should be monitored for fever until the end of the incubation period (>13 days). Those who develop symptoms should be isolated and tested.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

Acknowledgments. We thank Prof Scott Edmunds at the Beijing Genomics Institute in Shenzhen for his critical reading of the manuscript.

B. X. and W. C. are coprincipal investigators and jointly conceived of and designed the experiments. X. T., Y. D., H. W., K. K., X. H., H. M., S. Z., G. Z., N. C., B.-P. Z., H. C., A. Y., and G. L. isolated the virus and performed clinical virologic, serologic, epidemic, and data analysis. W. W., L. L., and F. M. performed the RT-PCR assays and virus sequencing.

Disclaimer. The sponsors of the study had no role in the study design, data collection, data analysis, data interpretation, or writing of this report.

Financial support. This work was sponsored by the Henan Medical Science Project (200702016), the China-Australia Health and HIV/AIDS Facility (EID35), and the Infectious Diseases Special Project, Minister of Health of China (2008ZX10004-103, 2009ZX10004-109).

Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Bianli X, Licheng L, Xueyong H, et al. Metagenomics analysis of fever, thrombocytopenia and leukopenia syndrome (FTLS) in Henan Province, china: discovery of a new bunyavirus. PLoS Pathog 2011; 7: e1002369.
2. Yu XJ, Liang MF, Zhang SY, et al. Fever with thrombocytopenia associated with a novel bunyavirus in China. New Engl J Med 2011; 364: 1523–32.
3. Shunan G, Da W. Study of clinical progress of Shuanghuanglian oral mixture. Chin J Information on Traditional Chin Med. 2005; 22:38–9.
4. Guidelines for the control of human granulocytic anaplasmosis. Beijing: Ministry of Health of the People's Republic of China, 2008. http://www.moh.gov.cn/publicfiles/business/htmlfiles/mohbgt/pw10805/200804/31429.htm. Accessed 29 July 2011.
5. Han K, Ma H, An X, et al. Early use of glucocorticoids was a risk factor for critical disease and death from pH1N1 infection. Clin Inf Dis 2011; 53:326–33.
6. Ma HL, He F, Wan JF, et al. Glucocorticoid and pyrazolone treatment of acute fever is a risk factor for critical and life-threatening human enterovirus 71 infection during an outbreak in China, 2008. Pediatr Infect Dis J 2010; 29:524–9.
7. Siegel JD, Rhinehart E, Jackson M, Chiarello L, and the Healthcare Infection Control Practices Advisory Committee. 2007 Guideline for isolation precautions: preventing transmission of infectious agents in healthcare settings. http://www.cdc.gov/ncidod/dhqp/pdf/isolation2007.pdf. Accessed 29 July 2011.
8. Bao C, Qi X, Wang H. A novel bunyavirus in China. N Engl J Med 2011; 365:862–5.
9. Bao C, Guo X, Qi X, et al. A family cluster of infections by a newly recognized bunyavirus in eastern China, 2007: further evidence of person-to-person transmission. Clin Inf Dis 2011; 53:1208–14.

別紙様式第2　　医薬品　　別紙3-1

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 2013 年 1 月 9 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	Global Alert and Response http://www.who.int/csr/disease/coronavirus_infections/update_20121221/en/index.html	公表国 サウジアラビア、ヨルダン、カタール	
販売名（企業名）						

研究報告の概要

WHO は、2012 年 10～12 月の間に、9 症例の新種のコロナウイルスによる重症急性呼吸器感染症の報告を受けている。カタール（2 例）、サウジアラビア（5 例）およびヨルダン（2 例）において感染確定症例が報告されている。すべての症例は重症で、5 例が死亡している。サウジアラビアでは 5 例すべてが確認症例である。最初の 2 例は、サウジアラビアの異なる地域に居住していた。2 例のうち 1 例は死亡している。残り 3 例は、同じ住居で生活している家族で、2 例が死亡している。他の 1 例の症状は、確定症例で観察された症状と類似していたが、この症例は回復し、ウイルス検出結果は陰性であった。ヨルダンでは 2 例が確認されている。2 例とも死亡している。2012 年 4 月に医療従事者集団に肺炎症状が発現しており、この際、症状を発現した症例から採取した保存試料を調べたところ、2 例の感染が確認された。

WHO は 2012 年 4 月にヨルダンで発生した感染症例について検討を行った。感染が確定した 2 例と関係があった医療従事者のうち肺炎症状を呈する人に対して調査を行い、次のような結果を得ている。①感染集団における初発症例は特定できなかった。②すべての症例は、肺炎に類似した著しい呼吸器症状を呈していた。感染可能性症例の症状は、概して、軽症であった。③この集団において、腎不全の患者はいなかった。④肺炎を呈した 1 例は心膜炎を有していたことが判明した。この患者は確定症例で、転帰は死亡であった。2 番目の確定患者は、重度の呼吸器疾患の合併症として播種性血管内凝固症候を発現した。転帰は死亡であった。⑤暴露経路は不明であった。⑥確定症例および可能性症例の中で、旅行経験および動物との接触経験を有する者はいなかった。

確定症例および感染可能性症例の家族メンバーの大半、また、確定症例と接触があった医療関係者の大半は、呼吸器症状を発現しなかった。一方、個人的な接触があった家族の少なくとも二人、および、医療介護を提供した数名が肺炎症状を示しており、このことは、ヒト - ヒト感染の可能性を示唆している。しかし、共通の感染源への暴露があった可能性を完全に排除することはできない。

この新種ウイルスは SARS コロナウイルスの遠縁に該当するが、両者は異なっている。現時点の情報から判断し、この新種ウイルスは、SARS コロナウイルスと異なり、ヒト集団内で容易に感染したり、持続的に感染することはないようである。

使用上の注意記載状況・その他参考事項等

BYL-2013-0416

Global Alert and Response
http://www.who.int/csr/disease/coronavirus_infections/update_20121221/en/index.html

報告企業の意見	今後の対応
新種のコロナウイルスによる重症急性呼吸器感染症の報告である。現時点で、持続的にヒトから人に感染する危険性は低いと考えられているが、WHO は注視が必要であるとしている。 コージネイト FS の製造工程における病原体除去・不活化処理は，脂質エンベロープをもつウイルス，および，エンベロープを持たないウイルスに対しても有効であることが報告されている。従って新種のコロナウイルスが本剤に混入する可能性は極めて低いと考えられる。	現時点で新たな安全対策上の措置を講じる必要はないと考える。 今後も、新規人畜共通感染症や新たなウイルス感染症に関する情報収集に努める。

MedDRA バージョン 15.1

BYL-2013-0416

World Health Organization

Global Alert and Response (GAR)

Background and summary of novel coronavirus infection – as of 21 December 2012

Coronavirus Infections

More on coronavirus infections

Over the past three months, WHO has received reports of nine cases of human infection with a novel coronavirus. Coronaviruses are a large family of viruses; different members of this family cause illness in humans and animals. In humans, these illnesses range from the common cold to infection with Severe Acute Respiratory Syndrome (SARS) coronavirus (SARS CoV).

This summary provides the latest information on all reported cases and provides details of a WHO mission to Jordan, which has concluded since the last web update.

Thus far, the laboratory confirmed cases have been reported by Qatar (two cases), Saudi Arabia (five cases) and Jordan (two cases). All patients were severely ill, and five have died.

A total of five confirmed cases have been reported from Saudi Arabia. The first two are not linked to each other and lived in different parts of the country; one of these has died. Three other confirmed cases are epidemiologically linked and occurred in one family living within the same household; two of these have died. One additional family member in this household also became ill, with symptoms similar to those of the confirmed cases. This person has recovered and tested negative, by polymerase chain reaction (PCR) tests, for the virus.

Two confirmed cases have been reported in Jordan. Both of these patients have died. These cases were discovered through testing of stored samples from a cluster of pneumonia cases in health care workers that occurred in April 2012.

In November 2012 staff from WHO Headquarters and the Eastern Mediterranean Regional Office were invited to Jordan to assess severe acute respiratory infection (SARI) surveillance and infection prevention and control measures, and to review the April 2012 outbreak. The mission included hospital site visits, interviews with patients, relatives and caregivers, and review of case files. In addition to the two previously confirmed cases, a number of health care workers with pneumonia associated with the cases were also included in the review and are now considered probable case.

The main findings of this mission are:

- The index case among this cluster could not be determined.
- All patients had significant respiratory disease presenting as pneumonia. Disease was generally milder in the unconfirmed probable cases. One patient who is a probable case had symptoms that were mild enough to be managed at home and was not admitted to hospital.
- No patient in this cluster had renal failure.
- One patient presented with pneumonia and was discovered to also have pericarditis. This patient had laboratory confirmation of infection and has died.
- A second patient developed disseminated intravascular coagulation as a complication of severe respiratory disease. This patient also had laboratory confirmation of infection and has died.
- The method of exposure is uncertain.
- There was no history of travel or contact with animals among confirmed or probable cases.

Most family members and health care workers who were closely exposed to confirmed and probable cases did not develop respiratory disease. However, the appearance of pneumonia in some who provided care and in at least two family members with direct personal contact increases the suspicion that person-to-person transmission may have occurred. The possibility of exposure to a common source has not been definitively excluded. Further investigation with serological testing (when it becomes available) to confirm additional cases may help determine the types of exposures that result in infection.

The current understanding of this novel virus is that it can cause a severe, acute respiratory infection presenting as pneumonia. The additional unconfirmed probable cases in Jordan indicate that milder presentations may also be a part of the clinical appearance associated with infection. Acute renal failure has occurred in five of the nine confirmed cases but was not a prominent feature of the Jordanian cluster. In addition, pericarditis and disseminated intravascular coagulation have now been seen in two confirmed cases.

WHO recognizes that the emergence of a new coronavirus capable of causing severe disease raises concerns because of experience with SARS. Although this novel coronavirus is distantly related to the SARS CoV, they are different. Based on current information, it does not appear to transmit easily or sustainably between people, unlike the SARS virus.

WHO has closely monitored the situation since detection of the first case and has been working with partners to ensure a high degree of preparedness should the new virus be found to be sufficiently transmissible to cause community outbreaks. Some viruses are able to cause limited human-to-human transmission under condition of close contact, as occurs in families, but are not transmissible enough to cause larger community outbreaks.

Actions taken by WHO in coordination with national authorities and technical partners include the following:

- Investigations are ongoing to determine the likely source of infection and the route of exposure. Close contacts of confirmed cases are being identified and followed up.

- An interim surveillance recommendation has been updated to assist clinicians to determine which patients should undergo laboratory testing for the presence of novel coronavirus.
- Laboratory assays for the virus have been developed. Reagents and other materials for testing are available, as are protocols, algorithms and reference laboratory services. WHO has activated its laboratory network to assist in testing and other services. WHO has now issued preliminary guidance for laboratory biorisk management.
- The three affected countries either have already or are in the process of acquiring the capacity to test for the novel coronavirus in national laboratories and have enhanced their surveillance activities according to WHO guidance along with other countries in the area.
- WHO has created a webpage for coronavirus infections, with guidance for surveillance, infection control, biorisk management, and laboratory testing, which can be found at: http://www.who.int/csr/disease/coronavirus_infections/en/index.html

Based on the current situation and available information:

- WHO encourages all Member States to continue their surveillance for severe acute respiratory infections (SARI) and to carefully review any unusual patterns.
- Further, testing for the new coronavirus of patients with unexplained pneumonias should be considered, especially in persons residing in or returning from the Arabian peninsula and neighboring countries. Any new cases should be promptly reported both to national health authorities and to WHO.
- When collecting specimens for testing, priority should be given to collection of lower respiratory tract specimens such as sputa and endotracheal aspirates (for intubated patients).
- In addition, any clusters of SARI or SARI in health care workers should be thoroughly investigated, regardless of where in the world they occur. These investigations will help determine whether the virus is distributed more widely in the human population beyond the three countries that have identified cases.
- Health care workers should be advised to scrupulously adhere to standard infection control precautions for all patients. Droplet precautions should be added to standard precautions for any patient known or suspected to have an acute respiratory infection, including patients with suspected or confirmed infection with novel coronavirus. Airborne precautions should be used for aerosol-generating procedures, including intubation and related interventions. Details can be found on the website listed above.
- WHO does not advise special screening at points of entry with regard to this event nor does it recommend that any travel or trade restrictions be applied.

WHO continues to monitor this situation closely. Unless information is received that changes our understanding of this virus and the disease it causes, the next web update is expected to be posted during the second week of January 2013.

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
				2013. 2. 14	該当なし	

一般的名称	新鮮凍結人血漿	研究報告の公表状況		公表国
販売名(企業名)	新鮮凍結血漿-LR「日赤」(日本赤十字社) 新鮮凍結血漿-LR「日赤」成分採血(日本赤十字社) 新鮮凍結血漿-LR「日赤」120(日本赤十字社) 新鮮凍結血漿-LR「日赤」240(日本赤十字社) 新鮮凍結血漿-LR「日赤」480(日本赤十字社)		ProMED 20130213.1541531	英国ほか

研究報告の概要

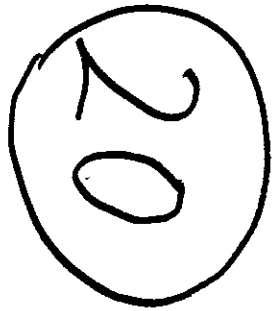
○新型コロナウイルス:英国、ヒトーヒト感染の疑い
新型コロナウイルス関連の重症下気道疾患症例
英国健康保護局(HPA)は2013年2月13日、新型コロナウイルス(NCoV)感染が既に確定している患者の家族1人に、NCoV感染が2013年2月11日に確認されたと発表した。この患者は、短期間の呼吸器症状を呈して2月9日に入院し、呼吸器感染症に罹患しやすい基礎疾患があった。最近の海外渡航歴はなく、現在は集中治療を受けている。
現時点で、NCoVによる重症肺炎の検査確定患者は全部で11人となった。
これまでの情報によると、この家族内でのNCoVのヒトーヒト感染が示唆される。
HPAは英国国際ガイドラインに従い、2患者と密接に接触した家族及び治療に携わった医療従事者の監視を継続中であると報告した。現在NCoVと一致する症状を呈する者はいない。
家族内でのNCoVヒトーヒト感染を考慮し、欧州疾病管理センターは2012年12月7日に発表されたリスクアセスメントの更新を現在進めている。

報告企業の意見	今後の対応
新型コロナウイルス(NCoV)感染確定患者と接触していた家族1人(渡航歴なし)にNCoV感染が確認され、家族内でのヒトーヒト感染が示唆されたとの報告である。	日本赤十字社では、輸血感染症対策として受付時に海外滞在歴の有無を確認し、帰国(入国)後4週間は献血不適としているほか、発熱などの体調不良者を献血不適としている。また、同様のウイルス性疾患である重症急性呼吸器症候群(SARS)患者または罹患の疑いがある場合や既往がある場合は献血不適とし、SARS患者または罹患疑いのある者と接触した場合は、発熱等の症状の有無に拘わらず、最後に接触した日から3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。

使用上の注意記載状況・その他参考事項等

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

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク



Published Date: 2013-02-13 18:16:29
Subject: PRO/AH/EDR> Novel coronavirus - Eastern Med. (04): UK, pers to pers trans susp
Archive Number: 20130213.1541531

NOVEL CORONAVIRUS - EASTERN MEDITERRANEAN (04): UK, PERSON TO PERSON TRANSMISSION SUSPECTED
****

A ProMED-mail post
http://www.promedmail.org
ProMED-mail is a program of the
International Society for Infectious Diseases
http://www.isid.org

In this report:

[1] HPA press release

[2] ECDC

[3] WHO GAR update

******

[1] HPA press release

Date: 13 Feb 2013
Source: HPA UK Press Release [edited]
http://www.hpa.org.uk/NewsCentre/NationalPressReleases/2013PressReleases/130213statementonlatestcoronaviruspatient/

The Health Protection Agency (HPA) can confirm a further case of novel coronavirus infection in a family member of the case announced on Monday [11 Feb 2013]. The patient, who is a UK resident, does not have any recent travel history and is currently receiving intensive care treatment at The Queen Elizabeth Hospital, Birmingham. It is understood that this patient has an existing medical condition that may make them more susceptible to respiratory infections. This latest case brings the total number of confirmed cases globally to 11, of which 3 have been diagnosed in the UK.

Professor John Watson, head of the respiratory diseases department at the HPA, said: "Confirmed novel coronavirus infection in a person without travel history to the Middle East suggests that person-to-person transmission has occurred and that it occurred in the UK. This case is a family member who was in close personal contact with the earlier case and who may have been at greater risk of acquiring an infection because of their underlying health condition. To date, evidence of person-to-person transmission has been limited. Although this case provides strong evidence for person to person transmission, the risk of infection in most circumstances is still considered to be very low. If novel coronavirus were more infectious, we would have expected to have seen a larger number of cases than we have seen since the 1st case was reported 3 months ago. However, this new development does justify the measures that were immediately put into place to prevent any further spread of infection and to identify and follow up contacts of known cases. We will continue to provide advice and support to healthcare workers looking after the patients and to contacts of both cases. In light of this latest case, we would like to emphasise that the risk associated with novel coronavirus to the general UK population remains very low. The HPA will continue to work closely with national and international health authorities and will share any further advice with health professionals and the public if and when more information becomes available."

Notes to editors:

Laboratory confirmed cases to date: 11
Saudi Arabia: 5 (3 deaths)
Jordan: 2 (2 deaths)
UK: 3 (1 patient from Qatar - receiving treatment, 2 patients from UK, 1 with recent travel to Pakistan and Saudi Arabia - both receiving treatment)
Germany: 1 (patient from Qatar - discharged)

Coronaviruses are causes of the common cold but can also include more severe illness, such as SARS (severe acute respiratory syndrome). This new coronavirus was 1st identified in September 2012 in a patient who died from a severe respiratory infection in June 2012. The virus has so far only been identified in a small number of cases of acute, serious respiratory illness who presented with fever, cough, shortness of breath, and breathing difficulties.

For further information, see the HPA's coronavirus web pages, which include a Q&A page on this topic [see http://www.hpa.org.uk/webw/HPAweb&Page&HPAwebAutoListName/Page/1317136202637].

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

*****

[2] ECDC Update
Date: 13 Feb 2013
Source: ECDC (European Centre for Disease Control) [edited]
http://ecdc.europa.eu/en/press/news/Lists/News/ECDC_DispForm.aspx?List=32e43ee8-e230-4424-a783-85742124029a&ID=844&RootFolder=%2Fen%2Fpress%2Fnews%2FLists%2FNews

Epidemiological update: Case of severe lower respiratory tract disease associated with a novel coronavirus:

On [13 Feb 2013], the HPA announced that one family contact of the previously-confirmed case reported on [11 Feb 2013] was laboratory-confirmed to be infected with the novel coronavirus (NCoV). This 2nd case from the same family was hospitalised on [9 Feb 2013] with a short history of respiratory symptoms. The patient has an existing medical condition that may make him more susceptible to respiratory infections. He does not have a recent travel history and is currently receiving intensive care treatment.

The cases have been notified through the EU alerting system for communicable diseases.

This brings the total of laboratory-confirmed cases of severe pneumonia caused by the NCoV to 11 globally (see table below).

The information available suggests human-to-human transmission of the NCoV in this family cluster.

The HPA reports that surveillance of family, close contacts of the 2 patients, and healthcare workers treating the 2 patients is ongoing, as per the UK National Guidelines. None are currently presenting with symptoms consistent with NCoV.

The HPA is also following-up regarding passengers who may have been exposed while flying with the case announced on [11 Feb 2013] and are in contact with the airline concerned.

In light of this human-to-human transmission of the NCoV within the family cluster, ECDC is now updating its risk assessment, previously published on [7 Dec 2012].

Case No: Date Onset / Age (years) / Sex / Probable place of infection / Date reported / Source / Outcome

1: April 2012 / 45/ F / Jordan** / 30 Nov 2012 / WHO/IHR / Dead

2: April 2012 / 25 / M / Jordan** / 30 Nov 2012 / WHO/IHR / Dead

3: 13 Jun 2012 / 60 / M / Kingdom of Saudi Arabia* / 20 Sep 2012 / Kingdom of Saudi Arabia, ProMED / Dead

4: 3 Sep 2012 / 49 / M / Qatar / Kingdom of Saudi Arabia*** / 22 Sep 2012 / HPA/WHO / Alive

5: NK / NK / NK / Kingdom of Saudi Arabia* / 4 Nov 2012 / Kingdom of Saudi Arabia, ProMED, SMJ / Alive

6: 12 Oct 2012 / 45 / M / Qatar
**** / 23 Nov 2012 / RKI/WHO / Alive

7: NK / NK / M / Kingdom of Saudi Arabia* / 19-23 Nov 2012 / Kingdom of Saudi Arabia, ProMED, WHO / Alive

8: 28 Oct 2012 / NK / M / Kingdom of Saudi Arabia* / 23 Nov 2012 / WHO / Dead

9: October 2012 / NK / M / Kingdom of Saudi Arabia* / 28 Nov 2012 / WHO / Dead

10: 24 Jan 2013 / 60 / M / Pakistan, Kingdom of Saudi Arabia*/ 8 Jan 2013 / EWRS / Alive, Hospitalised

11: 6 Feb 2013 / NK / M / United Kingdom* / 12 Feb 2013 / HPA / Alive, Hospitalised

* Part of family cluster
** Healthcare worker and part of outbreak linked to hospital
*** Patient transferred to UK

**** Patient transferred to Germany
NK: not known

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

******

[3] WHO GAR update
Date: 13 Feb 2013
Source: WHO GAR [edited]
http://www.who.int/csr/don/2013_02_13/en/index.html

Novel coronavirus infection - update [13 Feb 2013]:

The United Kingdom (UK) has informed WHO of another confirmed case of infection with the novel coronavirus (NCoV). The patient is a UK resident and a relative of the case announced on [11 Feb 2013].

The latest confirmed case does not have any recent travel history outside the UK and is currently hospitalized in an intensive care unit. It is understood that this patient has pre-existing medical conditions that may have increased susceptibility to respiratory infections.

Confirmed NCoV in a person without recent travel history indicates that infection was acquired in the UK. To date, evidence of person-to-person transmission has been limited. Although this case is suggestive of person-to-person transmission, on the basis of current evidence, the risk of sustained person-to-person transmission appears to be very low.

The Health Protection Agency (HPA) is following up on all close contacts (family and healthcare workers) who may have been exposed to either of these 2 new confirmed cases.

As of [13 Feb 2013], a total of 11 confirmed cases of human infection with NCoV have been notified to WHO, with no change in the number of fatalities i.e., 5 deaths since April 2012.

Based on the current situation and available information, WHO encourages all Member States to continue their surveillance for severe acute respiratory infections (SARI) and to carefully review any unusual patterns. Testing for the

new coronavirus should be considered in patients with unexplained pneumonias, or in patients with unexplained severe, progressive, or complicated respiratory illness not responding to treatment.

Any clusters of SARI or SARI in healthcare workers should be thoroughly investigated, regardless of where in the world they occur.

New cases and clusters of the NCoV should be reported promptly both to national health authorities and to WHO.

WHO does not advise special screening at points of entry with regard to this event nor does it recommend that any travel or trade restrictions be applied.

WHO continues to monitor the situation closely.

--
Communicated by:
ProMED-mail Rapporteur Marianne Hopp

[The above mentioned case of severe acute respiratory infection (SARI) is currently the 11th confirmed case of severe respiratory disease attributable to infection with the novel CoV 1st identified in a fatal case in Saudi Arabia (see prior ProMED-mail posts listed below). It is also the 3rd incident of infection with this novel CoV that occurred in a close contact of an earlier confirmed case, suggesting possible person to person transmission of the virus. There was a cluster of 3 confirmed cases in a family in Saudi Arabia in November 2012 and a cluster of 2 confirmed cases among ICU staff in a hospital in Jordan in May 2012. As stated clearly in the 3 reports of this update, evidence thus far does not seem to suggest an ease and facility of person-to-person contact of this organism as yet.

The table of cases presented in the ECDC report above is a very useful presentation and summary of the current publicly available information on the descriptive epidemiology of known confirmed cases of severe acute respiratory illness due to infection with this novel CoV. Information on exposure histories of each of the patients is not available (some of the earlier cases were reported to have had contact with farm animals in Saudi Arabia and Qatar, but similar information was not available on all cases). To date, cases that have been confirmed have been linked to geographic presence in the Middle East prior to onset of illness (Jordan, Saudi Arabia or Qatar; with one case also having visited Pakistan during the period prior to onset of illness). The absence of cases reported from other areas among individuals without history of contact with this region of the world may or may not reflect the true geographic distribution of this novel CoV, as there may be a bias against testing for this virus in the absence of such stated exposure history ("seek and ye shall find," or the corollary, "don't look and you won't find").

