Abstract# 2900 **Poster Board #-Session: 119-III**

The Risk of Transfusion-Transmitted Babesiosis Due to *Babesia microti* in Connecticut. Ritchard G. Cable,[1] Yan-Yun Wu*,[3] Stephanie Johnson*,[1] Kerri Dorsey*,[2] Russell Melmed*,[1] Jonathan Trouern-Trend*,[2] Shimian Zou*,[2] Laura Tonnetti*,[2] David Leiby*.[2] [1]*Blood Services, American Red Cross, Farmington, CT, USA;* [2]*Jerome H. Holland Laboratory, American Red Cross, Rockville, MD, USA;* [3]*Department of Laboratory Medicine, Yale University School of Medicine, New Haven, CT, USA.*

Babesia microti (Bm) is a tick-borne intra-erythrocytic parasite endemic in NE and the upper Midwest. Although primarily a tick-borne disease, Bm has been transmitted by transfusion in at least 50 documented cases. Symptoms include fever, hemolytic anemia, and thrombocytopenia, typically arising 2-8 weeks following transfusion. In order to assess the risk of Bm transmission by blood transfusion in Connecticut (CT), we tested a repository of donor and recipient samples collected in 2004-2007. *METHODS:* The repository consisted of frozen whole blood and serum samples collected generally 1, 3, 6, and 12 months after blood transfusions in a chronically transfused population, along with associated donor serum samples, collected at blood drives in CT. All recipient follow-up samples were screened for antibodies to *Babesia microti* by IFA, as were the initial samples of any seropositive recipient, using a 1:64 cut-off titer. If recipients tested IFA positive after being seronegative (seroconversion), corresponding donor sera were screened for Bm antibody to identify transfusion-transmission. Stored DNA from serial seroconverting recipient samples were also assessed by real-time PCR for Bm. We defined an evaluable transfusion for Bm as a platelet or RBC transfusion with at least one follow-up sample 14-180 days later. 107 recipients received evaluable transfusions. Altogether these recipients received 1920 evaluable RBC transfusions and 1634 evaluable platelet transfusions. *RESULTS:* All follow-up samples were seronegative for Bm except for a single follow-up sample in a recipient with sickle cell anemia transfused with 45 RBC over 24 months. This sample was reproducibly seropositive in 2 labs with a titer of 1:64 and was PCR negative. Blood samples 6 weeks before and 11 weeks after the seropositive sample were seronegative, but PCR +. To investigate, 11 earlier recipient samples taken 5-21 months before the seroconversion were tested and all were seronegative, although 2/11 were PCR + (one strongly positive). Donor serum samples from 18/21 RBC transfused prior to the strongly PCR + recipient sample were negative for Bm. Three donor samples were not available. The recipient reported no exposure to ticks and lived in a non-endemic area of Connecticut. The patient had received 41 units of red cells in the two years before enrollment in the study. There were no clinical symptoms attributable to Bm. *CONCLUSION:* This may be a case of transfusion-transmitted *Babesia microti*, despite our inability to identify a seropositive blood donor. However, the recipient may have acquired Bm from a tick bite or from earlier transfusions. The risk of *Babesia microti* transmission by transfusion in CT has thus been measured either as zero cases in 1920 RBC transfusions (95% CI 0.0 - 0.0016 per RBC) or as 1 case per 1920 RBC transfusions (0.005, CI 0.000013 - 0.0029 per RBC). A previous report (Gerber, et al. JID 1994; 170:231-234) directly measured the risk of transfusion transmission of *Babesia microti* in CT as 1 in 601 RBC (.0017). A recent risk estimate based on the prevalence of PCR positive CT donor samples is 1/1800 RBC (0.0006) (Cable RG, et al. Transfusion 2001: 41(suppl):12S-13S.) This current study of chronically transfused recipients is consistent with these earlier estimates.

Disclosure: No relevant conflicts of interest to declare.

