Transfusion Medicine and Hemotherapy

Case Report

Transfus Med Hemother 2016;43:198–202
DOI: 10.1159/000445195

Received: December 4, 2015
Accepted: March 2, 2016
Published online: May 3, 2016

Parvovirus B19 Passive Transmission by Transfusion of Intercept® Blood System-Treated Platelet Concentrate

Peter Gowland[a] Stefano Fontana[a] Martin Stolz[a] Nicola Andina[b] Christoph Niederhauser[a]

[a] Interregional Blood Transfusion SRC, Bern, Switzerland;
[b] University Clinic of Haematology and Central Haematology Laboratory, Bern University Hospital Inselspital, University of Bern, Bern, Switzerland

Keywords
Parvovirus B19 · Pathogen inactivation · Platelet transfusion · Transfusion-associated infections · Pathogen reduction

Summary
Background: Pathogen reduction methods for blood components are effective for a large number of viruses though less against small, non-enveloped viruses such as Parvovirus B19 (B19V). This article describes the passive transmission by transfusion of two B19V-contaminated pooled platelet concentrates (PCs) which were treated with the Intercept® blood pathogen reduction system. **Case Reports:** Two transfusion cases of B19V-contaminated Intercept-treated pooled PCs were described. Due to the analysis delay, the PCs were already transfused. The viral content of each donation was 4.87×10^{10} IU/ml in case 1and 1.46×10^{8} IU/ml in case 2. B19V (52 IU/ml) was detected in the recipient of the case 1 PC, whereas no virus could be detected in the case 2 PC recipient. A B19V IgM response and a transient boost of the underlying B19V IgG immune status and was observed in recipient 1. Recipient of the case 2 PC remained B19V IgG- and IgM-negative. B19V DNA sequence and phylogenetic analysis revealed a 100% homology between donor and recipient. **Conclusion:** This report describes passive B19V transmission by a PC with very high B19 viral load which elicited a transient boost of the B19V immunity, but not by a PC with a lower B19V content, suggesting that there is a B19 viral load threshold value at which B19V inactivation is exceeded.

Introduction

Human parvovirus B19 (B19V) is a common human pathogen that causes a variety of diseases with outcomes ranging from asymptomatic to severe symptoms, especially in immunocompromised patients. The majority of these are asymptomatic or mild childhood infections. However, other clinical outcomes may occur, e.g., erythema infectiosum in children, transient aplastic crisis, chronic pure red blood cell (RBC) aplasia, prolonged joint pain in elderly individuals, pregnancy complications (foetal hydrops or spontaneous abortion) as well as severe prolonged anaemia in immunocompromised haematological patients [1, 2].

B19V is mainly transmitted through the respiratory route; however, vertical transmission from mother to foetus as well as through blood and blood products has been reported. B19V is also a transfusion-transmitted agent because of the incomplete clearance of the virus after an acute infection, an extremely high viraemia in acutely infected individuals and the well-known resistance of the virus to many inactivation processes used during the manufacturing of blood-derived plasma derivatives and labile blood products [2–6]. B19V viraemia occurs approximately 1 week after primary infection and persists at high titres of up to 10^{14} viral particles/ml in plasma for approximately 5 days [2, 7].

Several cases of transfusion transmission of B19V have been reported, and many B19V-contaminated blood donations have been retrospectively or prospectively detected. B19V transfusion-transmitted infections (TTI), however, seem to be quite rare with RBCs and platelet concentrates (PCs) [4, 5, 8–10]. Apart from the common asymptomatic course of the disease and the fact that the infection is often not well recognised by many physicians, the most probable reason for the relatively few B19V TTIs is the presence of neutralising anti-B19V antibodies stemming either from the donation itself or being already present in the recipient [4]. The rela-

KARGER

Fax +49 761 4 52 07 14
Information@Karger.com
www.karger.com

1660–3796/16/0433–0198$39.50/0

Accessible online at:
www.karger.com/tmh

Dr. Peter Gowland
Interregional Blood Transfusion SRC:
Murtenstrasse 133
3008 Bern, Switzerland
peter.gowland@itransfusion.ch

tively common B19V contamination of plasma derivatives led to widespread adoption of B19V nucleic acid testing (NAT) screening of source and recovered plasma donations to detect high-titre viraemic units before pooling and fractionation in the pharmaceutical industry [2, 11–13].

As in many European countries, NAT of blood donors for B19V is not mandatory for the release of labile blood products in Switzerland but is routinely performed by the manufacturer of fractionation plasma [2]. Since 2007, all donations at the Interregional Blood Transfusion SRC Ltd (formerly Blood Transfusion Service (BTS) SRC Berne) were tested for B19V by NAT with a sensitivity limit of 10^6 IU/ml in the individual donation. Plasma derivatives that exceed this limit are excluded from the manufacturing plasma pools. Because this test is performed after the release of the labile products, stored infectious units are discarded by post-donation information procedures. Those units already transfused are traced by a look-back procedure.