The scientific community is eagerly awaiting the details of epidemiologic investigations conducted on the 11 previously confirmed cases of infection with the novel CoV, especially those addressing exposure to possible animal sources (bats, bat saliva and excrement, farm animals, etc.) and dates of contacts/dates of onset of previous clusters. In addition, information on field studies on bats and farm animals in the Middle Eastern countries addressing infection of animals with the novel CoV is eagerly awaited as well.

For the interactive HealthMap/ProMED map of the UK, see http://healthmap.org/r/1INY. For the interactive HealthMap/ProMED map of the Middle East, see http://healthmap.org/r/1HAJ. - Mod.MPP]

See Also

Novel coronavirus - Eastern Med. (03): Saudi comment 20130212.1540011
Novel coronavirus - Eastern Med. (02): UK ex Saudi Arabia, Pakistan 20130212.1539086
Novel coronavirus - Eastern Mediterranean: bat reservoir 20130122.1508656
2012

Novel coronavirus - Eastern Mediterranean (06): comments 20121225.1468821
Novel coronavirus - Eastern Mediterranean (05): WHO, transmission route 20121223.1465597
Novel coronavirus - Eastern Mediterranean (04): receptor charact. 20121211.1446670
Novel coronavirus - Eastern Mediterranean (03): research, ISARIC (UK) 20121208.1443486
Novel coronavirus - Eastern Mediterranean (02): diagnostics 20121207.1442473
Novel coronavirus - Eastern Mediterranean: WHO, Jordan, conf., RFI 20121130.1432498
Novel coronavirus - Saudi Arabia (19): Singapore: NOT 20121129.1430397
Novel coronavirus - Saudi Arabia (18): WHO, new cases, cluster 20121123.1421664
Novel coronavirus - Saudi Arabia (17): 4th case, RFI 20121121.1418018
Novel coronavirus - Saudi Arabia (16): whole genome sequence 20121114.1409556

Novel coronavirus - Saudi Arabia (15): new case 20121104.1391285
Novel coronavirus - Saudi Arabia (14): KSA MOH 20121022.1358297
Novel coronavirus - Saudi Arabia (13): history, collateral damage 20121021.1356623
Novel coronavirus - Saudi Arabia (12): RFI 20121019.1353615
Novel coronavirus - Saudi Arabia (11): clin. lab. & epi. investigations 20121004.1324712
Novel coronavirus - Saudi Arabia (10): WHO, revised case def. 20120930.1315960
Novel coronavirus - Saudi Arabia (09): real-time RT-PCR, addition 20120929.1315725
Novel coronavirus - Saudi Arabia (08): real-time RT-PCR assay 20120928.1314254
Novel coronavirus - Saudi Arabia (07): Eurosurveillance reports 20120928.1313337
Novel coronavirus - Saudi Arabia (06) 20120927.1311743
Novel coronavirus - Saudi Arabia (05): WHO, case def., nomenclature 20120926.1309747
Novel coronavirus - Saudi Arabia (04): RFI, Jordan, April 2012 20120925.1308001
Novel coronavirus - Saudi Arabia (03): UK HPA, WHO, Qatar 20120923.1305982
Novel coronavirus - Saudi Arabia (02): additional cases, RFI 20120923.1305931
Novel coronavirus - Saudi Arabia: human isolate 20120920.1302733
.....mpp/msp/dk

医薬品
医薬部外品　　研究報告　調査報告書
化粧品

識別番号・報告回数			報告日	第一報入手日 2013 年 1 月 17 日	新医薬品等の区分	厚生労働省処理欄

一般的名称	①②③⑨⑩pH4 処理酸性人免疫グロブリン ④⑤⑥⑦⑧ポリエチレングリコール処理人免疫グロブリン ⑪⑫人免疫グロブリン	研究報告の公表状況	共同通信社/2013/01/16	公表国 アメリカ
販売名 (企業名)	①日赤ポリグロビン N5%静注 0.5g/10mL（日本血液製剤機構） ②日赤ポリグロビン N5%静注 2.5g/50mL（日本血液製剤機構） ③日赤ポリグロビン N5%静注 5g/100mL（日本血液製剤機構） ④献血ヴェノグロブリン IH5%静注 0.5g/10mL（日本血液製剤機構） ⑤献血ヴェノグロブリン IH5%静注 1g/20mL（日本血液製剤機構） ⑥献血ヴェノグロブリン IH5%静注 2.5g/50mL（日本血液製剤機構） ⑦献血ヴェノグロブリン IH5%静注 5g/100mL（日本血液製剤機構） ⑧献血ヴェノグロブリン-IH ヨシトミ（日本血液製剤機構） ⑨日赤ポリグロビン N10%静注 5g/50mL（日本血液製剤機構） ⑩日赤ポリグロビン N10%静注 10g/100mL（日本血液製剤機構） ⑪グロブリン筋注 450mg/3mL「ベネシス」（日本血液製剤機構） ⑫グロブリン筋注 1500mg/10mL「ベネシス」（日本血液製剤機構）			

研究報告の概要	使用上の注意記載状況・その他参考事項等
米国疾病対策センター(CDC)当局者は 13 日、米国各地でインフルエンザ・ウイルスが急速に拡大していると指摘、感染リスクの高い人だけでなく国民全員がワクチンの接種を受けるよう呼び掛けた。CDC のウェブサイトによると、インフルエンザの広範な流行がみられるのは全米 50 州のうち 47 州と、前週の 41 州から拡大。生後 6 カ月以上の国民は例外なくワクチンを接種すべきだとし、幼児、妊婦のほか、ぜんそくや糖尿病などの既往症を抱えている人、65 歳以上の高齢者は特にワクチンが重要だと警告している。 一方、ニューヨーク州はインフルエンザの流行が過去最悪といわれる状態まで広がっており、クオモ同州知事は 12 日、公衆衛生非常事態宣言を発令した。同州ではこれまでにインフルエンザで幼児 2 人と高齢者 10 人が死亡している。今シーズンの感染者も 19,000 人以上と、前シーズンの 4,400 人から急増し、現在も 2,884 人が入院中という。 17	代表として献血ヴェノグロブリン IH5%静注 0.5g/10mL の記載を示す。 2. 重要な基本的注意 (1)本剤の原材料となる献血者の血液については、HBs 抗原、抗 HCV 抗体、抗 HIV-1 抗体、抗 HIV-2 抗体及び抗 HTLV-I 抗体陰性で、かつ ALT（GPT）値でスクリーニングを実施している。更に、プールした試験血漿については、HIV-1、HBV 及び HCV について核酸増幅検査（NAT）を実施し、適合した血漿を本剤の製造に使用しているが、当該 NAT の検出限界以下のウイルスが混入している可能性が常に存在する。本剤は、以上の検査に適合した血漿を原料として、Cohn の低温エタノール分画で得た画分からポリエチレングリコール 4000 処理、DEAE セファデックス処理等により人免疫グロブリンを濃縮・精製した製剤であり、ウイルス不活化・除去を目的として、製造工程において

医薬品
医薬部外品　　研究報告　調査報告書
化粧品

報告企業の意見	今後の対応	
インフルエンザウイルス（influenza virus）は、オルトミクソウイルス科に属するA型インフルエンザウイルス（influenzavirus A）、B型インフルエンザウイルス（influenzavirus B）、C型インフルエンザウイルス（influenzavirus C）の3属を指す。ウイルスの大きさは直径80～120nmの球形粒子で、エンベロープを有するRNAウイルスで、万一原料血漿にインフルエンザウイルスが混入したとしても、Human immunodeficiency virus-1（HIV-1）、或いはBVDVをモデルウイルスとしたウイルスクリアランス試験成績から、本剤の製造工程において不活化・除去されると考えている。	本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。	60℃、10時間の液状加熱処理、ウイルス除去膜によるろ過処理及びpH3.9～4.4の条件下での液状インキュベーション処理を施しているが、投与に際しては、次の点に十分注意すること。

米当局が全国民にインフル・ワクチン接種を呼び掛け　ＮＹ州は非常事態宣言

共同通信社 1月16日(水)配信

【ワシントンＤＰＡ＝共同】米疾病対策センター（ＣＤＣ）当局者は１３日、米国各地でインフルエンザ・ウイルスが急速に拡大していると指摘、感染リスクの高い人だけでなく国民全員がワクチンの接種を受けるよう呼び掛けた。

ＣＤＣのウェブサイトによると、インフルエンザの広範な流行がみられるのは全米５０州のうち４７州と、前週の４１州から拡大。生後６カ月以上の国民は例外なくワクチンを接種すべきだとし、幼児、妊婦のほか、ぜんそくや糖尿病などの既往症を抱えている人、６５歳以上の高齢者は特にワクチンが重要だと警告している。

一方、ニューヨーク州はインフルエンザの流行が過去最悪といわれる状態まで広がっており、クオモ同州知事は１２日、公衆衛生非常事態宣言を発令した。同州ではこれまでにインフルエンザで幼児２人と高齢者１０人が死亡している。今シーズンの感染者も１万９０００人以上と、前シーズンの４４００人から急増し、現在も２８８４人が入院中という。

クオモ知事はこうした事態に対処するため、薬剤師が１８歳以下の子どもにワクチンを接種することを認可した。同州の薬剤師は従来、１８歳以上の人にだけに接種することが許可されていたが、今後３０日間はその範囲が生後６カ月以上に拡大される。

マサチューセッツ州ボストン保健当局も９日、インフルエンザの急拡大を受けて公衆衛生非常事態を宣言しており、他の州当局も事態を注視するとともに、住民にワクチン接種を呼び掛けている。

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
				2012. 10. 20	該当なし	

一般的名称	新鮮凍結人血漿	研究報告の公表状況	Grard G, Fair JN, Lee D, Slikas E, Steffen I, Muyembe JJ, Sittler T, Veeraraghavan N, Ruby JG, Wang C, Makuwa M, Mulembakani P, Tesh RB, Mazet J, Rimoin AW, Taylor T, Schneider BS, Simmons G, Delwart E, Wolfe ND, Chiu CY, Leroy EM. PLoS Pathog. 2012 Sep;8(9):e1002924. doi: 10.1371/journal.ppat.1002924. Epub 2012 Sep 27.	公表国
販売名(企業名)	新鮮凍結血漿-LR「日赤」(日本赤十字社) 新鮮凍結血漿-LR「日赤」成分採血(日本赤十字社) 新鮮凍結血漿-LR「日赤」120(日本赤十字社) 新鮮凍結血漿-LR「日赤」240(日本赤十字社) 新鮮凍結血漿-LR「日赤」480(日本赤十字社)				ガボン共和国

研究報告の概要

○中央アフリカの急性出血熱に関連する新規ラブドウイルス
大規模シークエンシングにより、アフリカのコンゴ民主共和国マンガラ村において2009年に発生した急性出血熱患者3人に関連する新規ラブドウイルス(Bas-Congoウイルス:BASV)を発見した。3週間の間に報告されたこれらの症例は高熱、粘膜出血を突然発症し、さらに2人は3日以内に死亡した。唯一の生存者の急性期検体から1.09×10^{6}RNAコピー/mLの濃度でBASVが検出され、ゲノム配列の98.2%が読み取れた。系統樹解析の結果、BASVは他のラブドウイルスと離れており、アミノ酸の一致は34%未満であった。生存者及び同患者を直接担当した無症候の看護師1人(2人はいずれも医療従事者)から高値の中和抗体(1:1,000)が検出されたことから、BASVがヒトからヒトに伝播することが示唆された。本ウイルスの自然宿主動物あるいは媒介節足動物、正確な伝播様式はいまだに不明である。BASVは、アフリカにおいて急性出血熱の原因となる新たなヒト病原体である。

使用上の注意記載状況・その他参考事項等

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

血液を介するウイルス、
細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
中央アフリカで急性出血熱の原因となる新規のヒト病原体Bas-Congoウイルスが同定されたとの報告である。	今後も引き続き情報の収集に努める。

OPEN ACCESS Freely available online

PLOS | PATHOGENS

A Novel Rhabdovirus Associated with Acute Hemorrhagic Fever in Central Africa

Gilda Grard[1,2☯], Joseph N. Fair[3☯], Deanna Lee[4,5☯], Elizabeth Slikas[6], Imke Steffen[6], Jean-Jacques Muyembe[7], Taylor Sittler[4,5], Narayanan Veeraraghavan[4,5], J. Graham Ruby[8,9], Chunlin Wang[10], Maria Makuwa[7], Prime Mulembakani[7], Robert B. Tesh[11], Jonna Mazet[12], Anne W. Rimoin[13], Travis Taylor[3], Bradley S. Schneider[3], Graham Simmons[6], Eric Delwart[6], Nathan D. Wolfe[3], Charles Y. Chiu[4,5,14]*, Eric M. Leroy[1,2]*

1 Viral Emergent Diseases unit, Centre International de Recherches Médicales de Franceville, Franceville, Gabon, **2** MIVEGEC, UMR (IRD 224 - CNRS 5290 - UM1 - UM2), Institut de Recherche pour le Développement, Montpellier, France, **3** Global Viral Forecasting, Incorporated, San Francisco, California, United States of America, **4** Department of Laboratory Medicine, University of California, San Francisco, California, United States of America, **5** UCSF-Abbott Viral Diagnostics and Discovery Center, San Francisco, California, United States of America, **6** Blood Systems Research Institute, San Francisco, California, United States of America, **7** Institut National de Recherche Biomédicale, Kinshasa, Democratic Republic of the Congo, **8** Howard Hughes Medical Institute, Chevy Chase, Maryland, United States of America, **9** Department of Biochemistry, University of California, San Francisco, California, United States of America, **10** Department of Biochemistry, Stanford University, Stanford, California, United States of America, **11** Department of Pathology, University of Texas Medical Branch, Galveston, Texas, United States of America, **12** Department of Epidemiology, University of California at Davis, Davis, California, United States of America, **13** Department of Epidemiology, University of California at Los Angeles, Los Angeles, California, United States of America, **14** Department of Medicine, Division of Infectious Diseases, University of California, San Francisco, San Francisco, California, United States of America

Abstract

Deep sequencing was used to discover a novel rhabdovirus (Bas-Congo virus, or BASV) associated with a 2009 outbreak of 3 human cases of acute hemorrhagic fever in Mangala village, Democratic Republic of Congo (DRC), Africa. The cases, presenting over a 3-week period, were characterized by abrupt disease onset, high fever, mucosal hemorrhage, and, in two patients, death within 3 days. BASV was detected in an acute serum sample from the lone survivor at a concentration of 1.09×10^6 RNA copies/mL, and 98.2% of the genome was subsequently *de novo* assembled from ~140 million sequence reads. Phylogenetic analysis revealed that BASV is highly divergent and shares less than 34% amino acid identity with any other rhabdovirus. High convalescent neutralizing antibody titers of ≥1:1000 were detected in the survivor and an asymptomatic nurse directly caring for him, both of whom were health care workers, suggesting the potential for human-to-human transmission of BASV. The natural animal reservoir host or arthropod vector and precise mode of transmission for the virus remain unclear. BASV is an emerging human pathogen associated with acute hemorrhagic fever in Africa.

Citation: Grard G, Fair JN, Lee D, Slikas E, Steffen I, et al. (2012) A Novel Rhabdovirus Associated with Acute Hemorrhagic Fever in Central Africa. PLoS Pathog 8(9): e1002924. doi:10.1371/journal.ppat.1002924

Editor: David Wang, Washington University, United States of America

Received May 23, 2012; **Accepted** August 8, 2012; **Published** September 27, 2012

Funding: CIRMF is supported by the government of Gabon, Total-Fina-Elf Gabon, and the Ministère des Affaires Etrangères et Européennes de la France. This work is also partially financed by Global Viral Forecasting, graciously supported by the U.S. Department of Defense Armed Forces Health Surveillance Center, Division of Global Emerging Infections, Surveillance Operations (AFHSC GEIS), the Henry M. Jackson Foundation for the Advancement of Military Medicine, the Defense Threat Reduction Agency Cooperative Biological Engagement Program (DTRA-CBEP), Google.org, the Skoll Foundation, and the U.S. Agency for International Development (USAID) Emerging Pandemic Threats Program, PREDICT project, under the terms of Cooperative Agreement Number GHN-A-OO-09-00010-00. RBT is supported by NIH contract HHSN272201000040I/HHSN27200004/D04. ED is supported by R01-HL083254 and R01-HL105770 and by the Blood Systems Research Institute. CYC is supported by NIH grants R56-AI089532 and R01-HL105704, as well as an Abbott Viral Discovery Award. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. The contents of the manuscript are the responsibility of the authors and do not necessarily reflect the views of the United States Government.

Competing Interests: The authors have filed a patent application related to BASV. This does not alter the authors' adherence to all PLOS Pathogens policies on sharing data and materials.

* E-mail: eric.leroy@ird.fr (EML); charles.chiu@ucsf.edu (CYC)

☯ These authors contributed equally to this work.

Introduction

Viral hemorrhagic fever (VHF) encompasses a group of diseases characterized by fever, malaise, bleeding abnormalities, and circulatory shock [1,2,3]. Quality research on these infections is hindered by the fact that they are sporadic and often occur in geographically remote and politically unstable regions of the developing world. Most VHF diseases are associated with a short incubation period (2–21 days), abrupt onset, rapid clinical course, and high mortality, placing VHF agents amongst the most virulent human pathogens [4]. All known VHFs are zoonoses, and to date have been attributed to only four families of enveloped, single-stranded RNA viruses – *Arenaviridae*, *Bunyaviridae*, *Filoviridae* and *Flaviviridae*. Viruses from these families have caused major deadly outbreaks on the African continent (Fig. 1). Lassa fever virus (*Arenaviridae*) causes an estimated 500,000 cases each year in West Africa [5]. Crimean-Congo hemorrhagic fever (CCHF) and Rift Valley Fever viruses (*Bunyaviridae*) are associated with outbreaks in

Author Summary

We used deep sequencing, a method for generating millions of DNA sequence reads from clinical samples, to discover a novel rhabdovirus (Bas-Congo virus, or BASV) associated with a 2009 outbreak of 3 human cases of acute hemorrhagic fever in Mangala village, Democratic Republic of Congo (DRC), Africa. The cases, presenting over a 3-week period, were characterized by abrupt disease onset, high fever, bloody vomiting and diarrhea, and, in two patients, death within 3 days. BASV was present in the blood of the lone survivor at a concentration of over a million copies per milliliter. The genome of BASV, assembled from over 140 million sequence reads, reveals that it is very different from any other rhabdovirus. The lone survivor and a nurse caring for him (with no symptoms), both health care workers, were found to have high levels of antibodies to BASV, indicating that they both had been infected by the virus. Although the source of the virus remains unclear, our study findings suggest that BASV may be spread by human-to-human contact and is an emerging pathogen associated with acute hemorrhagic fever in Africa.

West, South and East Africa [6]. Ebola and Marburg viruses (*Filoviridae*) have caused several sporadic human outbreaks with high mortality (50–90%) in Central Africa, where they have also decimated local great ape populations [7]. Yellow fever and dengue viruses (*Flaviviridae*) are widely distributed throughout Sub-Saharan Africa where they cause both endemic and sporadic epidemic diseases in human populations [8].

Rhabdoviruses are members of the family *Rhabdoviridae* and order *Mononegavirales* and are enveloped viruses with single-stranded, negative-sense RNA genomes [9]. Their genomes encode at least five core proteins in the following order: 3′-nucleoprotein (N), phosphoprotein (P), matrix protein (M), glycoprotein (G) and large protein, or RNA-dependent RNA polymerase (L)-5′ (N-P-M-G-L). Rhabdoviruses are currently divided into six genera, with the two genera *Ephemerovirus* and *Vesiculovirus*, together with about 130 unclassified viruses, forming the dimarhabdovirus supergroup ("dipteran mammal-associated rhabdovirus") [10]. Notably, although rhabdoviruses span all continents and exhibit a wide host range, infecting plants, invertebrates, vertebrate animals, and humans, relatively few are known to cause human infections. Rabies virus (RABV) and related viruses from the *Lyssavirus* genus and Chandipura virus (CHPV) from the *Vesiculovirus* genus are known to cause acute encephalitis syndromes [11,12]. Other viruses from the genus *Vesiculovirus* cause vesicular stomatitis (mucosal ulcers in the mouth) and "flu-like" syndromes in both cattle and humans [13].

Unbiased next-generation or "deep" DNA sequencing is an emerging method for the surveillance and discovery of pathogens in clinical samples [14]. Unlike polymerase chain reaction (PCR), deep sequencing does not rely on the use of target-specific primers. Thus, the technique is particularly useful for the identification of novel pathogens with high sequence divergence that would elude detection by conventional PCR assays. Deep sequencing has been used previously to discover a new hemorrhagic fever-associated arenavirus from southern Africa, Lujo virus [15], as well as a new polyomavirus in human Merkel cell carcinoma [16]. With the depth of sequence data now routinely extending to >100 million reads, *de novo* genome assembly of novel viruses directly from primary clinical samples is feasible, as demonstrated by assembly of the 2009 pandemic influenza H1N1 virus genome from a single patient's nasal swab without the use of a reference sequence [17]. Here we report the critical role of deep sequencing in the discovery of a novel rhabdovirus associated with a small outbreak of fulminant hemorrhagic fever in the remote village of Mangala, Bas-Congo province, Democratic Republic of Congo (DRC), between May 25 and June 14, 2009.

Results

Case Reports from an Acute Hemorrhagic Fever Outbreak

Patient 1. The first case was a 15-year-old boy who presented to the health center in Mangala village (Boma Bungu Health Zone) on May 25, 2009 with malaise, epistaxis (nose bleeding), conjunctival injection, gingival bleeding, hematemesis (vomiting with blood), and watery diarrhea with blood (Table 1). No fever or respiratory symptoms were noted. Hemorrhagic symptoms initially appeared on May 24, and the patient died 2 days later from sudden circulatory collapse. The patient lived in the Tshela neighborhood of Mangala village and attended the local public school. All close contacts were monitored for 21 days, and none developed any signs of illness.

Patient 2. The second case was a 13-year-old girl. She attended the same public school as Patient 1 but was in a different class. She also lived in the Tshela neighborhood of Mangala village, about 50 meters from Patient 1's house. They knew each other but had no known face-to-face contact during the previous weeks. This patient presented to the health center on June 5, 2009 with headache, fever, abdominal pain, epistaxis, conjunctival injection, mouth bleeding, hematemesis, and diarrhea with blood. She was examined by a nurse and received acetaminophen and dipyrone for fever and quinine for possible malaria. Symptoms appeared on June 4, and the patient died suddenly on June 7, three days after onset. None of her close contacts developed symptoms during the 21 days of monitoring after her death.

Patient 3. The third case was a male nurse aged 32 years working in the health center visited by Patients 1 and 2. His disease appeared suddenly on June 13, 2009 with epistaxis, ocular and oral hemorrhage, hematemesis, and diarrhea with blood. Two days after the onset of hemorrhagic symptoms, he developed fever, anorexia, headache, fatigue, and abdominal pain. He was transferred to the regional general hospital of Boma (Fig. 1), a city of about 200,000 inhabitants, where a serum sample was obtained on June 15, just prior to treatment with fluid resuscitation, blood transfusion, and empiric antibiotics. Laboratory tests for malaria, tuberculosis, dengue, and bacterial sepsis were negative, and the patient recovered spontaneously a few days later. All persons in Mangala and Boma who had contact with Patient 3 were monitored for 21 days, and none became ill. Patient 3, like the two other patients, lived in the Tshela neighborhood of Mangala village, about 50 meters from Patients 1 and 2. Importantly, patient 3 was directly involved in the care of Patients 1 and 2 when they presented to the health center with hemorrhagic symptoms.

No disease outbreaks had been reported in the past in Boma Bungu Health Zone with the exception of a cholera diarrheal outbreak in 2006, and, notably, no cases of hemorrhagic disease had previously been reported. In addition, although DRC is a country endemic for filovirus infection (Fig. 1), no outbreaks of Ebola or Marburg fever have ever been described in Bas-Congo province. No animal die-offs or other unusual events in association with these cases were noted.