別紙様式第2-1

No. 10

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	機構処理欄
				2008. 5. 26	該当なし	
一般的名称	(製造承認書に記載なし)		研究報告の公表状況	Matsukura H, Shibata S, Tani Y, Shibata H, Furuta RA. Transfusion. 2008 May;48(5):1036-7.	公表国	
販売名(企業名)	合成血「日赤」(日本赤十字社) 照射合成血「日赤」(日本赤十字社)				日本	

	研究報告の概要	使用上の注意記載状況・その他参考事項等
研究報告の概要	○献血適格者におけるヒトパルボウイルスB19の持続感染 ヒトパルボウイルスB19持続感染の自然経過の特徴を明らかにするための長期的研究を実施した。日本では、全ての献血血液にRHA法によるB19抗原検査を行っている。この方法を用いて、1997年〜1999年に大阪の献血者979,052人からB19感染102例を特定した。102名のうち、次の献血に訪れた20名(男性15名、女性5名;平均年齢34.3歳)から血漿検体を採取し、ウイルス力価及びB19 IgG・IgM抗体力価を測定することができた。B19 DNAについてはTaqMan PCR法、B19抗体については酵素免疫測定法を使用した。B19抗原陽性の血液は不適として廃棄されるため、初回献血時についてはこれらの検査を行っていない。平均フォローアップ期間は838日(範囲、101〜1749日)だった。 血漿B19 DNAは、最初の6ヶ月間で急速に減少し、その後も減少は続いたが検出不能にはならなかった。B19抗体については、IgG、IgM両方が検出された9名ではIgMが検出不能となったが、他の9名ではIgGのみが検出され、IgMは2度目の献血の前に検出限界以下まで低くなったと考えられた。残り2名の献血者は調査期間の最後までIgMが検出可能だった(729日、743日)。B19抗体の当初の分析結果は異なるパターンを示したが、一度感染が成立すると、B19 IgGは20人の献血者全員で持続した。 これまでの研究結果と同様、本長期研究において献血者のB19持続感染が観察された。フォローアップ期間中、20名の献血者は高値のB19 IgGと低いウイルス力価を維持していたが、B19感染の症状を報告した者はいなかった。本研究のデータは、B19急性感染後の血漿ウイルス力価は約1年で10^4IU/mL未満、約2年で10^3IU/mL未満まで下がることを示された。ここで観察されたウイルス力価の動態は、B19 NATが実施できない状況において、より適切な献血者の選択に役立つだろう。これらの予備的な知見を実証するために、より大規模な研究が望まれる。	合成血「日赤」 照射合成血「日赤」 血液を介するウイルス、細菌、原虫等の感染 vCJD等の伝播のリスク

報告企業の意見	今後の対応
大阪のヒトパルボウイルスB19陽性献血者20名のB19 DNA、IgG・IgM抗体を長期間フォローアップしたところ、B19持続感染が観察されたが、B19感染の症状を報告した者はいなかった。B19急性感染後の血漿ウイルス力価は約1年で10^4IU/mL未満、約2年で10^3IU/mL未満まで下がることが示されたとの報告である。	今後も引き続き、ヒトパルボウイルスB19に関する新たな知見及び情報の収集に努める。日本赤十字社では、以前よりRHA法によるB19抗原検査を導入、ウイルス量の多い血液を排除している。また、2008年には感度向上のため検査法をCLEIA法に変更した。