Initial Sample Collection and Diagnostic Testing

A cluster of three human cases of typical acute hemorrhagic fever occurred between May 25 and June 13, 2009 in Mangala village,

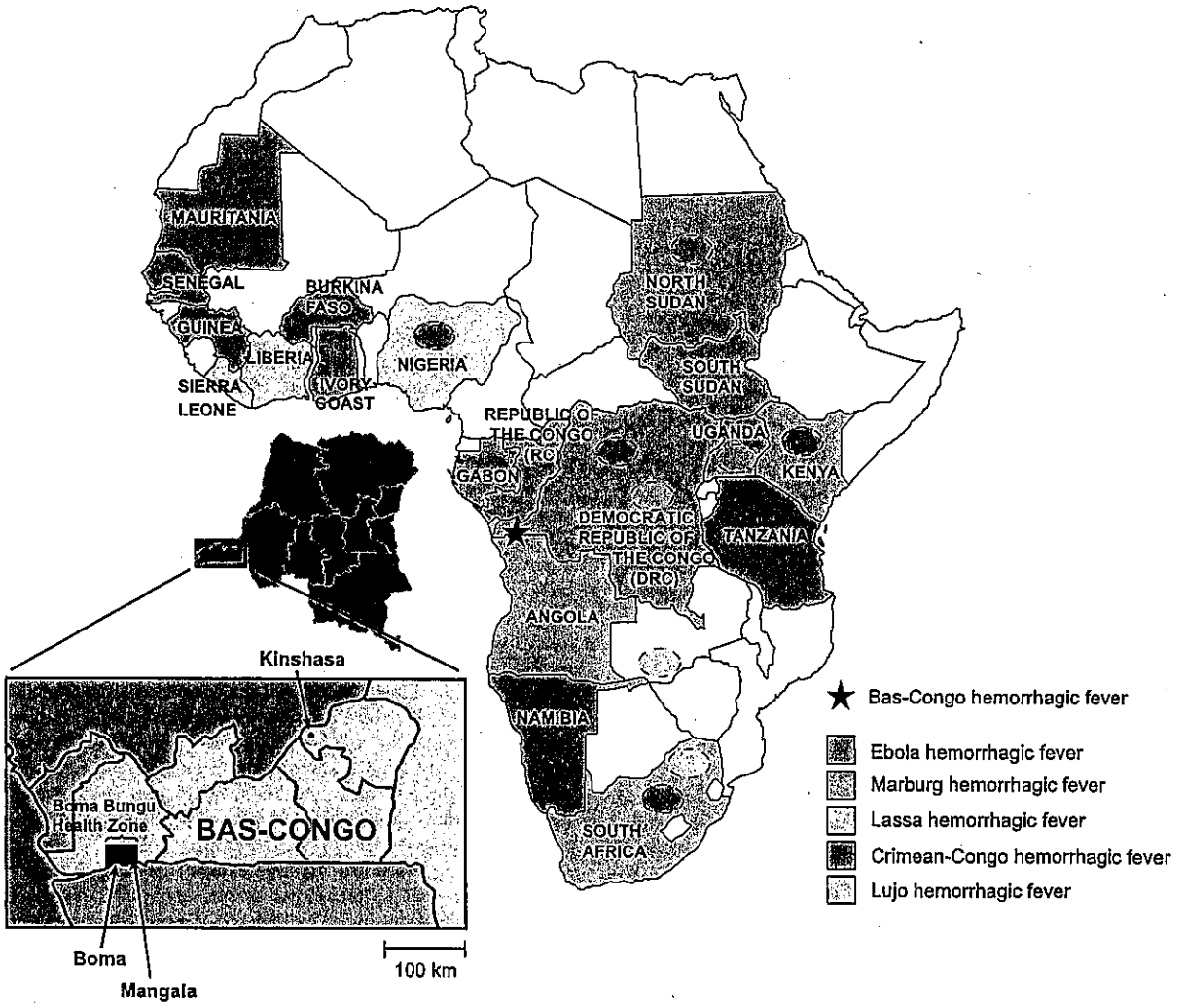


Figure 1. Map of Africa showing countries that are affected by viral hemorrhagic fever (VHF) outbreaks. Ebola VHF is pictured in orange, Marburg VHF in green, Crimean-Congo HF in violet, Lujo VHF in pink, and Lassa VHF in blue. Yellow fever and dengue VHF, which exhibit a wide geographic distribution throughout Sub-Saharan Africa, are not shown. Mangala village, located in the Bas-Congo province in DRC, is represented by a red star.
doi:10.1371/journal.ppat.1002924.g001

located in a remote tropical forest region in Central Africa. Cases were characterized by abrupt disease onset, high fever of >39°C when present, overt hemorrhagic symptoms with epistaxis, conjunctival injection, mouth and gastrointestinal bleeding, followed by death within 3 days of symptom onset in two patients (Table 1). The first patient, who died <48 hours after presentation, exhibited hemorrhagic symptoms without a documented fever, and only the third adult patient recovered from his illness. All three patients lived within a 2500-m^2 area in the same neighborhood of Mangala, a remote village in Bas-Congo province of DRC (Fig. 1). The first two patients died rapidly in Mangala village, and no blood samples were collected. A blood sample was collected from the third surviving patient three days after symptom onset and sent to Centre International de Recherches Médicales de Franceville (CIRMF) for etiological diagnosis. The sample tested negative by TaqMan real-time PCR assays for all viruses known to cause acute hemorrhagic fever in Africa (data not shown).

Discovery and Genome Assembly of the BASV Rhabdovirus

To identify a potential causative pathogen in the third surviving patient with unknown hemorrhagic fever, RNA extracts from the serum sample were analyzed using unbiased deep sequencing (Fig. 2). The initial Roche 454 pyrosequencing library yielded a total of 4,537 sequence reads, of which only a single 220 bp read (0.022%) aligned with any annotated viral protein sequence in GenBank. The translation product showed similarity to a segment of the L protein, or RNA-dependent RNA polymerase, from Tibrogargan and Coastal Plains rhabdoviruses, with 41% identity to Coastal Plains virus (GenBank ADG86364; BLASTx E-score of 2×10^{-6}). This finding suggested the presence of a novel, highly divergent rhabdovirus in the patient's serum. Attempts to extend the initial sequence by primer walking or PCR using rhabdovirus consensus primers failed due to limited sample availability; thus, we resorted to ultra-deep sequencing on an Illumina HiSeq 2000.

Table 1. Demographics of and clinical symptoms developed in the three patients suspected to be infected by Bas-Congo virus (BASV).

	Patient 1	Patient 2	Patient 3
Sex	Male	Female	Male
Age	15	13	32
Village	Mangala	Mangala	Mangala
Neighborhood	Tshela	Tshela	Tshela
Occupation	Schoolboy	Schoolgirl	Nurse
Disease onset	May 24	June 4	June 13
Time until death	2 days	3 days	survived
Fever (T>39°C)	No	Yes	Yes
Weakness	No	No	Yes
Malaise	Yes	No	No
Headache	No	Yes	Yes
Abdominal pain	No	Yes	Yes
Epistaxis (nose bleeding)	Yes	Yes	Yes
Ocular hemorrhage/conjunctival injection (eye bleeding)	Yes	Yes	Yes
Oral hemorrhage (mouth bleeding)	Yes	Yes	Yes
Hemorrhagic vomiting	Yes	Yes	Yes
Hemorrhagic diarrhea	Yes	Yes	Yes

doi:10.1371/journal.ppat.1002924.t001

Out of the 140,164,344 reads generated from Illumina sequencing, 4,063 reads (0.0029%) had nucleotide or protein homology to rhabdoviruses with an E-score of $<10^{-5}$. These reads were used as "seeds" for iterative *de novo* assembly, resulting in construction of an estimated 98.2% of the genome of the novel rhabdovirus. We provisionally named this rhabdovirus BASV, or Bas-Congo virus, referring to the province from which the outbreak originated.

The coverage of BASV achieved by deep sequencing was at least 10-fold across nearly the entire genome and included 29,094 reads out of ~140 million (0.021%) (Fig. 2). The viral load in the patient's serum was 1.09×10^6 RNA copies/mL by quantitative RT-PCR. The only moderately high titer is consistent with the fact that the sampled patient was a survivor of BASV infection and would thus be anticipated to have relatively lower viral titers in the blood, as also seen for survivors of Ebola virus infection [18].

Cultivation of the patient's serum in Vero, BHK, LLC-MK_2 (rhesus monkey kidney), CCL-106 (rabbit kidney) and C6/36 (*Aedes albopictus* mosquito) cell cultures failed to show cytopathic effect, and serial quantitative BASV RT-PCR assays on primary and passaged cell culture supernatants turned negative. Subsequent electron microscopy of inoculated cell cultures was negative for viral particles. In addition, no illnesses or deaths occurred in suckling mice inoculated intracerebrally with the BASV-positive serum and observed over 14 days.

Phylogenetic Analysis of BASV and Comparison with other Rhabdoviruses

Phylogenetic trees reveal that BASV belongs to the *dimarhabdoviridae* supergroup and is distantly related to members of the Tibrogargan group and the *Ephemerovirus* genus, although it clusters separately from other rhabdoviruses in an independent deeply rooted branch (Figs. 3 and 4; Fig. S1). Comparative analysis of the concatenated BASV proteins with representative dimarhabdoviruses reveals very low overall amino acid pairwise identity of 25.0 to 33.7%, depending on the virus (Fig. 5). Notably, BASV diverges significantly from either of the two main recognized human pathogens among rhabdoviruses, rabies virus or Chandipura virus.

The sequence divergence of BASV relative to other rhabdoviruses is also correlated with differences in genome structure (Fig. 5). The prototype genome organization of rhabdoviruses, found in lyssaviruses, is N-P-M-G-L. However, molecular analysis of novel rhabdoviruses has often revealed more complex genomes, with up to 10 additional open reading frames (ORF) located within an existing gene or interposed between the five core genes [19,20,21]. Rhabdoviruses from the Tibrogargan group (TIBV and CPV) share a distinctive genome structure with three additional genes, two between M and G (U1 and U2) and one between G and L (U3) [22]. Interestingly, BASV also has these three additional genes (U1–U3), confirming the phylogenetic relationship and overall structural similarity to the Tibrogargan group viruses. Based on their size, the U3 proteins of TIBV, CPV, and presumably BASV are candidate viroporins [22]. BASV is more distant structurally and phylogenetically from the Ephemero and Hart Park Group rhabdoviruses (Figs. 3 and 4), which do not contain U1 or U2 genes, but rather an additional two or three genes between G and L (including a putative U3 viroporin in BEFV referred to as the alpha-1 protein) (Fig. 5, asterisk). Moussa virus (MOUV), another rhabdovirus recently discovered in Africa (Fig. 4), does not contain any accessory genes but instead, shares the prototype N-P-M-G-L rhabdovirus structure [23].

BASV Serological Testing of the Case Patient and Close Contacts

To confirm that BASV is infectious to humans, convalescent sera were collected in early 2012 from surviving Patient 3 as well as five additional health care workers from Mangala identified as close contacts and tested in a blinded fashion for the presence of neutralizing antibodies to BASV (Fig. 6). Two of the six sera tested strongly positive with 50% protective doses between 1:1,000 and

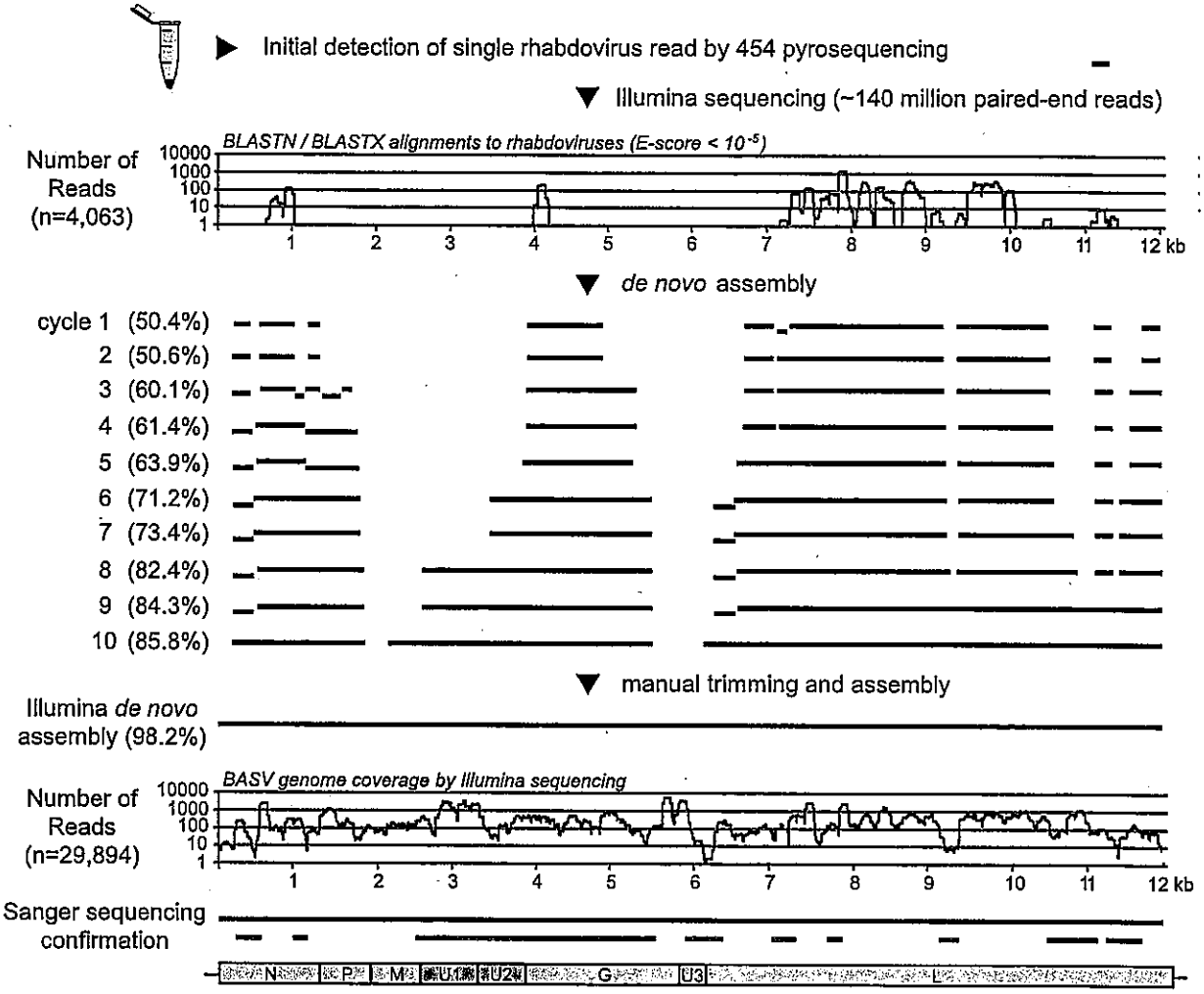


Figure 2. Deep sequencing and whole-genome *de novo* assembly of BASV. After initial discovery of BASV from a single 454 pyrosequencing read, 98.2% of the BASV genome was assembled *de novo* from >140 million paired-end Illumina reads. The horizontal lines (red) depict regions of the genome successfully assembled at the end of each cycle. PCR and Sanger sequencing were performed to confirm the assembly and genomic organization of BASV (green lines).
doi:10.1371/journal.ppat.1002924.g002

1:5,000 (Figs. 6A and 6F). Moreover, the observed neutralization was highly specific for BASV-G, since no neutralization was observed with pseudoviruses harboring the vesicular stomatitis virus glycoprotein (VSV-G). One of the neutralizing sera had been collected from surviving Patient 3 (Fig. 6A, "Patient 3"), whereas the other serum sample, containing even higher titers, corresponded to an asymptomatic nurse directly caring for Patient 3 during his period of acute hemorrhagic illness (Fig. 6F, "Contact 5"). Specifically, Contact 5 was the primary health care provider to Patient 3 at the health center and during his transfer to the general hospital at Boma. All 6 individuals, including Patient 3, tested negative for BASV viremia by specific RT-PCR (data not shown).

Epidemiological Screening for BASV in the DRC

BASV was not detected by PCR in 43 serum samples from other unknown cases or outbreaks of hemorrhagic fever reported in the DRC from 2008–2010 (Fig. 7A, pink). Five of these 43 samples originated from the Bas-Congo outside of Mangala village and the Boma Bungu Health Zone. In total, the unknown hemorrhagic cases/outbreaks spanned 9 of the 11 provinces in the DRC, and all 43 samples also tested negative by PCR for the known hemorrhagic fever viruses circulating in Africa (data not shown). Fifty plasma samples collected from randomly selected blood donors in the Kasai-Oriental province of DRC (Fig. 7A, star; Table S2) were also screened and found to be negative for BASV-neutralizing antibodies (Fig. 7B).

Discussion

Among more than 160 species of rhabdoviruses identified to date, fewer than 10 have been isolated from humans [24]. In addition, while human infection by rhabdoviruses has previously been associated with encephalitis, vesicular stomatitis, or "flu-like" illness, the discovery of BASV is the first time that a member of the *Rhabdovirus* family has been associated with hemorrhagic fever in humans with a fulminant disease course and high fatality rate. To our knowledge, this is also the first successful demonstration of

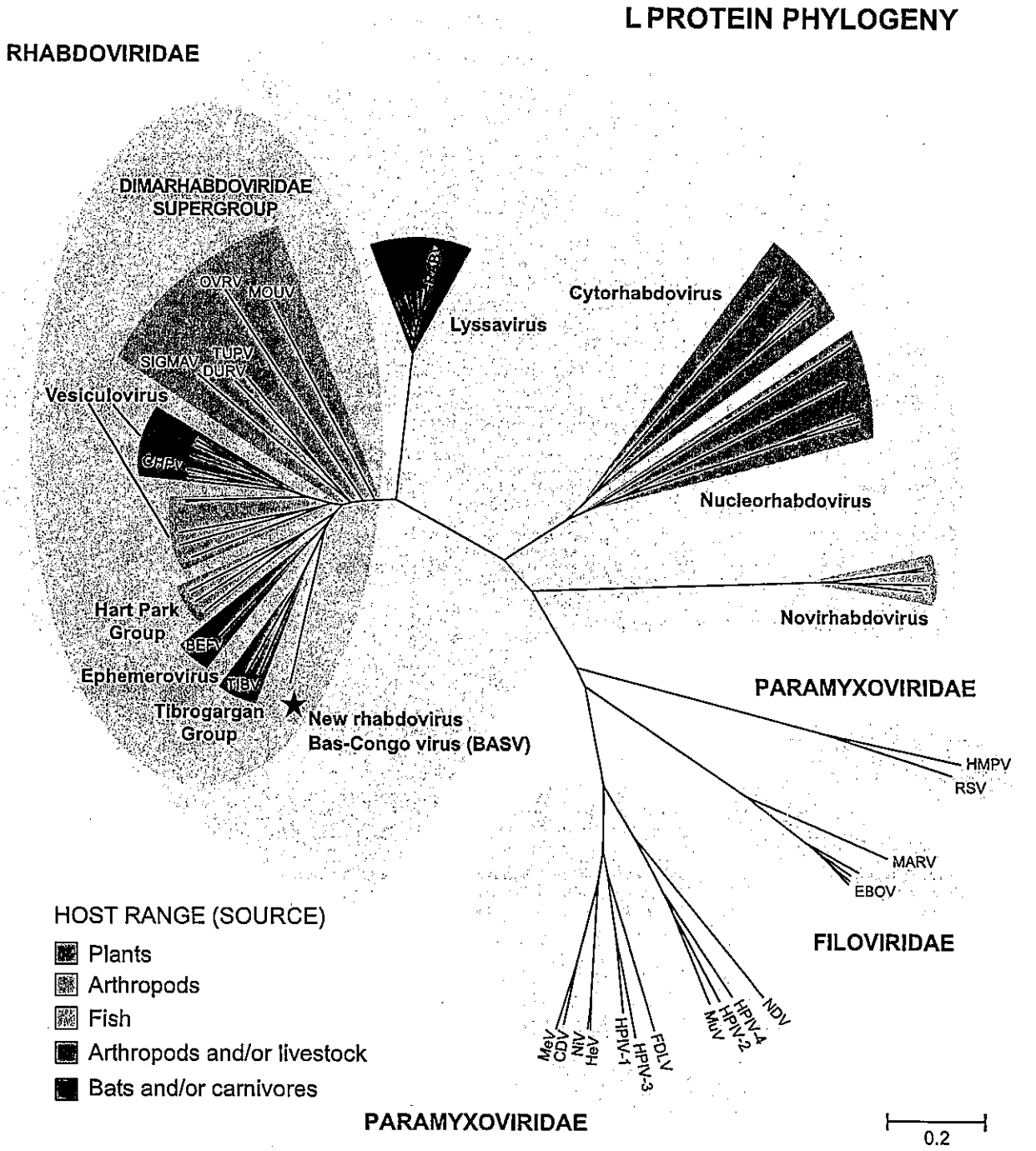


Figure 3. Phylogenetic analysis of the L proteins of BASV and other viruses in the order *Mononegavirales*. The host from which each virus was isolated is represented by a specific color. To generate the *Mononegavirales* (*Rhabdoviridae*, *Filoviridae* and *Paramyxoviridae*) phylogeny trees, all complete sequences of the large (L) protein, or RNA-dependent RNA polymerase (2000–2300 amino acids in length) were downloaded from GenBank. Abbreviations and accession numbers used for the phylogenetic analysis are provided in Methods.
doi:10.1371/journal.ppat.1002924.g003

de novo assembly of a novel, highly divergent viral genome in the absence of a reference sequence and directly from a primary clinical sample by unbiased deep sequencing.

Several lines of evidence implicate BASV in the hemorrhagic fever outbreak among the 3 patients in Mangala. First, this virus was the only credible viral pathogen detected in the blood of the lone survivor during his acute hemorrhagic illness by exhaustive deep sequencing of over 140 million reads. Analysis of the Illumina deep sequencing reads for the presence of other viral pathogens yielded only endogenous flora or confirmed laboratory contaminants (Table

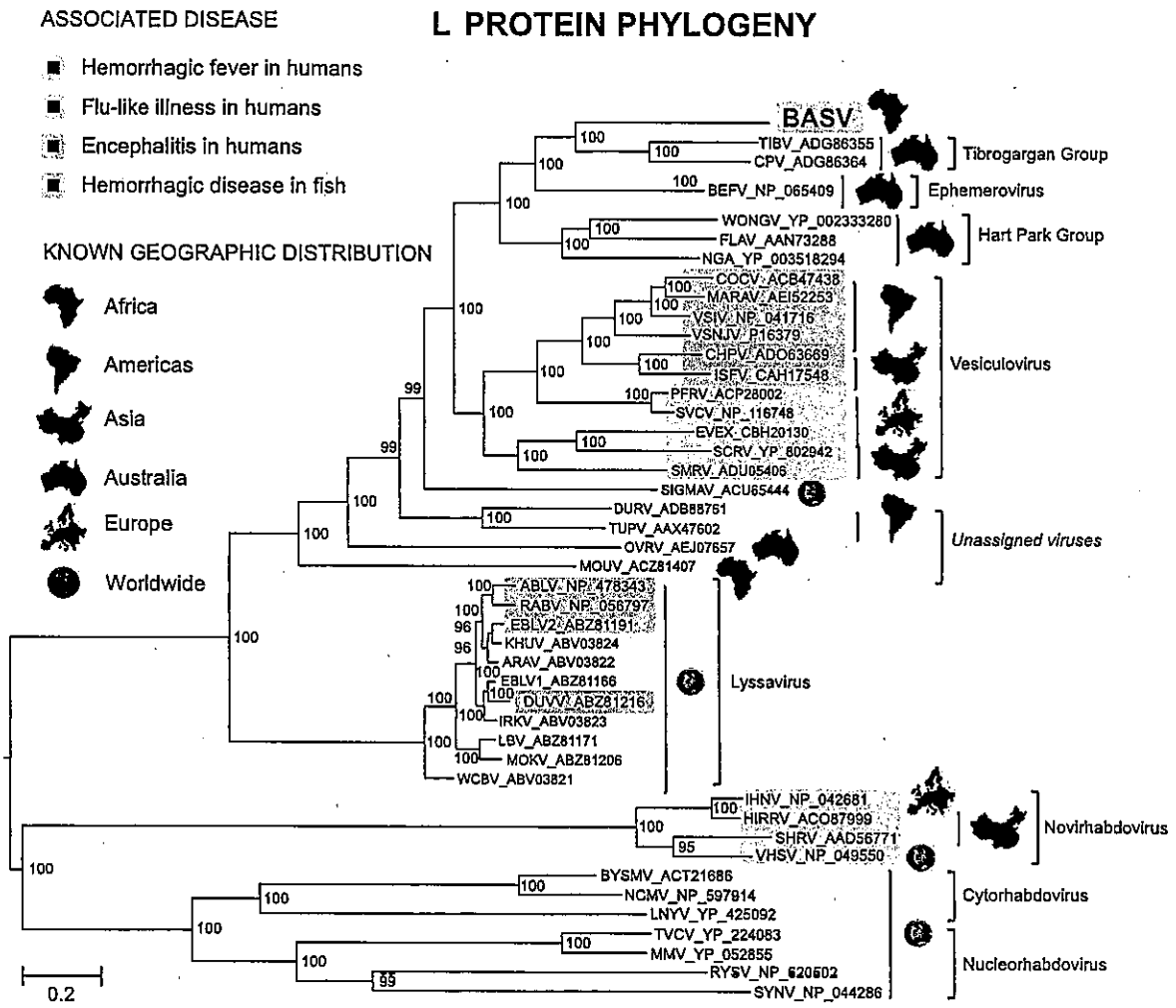


Figure 4. Phylogenetic analysis of the L proteins of BASV and other rhabdoviruses. The geographic distribution for each virus or group of viruses is indicated with a specific icon, while diseases associated with infection by certain rhabdoviruses are indicated by specific colors. Abbreviations and accession numbers used for the phylogenetic analysis are provided in Methods.
doi:10.1371/journal.ppat.1002924.g004

S1 and Fig. S2). Some enteric pathogens, such as E. coli O157:H7, Campylobacter, Shigella, and Salmonella, are diagnosed through fecal laboratory testing and not blood, and have been associated with hemorrhagic diarrhea [25]. However, these outbreaks are typically foodborne and associated with larger clusters and much greater numbers of clinical cases than reported here [26,27,28]. Furthermore, enteric diarrheal cases rarely present with systemic symptoms such as fever or generalized mucosal hemorrhage, with bleeding most often limited to the gastrointestinal tract, and overall mortality rates are generally low [26]. Thus, the clinical syndrome observed in 3 patients with hemorrhagic fever in the DRC, a region endemic for viral hemorrhagic fevers, is much more consistent with infection by a VHF disease agent. BASV is a plausible hemorrhagic fever candidate because it is a novel, highly divergent infectious virus, thus of unknown pathogenicity, and was detected at a titer of >1 million copies/mL in blood from an acutely ill individual. In addition, there is ample precedent for hemorrhagic disease from rhabdoviruses, as members of the genus *Novirhabdovirus* cause severe hemorrhagic septicemia in fresh and saltwater fish worldwide [29] (Fig. 4). The detection of BASV seropositivity in an asymptomatic close contact (Fig. 6) is not surprising given that up to 80% of patients infected with Lassa virus do not exhibit any hemorrhagic fever symptoms [30,31].