LETTERS TO THE EDITOR

Persistent infection by human parvovirus B19 in qualified blood donors

Persistent parvovirus B19 infection with a low viral load has been reported in immunocompromised and in immunocompetent individuals (reviewed in Parsyan and Candotti[1]). Large cross-sectional studies using highly sensitive DNA amplification methods have also demonstrated persistent B19 infection.[2] Recently, Lefere and colleagues[3] conducted a longitudinal study of nonimmunodeficient patients who were multitransfused with red blood cells, demonstrating that asymptomatic chronic B19 infections may persist for a long period.[3] To characterize the natural course of persistent B19 infections, we conducted the following longitudinal study using an in-house TaqMan polymerase chain reaction method for B19 DNA and enzyme immunoassays to detect B19 immunoglobulin M (IgM) and immunoglobulin G (IgG; Denka Seiken, Tokyo, Japan). This study was approved by the ethical Committee of the Japanese Red Cross Osaka Blood Center. In Japan, all donated blood is tested for B19 infection with an in-house receptor-mediated hemagglutination method that detects B19 antigen as a marker of a high viremic stage of infection (cutoff, approx. 2.5×10^{10} IU/mL B19 DNA; data not shown). Using this method, we identified 102 cases of B19 infection among 979,052 blood donors in Osaka between 1997 and 1999. We were able to test the plasma samples of 20 of these 102 donors (15 male, 5 female; mean age, 34.3 years) when they returned for subsequent blood donations for viral load and B19-specific IgG and IgM. We did not examine the donors at their first visit because B19 antigen-positive blood was automatically disqualified and disposed. The mean duration of follow-up was 838 days (range, 101-1749 days). The results of sequential viral load testing for all donors are shown in Fig. 1A. In the first 6 months, we observed a rapid decline in plasma B19 DNA, which decreased continuously, but never became undetectable. Median plasma B19 viral loads for samples tested within every 6 months are shown in Fig. 1B. We analyzed the B19 antibody for all donors during the study period (Fig. 2A). For 9 donors (Donors 1-9) with both IgG and IgM, IgM became undetectable, while for 9 others (Donors 10-18), only B19 IgG was detected, presumably because B19 IgM had decreased to an undetectable level before the second visit. The remaining 2 donors (Donors 19 and 20) had B19 IgM–detectable until the last visit (at 729 and 743 days). Although the initial profile for B19 antibodies showed different patterns, once established, B19-specific IgG persisted in all 20 donors. Summaries for 3 representative cases corresponding to each of these patterns for IgM, IgG, and viral load are presented in Fig. 2B.

Consistent with previous studies that suggest that B19 DNA may persist for a long period in immunocompetent individuals,[3-5] we observed persistent B19 infection in healthy blood donors in the present longitudinal study. During the follow-up period, none of the 20 infected blood donors reported symptoms of B19 infection, although they retained high levels of B19 IgG and low viral load. Our data suggest that in healthy individuals, the B19 plasma viral load declines to below 10^4 IU per mL in approximately 1 year and to 10^3 IU per mL in approximately 2 years after an acute (high viremia) infection. The patterns of plasma B19 viral load observed in our study may be useful for identifying more suitable blood donors in circumstances where B19 NAT is unavailable. We encourage further studies with a larger sample size to validate these preliminary findings.

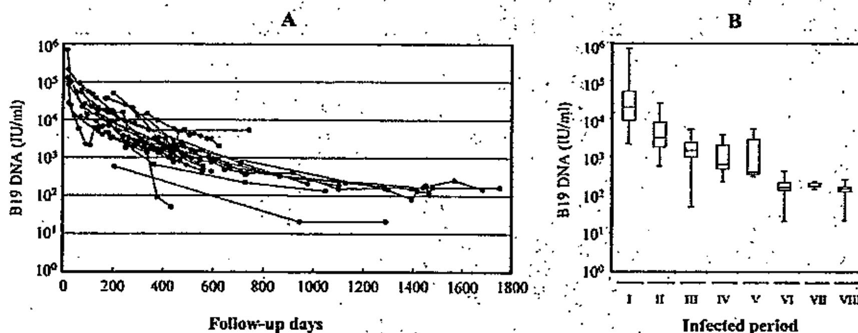

Fig. 1. (A) Changes in plasma B19 viral load in healthy blood donors after an acute B19 infection. Each line represents plasma B19 DNA of the same donor. Time 0 was defined as the first test visit when positive results were obtained for B19 antigen (high viremic phase). (B) Plasma B19 viral loads for all cases by 6-month intervals. Medians of the plasma B19 viral load with its 75th (top of the box) and 25th (bottom of the box) percentiles in each category were indicated. I = 0 to 0.5 years (0-182 days); II = 0.5 to 1.0 years (183-365 days); III = 1.0 to 1.5 years (366-549 days); IV = 1.5 to 2.0 years (550-730 days); V = 2.0 to 2.5 years (731-914 days); VI = 2.5-3.0 years (915-1096 days); VII = 3.0-3.5 years (1096-1279 days); VIII = at least 3.5 years (≥1280 days).

Harumichi Matsukura, BS
Sachiko Shibata, MT
Yoshihiko Tani, MD, PhD
Hirotoshi Shibata, MD, PhD
Rika A. Furuta, PhD
e-mail: furuta@osaka.bc.jrc.or.jp
Japanese Red Cross
Osaka Blood Center
Osaka, Japan

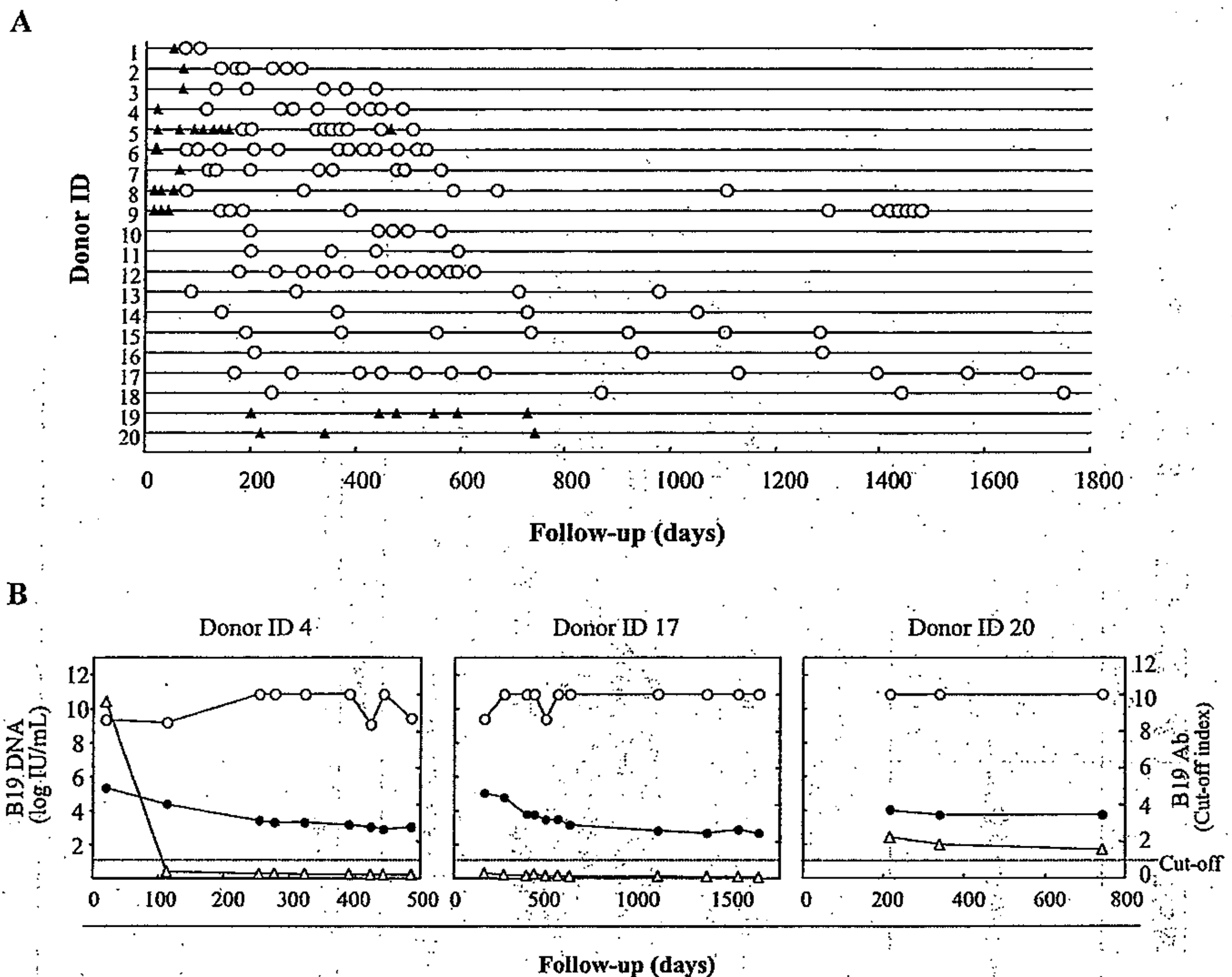

Fig. 2. (A) B19 IgM and IgG for individual donors at follow-up visits. (▲) Positive for both IgM and IgG; (O) positive for IgG. (B) Representative cases for three patterns of test results. Changes in viral load (●), IgM (△), and IgG (O). Donors correspond to those in A.