Prior to the BASV outbreak, no hemorrhagic disease cases had been reported in Boma Bungu Health Zone. BASV was also not detected in 43 serum samples from unknown, filovirus-negative cases or outbreaks of hemorrhagic fever from 2008–2010 spanning 9 of the 11 provinces in the DRC (Fig. 7A). In addition, a serosurvey of 50 random blood donors from Kasai-Oriental province in central DRC was negative for prior exposure to BASV (Fig. 7B). Taken together, these data suggest that the virus may have emerged recently and locally from Boma Bungu in Bas-Congo, DRC.

We were unable to isolate BASV despite culturing the RNA-positive serum in a number of cell cultures and inoculation into suckling mice. One explanation for these negative findings may be that the virus inoculation titers of <50 μL were insufficient, although this is surprising given the concentration of >1 million copies per mL of BASV in blood from the lone survivor. A more likely explanation is viral inactivation resulting from the lack of

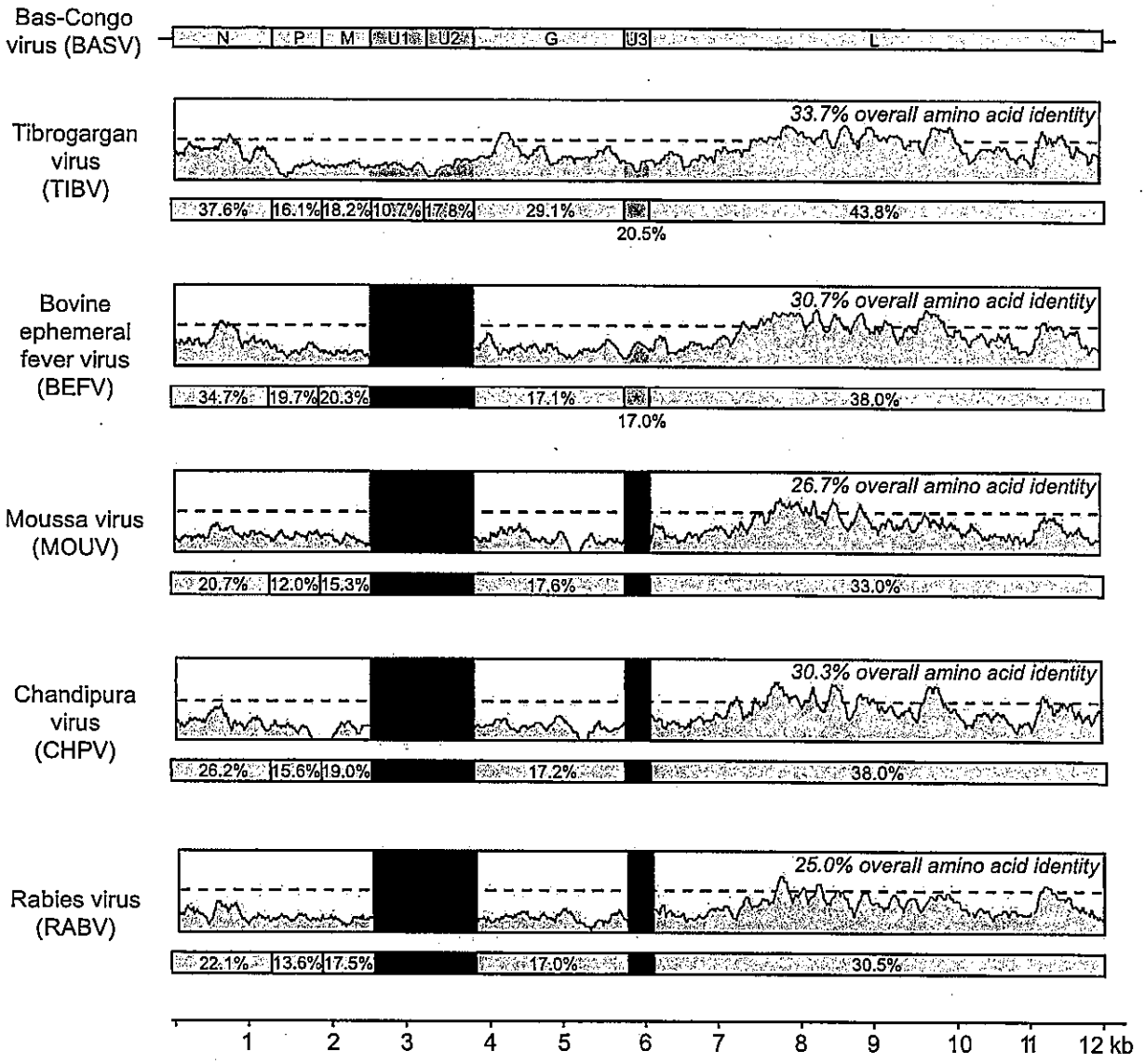


Figure 5. Schematic representation of the genome organization of BASV and its protein similarity plot compared to representative rhabdoviruses. The similarity plots are generated by aligning the concatenated rhabdovirus proteins and calculating scanning amino acid pairwise identities using a window size of 50 bp. The horizontal bar under each similarity plot shows the percent identity of the rhabdovirus protein relative to its corresponding protein in BASV. Genes coding for the 5 core rhabdovirus proteins are shown in green, while the accessory U1, U2, or U3 genes are shown in blue. Black bars correspond to accessory proteins which are not present in the genome. Note that BEFV contains 3 genes between G and L; only the alignment between the alpha-1 protein of BEFV and the U3 protein of BASV is shown (asterisk). The x-axis refers to the nucleotide position along the ~12 kb genome of BASV.
doi:10.1371/journal.ppat.1002924.g005

adequate cold chain facilities in remote Boma Bungu. Viral RNA can often still be detected by RT-PCR in sera that is culture-negative [32]. In support of this premise, we have observed that the BASV-G/VSVΔG-GFP pseudotyped virus efficiently infects and replicates in a variety of insect and mammalian (including human) cell lines (Steffen, *et al.*, manuscript in preparation). In the absence of a positive culture, a "reverse genetics" approach to produce recombinant BASV particles, if successful, would greatly facilitate further study of the virus, as established previously for other rhabdoviruses such as VSV [33].

Based on our findings, some speculations on the origin of and routes of transmission for BASV can be made. All 3 patients became ill with acute hemorrhagic fever over a 3-week period within the same 2500-m^2 area of Mangala village, suggesting that all 3 cases were infected with the same pathogen. Waterborne or airborne transmission would be expected to result in more numerous cases than the 3 reported. There were no reports of animal die-offs that would suggest potential exposures to infected wild animals or livestock. Taken together, these observations suggest that an unknown arthropod vector could be a plausible source of infection by BASV. This hypothesis is consistent with the phylogenetic and structural relationship of BASV to rhabdoviruses in the Tibrogargan group and *Ephemerovirus* genus, which are transmitted to cattle and buffalo by *Culicoides* biting midges [9]. In

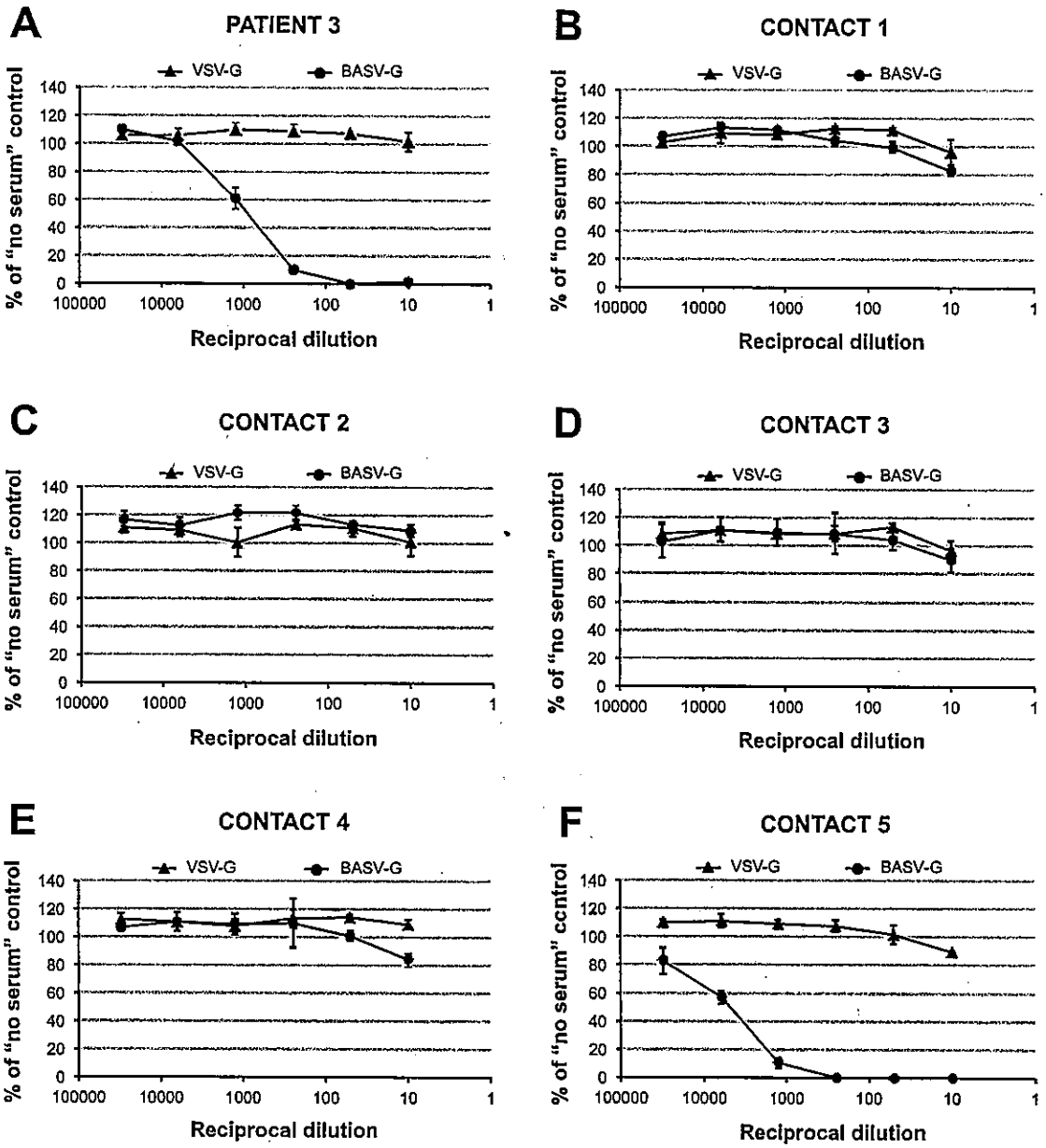
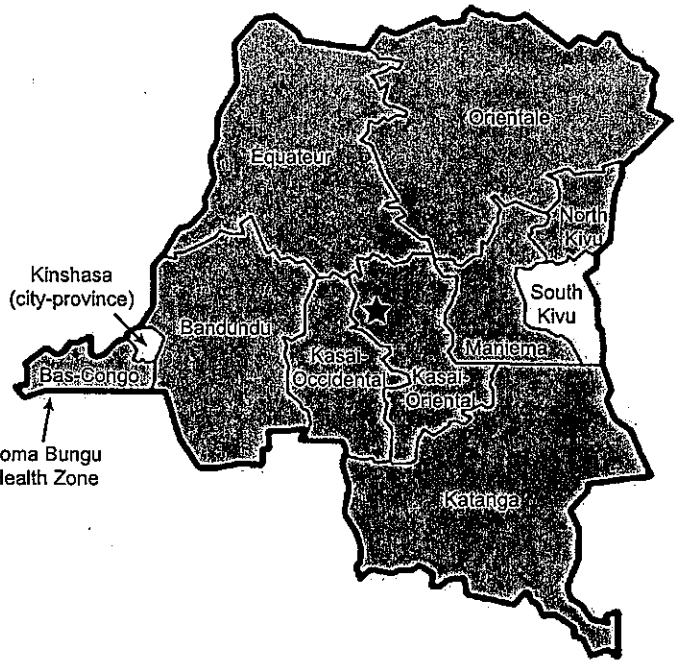


Figure 6. Detection of antibodies to BASV by serum neutralization of VSVΔG-GFP pseudotypes. Infectivities of VSVΔGFP pseudotypes bearing the glycoproteins of VSV or BASV, respectively, after incubation with 5-fold serial dilutions (1:10, 1:50, 1:250, 1:1,250, 1:6,250, 1:31,250) of sera from six individuals are depicted as percent of infectivity in the absence of serum. The six individuals tested include a patient with hemorrhagic fever (panel A, "Patient 3"), the nurse directly caring for him (panel F, "Contact 5"), and other health care workers in Mangala village (panels B–E). All data points represent the average of triplicate assays; error bars indicate standard deviations. Similar results were obtained in an independent experiment using murine leukemia virus (MLV)-based pseudotypes (data not shown).
doi:10.1371/journal.ppat.1002924.g006

addition, the recent discovery of Moussa virus (MOUV), isolated from *Culex* mosquitoes in Cote d'Ivoire, Africa [23], implies the presence of hitherto unknown arthropod vectors for rhabdoviruses on the continent. Nevertheless, at present, we cannot exclude the possibility of other zoonotic sources for the virus or even nosocomial bloodborne transmission (as Patients 1 and 2 have not clearly been established to be BASV cases by serology or direct detection), and the natural reservoir and precise mode of transmission for BASV remain unknown. A community-based serosurvey in Boma Bungu and an investigation to track down potential arthropod or mammalian (e.g. rodents and bats) sources for BASV are currently underway.

Although we cannot exclude the possibility of independent arthropod-borne transmission events, our epidemiologic and serologic data do suggest the potential for limited human-to-human transmission of BASV. Patient 3, a nurse, had directly taken care of Patients 1 and 2 at the health center, and another nurse (Contact 5), who had taken care of Patient 3 (but not

A



Provinces in DRC, Africa reporting unknown hemorrhagic fever cases or outbreaks from 2008-2010

★ Geographical origin of 50 serum samples from DRC, Africa from randomly selected healthy blood donors tested for neutralizing antibodies to BASV

B

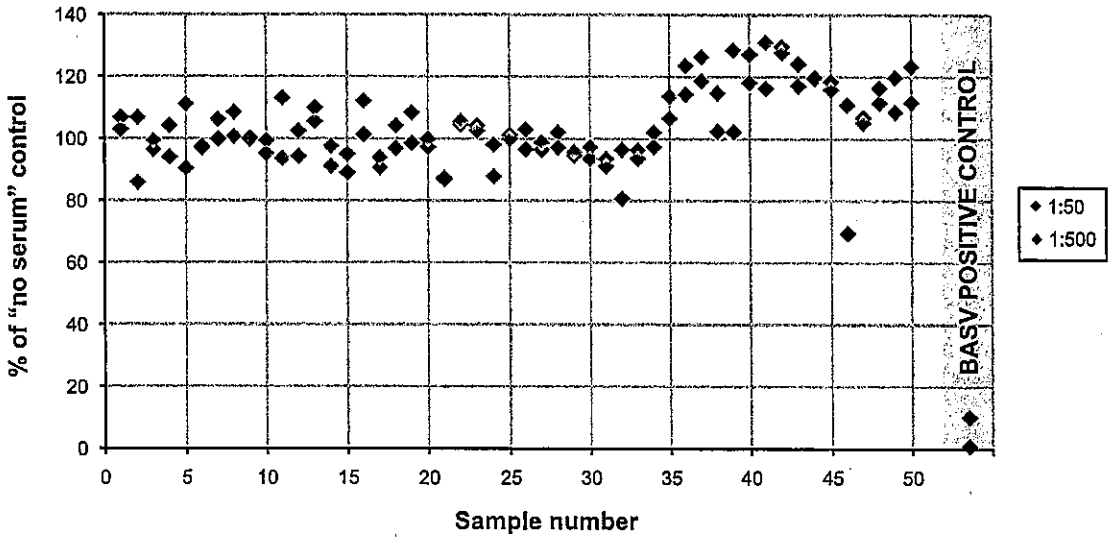


Figure 7. BASV Screening in DRC, Africa. (A) All 43 serum samples corresponding to unknown hemorrhagic fever cases or outbreaks in 2008–2010 from 9 provinces in DRC (pink) tested negative for BASV by PCR. (B) Sera from 50 donors in Kasai-Oriental province, DRC (Panel A, star) were tested for BASV-neutralizing antibodies. Sera at 1:50 (dark blue) or 1:500 dilution (light blue) were tested. Serum from the surviving Patient 3 was included as a positive control (grey shaded area). Data points represent an average of duplicate assays.
doi:10.1371/journal.ppat.1002924.g007

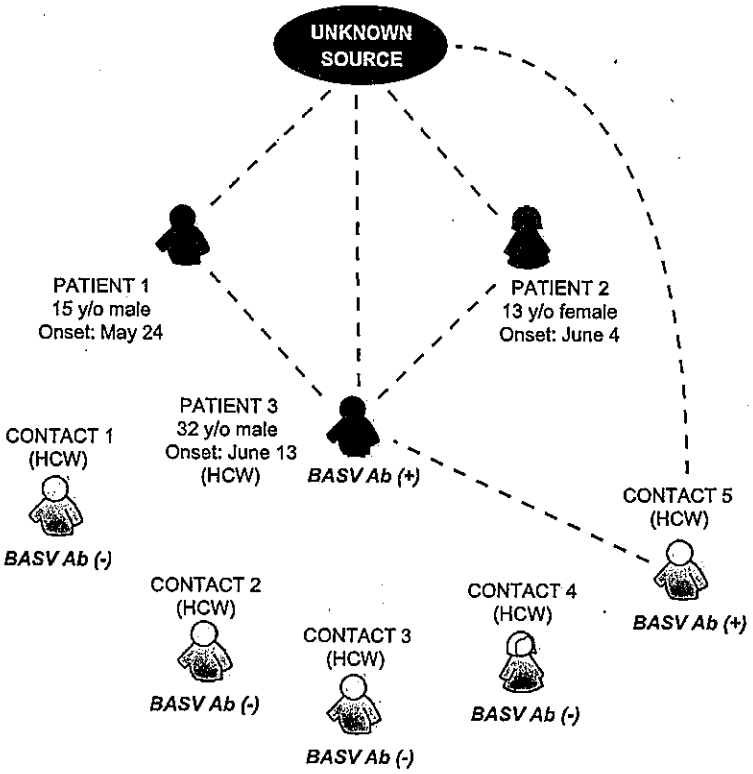


Figure 8. Proposed model for BASV transmission during the hemorrhagic fever outbreak in Mangala. Patients presenting with symptoms of acute hemorrhagic fever are depicted in red. Dashed red lines represent potential routes of BASV transmission. Contacts 1 through 5 are health care workers at the local health center in Mangala village. Abbreviations: HCW, health care worker; y/o, year-old; Ab, antibody.
doi:10.1371/journal.ppat.1002924.g008

Patients 1 or 2) had serologic evidence of asymptomatic BASV infection. We present a hypothetical model for BASV transmission during the hemorrhagic fever outbreak in which the initial infection of two children in Mangala (Patients 1 and 2) was followed by successive human-to-human transmission events involving two healthcare workers (Patient 3 and Contact 5) (Fig. 8). This pattern of transmission from the community to health care workers is also commonly seen in association with outbreaks of Ebola and Crimean-Congo hemorrhagic fever [6,34].

While rhabdoviruses are distributed worldwide, some authors have suggested that the *Rhabdoviridae* family probably originated from tropical regions of the Old or New World [9]. The discovery of BASV in Central Africa suggests that additional rhabdoviruses of clinical and public health importance likely await identification, especially in these poorly investigated geographic regions. Active epidemiological investigation and disease surveillance will be needed to fully ascertain the clinical and public health significance of BASV infection in humans, as well as to prepare for potentially larger human outbreaks from this newly discovered pathogen.

Methods

Ethics Statement

Written informed consent for publication of their case reports was obtained from the sole survivor of the hemorrhagic fever outbreak and the parents of the two deceased children. Written informed consent was obtained from the surviving patient and 5 of his close contacts for analysis of the serum samples reported in this study. Samples were analyzed under protocols approved by the institutional review boards of University of California, San Francisco, the University of Texas Medical Branch, and the National Institute of Biomedical Research (INRB) and CIRMF in Gabon, and the Institutional Animal Care and Use Committee (IACUC) of the University of Texas Medical Branch.

Diagnostic Samples

No diagnostic samples were available from Patient 1 or Patient 2. Blood was collected in a red top serum tube from Patient 3 on June 16, during the acute phase, three days after hemorrhagic onset. The sample was transported at 4°C to the BSL-4 facility at CIRMF. Serum was obtained by centrifugation at 2300 rpm for 10 min. No other acute samples from Patient 3 were available. In January of 2012 (~2.5 years after the outbreak), convalescent sera were collected from Patient 3 and close contacts (other workers at the health center) for BASV neutralization testing. Forty-three serum samples from other unknown hemorrhagic fever cases or outbreaks representing 9 of 11 provinces in the DRC were available for BASV PCR testing (Fig. 7A). Fifty available plasma samples from random blood donors (median age 27.5 years; age range 1–76 years) in Kasai Oriental province, DRC, were also tested for antibodies to BASV (Fig. 7A and B; Table S2).

Nucleic Acid Extraction and Viral PCR Testing

RNA was extracted from 140 µl of serum using the QIAamp viral RNA mini kit (Qiagen). Taqman real-time reverse-transcription-PCR (RT-PCR) testing for known hemorrhagic fever viruses was performed using primers and probes specific for Marburg

virus (MARV), all four species of Ebola virus (Zaire, ZEBOV; Sudan, SEBOV; Côte d'Ivoire, CIEBOV, and Bundibugyo, BEBOV), Crimean-Congo hemorrhagic fever virus (CCHFV), Yellow fever virus (YFV), Dengue virus (DENV), Rift Valley fever virus (RVFV) and Chikungunya virus (CHIKV) (available upon request).

Discovery of the BASV Rhabdovirus by 454 Pyrosequencing

200 µL of serum sample were inactivated in 1 mL of TRIzol (Invitrogen), and nucleic acid extraction and purification were performed according to the manufacturer's instructions. Roche 454 pyrosequencing using randomly amplified cDNA libraries was performed as described previously [35]. Viral sequences were identified using BLASTn or BLASTx by comparison to the GenBank nonredundant nucleotide or protein database, respectively (E-score cutoff = 10^{-5}).

De novo Assembly of the BASV Genome by Illumina Sequencing

To recover additional BASV sequence, two sets of cDNA libraries were prepared from DNase-treated extracted RNA using a random PCR amplification method as described previously [36], or random hexamer priming according to the manufacturer's protocol (Illumina). The libraries were then pooled and sequenced on two lanes of an Illumina HiSeq 2000. Raw Illumina sequences consisting of 100 base pair (bp) paired-end reads were filtered to exclude low-complexity, homopolymeric, and low-quality sequences, and directly compared using BLASTn or BLASTx alignments to a library consisting of all rhabdovirus sequences in GenBank. The initial read obtained by 454 pyrosequencing as well as other reads aligning to rhabdoviruses were then inputted as "seeds" into the PRICE *de novo* assembler [37] (Fig. 2), with a criterion of at least 85% identity over 25-bp to merge two fragments. *De novo* assembly of the BASV genome was performed iteratively using PRICE and the Geneious software package (Biomatters) [38]. The near-complete whole genome sequence of the novel rhabdovirus (~98.2% based on protein homology to other rhabdoviruses) was determined to at least 3× redundancy by *de novo* assembly as well as PCR and Sanger sequencing of low-coverage regions. Sanger sequencing was also performed to verify the accuracy of the assembly and confirm the genomic organization of BASV (Fig. 2).

Deep Sequencing Analysis of the BASV Serum Sample for Other Pathogens

Rapid classification of the ~140 million 100-bp paired-end Illumina reads was performed using a modified cloud computing-based computational analysis pipeline [17] (Veeraraghavan, Sittler, and Chiu, manuscript in preparation). Briefly, reads corresponding to human sequences were taxonomically classified using SOAP and BLAT software [39,40]. Other reads were then identified using BLASTn or BLASTx by comparison to GenBank-derived reference databases (E-score cutoff = 10^{-5}).

PCR Quantitation of BASV Burden

To estimate the viral load in the patient's serum, we first designed a set of specific PCR primers for detection of BASV targeting the L protein, BASV-F (5′- CGCTGATGGTTTTT-GACATGGAAGTCC-3′)/BASV-R (5′-TAAACTTCCTCTC-TCCTCTAG-3′), for use in a SYBR-Green real-time quantitative RT-PCR assay. A standard curve for the assay was constructed as described previously [36]. The viral load in the patient's serum was determined by comparison to the standard curve.

Structural Features and Phylogenetic Analysis

Predicted open reading frames (ORFs) in the BASV genome were identified with Geneious [38]. Multiple sequence (Figs. 3 and 4; Fig. S1) and pairwise (Fig. 5) alignments of BASV proteins relative to corresponding proteins from other rhabdoviruses were calculated using MAFFT (v6.0) with the E-INS-i option and at default settings [41]. To generate the phylogeny trees, all rhabdoviruses in GenBank were included as well as representative members of other families within the order *Mononegavirales*. Bayesian tree topologies were assessed with MrBayes V.32 software (20,000 sampled trees; 5,000 trees discarded as burn-in) [42]. Convergence was confirmed by the PSRF statistic in MrBayes, as well as by visual inspection of individual traces using TRACER from the BEAST software package [43]. Trees were visualized after midpoint rooting with FigTree V1.31 [43].

Virus Cultivation in Cell Cultures or Suckling Mice

Initial attempts were made to culture the virus using a total of 200 µL of BASV-positive serum inoculated onto confluent monolayers of Vero E6 and C6/36 (*Aedes albopictus* mosquito) cells in 6-well plastic tissue culture plates at 37°C and 28°C, respectively, in a 5% CO_2 environment as previously described [44]. From 20–50 µL of serum were used to inoculate the cells, which were examined daily for cytopathic effect (CPE) at days 5, 7, and 14. Supernatants were harvested and two additional blind passages were performed, each passage followed by 14 days of observation for CPE. Cell culture supernatants were also monitored for evidence of viral replication by quantitative RT-PCR.

Using the remaining 100 uL of BASV-positive serum, further attempts were made to culture the virus in 5 cell lines and in suckling mice. The serum sample was split in half and diluted 1:20 or 1:10 in phosphate-buffered saline with 20% fetal bovine serum (FBS) to allow sufficient volume to inoculate cell cultures or mice, respectively. The first diluted sample was inoculated intracerebrally into a litter (n = 12) of 1 day old mice. Pups were observed daily for 14 days for lethality or signs of clinical illness. The second diluted sample was inoculated into 12.5 cm^2 tissue culture flasks of Vero, BHK, LLC-MK_2 (rhesus monkey kidney), CCL-106 (rabbit kidney) and C6/36 cells. Vertebrate cells were held at 37°C for 14 days and observed for evidence of CPE. Mosquito cells were maintained at 28°C for 10 days. Since no CPE was observed in any of the cultures, cells were subsequently fixed for transmission electron microscopy to see if viral particles could be visualized [45].

Construction of VSVΔG-GFP Pseudotypes and BASV Serum Neutralization Testing

A pseudotype system based on a vesicular stomatitis virus (VSV) construct carrying a reporter gene for green fluorescent protein (VSVΔG-GFP) and bearing the predicted synthesized BASV glycoprotein (BASV-G) was used to generate a serum neutralization assay for BASV. Briefly, the predicted BASV glycoprotein (BASV-G) was synthesized (Genscript) and subcloned into the pCAGGS expression plasmid. Human embryonic kidney 293T cells were seeded (DMEM + 10% FBS + penicillin/streptomycin + Glutamax (Gibco) + non-essential amino acids (Gibco)) in 10 cm culture dishes 24 hours prior to transfection. Cells were transfected with 20 µg BASV-G, VSV-G, or empty pCAGGS DNA per dish following a calcium phosphate transfection protocol [46]. The culture medium was replaced 15 hours post-transfection and cells were stimulated with 6.2 mM valproic acid for 4 hours before the medium was replaced again. At 36 hours post-transfection the transfected cells were infected with VSVΔG-GFP/VSV-G pseu-

dotypes at a multiplicity of 0.1–0.3. The inoculum was removed after 4 hours and replaced by fresh culture medium. At 24 hours post-infection, infectious supernatants were harvested, filtered through 0.45 µm filters, and concentrated 10-fold by centrifugation through a 100-kDA filter (Millipore). Concentrated viruses were aliquoted and stored at −80°C.

For serum neutralization testing, human hepatoma Huh-7 cells were seeded (DMEM +10% FBS + penicillin/streptomycin + Glutamax (Gibco) + non-essential amino acids (Gibco)) in 48-well plates 24 hours prior to infection. Per well 10 µl of pseudovirus harboring either BASV-G or VSV-G (adjusted to obtain 25–50% infection of target cells) was mixed with 10 µl of the respective serum dilution and incubated for 45 minutes at 37°C. Subsequently, the mix was added to the target cells (performed in triplicate) and cells were incubated for 24 hours at 37°C. The infected cells were detached with trypsin and washed with PBS before fixing with 2% paraformaldehyde for 1 hour at room temperature. GFP expression in infected cells was quantified by flow cytometry using a LSR II (BD Biosciences) and the collected data was analyzed with FlowJo software (TreeStar).

Abbreviations and Nucleotide Sequence Accession Numbers

The annotated, nearly complete sequence of BASV has been submitted to GenBank (accession number JX297815). Deep sequencing reads have been submitted to the NCBI Sequence Read Archive (accession number SRA056894). Accession numbers used for the phylogenetic analyses in Figs 3, 4, and S1 are listed as follows, in alphabetical order: ABLV, Australian bat lyssavirus (NP_478343); ARAV, Aravan virus (ABV03822), BEFV, Bovine ephemeral fever virus (NP_065409); BYSMV, Barley yellow striate mosaic virus (BYSMV); CDV, Canine distemper virus (AAR32274); CHPV, Chandipura virus (ADO63669); CPV, Coastal Plains virus (ADG86364); COCV, Cocal virus (ACB47438); DURV, Durham virus (ADB88761); DUVV, Duvenhage virus (ABZ81216); EBLV1, European bat lyssavirus 1 (ABZ81166), EBLV2, European bat lyssavirus 2 (ABZ81191); EBOV, Ebola virus (AAG40171, AAA79970, BAB69010); EVEX, Eel virus European X virus (CBH20130); FDLV, Fer-de-lance virus (NP_899661); FLAV, Flanders virus (AAN73288); HeV, Hendra virus (NP_047113); HIRRV, Hirame rhabdovirus (ACO87999); HMPV, Human metapneumovirus (L_HMPVC); HPIV-1, Human parainfluenza virus type 1 (AA A69579); HPIV-2, Human parainfluenza virus type 2 (CAA 40788); HPIV-3, Human parainfluenza virus type 3 (AAA46854); HPIV-4, Human parainfluenza virus type 4 (BAJ11747); INHV, Infectious hematopoietic necrosis virus (NP_042681); IRKV, Irkut virus (ABV03823); ISFV, Isfahan virus (CAH17548); KHUV, Khujand virus (ABV03824); LBV, Lagos bat virus (ABZ81171); LNYV, Lettuce necrotic yellows virus (YP_425092); MARAV, Maraba virus (AEI52253); MARV, Marburg virus (YP_001531159); MeV, Measles virus (AF266288); MMV, Maize mosaic virus (YP_052855); MOKV, Mokala virus (ABZ81206); MOUV, Moussa virus (ACZ81407); MUV, Mumps virus (AF 201473); NCMV, Northern cereal mosaic virus (NP_597914); NDV, Newcastle disease virus (ADH10207); NGAV, Ngaingan virus (YP_003518294); NiV, Nipah virus (AAY43917); OVRV, Oak Vale rhabdovirus (AEJ07657); PFRV, Pike fry rhabdovirus (ACP28002); RABV, Rabies virus (NP_056797); RSV, Respiratory syncytial virus (NP_056866); RYSV, Rice yellow stunt rhabdovirus (NP_620502); SIGMAV, Sigma virus (ACU65444); SCRV, Siniperca chuatsi rhabdovirus (YP_802942); SHRV, Snakehead virus (AAD56771); SMRV, Scophthalmus maximus rhadovirus (ADU05406); SVCV, Spring viremia of carp virus (NP_116748); SYNV, Sonchus yellow net virus (NP_044286); TIBV, Tibrogargan virus (ADG86355); TUPV, Tupaia virus (AAX47602); TVCV, Tomato vein clearing virus (YP_224083); VHSV, Viral hemorrhagic septicemia virus (NP_049550); VSIV, Vesicular stomatitis virus, Indiana (NP_041716); VSNJV, Vesicular stomatitis virus, New Jersey (P16379); WCBV, West Caucasian bat virus (ABV03821); WONGV, Wongabel virus (YP_002333280).

Supporting Information

Figure S1 Phylogenetic analysis of the N, P, M, and G proteins of BASV and other rhabdoviruses. Each phylogenetic tree is rooted by using the corresponding protein from human parainfluenza virus type 1 (HPIV-1), a paramyxovirus, as an outgroup. Abbreviations and accession numbers used for the phylogenetic analysis are provided in Methods.
(TIF)

Figure S2 Confirmation of laboratory contamination by rotavirus and absence of rotavirus in BASV serum by specific PCR. An RT-PCR assay for detection of Group A rotaviruses was performed using primers NSP3F (5′-ACCATCTWCACRTRACCCTCTATGAG-3′) and NSP3R (5′-GGTCACATAACGCCCCTATAGC-3′), which generate an 87-bp amplicon (Freeman, et al., (2008) J Med Virol 80: 1489–1496). PCR conditions for the assay were 30 min at 50°C, 15 min at 95°C for the reverse transcription step followed by 40 cycles of 95°C, 30 s/55°C, 30 s/72°C, 30 s and 72°C/7 min for the final extension. PCR products are visualized by gel electrophoresis, using a 2% agarose gel and 1 kB ladder. Rotavirus is readily detected in extracted RNA from a stool sample taken from an ongoing study of viral diarrhea in the laboratory (lane 1), but not in two separate aliquots of extracted nucleic acid from the BASV serum sample (lanes 2 and 3).
(TIF)

Table S1 Viral reads in the deep sequencing data corresponding to the BASV-positive serum sample.
(DOCX)

Table S2 Demographics of 50 blood donors from Kasai-Oriental province, DRC, randomly selected for BASV antibody screening.
(DOCX)

Acknowledgments

The authors thank the national and international teams involved in the control of suspected hemorrhagic fever cases that occurred in 2009 in Democratic Republic of the Congo (DRC). The national teams are members of the DRC Ministry of Health and the National Institute of Biomedical Research (INRB). The international teams are epidemiological and medical experts of the World Health Organization (WHO) and the NGO 'Médecins Sans Frontières'. We thank all those involved in sample collection and case reporting, especially Etienne Mukendi at the Cellule de Surveillance Epidemiologique, Bas-Congo, DRC. We are also grateful to A Délicat and P Engandja from Centre International de Recherches de Franceville (CIRMF), Gabon, Matthew LeBreton at Global Viral Forecasting, Incorporated, Nicole A. Hoff at University of California, Los Angeles, Thomas Geisbert at University of Texas Medical Branch, as well as Samia Naccache, Rick Hsu and Yasamin Mohammadi at University of California, San Francisco (UCSF), for technical assistance during this work.

Author Contributions

Conceived and designed the experiments: GG JNF DL GS ED NDW CYC EML. Performed the experiments: GG DL ES IS RBT. Analyzed the

data: GG JNF DL J-JM NV MM PM GS ED NDW CYC EML. Contributed reagents/materials/analysis tools: TS JGR CW RBT JM AWR TT BSS GS ED NDW CYC EML. Wrote the paper: GG JNF DL IS RBT GS ED NDW CYC EML. Obtained consents from patients and their families: PM.

References

1. Bray M (2005) Pathogenesis of viral hemorrhagic fever. Curr Opin Immunol 17: 399–403.
2. Carrion R, Jr., Patterson JL (2011) Vaccines against viral hemorrhagic fevers: non-human primate models. Hum Vaccin 7: 667–673.
3. Geisbert TW, Jahrling PB (2004) Exotic emerging viral diseases: progress and challenges. Nat Med 10: S110–121.
4. Borio L, Inglesby T, Peters CJ, Schmaljohn AL, Hughes JM, et al. (2002) Hemorrhagic fever viruses as biological weapons: medical and public health management. JAMA 287: 2391–2405.
5. Fichet-Calvet E, Rogers DJ (2009) Risk maps of Lassa fever in West Africa. PLoS Negl Trop Dis 3: e388.
6. Ergonul O (2006) Crimean-Congo haemorrhagic fever. Lancet Infect Dis 6: 203–214.
7. Leroy EM, Gonzalez JP, Baize S (2011) Ebola and Marburg haemorrhagic fever viruses: major scientific advances, but a relatively minor public health threat for Africa. Clin Microbiol Infect 17: 964–976.
8. Monath TP (2007) Dengue and yellow fever–challenges for the development and use of vaccines. N Engl J Med 357: 2222–2225.
9. Kuzmin IV, Novella IS, Dietzgen RG, Padhi A, Rupprecht CE (2009) The rhabdoviruses: biodiversity, phylogenetics, and evolution. Infect Genet Evol 9: 541–553.
10. Fu ZF (2005) Genetic comparison of the rhabdoviruses from animals and plants. Curr Top Microbiol Immunol 292: 1–24.
11. Gurav YK, Tandale BV, Jadi RS, Gunjikar RS, Tikute SS, et al. (2010) Chandipura virus encephalitis outbreak among children in Nagpur division, Maharashtra, 2007. Indian J Med Res 132: 395–399.
12. Warrell MJ, Warrell DA (2004) Rabies and other lyssavirus diseases. Lancet 363: 959–969.
13. Rodriguez LL (2002) Emergence and re-emergence of vesicular stomatitis in the United States. Virus Res 85: 211–219.
14. Tang P, Chiu C (2010) Metagenomics for the discovery of novel human viruses. Future Microbiol 5: 177–189.
15. Briese T, Paweska JT, McMullan LK, Hutchison SK, Street C, et al. (2009) Genetic detection and characterization of Lujo virus, a new hemorrhagic fever-associated arenavirus from southern Africa. PLoS Pathog 5: e1000455.
16. Feng H, Shuda M, Chang Y, Moore PS (2008) Clonal integration of a polyomavirus in human Merkel cell carcinoma. Science 319: 1096–1100.
17. Greninger AL, Chen EC, Sittler T, Scheinerman A, Roubinian N, et al. (2010) A metagenomic analysis of pandemic influenza A (2009 H1N1) infection in patients from North America. PLoS One 5: e13381.
18. Towner JS, Rollin PE, Bausch DG, Sanchez A, Crary SM, et al. (2004) Rapid diagnosis of Ebola hemorrhagic fever by reverse transcription-PCR in an outbreak setting and assessment of patient viral load as a predictor of outcome. J Virol 78: 4330–4341.
19. Allison AB, Palacios G, Travassos da Rosa A, Popov VL, Lu L, et al. (2011) Characterization of Durham virus, a novel rhabdovirus that encodes both a C and SH protein. Virus Res 155: 112–122.
20. Gubala AJ, Proll DF, Barnard RT, Cowled CJ, Crameri SG, et al. (2008) Genomic characterisation of Wongabel virus reveals novel genes within the Rhabdoviridae. Virology 376: 13–23.
21. Springfeld C, Darai G, Cattaneo R (2005) Characterization of the Tupaia rhabdovirus genome reveals a long open reading frame overlapping with P and a novel gene encoding a small hydrophobic protein. J Virol 79: 6781–6790.
22. Gubala A, Davis S, Weir R, Melville L, Cowled C, et al. (2011) Tibrogargan and Coastal Plains rhabdoviruses: genomic characterization, evolution of novel genes and seroprevalence in Australian livestock. J Gen Virol 92: 2160–2170.
23. Quan PL, Junglen S, Tashmukhamedova A, Conlan S, Hutchison SK, et al. (2010) Moussa virus: a new member of the Rhabdoviridae family isolated from Culex decens mosquitoes in Cote d'Ivoire. Virus Res 147: 17–24.
24. Fu ZF (2005) The world of rhabdoviruses. Berlin; New York: Springer. vi, 208 p.
25. Brooks JT, Shapiro RL, Kumar L, Wells JG, Phillips-Howard PA, et al. (2003) Epidemiology of sporadic bloody diarrhea in rural Western Kenya. Am J Trop Med Hyg 68: 671–677.
26. (2011) Vital signs: incidence and trends of infection with pathogens transmitted commonly through food–foodborne diseases active surveillance network, 10 U.S. sites, 1996–2010. MMWR Morb Mortal Wkly Rep 60: 749–755.
27. Daniels NA, MacKinnon L, Rowe SM, Bean NH, Griffin PM, et al. (2002) Foodborne disease outbreaks in United States schools. Pediatr Infect Dis J 21: 623–628.
28. Rangel JM, Sparling PH, Crowe C, Griffin PM, Swerdlow DL (2005) Epidemiology of Escherichia coli O157:H7 outbreaks, United States, 1982–2002. Emerg Infect Dis 11: 603–609.
29. Hoffmann B, Beer M, Schutze H, Mettenleiter TC (2005) Fish rhabdoviruses: molecular epidemiology and evolution. Curr Top Microbiol Immunol 292: 81–117.
30. (2004) Imported Lassa fever–New Jersey, 2004. MMWR Morb Mortal Wkly Rep 53: 894–897.
31. Leroy EM, Baize S, Volchkov VE, Fisher-Hoch SP, Georges-Courbot MC, et al. (2000) Human asymptomatic Ebola infection and strong inflammatory response. Lancet 355: 2210–2215.
32. Levi JE, Tateno AF, Machado AF, Ramalho DC, de Souza VA, et al. (2007) Evaluation of a commercial real-time PCR kit for detection of dengue virus in samples collected during an outbreak in Goiania, Central Brazil, in 2005. J Clin Microbiol 45: 1893–1897.
33. Stanifer ML, Cureton DK, Whelan SP (2011) A recombinant vesicular stomatitis virus bearing a lethal mutation in the glycoprotein gene uncovers a second site suppressor that restores fusion. J Virol 85: 8105–8115.
34. Guimard Y, Bwaka MA, Colebunders R, Calain P, Massamba M, et al. (1999) Organization of patient care during the Ebola hemorrhagic fever epidemic in Kikwit, Democratic Republic of the Congo, 1995. J Infect Dis 179 Suppl 1: S268–273.
35. Victoria JG, Kapoor A, Li L, Blinkova O, Slikas B, et al. (2009) Metagenomic analyses of viruses in stool samples from children with acute flaccid paralysis. J Virol 83: 4642–4651.
36. Chen EC, Yagi S, Kelly KR, Mendoza SP, Maninger N, et al. (2011) Cross-species transmission of a novel adenovirus associated with a fulminant pneumonia outbreak in a new world monkey colony. PLoS Pathog 7: e1002155.
37. Earl D, Bradnam K, St John J, Darling A, Lin D, et al. (2011) Assemblathon 1: a competitive assessment of de novo short read assembly methods. Genome Res 21: 2224–2241.
38. Drummond A, Ashton B, Cheung M, Heled J, Kearse M, et al. (2010) Geneious v5.3.4. Geneious website. Available from http://www.geneious.com. Accessed 2012 Aug 16.
39. Kent WJ (2002) BLAT–the BLAST-like alignment tool. Genome Res 12: 656–664.
40. Li R, Yu C, Li Y, Lam TW, Yiu SM, et al. (2009) SOAP2: an improved ultrafast tool for short read alignment. Bioinformatics 25: 1966–1967.
41. Katoh K, Kuma K, Toh H, Miyata T (2005) MAFFT version 5: improvement in accuracy of multiple sequence alignment. Nucleic Acids Res 33: 511–518.
42. Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, et al. (2012) MrBayes 3.2: Efficient Bayesian Phylogenetic Inference and Model Choice Across a Large Model Space. Syst Biol 61: 539–42.
43. Drummond AJ, Rambaut A (2007) BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evol Biol 7: 214.
44. Grard G, Biek R, Muyembe Tamfum JJ, Fair J, Wolfe N, et al. (2011) Emergence of divergent zaire ebola virus strains in democratic republic of the congo in 2007 and 2008. J Infect Dis 204 Suppl 3: S776–784.
45. Mihindukulasuriya KA, Nguyen NL, Wu G, Huang HV, da Rosa AP, et al. (2009) Nyamanini and midway viruses define a novel taxon of RNA viruses in the order Mononegavirales. J Virol 83: 5109–5116.
46. Kingston RE, Chen CA, Rose JK (2003) Calcium phosphate transfection. Curr Protoc Mol Biol Chapter 9: Unit 9 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	新医薬品等の区分	総合機構処理欄
				2012. 10. 20	該当なし	

一般的名称	新鮮凍結人血漿	研究報告の公表状況		公表国
販売名(企業名)	新鮮凍結血漿-LR「日赤」(日本赤十字社) 新鮮凍結血漿-LR「日赤」成分採血(日本赤十字社) 新鮮凍結血漿-LR「日赤」120(日本赤十字社) 新鮮凍結血漿-LR「日赤」240(日本赤十字社) 新鮮凍結血漿-LR「日赤」480(日本赤十字社)		ProMED 20120929.1315179	オーストラリア

研究報告の概要

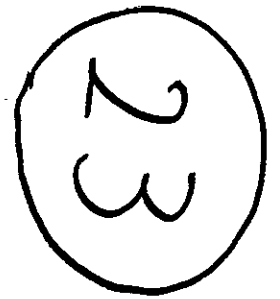
○ロスリバーウイルスーオーストラリア(西オーストラリア州)
今年(2012年)西オーストラリア州で重大な蚊媒介性疾患が流行しており、ロスリバーウイルス感染者については3年前の5倍に増加している。生涯継続する後遺症を遺すこともあるこのウイルスに、2011年から2012年にかけて州全域で1,570人が感染した。マンジュラの衛生官は、2011年ー2012年の蚊のシーズンは史上最悪であるとした。西オーストラリア州のロスリバーウイルス感染者は2009年ー2010年の332人から2010年ー2011年には2倍以上の770人になった。過去2年間、ラニーニャ現象によって蚊が多く発生したことが原因であるとのこと。

使用上の注意記載状況・その他参考事項等

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

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
西オーストラリア州においてロスリバーウイルス感染者が3年前の5倍に増加しているとの報告である。	日本赤十字社では、輸血感染症対策として問診時に海外滞在歴の有無を確認し、帰国(入国)後4週間は献血不適としている。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。



Published Date: 2012-09-29 11:34:14
Subject: PRO/AH/EDR> Ross River virus - Australia (05): (WA)
Archive Number: 20120929.1315179

ROSS RIVER VIRUS - AUSTRALIA (05): (WESTERN AUSTRALIA)
****

A ProMED-mail post
http://www.promedmail.org
ProMED-mail is a program of the
International Society for Infectious Diseases
http://www.isid.org

Date: Sat 29 Sep 2012

Source: The West [edited]

http://au.news.yahoo.com/thewest/a/-/breaking/14991504/huge-rise-in-ross-river-cases/

Outbreaks of a serious mosquito-borne disease have exploded in WA [Western Australia state] this year [2012], with 5 times more people contracting Ross River virus than 3 years ago. The virus, which can leave victims with a lifetime of debilitating symptoms and side-effects, infected 1570 people across the State in 2011-2012.

Public health officials in Mandurah branded the 2011-2012 mosquito season the worst on record.

WA cases of Ross River virus reached 332 in 2009-2010 and the number more than doubled to 770 in 2010-2011.

Department of Health entomologist Peter Neville said there had been more mosquitoes over the past 2 years. "It's largely to do with weather events," he said. "Over the last 2 years we have been under La Nina weather conditions." Those conditions meant more rain and higher minimum temperatures, leading to more mosquitoes. He said there was a spike in Ross River virus cases every 3-4 years.

Infected people get a fever, headaches, rashes and painful, swollen joints. "In some cases it can last up to 12 months," Dr Neville said. "In some people, it can be quite devastating. The virus can reduce people's capacity to work. It's quite debilitating."