REFERENCES

1. Parsyan A, Candotti D. Human erythrovirus B19 and blood transfusion—an update. Transfus Med 2007;17:263-78.
2. Kleinman SH, Glynn SA, Lee TH, Tobler L, Montalvo L, Todd D, Kiss JE, Shyamala V, Busch MP; National Heart, Lung, Blood Institute Retrovirus Epidemiology Donor Study (REDS-II). Prevalence and quantitation of parvovirus B19 DNA levels in blood donors with a sensitive polymerase chain reaction screening assay. Transfusion 2007; 47:1756-64.
3. Lefrère JJ, Servant-Delmas A, Candotti D, Mariotti M, Thomas I, Brossard Y, Lefrère F, Girot R, Allain JP, Laperche S. Persistent B19 infection in immunocompetent individuals: implications for transfusion safety. Blood 2005; 106:2890-5.
4. Candotti D, Etiz N, Parsyan A, Allain JP. Identification and characterization of persistent human erythrovirus infection in blood donor samples. J Virol 2004;78:12169-78.
5. Norja P, Hokynar K, Aaltonen LM, Chen R, Ranki A, Partio EK, Kiviluoto O, Davidkin I, Leivo T, Eis-Hübinger AM, Schneider B, Fischer HP, Tolba R, Vapalahti O, Vaheri A, Söderlund-Venermo M, Hedman K. Bioportfolio: lifelong persistence of variant and prototypic erythrovirus DNA genomes in human tissue. Proc Natl Acad Sci U S A 2006; 103:7450-3.

New cell lines express HNA-1c, -4a, -4b, -5a, or -5b for identification of HNA antibodies

Antibodies to human leukocyte antigens (HLAs) or human neutrophil antigens (HNAs) are regarded to be the principal causes of nonhemolytic transfusion reactions, including transfusion-related acute lung injury. Although flow cytometric (FCM) analysis using panels of phenotyped neutrophils is widely used to detect and identify antibodies to HNAs, FCM is time-consuming and

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識別番号・報告回数		回	報告日 年　月　日	第一報入手日 2008年4月16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	Inactivation of parvovirus B19 during STIM-4 vapor heat treatment of three coagulation factor concentrates Berting, A. et al, Transfusion, ahead of print	公表国 オーストリア		
販売名（企業名）							

研究報告の概要	使用上の注意記載状況・その他参考事項等
ヒトパルボウイルスB19（B19V）の感染性アッセイが可能となる以前は，モデルウイルスと呼ばれる動物パルボウイルス（例：マウスパルボウイルス）が，血漿タンパクの製造工程におけるウイルス不活化の確認に使用されており，一般的にパルボウイルスは熱不活化に耐性があると考られていた。しかし，最近の知見より，B19Vは動物パルボウイルスよりも熱に弱いことが明らかになってきた。 本文献は，数種の血液凝固因子製剤においてSTIM-4蒸気加熱処理装置を用いた不活化処理を行い，B19Vとモデルウイルスとして用いられていたマウス微小ウイルス（MMV）間での不活化効果の比較を行っている。 血液凝固因子製剤の中間体の種類に関わらず，試験に用いたB19V（遺伝子1型，2型）はいずれも動物パルボウイルスと比較して，STIM-4蒸気加熱処理工程によって効果的に不活化された（Log減少ファクター，3.5～4.8）。これより，蒸気加熱処理によるB19Vの効果的な不活化が示唆され，B19Vに対するSTIM-4蒸気過熱処理を行った血液凝固因子製剤の安全性が高まると考えられた。	BYL-2008-0320