A report to the City of Mandurah this week revealed the council has struggled against mosquitoes. "The continuation of the La Nina weather event resulted in local weather and tide behaviour that made mosquito management very difficult due to consistent inundation of breeding sites and the frequent hatching of salt-marsh mosquito larvae," environmental health officer Brendan Ingle wrote.

Ross River virus cases in the Peel region soared from 68 in 2009-2010 to 206 in 2011-2012.

Mandurah residents complained this week that swarms of mosquitoes make it impossible for them to go outside and warned the city's reputation was being harmed.

Mandurah mayor Paddi Creevey said the council had quadrupled the amount of insecticide sprayed to kill mosquito larvae. "What we can't control is the

El Nino/La Nina effect, and when those tides stay up and they inundate the breeding areas, no amount of spraying will kill them," she said.

People are urged to be especially vigilant about mosquitoes at dawn and dusk. [They are advised to] wear long, loose clothing and apply insect repellent.

[Byline: Angela Pownall]

--

Communicated by:

ProMED-mail Rapporteur Kunihiko Iizuka

[Ross River virus infections in Western Australia have increased significantly in 2012. In the 1st 2 months of 2012, there were 511 cases state-wide. The above report indicates that there have been 1570 cases in the 2011-2012 transmission season. Ross River virus is a zoonotic alphavirus transmitted by a wide range of mosquitoes including _Aedes_ and _Culex_species, and causes acute polyarthritis in humans. - Mod.TY

A HealthMap/ProMED-mail map can be accessed at: http://healthmap.org/r/21Bk.]

See Also

Ross River virus - Australia (04) (VI) 20120421.1109313
Ross River virus - Australia (03): (VI) 20120419.1107581
Ross River virus - Australia (WA, TA) 20120325.1079874
Ross River virus - Australia (WA) 20120302.1059212
.....jw/ty/mj/ejp/jw

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
				2013. 2. 20	該当なし	

一般的名称	新鮮凍結人血漿	研究報告の公表状況		公表国
販売名(企業名)	新鮮凍結血漿-LR「日赤」(日本赤十字社) 新鮮凍結血漿-LR「日赤」成分採血(日本赤十字社) 新鮮凍結血漿-LR「日赤」120(日本赤十字社) 新鮮凍結血漿-LR「日赤」240(日本赤十字社) 新鮮凍結血漿-LR「日赤」480(日本赤十字社)		ProMED 20130215.1544648	オーストラリア

研究報告の概要

○オーストラリアコウモリリッサウイルスーオーストラリア
クイーンズランド州北部在住の8歳の男児が、恐らくオーストラリアコウモリリッサウイルスを保有しているコウモリまたはオオコウモリにより咬傷あるいは擦過傷を受け、ウイルスに感染し、現在危篤状態である。これは狂犬病様のオーストラリアコウモリリッサウイルスに感染した3人目の確定症例である。過去(1996年と1998年)に感染した2人は死亡した。
ウイルスへの暴露から発症までの期間は様々であり、ヒトにおける既知の2症例のうち、1例はコウモリに咬まれてから数週間後、もう1例は2年以上経過してから発症している。このウイルスは麻痺、せん妄、痙攣をもたらし、通常呼吸麻痺により死亡する。ヒトーヒト感染は理論上可能であるが、極めて起こりにくいと考えられる。男児の家族は現時点で症状は見られていないが、予防措置を受けた。
クイーンズランド州の保健担当官は、全てのオーストラリアのコウモリがこのウイルスを保有している可能性があり、ウイルスからの最良の防護策はコウモリやオオコウモリとの接触を避けることであると述べた。

使用上の注意記載状況・その他参考事項等

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

血液を介するウイルス、
細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
オーストラリアのクイーンズランド州在住の男児がオーストラリアコウモリリッサウイルスに感染し重篤となっており、これはこのウイルスへの感染が確認された3例目であるとの報告である。	日本赤十字社では、輸血感染症対策として受付時に海外滞在歴の有無を確認し、帰国(入国)後4週間は献血不適としているほか、発熱などの体調不良者を献血不適としている。また、動物に噛まれた場合は傷が治癒してから3カ月間献血不適としている。今後も情報の収集に努める。

Published Date: 2013-02-15 17:55:17
Subject: PRO/AH/EDR> Australian bat lyssavirus - Australia: (QL) 3rd victim
Archive Number: 20130215.1544648

AUSTRALIAN BAT LYSSAVIRUS - AUSTRALIA: (QUEENSLAND) THIRD VICTIM
****

A ProMED-mail post
http://www.promedmail.org
ProMED-mail is a program of the
International Society for Infectious Diseases
http://www.isid.org

Date: Fri 15 Feb 2013
Source: World News, AAP report [edited]
http://www.sbs.com.au/news/article/1737424/Qld-boy-ill-with-rabies-like-bat-virus

Queensland boy ill with rabid-like virus

A north Queensland boy was likely bitten or scratched by a bat or a flying fox carrying Australian bat lyssavirus, and he's now critically ill in hospital. An 8-year-old Queensland boy is critically ill with the bat-borne virus that causes fits [seizures], paralysis and death. It's only the 3rd confirmed case of the rabies-like Australian bat lyssavirus recorded in the country. The other 2 victims, both infected in Queensland, died.

It's assumed the north Queensland boy was bitten or scratched by a bat or a flying fox carrying the deadly virus. It's believed he was infected about 2 months ago and a few weeks ago developed a brain infection that led to fits [seizures]. He's now so unwell he cannot give doctors any clues about how he came to be infected. "We're not sure of the circumstances because the child is now too sick to tell us," Queensland Chief Health Officer Jeannette Young told reporters on Friday [15 Feb 2013].

"He's critically unwell. The previous 2 cases did not survive and the vast majority of people who contract rabies [rabies virus and Australian bat lyssavirus are distinct virus species - Mod.CP] overseas die, unfortunately. "The other 2 cases were recorded in 1996 and 1998.

Dr Young said the family was incredibly distressed given the prognosis for the boy. The time from exposure to the virus to the development of symptoms is variable. Of the 2 known human cases, one became ill several weeks after being bitten by a bat while the other became ill more than 2 years after a bat bite. The virus causes paralysis, delirium and convulsions. Death is usually caused by respiratory paralysis. It's theoretically possible that the virus could be passed from human to human but that is considered very unlikely. And so far the boy's family has not shown any signs of symptoms but they've been given post-exposure drugs [rabies virus vaccine?] as a safeguard.

Dr Young said it's assumed any bat in Australia could carry the disease, and bat behaviour is not an accurate guide to infection. She said the best protection against the virus was to avoid handling any bat or flying fox. "Only people who have been trained in the care of bats, and who have been vaccinated against rabies, should ever handle bats or flying foxes," she said. "It is important to also encourage young children to never handle bats, particularly if they should come across a sick or injured one."

Federal MP Bob Katter, who holds the north Queensland seat of Kennedy, says it's time to revisit the idea of culling bats. The independent MP has long supported culling because bats spread disease, ruin farmers' crops and are a pest. He says Premier Campbell Newman has broken a pre-election promise to do something about bat colonies that have invaded some Queensland towns. "Clearly the Liberal National Party puts the welfare of bats over the

lives of human beings," Mr Katter told reporters on Friday. He said bat populations were out of control thanks to laws preventing farmers and others from killing them.

--
Communicated by:
Gert van der Hoek
Senior Moderator
FluTrackers.com
http://www.flutrackers.com/forum/index.php

[Gert van der Hoek is thanked for drawing attention to this report.

Australian bat lyssavirus [ABLV} is classified as a district species in the genus _Lyssavirus_ of the family _Rhabdoviridae_. It is closely related to rabies virus, but restricted to bats. It is antigenically similar enough to be neutralised by standard anti-rabies virus vaccine which can be used for post-exposure prophylaxis if administered before the onset of symptoms of disease. However in the present case in view of the lapse of time between exposure and appearance of symptoms it is unlikely that post-exposure prophylaxis could be successful.

According to Queensland Health (http://access.health.qld.gov.au/hid/InfectionsandParasites/ViralInfections/australianBatLyssavirus_fs.asp): Australian bat lyssavirus (ABLV) is a virus that can be transmitted from bats to humans, causing serious illness. The virus was 1st identified in 1996 and has been found in 4 kinds of flying foxes/fruit bats and one species of insect-eating microbat. Evidence of previous infection has been found in blood tests from a number of other bat species. It is therefore assumed that any bat in Australia could potentially carry the virus.

Since November 1996, 2 people have died as a result of ABLV infection after being bitten by bats. ABLV is one of 12 types of lyssavirus which are found around the world. ABLV is the only one of these known to occur in Australia. ABLV infection in humans causes a serious illness which results in paralysis, delirium, convulsions and death. Death is usually due to respiratory paralysis. Transmission of the virus from bats to humans is thought to usually be by a scratch or bite, but also potentially by being exposed to bat saliva through the eyes, nose or mouth (mucous membrane exposure). ABLV is unlikely to survive outside the bat for more than a few hours, especially in dry environments that are exposed to sunlight. The time from exposure to the virus to the development of symptoms is variable; of the 2 other known human cases of ABLV infection, one became ill several weeks after being bitten by a bat while the other became ill more than 2 years after a bat bite.

There is no available treatment for ABLV. In all potential exposures to ABLV (bites, scratches, mucous membrane exposures), seek medical advice immediately, even if you have been vaccinated. Proper cleansing of the wound is the single most effective measure for reducing transmission. If bitten or scratched, immediately wash the wound thoroughly with soap and water for at least 5 minutes. If available, an antiseptic with anti-virus action such as povidone-iodine, iodine tincture, aqueous iodine solution or alcohol (ethanol) should be applied after washing. If bat saliva contacts the eyes, nose or mouth, it is necessary to flush the area thoroughly with water. Seek medical attention as soon as possible. The best protection against being exposed to the virus is for members of the community to avoid handling any bat or flying fox.

Anyone who has been potentially exposed to ABLV, and has never received pre-exposure vaccination, will require an injection of rabies immunoglobulin and a series of 4 rabies vaccine injections over one month (on days zero, 3, 7, and 14). Queensland Health will fund these injections, which are called 'post-exposure prophylaxis,' and your local public health unit will arrange for these injections to be delivered to your GP or hospital. These injections are recommended for anyone who has been exposed to ABLV, regardless of how long ago the exposure occurred. People with a weakened immune system will require a further (5th) dose of vaccine given at day 28 and follow up blood tests to confirm their immunity. Post-exposure vaccination may be delayed for up to 48 hours if the bat is available for testing, without placing other people at risk of exposure.

A map of Australia, shooing the location of Queensland can be accessed at: http://mapsof.net/map/australia-states-rs01#.UR6fsaXEIac. - Mod.CP
A HealthMap/ProMED-mail map can be accessed at: http://healthmap.org/r/1z_*.]

See Also

2011

Australian bat lyssavirus - Australia (02): (VI) flying fox 20110714.2130
Australian bat lyssavirus - Australia: (VI) flying fox 20110526.1601
2010

Australian bat lyssavirus - Australia: (QL) flying fox, human exp., corr. 20100107.0074
Australian bat lyssavirus - Australia: (QL) flying fox, human exp 20100106.0061
2009

Australian bat lyssavirus, human, susp. - Australia (NSW) 20090320.1122
Australian bat lyssavirus, flying fox - Australia (QLD) 20041111.3050
.....cp/ejp/dk

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
				2012. 11. 19	該当なし	

一般的名称	新鮮凍結人血漿	研究報告の公表状況		公表国
販売名（企業名）	新鮮凍結血漿-LR「日赤」（日本赤十字社） 新鮮凍結血漿-LR「日赤」成分採血（日本赤十字社） 新鮮凍結血漿-LR「日赤」120（日本赤十字社） 新鮮凍結血漿-LR「日赤」240（日本赤十字社） 新鮮凍結血漿-LR「日赤」480（日本赤十字社）		WHO Global Alert and Response (GAR); 8 October 2012	WHO

研究報告の概要

○コンゴ民主共和国におけるエボラアウトブレイク－最新情報
2012年10月7日現在、49人（確定患者は31人、可能性の高い患者は18人）のエボラ出血熱患者がコンゴ民主共和国で報告されている。うち24人（確定患者は10人、可能性の高い患者は14人）が死亡した。これらは同国のOrientale州、Haut Uélé地区のIsiroとViadanaから報告された。コンゴ民主共和国保健省は引き続き国の対策本部下で関係機関と連携して、感染連鎖のあらゆる可能性を調査し、アウトブレイクを止めるための適切な対策を講じている。最初の検体はウガンダウイルス研究所で検査され、ウイルスが確認された。CDCはアウトブレイクの早期にIsiroに検査施設を設置し、カナダ公衆衛生局は移動検査施設を用いて、現場での迅速な診断の支援を継続している。

使用上の注意記載状況・その他参考事項等

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

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
コンゴ民主共和国でエボラ出血熱のアウトブレイクがあり、2012年10月7日現在49人の患者が報告され、そのうち24人が死亡したとの報告である。	日本赤十字社では、輸血感染症対策として受付時に海外滞在歴の有無を確認し、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。今後も引き続き情報の収集に努める。

World Health Organization

Global Alert and Response (GAR)

Ebola outbreak in Democratic Republic of Congo – update

Share Print

8 OCTOBER 2012 - As of 7 October 2012, 49 cases (31 laboratory confirmed, 18 probable) with Ebola haemorrhagic fever (EHF) have been reported in the Democratic Republic of Congo (DRC). Of these, 24 have been fatal (10 confirmed, 14 probable).

The cases reported are from Isiro and Viadana health zones in Haut-Uélé district in Province Orientale.

Ministry of Health (MoH) continues to work with partners, under the National Task Force to identify all possible chains of transmission of the illness and ensure that appropriate measures are taken to interrupt transmission and stop the outbreak. The task force includes Médecins Sans Frontières (MSF); the International Federation of Red Cross and Red Crescent Societies (IFRC); US Agency for International Development (USAID); US Centers for Disease Control and Prevention (CDC); and the United Nations Children's Fund (UNICEF) and WHO.

Response operations continue in the areas of coordination; Infection Prevention and Control (IPC); surveillance and epidemiology; case management; public information and social mobilization; psychosocial support; anthropological analysis; and logistics.

WHO and the Global Outbreak Alert and Response Network (GOARN) have deployed experts to support operational response, including establishment of a field laboratory and in the area of infection prevention and control in health care settings.

Initial samples were tested and confirmed by Uganda Virus Research Institute (UVRI). CDC established a field laboratory in Isiro in the beginning of the outbreak and Public Health Agency of Canada (PHAC) is continuing to provide support on rapid diagnosis in the field with their mobile laboratory facilities in Isiro.

Ongoing activities in Isiro and neighbouring areas include: training of health care workers on IPC in health care facilities, provision of support on case management, strengthening surveillance, working with traditional healers in raising awareness about EHF, providing psychosocial support to affected families, and conducting outreach to schools.

With respect to this event, WHO does not recommend any travel or trade restrictions to be applied to the DRC.

General information on controlling infection of EHF in health-care settings

Human-to-human transmission of the Ebola virus is primarily associated with direct contact with blood and body fluids. Transmission to healthcare

Related links

Ebola haemorrhagic fever
Fact sheet

Interim infection control recommendations for care of patients with suspected or confirmed filovirus (Ebola, Marburg) haemorrhagic fever

workers has been reported when appropriate infection control measures have not been observed.

Health-care workers caring for patients with suspected or confirmed Ebola virus need to apply infection control measures to avoid any exposure to the patient's blood and body fluids and/or direct unprotected contact with the possibly contaminated environment. In addition, it is important that Standard Precautions, particularly hand hygiene, the use of gloves and other personal protective equipment, safe injection practices and other measures are applied to all patients in all health care settings at all times.

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
				2013. 2. 6	該当なし	

一般的名称	新鮮凍結人血漿	研究報告の公表状況		公表国
販売名(企業名)	新鮮凍結血漿-LR「日赤」(日本赤十字社) 新鮮凍結血漿-LR「日赤」成分採血(日本赤十字社) 新鮮凍結血漿-LR「日赤」120(日本赤十字社) 新鮮凍結血漿-LR「日赤」240(日本赤十字社) 新鮮凍結血漿-LR「日赤」480(日本赤十字社)		ProMED 20130205.1530748	バングラデシュ

研究報告の概要

○ニパウイルス脳炎ーバングラデシュ
2012ー13年の冬、バングラデシュで再流行した致死性のニパウイルスによって、2013年2月3日現在、感染者12人中10人(首都の2人を含む)が死亡した。バングラデシュの疫学疾病対策研究所(IEDCR)によると、死亡者はダッカ県、パブナ県、ナトール県から各2名、ラジバリ県、ジェナイダ県、ナオガオン県、ラジシャヒ県から各1名が報告されている。ダッカ県の死亡者2名は、ミメシング県バールカでナツメヤシの生ジュースを飲んだと報告している。IEDCRの担当官は、感染したコウモリの尿や唾液で汚染された生のナツメヤシジュースや果物を飲食しないように警告した。患者を介護する者も予防策をとる必要がある。
バングラデシュでのアウトブレイクではこれまでに感染者180人中139人が死亡している。

報告企業の意見	今後の対応
2012ー13年の冬にバングラデシュで再流行したニパウイルスにより、2013年2月3日現在、感染者12人中10人が死亡したとの報告である。	日本赤十字社では、輸血感染症対策として受付時に海外滞在歴の有無を確認し、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。今後も引き続き情報の収集に努める。

使用上の注意記載状況・その他参考事項等

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

血液を介するウイルス、
細菌、原虫等の感染
vCJD等の伝播のリスク

26

Published Date: 2013-02-05 17:56:11
Subject: PRO/AH/EDR> Nipah encephalitis, human - Bangladesh (03)
Archive Number: 20130205.1530748

NIPAH ENCEPHALITIS, HUMAN - BANGLADESH (03)
****
A ProMED-mail post
http://www.promedmail.org
ProMED-mail is a program of the
International Society for Infectious Diseases
http://www.isid.org

Date: Tue 5 Feb 2013
Source: Financial Express [edited]
http://www.thefinancialexpress-bd.com/index.php?ref=MjBfMDJfMDVfMTNfMV84OF8xNTkxODg=
After staging a comeback in the country this winter, deadly Nipah virus has so far claimed the lives of 10 people, including 2 in the capital, out of 12 infected as of 3 Feb [2013], reports UNB (United News of Bangladesh). Among the casualties, there have been 2 from each of Dhaka, Pabna, and Natore and one from each of Rajbari, Jhenaidah, Naogaon, and Rajshahi, according to Bangladesh's Institute of Epidemiology Diseases Control and Research (IEDCR). It says 2 of the victims of Dhaka consumed raw date palm juice from Bhaluka, Mymensingh.
Dr Mushtuq Hossain, the principal scientific officer (medical sociology) of IEDCR, told UNB on Monday [4 Feb 2013] that those died and who have been affected by the deadly Nipah virus consumed raw date palm juice. Mushtuq cautioned that no one should drink raw date palm juice and fruits that were partly eaten by animals earlier. "One should drink date juice after its proper boiling and eat raw fruit after washing it properly."
Asked about the symptoms of the disease, Mushtuq said high fever, senseless talking, acute chest pain, respiratory problems, and severe headache are the symptoms of the disease. "If any patient has those symptoms he or she should be admitted to nearby hospital for treatment." Mushtuq added that the attendants of the Nipah virus affected patients should take precautionary steps while taking care of the patients as the virus is contagious.
Human Nipah virus (NiV) infection, an emerging zoonotic disease, was 1st recognised in a large outbreak of 276 reported cases in Malaysia and Singapore from September 1998 through May 1999.
NiV is a highly pathogenic paramyxovirus belonging to genus Henipavirus. It is an enveloped RNA virus.
But now, according to Mushtuq, the disease is only found in Bangladesh. Some 139 patients out of 180 infected people so far died in the outbreak of the disease in Bangladesh.
-- communicated by: ProMED-mail from HealthMap alerts <promed@promedmail.org>

[The number of Nipah virus infections and deaths continues to slowly rise so far this year (2013). Curiously, the above report makes no mention of the source of palm sap or fruit contamination. The reservoir hosts of Nipah virus are giant fruit bats (_Pteropus_ species). They shed virus, particularly during the breeding season when pregnant or lactating. Because these sporadic cases occur in geographically scattered areas, increasing the degree of public awareness, and need for either boiling the sap or preventing access to sap collection vessels by the bats by placing barriers around the jars, is a major public health education challenge. - Mod.TY
An image of a _Pteropus_ fruit bat can be found at http://rpmedia.ask.com/ts?u=/wikipedia/commons/thumb/3/3d/Pteropus_giganteus_fg01.JPG/180px-Pteropus_giganteus_fg01.JPG.
A HealthMap/ProMED-mail map can be accessed at http://healthmap.org/r/1yvE.]

See Also

Nipah encephalitis - Bangladesh (02) 20130128.1518442
Nipah encephalitis - Bangladesh: 20130124.1513132
2012

Nipah encephalitis, human - Bangladesh (03): (JI): 20120212.1040138
Nipah encephalitis, human - Bangladesh (JI) (02), Susp.: 20120128.1024955

Nipah encephalitis, human - Bangladesh: (JI) 20120125.1022056
2011

Nipah encephalitis, human - Bangladesh: (RP) (05) 20110308.0756
.....sb/sh/ty/ejp/sh

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
				2013. 2. 15	該当なし	

一般的名称	新鮮凍結人血漿	研究報告の公表状況		公表国
販売名(企業名)	新鮮凍結血漿-LR「日赤」(日本赤十字社) 新鮮凍結血漿-LR「日赤」成分採血(日本赤十字社) 新鮮凍結血漿-LR「日赤」120(日本赤十字社) 新鮮凍結血漿-LR「日赤」240(日本赤十字社) 新鮮凍結血漿-LR「日赤」480(日本赤十字社)		Krause PJ, Narasimhan S, Wormser GP, Rollend L, Fikrig E, Lepore T, Barbour A, Fish D. N Engl J Med. 2013 Jan 17;368(3):291-3. doi: 10.1056/NEJMc1215469.	米国

研究報告の概要

○米国におけるヒト*Borrelia miyamotoi*感染

回帰熱を引き起こすスピロヘータの一種である*Borrelia miyamotoi*は、ライム病を媒介する全ダニ種から検出されている。2001年にコネチカット州でシカダニから検出されて以来、米国のライム病が浸淫している全地域で検出されるようになった。ヒトにおける初めての*B.miyamotoi*感染は2011年ロシアで報告された。現在、米国にも*B.miyamotoi*感染が存在する証拠及び感染率について報告する。

1990年～2010年、ライム病浸淫地域在住者の3群(第1群;ロードアイランド州及びマサチューセッツ州でダニ媒介性感染症の血清検査を受けた584人、第2群;ニューイングランド州南部でライム病が疑われた277人、第3群;ニューヨーク州南部でウイルス感染様症状を呈しライム病検査を行った14人)から採取した血清保管検体について、ELISA法とウエスタンブロット法を用いて*B.miyamotoi*のGlpQタンパク質抗体の検出を行った。

結果、抗体陽性率は第1群で1.0%、第2群で3.2%、第3群で21.0%であった(3群間の比較でP<0.001)。第2群の1人及び第3群の2人の回復期抗体価は、急性期の抗体価と比べて4倍以上であった。この所見から、これらの患者は最近*B.miyamotoi*に感染したと考えられる。この3人はいずれも免疫不全ではない。ウイルス感染様症状を呈していた症候性患者は全員、ドキシサイクリンまたはアモキシシリンが投与された。

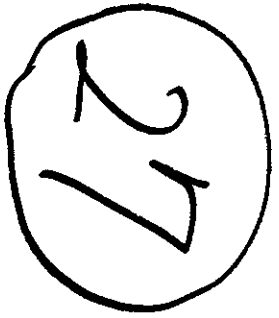
今回、18人から*B.miyamotoi*抗体が検出された。これは米国のライム病浸淫地域で*B.miyamotoi*感染が広がっている可能性を示唆している。

使用上の注意記載状況・その他参考事項等

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

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
米国のライム病が浸淫している地域の在住者における血清検体から、回帰熱を引き起こす*Borrelia miyamotoi*の抗体が検出され、*B.miyamotoi*感染がこれらの地域で広がっている可能性が示唆されるとの報告である。	国内外のダニ媒介性感染症について、今後も引き続き情報の収集に努める。



tory tract infections cautioning against their misuse.[3] Linezolid may become subject to similar misuse by physicians who prescribe it for the treatment of undiagnosed infections, as has been reported.[4] This observation is consistent with our own at a tertiary care hospital in India. In the recent guidelines on pneumonia from India, we have called for restrictions on the use of linezolid.[5] It is desirable that future guidelines on respiratory and other infections advise against its use early in the course of an infection.

Sahajal Dhooria, M.D.
Ritesh Agarwal, M.D., D.M.
Digamber Behera, M.D.