報告企業の意見	今後の対応
加熱処理によるウイルス不活化の程度は各製剤によって左右されるため，製剤毎に確認する必要があると考える。 弊社のポリグロビンNの製造に使用されているプール血漿においては，B19Vに対するNATを実施し，10E5 IU/mL以上が確認された場合は，そのプール血漿を製造工程から除去している。感染リスクを完全に排除することはできないが，伝播の可能性は非常に低いと考える。	今後とも利用可能なB19Vの検出方法の改善に関する情報収集に努める。

HEMOSTASIS

Inactivation of parvovirus B19 during STIM-4 vapor heat treatment of three coagulation factor concentrates

Andreas Berting, Jens Modrof, Ulrike Unger, Matthias Gessner, Andreas Klotz, Gerhard Poelsler, and Thomas R. Kreil

BACKGROUND: To enhance the viral safety margins, nanofiltration has been widely integrated into the manufacturing process of plasma-derived medicinal products. Removal of smaller agents such as parvovirus B19 (B19V) by filtration, however, is typically less efficient. Because recent investigations have demonstrated that B19V may be more heat sensitive than animal parvoviruses, the potential B19V inactivation by a proprietary vapor heating procedure (STIM-4) as incorporated into the manufacturing processes of several nanofiltered coagulation factor concentrates was investigated.
STUDY DESIGN AND METHODS: An infectivity assay based on quantitative reverse transcription–polymerase chain reaction (TaqMan, Applied Biosystems) detection of B19V mRNA after inoculation of a permissive cell line (UT7 Epo S1 cells) was used to investigate the virus inactivation capacity of the STIM-4 vapor heat treatment as used during the manufacture of nanofiltered second-generation Factor VIII inhibitor-bypassing activity (FEIBA), F IX complex, and FVII products.
RESULTS: In contrast to animal parvoviruses, both B19V genotypes investigated, that is, 1 and 2, were shown to be surprisingly effectively inactivated by the STIM-4 vapor heat treatment process, with mean log reduction factors of 3.5 to 4.8, irrespective of the product intermediate tested.
CONCLUSION: The newly demonstrated effective inactivation of B19V by vapor heating, in contrast to the earlier used animal parvoviruses, results in significant B19V safety margins for STIM-4–treated coagulation factor concentrates.

To further enhance the safety margins of plasma-derived medicinal products against any residual virus safety concerns, manufacturers have continuously sought to implement dedicated virus reduction steps into the manufacturing processes of these products. Once robustly established and widely available, nanofiltration has frequently been considered an option for this purpose (for review see Burnouf and Radosevich[1]).

In selecting the appropriate pore sizes of these filters, commercially available between 15 and 75 nm, a delicate balance needs to be struck between maintaining an appropriate yield of the respective product intermediate while effectively removing viruses. Especially for larger-molecular-weight protein preparations the removal of smaller viruses has thus been difficult,[2] unless virus antibodies present in the intermediate increased the effective filtration size of a virus by formation of virus–antibody complexes,[3,4] or specific product formulations contributed to virus removal by inducing virus aggregation.[5] Particularly parvovirus B19 (B19V), currently the only known parvovirus associated with significant pathogenicity for humans, can thus often not be efficiently removed from larger-molecular-weight biologic entities of medicinal importance by these procedures.

B19V contaminates human blood or plasma donations, at reported frequencies of 1 in 800-5950[6,7] and levels

ABBREVIATIONS: B19V = parvovirus B19; FEIBA = Factor VIII inhibitor-bypassing activity; LRF(s) = log reduction factor(s); MMV = mice minute virus; NF/VH = nanofiltered and vapor heat treated.

From the Global Pathogen Safety and Molecular Biology, Baxter BioScience, Vienna, Austria.

Address reprint requests to: Thomas R. Kreil, PhD, Baxter BioScience, Benatzkygasse 2-6, A-1221 Vienna, Austria; e-mail: thomas_kreil@baxter.com.

Received for publication September 24, 2007; revision received November 19, 2007, and accepted November 24, 2007.

doi: 10.1111/j.1537-2995.2008.01662.x

TRANSFUSION ;**:**-**.