Postgraduate Institute of Medical Education and Research
Chandigarh, India
riteshpgi@gmail.com

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

1. Singla R, Caminero JA, Jaiswal A, et al. Linezolid: an effective, safe and cheap drug for patients failing multidrug-resistant tuberculosis treatment in India. Eur Respir J 2012;39:956-62.
2. Adriaenssens N, Coenen S, Versporten A, et al. European Surveillance of Antimicrobial Consumption (ESAC): outpatient quinolone use in Europe (1997-2009). J Antimicrob Chemother 2011;66:Suppl 6:vi47-vi56.
3. Migliori GB, Langendam MW, D'Ambrosio L, et al. Protecting the tuberculosis drug pipeline: stating the case for the rational use of fluoroquinolones. Eur Respir J 2012;40:814-22.
4. Aubin G, Lebland C, Corvec S, et al. Good practice in antibiotic use: what about linezolid in a French university hospital? Int J Clin Pharm 2011;33:925-8.
5. Gupta D, Agarwal R, Aggarwal AN, et al. Guidelines for diagnosis and management of community- and hospital-acquired pneumonia in adults: Joint ICS/NCCP(I) recommendations. Lung India 2012;29:Suppl 2:S27-S62.

DOI: 10.1056/NEJMc1214183

TO THE EDITOR: Lee et al. report that 82% of the patients with XDR tuberculosis who were treated with linezolid had drug-related toxicity. This prompted the authors to ask for careful drug monitoring by means of conventional blood analysis. Can we do more? Preliminary evidence[1-4] has suggested the potential relation between the pharmacokinetics of linezolid and its tolerability, providing the rationale for targeting linezolid dosage on the basis of its plasma concentrations — that is, therapeutic drug monitoring. Further study is warranted to determine whether therapeutic drug monitoring can serve as a predictive tool to improve the safety of patients requiring long-term therapy with linezolid.

Dario Cattaneo, Pharm.D., Ph.D.
Giovanna Orlando, M.D., Ph.D.
Laura Cordier, M.D.

Luigi Sacco University Hospital
Milan, Italy
orlando.giovanna@hsacco.it

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

1. Tsuji Y, Hiraki Y, Mizoguchi A, et al. Pharmacokinetics of repeated dosing of linezolid in a hemodialysis patient with chronic renal failure. J Infect Chemother 2008;14:156-60.
2. Matsumoto K, Takeshita A, Ikawa K, et al. Higher linezolid exposure and higher frequency of thrombocytopenia in patients with renal dysfunction. Int J Antimicrob Agents 2010;36:179-81.
3. Sasaki T, Takane H, Ogawa K, et al. Population pharmacokinetic and pharmacodynamic analysis of linezolid and a hematologic side effect, thrombocytopenia, in Japanese patients. Antimicrob Agents Chemother 2011;55:1867-73.
4. Pea F, Viale P, Cojutti P, Del Pin B, Zamparini E, Furlanut M. Therapeutic drug monitoring may improve safety outcomes of long-term treatment with linezolid in adult patients. J Antimicrob Chemother 2012;67:2034-42.

DOI: 10.1056/NEJMc1214183

Human *Borrelia miyamotoi* Infection in the United States

TO THE EDITOR: *Borrelia miyamotoi*, a spirochete that is genetically related to the species of borrelia that cause relapsing fever, has been detected in all tick species that are vectors of Lyme disease.[1,2] It was detected in *Ixodes scapularis* ticks from Connecticut in 2001 and subsequently has been detected in all areas of the United States where Lyme disease is endemic. The first human cases of *B. miyamotoi* infection were reported in Russia in 2011.[3] We now provide evidence of *B. miyamotoi* infection and the prevalence of this infection among people in the United States.

Enzyme-linked immunosorbent assays and confirmatory Western blot assays of archived serum samples obtained from three groups of patients who were living in areas where Lyme disease was endemic between 1990 and 2010 were used to detect antibody against *B. miyamotoi* GlpQ protein (an antigen that is nonreactive to *B. burgdorferi* antibody).[4] Group 1 consisted of 584 patients who participated in serologic surveys for tickborne infections on Block Island and Prudence Island, Rhode Island, and Brimfield, Massachusetts. Patients in the serologic survey were healthy at the time of blood sampling and were enrolled during the spring and autumn of each year. Group 2 included 277 patients from southern New England who were evaluated for

Table 1. Serologic and Clinical Characteristics of *Borrelia miyamotoi* Infection in Study Patients.*

Group, Patient No., and Serum Phase†	Assay Method			Coinfection‡	No. of Symptoms
	ELISA	Western Blot			
		IgM	*IgG*		
Group 1					
Patient 1	Positive at 1:320 dilution	Positive	Positive	None	None
Patient 2	Positive at 1:320 dilution	Positive	Negative	None	None
Patient 3	Positive at 1:320 dilution	Positive	Positive	None	None
Patient 4	Positive at ≥1:320 dilution§	Not done	Positive	None	None
Patient 5	Positive at ≥1:320 dilution§	Not done	Positive	None	None
Patient 6	Positive at 1:320 dilution	Positive	Positive	None	None
Group 2					
Patient 7	Positive at ≥1:320 dilution§	Not done	Positive	None	5
Patient 8	Positive at 1:320 dilution	Negative	Positive	None	9
Patient 9	Positive at 1:320 dilution	Negative	Positive	None	8
Patient 10	Positive at ≥1:320 dilution§	Not done	Positive	None	6
Patient 11	Positive at ≥1:320 dilution§	Not done	Positive	None	3
Patient 12	Positive at 1:1280 dilution	Negative	Positive	Lyme disease	4
Patient 13	Positive at 1:320 dilution	Negative	Positive	Lyme disease	Uncertain
Patient 14	Positive at 1:320 dilution	Positive	Positive	Lyme disease	Uncertain
Patient 15					
Acute	Negative at 1:160 dilution	Negative	Negative	Babesiosis	12
Convalescent	Positive at 1:1280 dilution	Positive	Positive		
Group 3					
Patient 16	Positive at 1:1280 dilution	Positive	Positive	None	5
Patient 17					
Acute	Negative at 1:80 dilution	Positive	Negative	None	10
Convalescent	Positive at 1:320 dilution	Positive	Positive		
Patient 18					
Acute	Negative at 1:80 dilution	Positive	Positive	Lyme disease	12
Convalescent	Positive at 1:320 dilution	Negative	Positive		

* ELISA denotes enzyme-linked immunosorbent assay.
† See the text for the definition of the various groups.
‡ The diagnosis of Lyme disease was based on a typical erythema migrans skin lesion in Patients 12, 13, 14, and 18. Patients 8 and 16 had an atypical erythema migrans skin lesion (<5 cm in diameter).
§ Tests to determine the presence of antibody in serum dilutions greater than 1:320 were not performed.

suspected Lyme disease. Group 3 consisted of 14 patients from southern New York who were evaluated at a Lyme disease clinic with a viral-like illness in the late spring or summer; these patients did not have symptoms or signs suggestive of an upper respiratory tract infection or gastroenteritis.

The seroprevalence was 1.0% in group 1, 3.2% in group 2, and 21.0% in group 3 ($P<0.001$ for comparisons among the three groups). In one patient in group 2 and two patients in group 3, the antibody titer was at least four times as high in the convalescent serum samples as in the acute serum samples; these findings suggest that these patients were recently infected with *B. miyamotoi* (Table 1). All symptomatic patients presented with a viral-like illness and were treated with doxycycline or amoxicillin. Unlike

the patient with well-documented *B. miyamotoi* infection described by Gagliotta et al.[5] elsewhere in this issue of the *Journal*, none of the three patients with evidence of recent *B. miyamotoi* infection in our study were immunocompromised. One patient had *B. miyamotoi* seroconversion and no erythema migrans skin lesion or laboratory evidence of human granulocytic anaplasmosis coinfection (Patient 17). This patient had a temperature of 39.4°C, chills, sweats, a headache, neck stiffness, fatigue, myalgias, arthralgias, abdominal pain, a cough, a sore throat, and right inguinal lymphadenopathy. He was treated successfully with 14 days of doxycycline. The identification of *B. miyamotoi* antibody in 18 of our study patients, including seroconversion associated with symptoms in 3 patients, suggests that *B. miyamotoi* infection may be prevalent in areas where Lyme disease is endemic in the United States.

Peter J. Krause, M.D.
Yale School of Public Health
New Haven, CT
peter.krause@yale.edu

Sukanya Narasimhan, Ph.D.
Yale School of Medicine
New Haven, CT

Gary P. Wormser, M.D.
New York Medical College
Valhalla, NY

Lindsay Rollend, M.P.H.
Yale School of Public Health
New Haven, CT

Erol Fikrig, M.D.
Yale School of Medicine
New Haven, CT

Timothy Lepore, M.D.
Nantucket Cottage Hospital
Nantucket, MA

Alan Barbour, M.D.
University of California, Irvine
Irvine, CA

Durland Fish, Ph.D.
Yale School of Public Health
New Haven, CT

The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute of Allergy and Infectious Diseases or the National Institutes of Health.

Supported by grants from the National Institute of Allergy and Infectious Diseases at the National Institutes of Health (R21AI088079, to Drs. Krause and Fish), the Gordon and Llura Gund Foundation (to Drs. Krause and Lepore), the G. Harold and Leila Y. Mathers Foundation (to Dr. Fish), and the Howard Hughes Medical Institute (to Dr. Fikrig).

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

1. Fukunaga M, Takahashi Y, Tsuruta Y, et al. Genetic and phenotypic analysis of *Borrelia miyamotoi* sp. nov., isolated from the ixodid tick *Ixodes persulcatus*, the vector for Lyme disease in Japan. Int J Syst Bacteriol 1995;45:804-10.
2. Scoles GA, Papero M, Beati L, Fish D. A relapsing fever group spirochete transmitted by *Ixodes scapularis* ticks. Vector Borne Zoonotic Dis 2001;1:21-34.
3. Platonov AE, Karan LS, Kolyasnikova NM, et al. Humans infected with the relapsing fever spirochete *Borrelia miyamotoi*, Russia. Emerg Infect Dis 2011;17:1816-23.
4. Schwan TG, Schrumpf ME, Hinnebusch BJ, Anderson DE Jr, Konkel ME. GlpQ: an antigen for serological discrimination between relapsing fever and Lyme borreliosis. J Clin Microbiol 1996;34:2483-92.
5. Gugliotta JL, Goethert HK, Berardi VP, Telford SR III. Meningoencephalitis from *Borrelia miyamotoi* in an immunocompromised patient. N Engl J Med 2013;368:240-5.

DOI: 10.1056/NEJMc1215469

Checklists for Invasive Procedures

TO THE EDITOR: In recent years, the World Health Organization (WHO) has undertaken a number of global and regional initiatives to improve the safety of surgical care. Its 2008 Safe Surgery Saves Lives campaign introduced the concept of a checklist, which was intended to identify and control risk during each of the three phases of an operation: before induction of anesthesia ("sign-in"), before incision of the skin ("time-out"), and before the patient leaves the operating room ("sign-out"). It has been well received by the spectrum of health care professionals in the operating room[1] and has been shown to reduce mortality and morbidity.[2]

However, the concept has faltered in moving beyond the operating room, despite the rapidly expanding list of invasive procedures now taking place in nonsurgical, interventional specialties. The same sign-in, time-out, and sign-out phases are eminently applicable to procedures performed in the endoscopy suite, the cardiac catheter laboratory, and interventional radiology rooms. These patients are deserving of the same safety considerations that are being afforded to those undergoing an operation; the essential objectives listed by the WHO include appropriate consent, appropriate personnel and equipment, correct procedural site, avoidance of known al-

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
				2012. 10. 20	該当なし	

一般的名称	新鮮凍結人血漿	研究報告の公表状況		公表国
販売名(企業名)	新鮮凍結血漿-LR「日赤」(日本赤十字社) 新鮮凍結血漿-LR「日赤」成分採血(日本赤十字社) 新鮮凍結血漿-LR「日赤」120(日本赤十字社) 新鮮凍結血漿-LR「日赤」240(日本赤十字社) 新鮮凍結血漿-LR「日赤」480(日本赤十字社)		Bautista G, Ramos A, Forés R, Regidor C, Ruiz E, de Laiglesia A, Navarro B, Bravo J, Portero F, Sanjuan I, Fernández MN, Cabrera R. Transpl Infect Dis. 2012 Oct;14(5):496-501. doi: 10.1111/j.1399-3062.2012.00735.x. Epub 2012 May 1.	スペイン

研究報告の概要

○臍帯血移植レシピエントにおけるトキソプラズマ症
トキソプラズマ症は臍帯血移植(CBT)レシピエントのような免疫不全患者に影響を及ぼす重篤な日和見感染症である。この病院で治療された4人及び文献から収集した5人(計9人)のトキソプラズマ症CBT患者について再評価した。
この病院におけるトキソプラズマ症の割合はCBTレシピエントで6%、同種造血幹細胞移植レシピエントで0.2%であった(P<0.001)。5人(56%)は播種性トキソプラズマ症、4人(44%)は中枢神経系への限局性感染であった。9人のうち5人(56%)において、トキソプラズマ症の発症の前にサイトメガロウイルスの複製が確認された。7人(78%)は以前、移植片対宿主病を発症した。播種性疾患を呈した患者は全てトキソプラズマ感染症により死亡した。彼らの移植前の血清学検査結果は、陽性1人、陰性3人、不明1人であった。播種性患者5人のうち1人のみ、トキソプラズマ予防薬コトリモキサゾールを受けていた。CBTレシピエントにおける播種性トキソプラズマ症の死亡率は容認出来ないほどに高いことが示された。これらの患者の多くは血清学的検査で陰性となり、臨床症状が明確ではないため診断が難しい。CBTレシピエントにおいて、より良い診断検査と予防戦略が必要とされる。

使用上の注意記載状況・その他参考事項等

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

血液を介するウイルス、
細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
臍帯血移植では同種造血幹細胞移植に比べてトキソプラズマ症の発症率が非常に高いことが示されたとの報告である。	日本赤十字社では輸血感染症対策として、トキソプラズマ症の既往がある場合は完全に治癒して一定期間が経過するまで献血不適としている。今後も情報の収集に努める。

18

Transplant Infectious Disease, ISSN 1398-2273

Toxoplasmosis in cord blood transplantation recipients

G. Bautista, A. Ramos, R. Forés, C. Regidor, E. Ruiz, A. de Laiglesia, B. Navarro, J. Bravo, F. Portero, I. Sanjuan, M.N. Fernández, R. Cabrera. Toxoplasmosis in cord blood transplantation recipients.
Transpl Infect Dis 2012: 14: 496–501. All rights reserved

Abstract: Toxoplasmosis is a devastating opportunistic infection that can affect immunocompromised patients such as cord blood transplantation (CBT) recipients. The clinical characteristics of 4 toxoplasmosis CBT patients treated at our institution are reviewed, together with 5 cases collected from the literature. The rate of toxoplasmosis in our hospital was 6% in CBT recipients and 0.2% in other types of allogeneic hematopoietic stem cell transplantation ($P < 0.001$). Five patients (56%) presented disseminated toxoplasmosis and 4 patients (44%) had localized infection in the central nervous system. In 5 of the 9 patients considered (56%), cytomegalovirus viral replication had been detected before the clinical onset of toxoplasmosis. Seven patients (78%) had previously developed graft-versus-host disease. All patients who exhibited disseminated disease died due to *Toxoplasma* infection. Pre-transplant serology was positive in 1 patient, negative in 3 patients, and not performed in another. Only 1 of these 5 patients with disseminated disease had received *Toxoplasma* prophylaxis with cotrimoxazole. It could be concluded that mortality in CBT patients with disseminated toxoplasmosis is unacceptably high. The negative results of serology in the majority of these cases, and its unspecific clinical presentation, makes diagnosis exceedingly difficult. Better diagnostic tests and prophylaxis strategy are needed in CBT recipients.

G. Bautista[1], A. Ramos[2], R. Forés[1], C. Regidor[1], E. Ruiz[1], A. de Laiglesia[1], B. Navarro[1], J. Bravo[1], F. Portero[3], I. Sanjuan[1], M.N. Fernández[1], R. Cabrera[1]

[1]Department of Hematology, Hospital Universitario Puerta de Hierro, Majadahonda, Madrid, Spain, [2]Department of Internal Medicine, Infectious Disease Unit, Hospital Universitario Puerta de Hierro, Majadahonda, Madrid, Spain, [3]Department of Microbiology, Hospital Universitario Puerta de Hierro, Majadahonda, Madrid, Spain

Key words: toxoplasmosis; cord blood stem cell transplantation; sulfadiazine; pyrimethamine; cytomegalovirus; graft-versus-host disease

Correspondence to:
Antonio Ramos, Department of Internal Medicine, Infectious Diseases Unit, Hospital Universitario Puerta de Hierro, Universidad Autónoma de Madrid, Maestro Rodrigo no. 2, 28220 Majadahonda, Madrid, Spain
Tel: 34 911914362
Fax: 34 911916807
E-mail: aramos220@gmail.com

Received 17 July 2011, revised 1 November 2011, accepted for publication 26 December 2011

DOI: 10.1111/j.1399-3062.2012.00735.x
Transpl Infect Dis 2012: 14: 496–501

Toxoplasmosis is a devastating opportunistic infection in hematopoietic stem cell transplant (HSCT) patients that is caused by the protozoan *Toxoplasma gondii,* and is associated with high mortality (1). It can induce symptoms limited to the central nervous system, lung, heart, and eyes, or cause disseminated infection (2). Its incidence shows marked geographical variations, being higher in Southern Europe in comparison with other developed regions (3). Primary infection in immunocompetent hosts leads to latency of the parasite as cysts in muscle and other tissues (4). Toxoplasmosis in HSCT recipients usually results from the reactivation of latent infection rather than being due to primary infection. A few cases have been reported in HSCT recipients with negative *Toxoplasma* antibody titers, suggesting transmission of infection via marrow or blood products (5).

Cord blood transplantation (CBT) is associated with prolonged and severe impairment of cellular immunity and is considered an important risk factor for *Toxoplasma* infection and other opportunistic infections (1, 6–8). The experience of toxoplasmosis in CBT patients communicated in the literature up to now has consisted of reports of isolated cases (1, 6, 9, 10).

We report 4 cases of toxoplasmosis in CBT recipients treated in our institution and review similar previously reported cases in HSCT patients.

Methods

In our institution, some eligible patients have received a single unit of CBT following myeloablative conditioning, supported by the co-infusion of a relatively

low number of T-cell-depleted, mobilized hematopoietic stem cells (MHSC) from a third-party donor ("dual" CBT). This strategy results in early recovery of circulating granulocytes and high rates of CB engraftment and full chimerism, making CBT with single units of relatively low content feasible in adults (11). A minimum of 1.5×10^7 total nucleated cells, and 0.1×10^6 CD34+ cells/kg recipient body weight before freezing, were infused. Third-party donors were selected based on their suitability to donate and undergo an MHSC collection procedure with granulocyte colony-stimulating factor mobilization, negative serological cross-match with the patient, cytomegalovirus (CMV) serology, age, and gender. For most patients, the preparative regimen consisted of fractionated total body irradiation to a total of 10 Gy in 5 doses over 3 days (−8 to −6) with lungs shielded at 8 Gy; fludarabine, total dose of 120 mg/m^2 (30 mg/m^2/day intravenous [IV], days −5 to −2); cyclophosphamide 120 mg/kg total dose (60 mg/kg/day IV over 1 h, days −3 and −2); and equine antithymocyte globulin (Lymphoglobuline, Imtix-Sangstat, Lyon, France) 30 mg/kg on day −1, versus Thymoglobulin (Genzyme, Cambridge, Massachusetts, USA) 0.5 mg/kg day −3 and 2 mg/kg/d days −2 and −1. Busulfan at a total oral dose of 8 mg/kg (6.4 mg/kg IV after 2004) substituted for total body irradiation when the latter was contraindicated. Patients were nursed in positive pressure air-filtered rooms. Gut decontamination using ciprofloxacin was initiated on day −8 and continued until the absolute neutrophil count (ANC) dropped below 0.5×10^9/L, when patients were switched to IV meropenem. Patients also received daily cotrimoxazole (1200/240 mg/12 h IV from day −8 to −1), and 3 times per week from CB engraftment until day +180. After May 2004 (when the third case was diagnosed) chemoprophylaxis against toxoplasmosis was changed to pre-transplant cotrimoxazole, oral azithromycin 1 g twice a week until CB engraftment, and then pyrimethamine/sulfadoxine (Fansidar®; Roche Pharmaceuticals, Nutley, New Jersey, USA) and folinic acid, continued until day +180. Patients also received fluconazole from day −8 until ANC recovery, immunoglobulin (400 mg/kg weekly from day −3 to +60), and acyclovir (200 mg/8h from day −8 to +35, when it was switched to the oral route. The enzyme-linked fluorescence assay (VIDAS®, bioMérieux Inc., Marcy l'Etoile, France) technique was employed for *Toxoplasma* IgG antibody determination.

A definition of possible, probable, and definite toxoplasmosis had previously been proposed (12). Patients who had clinical and radiological evidence suggestive of brain *Toxoplasma* disease plus a positive polymerase chain reaction (PCR) test from cerebrospinal fluid (CSF) (or other biological specimen), but who had no histological confirmation, were classified as having probable *Toxoplasma* disease. Disseminated toxoplasmosis was defined as clinical, radiological, or histological evidence of disease affecting >1 organ. Samples of CSF (0.5 mL) were concentrated by centrifuging at 1800 3 *g* for 10 min. The samples were incubated and shaken in 100-mL portions of lysis buffer (10 mM Tris-HCl [pH 8.3], 1.5 mM $MgCl_2$, 50 mM KCl, 0.1 mg of gelatin per mL, 0.5% Tween 20, 20 mg of proteinase K) at 55°C for 90 min. After inactivating the proteinase K at 94°C for 10 min, the suspension was centrifuged at 12,000 rpm for 5 min, and the supernatant, which contained the DNA, was moved to a new tube. *T. gondii* infections were initially confirmed by nested PCR amplification of the repetitive and conserved gene B1 (13).

Toxoplasma disease was considered the main cause of death when no other relevant complication occurred prior to death. Patients were considered to have died from another cause if they had responded to therapy before death from an unrelated complication. Patients were considered evaluable for response to anti-*Toxoplasma* therapy if they completed at least 4 days of treatment. Response to therapy was defined as an improvement of clinical signs and symptoms attributable to *Toxoplasma* disease despite residual findings in physical examinations or imaging studies.

A search of literature was conducted in MEDLINE to find documented cases of toxoplasmosis in CBT recipients between 1980 and March 2011. The key words used were "cord blood stem cell transplantation," "hematopoietic stem cell transplantation," "toxoplasmosis," and "*Toxoplasma*." Care was taken to exclude cases likely to reflect duplicate reporting. Six cases were detected. One case was not included in the study owing to paucity of clinical information (8).

A comparison was undertaken of the clinical characteristics of patients who developed toxoplasmosis with those without the disease in our cohort. In the case of patients who received >1 transplant, only the clinical features of the first transplant were considered.

Continuous variables were compared with the Student *t*-test or the Mann–Whitney test when a normal distribution could not be assumed. Categorical data were compared with the chi-square, chi-square for trends, or Fisher's exact test when appropriate.

Results

Since our institution began the CBT program, 75 transplants have been performed in 70 patients

(5 patients received a second transplant due to failure of the first CBT which, in all cases, occurred during the first 3 months). Four cases of toxoplasmosis were diagnosed in these patients between 1993 and 2007 (6%). This rate was higher than that observed in patients receiving other types of allogeneic HSCT treated in the same institution (0.2%, 1 case in 2008 out of 401 transplanted patients, $P < 0.001$). Hematological diseases that gave rise to CBT were acute lymphoblastic leukemia (2 patients), acute myeloid leukemia (1 patient), and accelerated phase-chronic myeloid leukemia (1 patient). Three patients suffered graft-versus-host disease (GvHD) before toxoplasmosis. CMV replication was demonstrated before the onset of the disease in all 4 cases. In 2 of them, there was also CMV disease (viral syndrome and esophagitis, 1 patient each). In our institution, there were no significant differences in age, gender, underlying disease, proportion of uncontrolled disease at transplantation or engraftment day in CBT between patients with and without toxoplasmosis. There were more cases of CMV replication during the first 6 weeks in patients who suffered from toxoplasmosis ($P = 0.148$). After May 2004 (when azithromycin prior to engraftment was added to the toxoplasmosis prophylaxis), the incidence of toxoplasmosis decreased from 15% (3 cases in 20 CBT patients) to 2% (1 case in 50 CBT, $P = 0.067$). Three patients (75%) had positive pre-transplant recipient serology and 1 patient had negative. Two third-party donors had positive serology, in another patient it was negative and, in the last one, the result was unknown. None of these patients who developed toxoplasmosis and had a positive (or unknown) pre-transplant or third-party donor serology received prophylaxis with trimethoprim/sulfamethoxazole or pyrimethamine/sulfadoxine ($P = 0.062$). Administration of these drugs was not commenced owing to their potential hematological toxicity in patients that did not achieve adequate CBT engraftment.

The clinical characteristics of these 4 patients, together with the other 5 patients included from the literature review, are shown in Table 1 (1, 6, 10, 11). The mean age was 29 years (range 7–53 years) and 5 patients (56%) were male. The underlying disease was acute leukemia in 7 patients (78%). A total of 5 patients (56%) presented disseminated toxoplasmosis and 4 (44%) with localized infection in the central nervous system. Of the 5 patients (56%) who presented with disseminated disease, only 1 had positive donor serology (they were negative in 3, and not reported in 1). All of them died due to *Toxoplasma* infection. One patient developed disseminated toxoplasmosis despite having had negative pre-transplant serology, negative third-party donor transplant serology, and having been infused seronegative hemotherapeutic products. In 5 of 9 patients (56%), CMV viral replication was detected before the clinical onset of toxoplasmosis. In all, 7 patients (78%) developed GvHD. Those patients who received adequate treatment for >4 days survived.

Discussion

The high incidence of toxoplasmosis in CBT patients in our institution is consistent with that reported in relation to other infections (6–8, 14–16). The high seroprevalence of toxoplasmosis in southern Europe, together with the increasing number of CBTs to be performed in the coming years, could lead to a significant rise in toxoplasmosis in these patients (3, 12, 15–17).

The timing of disease onset (+48 day) in CBT recipients was earlier than that observed in patients with other types of allogenic HSCT (10, 12). This finding could be related to distinctive features of CBT, such as the severe impairment of cellular immunity and the absence of specific immunity in the donor (10, 16, 17).

As observed in other HSCT patients, the development of GvHD seems related to the risk of toxoplasmosis (12). The intensification of immunosuppression for GvHD control could induce the reactivation of latent *Toxoplasma* infection (18–20). However, the difference in the frequency of occurrence of this complication between patients with and without toxoplasmosis in our institution was not significant. Antithymocyte globulin in the conditioning regimen and lymphocytopenia early after HSCT could increase the risk of developing toxoplasmosis (21, 22). Another interesting point is the analysis of the relationship between CMV replication and the subsequent development of toxoplasmosis. All 4 patients treated in our hospital showed viral replication before the onset of toxoplasmosis, which is compatible with the immunomodulatory effect of CMV replication in *Toxoplasma* reactivation, as has been seen in other opportunistic infections (23–26).

The proportion of disseminated disease in the 9 CBT cases described was similar to that detected in patients with other types of HSCT (1). The majority of "probable" CBT cases were diagnosed with PCR amplification of specific *T. gondii* antigens or DNA sequences in CSF (22, 27). This technique can be used in blood, CSF, and bronchoalveolar lavage fluid (22, 23, 27, 28). However, results in published studies

Summary of reported toxoplasmosis cases in cord blood transplantation (CBT) patients

Reference Case	Age/ gender	Underlying disease	Conditioning regimen	Previous CMV replication	Previous GvHD (Grade)	Pre-transplant recipient serology	Third-party donor serology[2]	Post-transplant prophylaxis	Time presentation (days)	Diagnostic certainty[3]	Disease location	Toxoplasmosis treatment >4 days	Diagnosis	Infection outcome	Patient status (cause of death)
Martino et al. (6)	30/M	ALL	NR	NR	Acute (2)	NR	–	TMP-SMZ	59	Probable	Brain	P-S	PCR	Recovery	Unknown
Martino et al. (6)	21/M	ALL	NR	NR	Acute (0)	NR	–	Atovaquone	45	Definite	Disseminated	No	PCR, autopsy	Failure	Death (toxo)
Goebel et al. (1)	7/M	ALL	TBI, CFM, ATG	No	Acute (2)	No	–	No	48	Definite	Disseminated	No	Autopsy	Failure	Death (toxo)
Delhaes et al. (10)	42/F	AML	BU, FLU	No	Acute (NR)	No	–	No	48	Definite	Disseminated	No	Bone marrow smear	Failure	Death (toxo)
Bautista et al. (11)	15/F	MDS	BU, CFM, ATG	Yes	No	Yes	–	No	35	Definite	Disseminated	P-S[4]	PCR in blood and BAL	Failure	Death (toxo)
Case 1 (PR)	21/M	ALL	FLU, BU, CTX, ATG	Yes	Acute (3)	Yes	Unknown	No	37	Probable	Brain	P-S	PCR in CSF	Recovery	Death (GvHD)
Case 2 (PR)	53/M	AML	FLU, BU, CTX, ATG	Yes	Acute (1)	No	No	No	70	Definite	Disseminated	No	Autopsy	Failure	Death (toxo)
Case 3 (PR)	29/F	AP-CML	FLU, TBI[1], CTX, ATG	Yes	Acute (1)	Yes	Yes	No	43	Probable	Brain	P-S	PCR in CSF	Recovery	Alive
Case 4 (PR)	45/F	ALL	FLU, TBI[1], CTX, ATG	Yes	No	Yes	Yes	Azithromycin	50	Probable	Brain	P-S	PCR in CSF	Recovery	Death (MOF)

[1]TBI: total body irradiation (10 Gy with 8 in the lung).
[2]Auxiliary mini-allogeneic transplantation ("dual" CBT).
[3]Probable: positive result of PCR of a blood sample and/or another sample from the organ involved with clinical signs and symptoms and radiological evidence of active disease. Definite: histological evidence of active toxoplasmosis.
[4]P-S treatment was stopped 12 days before death because of liver toxicity.
CMV, cytomegalovirus; GvHD, graft-versus-host disease; M, male; ALL, acute lymphoid leukemia; NR, not reported; TMP-SMZ, trimethoprim/sulfamethoxazole; BAL, bronchoalveolar lavage; P-S, pyrimethamine/sulfadoxine; PCR, polymerase chain reaction; toxo, toxoplasmosis; TBI, total body irradiation; CFM, cyclophosphamide; ATG, antithymocyte globulin; F, female; CSF, cerebrospinal fluid; AML, acute myeloid leukemia; BU, busulfan; FLU, fludarabine; MDS, myelodysplastic syndrome; CTX, cytoxan; AP-CML, accelerated phase-chronic myeloid leukemia; PR, present report; MOF, multiorgan failure.

Table 1

are difficult to judge because PCR techniques are not standardized clinical tests (29).

As could be expected, in our institution, a tendency to a more positive pre-transplant serological status was seen in recipients who developed toxoplasmosis than in the other patients. However, a significant proportion of these patients had previous negative *Toxoplasma* serology. This fact, also observed in other toxoplasmosis studies in HSCT patients, has been mainly attributed to primary infection suffered a few days after transplantation and to the fact that, in heavily pre-treated patients, the titers may drop just below the level of positivity (5, 30). However, this fact is intriguing because some patients had not left the hospital, had not eaten uncooked food, and had not received transfusions from seropositive donors, thereby putting the reliability of *Toxoplasma* serology results into question.

Altogether, 3 of the 9 cases presented (33%) developed the disease despite prophylaxis (31, 32). Some prophylaxis failures have been attributed to underdosing cotrimoxazole (double-strength, 2 or 3 times a week). One of the most remarkable results of this study was the finding of increased risk of toxoplasmosis in patients who should have received prophylaxis as recommended in the ASBMT guidelines (i.e., trimethoprim/sulfamethoxazole or pyrimethamine/sulfadoxine), but in whom it was not administered because of poor or delayed engraftment (5). In most cases, the rationale for not using these drugs is their bone marrow toxicity (5). Although little information is available about the role of azithromycin in the treatment and prevention of toxoplasmosis, we thought it could be effective in CBT (33). For this reason, in our institution, it was decided to prescribe post-transplant prophylaxis with azithromycin in seropositive recipients before the transplant engraftment, and a significant decrease in the incidence was noted (15% versus 2%) (34, 35).

The mortality from toxoplasmosis in the 9 CBT patients studied was quite high (56%); however, this was similar to that reported in other types of HSCT (36). Interestingly, all patients with disease located in the central nervous system survived, whereas all patients with disseminated forms died (12). The majority of patients who suffered from disseminated infection had a negative pre-transplant serology. In many of these patients, the diagnosis was achieved very late because of a lack of clinical suspicion due to negative serology and a non-specific clinical presentation. None of these patients received *Toxoplasma*-specific treatment for >4 days. Therefore, one of the biggest challenges in this field is to consider disseminated toxoplasmosis as a possible diagnosis, even when the serology is negative.

One strategy to improve this dramatic situation could be to perform periodic *Toxoplasma* genome determinations in blood using PCR amplification of *T. gondii* DNA in patients who, despite negative serology, are considered to have a higher risk of developing toxoplasmosis, such as those with GvHD or reactivation of an immunomodulating infection (1, 4, 10). However, the use of prophylaxis and/or preemptive therapy based on PCR presents several problems. This technique is not available in many hospitals and could produce false-positive and -negative results. In addition, preemptive therapy based on PCR did not prevent the development of 6 cases of toxoplasmosis among 16 patients with positive blood PCRs (6). Finally, the clinical meaning and prognosis of a positive *Toxoplasma* PCR in an asymptomatic patient is unknown (6).

One limitation of this study is that not all the relevant data were available in some of the cases, such as pre-transplantation *Toxoplasma* serology, prophylaxis administered, conditioning regimen, engraftment day, or previous CMV replication.

In summary, toxoplasmosis is an important infection in HSCT patients. A high mortality has been shown in the cases of disseminated toxoplasmosis in CBT studied. The negative results of serology in the majority of these cases and its unspecific clinical presentation make diagnosis exceedingly difficult. Better diagnostic tests are needed to identify recipients at risk of *Toxoplasma* disease in CBT. An improvement in *Toxoplasma* prophylaxis protocols is desirable in CBT recipients. The possible role of azithromycin in the prevention of toxoplasmosis in these patients should be analyzed in future studies.

Acknowledgements:

The authors thank Martin Hadley-Adams for assisting with the English language and preparation of the manuscript.

Conflict of interest: The authors declare no conflict of interest.

References

1. Goebel WS, Conway JH, Faught P, Vakili ST, Haut PR. Disseminated toxoplasmosis resulting in graft failure in a cord blood stem cell transplant recipient. Pediatr Blood Cancer 2007; 48: 222–226.

2. Derouin F, Pelloux H; ESCMID Study Group on Clinical Parasitology. Prevention of toxoplasmosis in transplant patients. Clin Microbiol Infect 2008; 14:1089–1101.
3. Held TK, Krüger D, Switala AR, et al. Diagnosis of toxoplasmosis in bone marrow transplant recipients: comparison of PCR-based results and immunohistochemistry. Bone Marrow Transplant 2000; 25: 1257–1262.
4. Stinson EB, Bieber CP, Griepp RB, Clark DA, Shumway NE, Remington JS. Infectious complications after cardiac transplantation in man. Ann Intern Med 1971; 74: 22–36.
5. Chandrasekar PH, Momin F. Disseminated toxoplasmosis in marrow recipients: a report of three cases and a review of the literature. Bone Marrow Transplant Team. Bone Marrow Transplant 1997; 19: 685–689.
6. Martino R, Bretagne S, Einsele H, et al. Early detection of *Toxoplasma* infection by molecular monitoring of *Toxoplasma gondii* in peripheral blood samples after allogeneic stem cell transplantation. Clin Infect Dis 2005; 40: 67–78.
7. Sauter C, Abboud M, Jia X, et al. Serious infection risk and immune recovery after double-unit cord blood transplantation without antithymocyte globulin. Biol Blood Marrow Transplant 2011; 17: 1460–1471. Epub 1 Mar 2011.
8. Parikh SH, Mendizabal A, Martin PL, et al. Unrelated donor umbilical cord blood transplantation in pediatric myelodysplastic syndrome: a single-center experience. Biol Blood Marrow Transplant 2009; 15: 948–955.
9. Liapis K, Baltadakis I, Balotis K, Karakasis D. Multisystem febrile illness in a cord-blood transplant recipient. Clin Infect Dis 2010; 51: 716–717.
10. Delhaes L, Mraz JC, Fréalle E, et al. Severe pulmonary toxoplasmosis after allo-SCT in two patients: from *Toxoplasma* genotyping to clinical management. Bone Marrow Transplant 2010; 45: 580–583.
11. Bautista G, Cabrera JR, Regidor C, et al. Cord blood transplants supported by co-infusion of mobilized hematopoietic stem cells from a third-party donor. Bone Marrow Transplant 2009; 43: 365–373.
12. Martino R, Maertens J, Bretagne S, et al. Toxoplasmosis after hematopoietic stem cell transplantation. Clin Infect Dis 2000; 31: 1188–1195.
13. Fuentes I, Rubio JM, Ramírez C, Alvar J. Genotypic characterization of *Toxoplasma gondii* strains associated with human toxoplasmosis in Spain: direct analysis from clinical samples. J Clin Microbiol 2001; 39: 1566–1570.
14. Parody R, Martino R, Rovira M, et al. Severe infections after unrelated donor allogeneic hematopoietic stem cell transplantation in adults: comparison of cord blood transplantation with peripheral blood and bone marrow transplantation. Biol Blood Marrow Transplant 2006; 12: 734–748.
15. Saavedra S, Sanz F, Jarque I, et al. Early infections in adult patients undergoing unrelated donor cord blood transplantation. Bone Marrow Transplant 2002; 30: 937–943.
16. Fricker-Hidalgo H, Bulabois CE, Brenier-Pinchart MP, et al. Diagnosis of toxoplasmosis after allogeneic stem cell transplantation: results of DNA detection and serological techniques. Clin Infect Dis 2009; 48: e9–e15.
17. Duband S, Cornillon J, Tavernier E, Dumollard JM, Guyotat D, Péoc'h M. Toxoplasmosis with hemophagocytic syndrome after bone marrow transplantation: diagnosis at autopsy. Transpl Infect Dis 2008; 10: 372–374.
18. Shaw MH, Reimer T, Sánchez-Valdepeñas C, et al. T cell-intrinsic role of Nod2 in promoting type 1 immunity to *Toxoplasma gondii*. Nat Immunol 2009; 10: 1267–1274.
19. Derouin F, Devergie A, Auber P, et al. Toxoplasmosis in bone marrow transplant recipients: report of seven cases and review. Clin Infect Dis 1992; 15: 267–270.
20. Sing A, Leitritz L, Roggenkamp A, et al. Pulmonary toxoplasmosis in bone marrow transplant recipients: report of two cases and review. Clin Infect Dis 1999; 29: 429–433.
21. Hunter CA, Remington JS. Immunopathogenesis of toxoplasmic encephalitis. J Infect Dis 1994; 170: 1057–1067.
22. Michel G, Thuret I, Chambost H, Scheiner C, Mary C, Perrimond H. Lung toxoplasmosis after HLA mismatched bone marrow transplantation. Bone Marrow Transplant 1994; 14: 455–457.
23. Nichols WG, Corey L, Gooley T, Davis C, Boeckh M. High risk of death due to bacterial and fungal infection among cytomegalovirus (CMV)-seronegative recipients of stem cell transplants from seropositive donors: evidence for indirect effects of primary CMV infection. J Infect Dis 2002; 185: 273–282.
24. Fisher RA. Cytomegalovirus infection and disease in the new era of immunosuppression following solid organ transplantation. Transpl Infect Dis 2009; 11: 195–202.
25. Pomeroy C, Filice GA, Hitt JA, Jordan MC. Cytomegalovirus-induced reactivation of *Toxoplasma gondii* pneumonia in mice: lung lymphocyte phenotypes and suppressor function. J Infect Dis 1992; 166: 677–681.
26. Galván Ramírez ML, Castillo-de-León Y, Espinoza-Oliva M, et al. Acute infection of *Toxoplasma gondii* and cytomegalovirus reactivation in a pediatric patient receiving liver transplant. Transpl Infect Dis 2006; 8: 233–236.
27. Small TN, Leung L, Stiles J, et al. Disseminated toxoplasmosis following T cell-depleted related and unrelated bone marrow transplantation. Bone Marrow Transplant 2000; 25: 969–973.
28. Saad R, Vincent JF, Cimon B, et al. Pulmonary toxoplasmosis after allogeneic bone marrow transplantation: case report and review. Bone Marrow Transplant 1996; 18: 211–212.
29. Brinkman K, Debast S, Sauerwein R, Ooyman F, Hiel J, Raemaekers J. *Toxoplasma* retinitis/encephalitis 9 months after allogeneic bone marrow transplantation. Bone Marrow Transplant 1998; 21: 635–636.
30. Aoun M, Georgala A, Mboumi K, et al. Changing the outcome of toxoplasmosis in bone marrow transplant recipients. Int J Antimicrob Agents 2006; 27: 570–572.
31. Derouin F, Gluckman E, Beauvais B, et al. *Toxoplasma* infection after human allogeneic bone marrow transplantation: clinical and serological study of 80 patients. Bone Marrow Transplant 1986; 1: 67–73.
32. Foot ABM, Garin YJF, Ribaud P, Devergie A, Derouin F, Gluckman E. Prophylaxis of toxoplasmosis infection with pyrimethamine/sulfadoxine (Fansidar) in bone marrow transplant recipients. Bone Marrow Transplant 1994; 14: 241–245.
33. Wiselka MJ, Read R, Finch RG. Response to oral and intravenous azithromycin in a patient with *Toxoplasma* encephalitis and AIDS. J Infect 1996; 33: 227–279.
34. Tabbara KF, Hammouda E, Tawfik A, Al-Omar OM, Abu El-Asrar AM. Azithromycin prophylaxis and treatment of murine toxoplasmosis. Saudi Med J 2005; 26: 393–397.
35. Godofsky EW. Treatment of presumed cerebral toxoplasmosis with azithromycin. N Engl J Med 1994; 330: 575–576.
36. Slavin MA, Meyers JD, Remington JS, Hackman RC. *Toxoplasma gondii* infection in marrow transplant recipients: a 20 year experience. Bone Marrow Transplant 1994; 13: 549–557.

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
				2012. 11. 16	該当なし	

一般的名称	新鮮凍結人血漿	研究報告の公表状況		公表国
販売名(企業名)	新鮮凍結血漿-LR「日赤」(日本赤十字社) 新鮮凍結血漿-LR「日赤」成分採血(日本赤十字社) 新鮮凍結血漿-LR「日赤」120(日本赤十字社) 新鮮凍結血漿-LR「日赤」240(日本赤十字社) 新鮮凍結血漿-LR「日赤」480(日本赤十字社)		ProMED 20121114.1409214	メキシコ

研究報告の概要

○ロッキー山紅斑熱ーメキシコ
メキシコ保健省はダニ媒介性のリケッチア感染症であるロッキー山紅斑熱の4症例を確定し、別の4症例を疑いありとした。Coahuila州Saltilloの4集落で既に防疫線が設けられた。確定した4症例のうち1例がSaltillo、1例がParras de la Fuente、2例がTorreonからで、疑い例は全てSaltilloでの発生であった。Valle de las Aves集落において少なくとも2人の女児が死亡するというこの緊急事態に直面し、当該集落及びLomas de Zapaliname、Pedregal、Nueva Imagenでは2012年11月10日・11日から予防措置が実施された。これらの地域は上下水道、舗装などが未整備で、河川がゴミで溢れ、犬などの繁殖地となり、これらの動物がヒトへの感染原因となるという。

使用上の注意記載状況・その他参考事項等

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

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
メキシコのCoahuila州でダニ媒介性のリケッチア感染症であるロッキー山紅斑熱が発生しているとの報告である。	日本赤十字社では、輸血感染症対策として受付時に海外滞在歴の有無を確認し、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ほか、リケッチア感染症の既往がある場合は完全に治癒して一定期間が経過するまで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。

Published Date: 2012-11-14 14:58:38
Subject: PRO/EDR> Rocky Mountain spotted fever - Mexico (02): (CA)
Archive Number: 20121114.1409214

ROCKY MOUNTAIN SPOTTED FEVER - MEXICO (02): (COAHUILA)
****

A ProMED-mail post
http://www.promedmail.org
ProMED-mail is a program of the
International Society for Infectious Diseases
http://www.isid.org

Date: Mon 12 Nov 2012

Source: Zocalo [in Spanish, machine trans., summ. & edited]

http://www.zocalo.com.mx/seccion/articulo/van-4-casos-de-infeccion-por-garrapatas-sospechan-otros-4

The Health Ministry has confirmed 4 cases of people with tickborne spotted fever and 4 more that are likely, so a sanitary cordon has already been implemented in 4 colonies of Saltillo [Coahuila]. Of the 4 confirmed cases, one is located in Saltillo, another in Parras de la Fuente, and 2 in Torreon, while the 4 probable cases are of the state capital [Saltillo].

Faced with this emergency, which has killed at least 2 girls in the Valle de las Aves colony in this sector and in the colonies of Lomas de Zapaliname, Pedregal, and Nueva Imagen, preventive measures were undertaken since last weekend [10-11 Nov 2012].

Luis Armando Hernandez Perez, head of Sanitary District no 8 of the Ministry of Health, said that these areas lack services such as water, sewage, pavement, and that nearby there is a stream full of garbage, which becomes a breeding ground so that dogs that inhabit the area fill with these animals and transmit them to humans.

To receive adequate treatment the patient must be attended within the 1st 7 days of when symptoms first appeared. Treatment is mainly based on antibiotics and in some cases the patient must remain hospitalized.

[byline: Aracely Gallegos]

--

communicated by:

ProMED-mail

<promed@promedmail.org>

[_Rickettsia rickettsii_ , the cause of Rocky Mountain spotted fever, has been identified in southern Canada, the USA, northern Mexico, Costa Rica, Panama, Brazil, and Argentina (1-6). Some synonyms for Rocky Mountain spotted fever in other countries include tick typhus, Tobia fever (Colombia), Sao Paulo fever and febre maculosa (Brazil), and fiebre manchada (Mexico).

References

---

1. Bustamante ME, Varela G. Distribucion de las rickettsiasis en Mexico. Rev Inst Salubr Enferm Trop. 1947; 8: 13-4.

2. Fuentes L. Ecological study of Rocky Mountain spotted fever in Costa Rica. Am J Trop Med Hyg. 1986; 35(1): 192-6 (abstract available at http://www.ncbi.nlm.nih.gov/pubmed/3080917).

3. Peacock MG, Ormsbee RA, Johnson K. Rickettsioses of Central America. Am J Trop Med Hyg. 1971; 20(6): 941-9.

4. Galvao MAM, Dumler JS, Mafra CL, et al. Fatal spotted fever rickettsiosis, Minas Gerais, Brazil. Emerg Infect Dis. 2003; 9(11): 1402-5 (available at http://www.cdc.gov/ncidod/EID/vol9no11/03-0193.htm).

5. Ripoll CM, Remondegui CE, Ordonez G, et al. Evidence of rickettsial spotted fever and ehrlichial infections in a subtropical territory of Jujuy, Argentina. Am J Trop Med Hyg. 1999; 61(2): 350-4 (available at http://www.ajtmh.org/cgi/reprint/61/2/350).

6. Zavala-Castro JE, Zavala-Velazquez JE, Walker DH, et al. Fatal human infection with _Rickettsia rickettsii_, Yucatan, Mexico. Emerg Infect Dis [serial on the Internet]. 2006 Apr [date cited] (available at http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3294703/). - Mod.LL

A HealthMap/ProMED-mail map can be accessed at: http://healthmap.org/r/45rQ.]

See Also

Rocky Mountain spotted fever - Brazil (03): (SP) 20121001.1318074
Rocky Mountain spotted fever - Mexico: (BN) 20120828.1268087
Rocky Mountain spotted fever - Brazil (02): (SP) 20120816.1247445
Rocky Mountain spotted fever - USA (05): (TN) increase 20120802.1225293
Rocky Mountain spotted fever - Brazil: (SP) 20120724.1213447
Rocky Mountain spotted fever - USA (04): (KS, IL, AR) 20120719.1206256
Rocky Mountain spotted fever - USA (03): (TN) 20120606.1157629
Rocky Mountain spotted fever - USA (02): (AZ) 20120411.1097210
Rocky Mountain spotted fever - USA: (MO), early susp. cases 20120404.1090349
Rickettsiosis - Mexico: comments 20120102.0008
2011

Rickettsiosis - Mexico: (Michoacan) 20111231.3724
Rocky Mountain spotted fever - USA: (OH) 20110811.2436
2010

Rickettsiosis - Mexico: (SO) 20100729.2547
2009

Rickettsiosis - Mexico: (BN) 20090821.2959
2003

Rickettsiosis - Mexico: background 20030810.1977
Rickettsiosis - Mexico (Durango): RFI 20030808.1958
Rocky Mountain spotted fever - Latin America 20030726.1828
Rocky Mountain spotted fever - Brazil (Sao Paolo) 20030725.1814
1997

Rocky Mountain spotted fever - Brazil (Minas Gerais) 19970903.1886
.....sb/ll/mj/sh