Pathogen reduction methods for blood components have been shown to be effective for a large number of pathogens including B19V [14, 15]. In 2011, Switzerland was the first country introducing nation-wide the pathogen reduction of PCs with amotosalen and UVA (Intercept® blood pathogen reduction system; Cerus Corporation, Amersfoort, Netherlands) [16]. The degree of B19V reduction achieved has been reported to range from 3.5 to 5 log of B19V by the infectivity assay or up to reach 6 logs as measured by polymerase chain reaction (PCR) inhibition [14, 15, 17].

Material and Methods

NAT screening for B19V is performed in pools of up to 480 donations on a twice weekly basis. The validated in-house quantitative NAT for B19V DNA for all 3 known genotypes has a 95% sensitivity limit in an individual donation of 32.7 IU/ml (95% CI 27.1–42.8 IU/ml). B19V-positive pool samples containing $>1 \times 10^4$ IU/ml B19V DNA are resolved to the single donation. Those donations with viral loads $>1 \times 10^6$ IU/ml B19V DNA are removed from the plasma fractionation process. The corresponding RBCs and PCs are also removed from the stock by post-donation information procedure. Already transfused PCs are traced by a look-back procedure in the patients. Our laboratory adheres strictly to precautionary measures to prevent sample contamination. Sample preparation, reagent preparation and amplification are strictly separated in different laboratories. Pooled PCs are manufactured by the buffy coat method out of 5 whole blood donations and routinely treated with amotosalen and UVA (Intercept blood pathogen reduction system) [18]. From the 5 pooled buffy coats there remains 125–130 ml of the original plasma in the final pathogen-reduced PC, thus from each involved donation approximately 25 ml rest plasma remains in the final product.

B19V ELISA and Immunoblot serology (IgG and IgM) was performed with commercial assays (DiaSorin Ireland Ltd., Dublin, Irland; MIKROGEN GmbH, Neuried, Germany). Epitope specificity of the IgG antibodies and avidity was investigated using the Immunoblot. B19V DNA sequence analysis was performed on a partial coding NS1/VP1 unique 936 bp PCR fragment of the B19V genome and were aligned using CLUSAL_W [19, 20]. Phylogenetic and molecular evolutionary analyses were conducted with MEGA version 6 using the nearest-neighbour joining method from a Kimura 2-parameter distance matrix [21].

Case Reports

B19V NAT is conducted in pools of up to 480 donations twice weekly, and positive pools are resolved to the single donation with further B19V NAT analyses. The delay in detecting the corresponding B19V-contaminated blood unit is approximately 5 days. Since B19V NAT is not mandatory for the release of labile blood products, including PCs, occasionally these products are transfused before a recall of the product is initiated. Here we report two cases of suspected transfusion-transmitted B19V transmissions.

Case 1

A 74-year-old female patient was transfused with a pooled PC which contained a PC from a B19V NAT-positive donation. The viral load of the B19V-contaminated donation was 4.87×10^{10} IU/ml. The donor, a 37-year-old repeat male donor, was unaware of his active B19V infection. Ten days post transfusion a B19V viral load of 52 IU/ml was measured in the plasma of the recipient as part of a look-back investigation. B19V DNA sequence and phylogenetic analysis of the partial NS1/VP1 unique 936 bp PCR fragment revealed a B19V genotype 1a for both viruses and a 100% homology between the B19V isolate from the donor and recipient (fig. 1). After 74 days B19V was undetectable in the recipient's blood. B19V IgG and IgM serology were conducted for the donor and patient's blood samples which were available. The original B19V-contaminated donation was both B19V IgG- and IgM-negative but the donor was strongly positive for B19V IgG and IgM at 52 days post donation, indicating a very recent B19V infection. Unfortunately no plasma sample was available from the recipient prior to or during the PC transfusion. At 10 days post transfusion the B19V IgG test was positive up to 1:1,000, whereas after 74 days a positive B19V IgG value was measured up to 1:100, suggesting a transient boost of an underlying B19V IgG immune status. Immunoblot analysis on both post-transfusion samples revealed IgG antibodies to the minor and major capsid antigens (VP1/2) as well as to the non-structural protein NS1, and the avidity was high, further suggesting a previous B19V immunity. B19V IgM antibodies were detected in an Immunoblot on the 10-day post-transfusion sample, but not in the follow-up sample.

Case 2

A 72-year-old male patient was transfused with a pooled PC which contained a PC from a B19V NAT-positive donation. The viral load of this contaminated donation was 1.46×10^8 IU/ml. The donor, a 41-year-old repeat male donor, was unaware of his active B19V infection, and no B19V IgG or IgM antibodies were detected in the B19V-contaminated donation. At 52 days post donation the donor had, as expected, seroconverted for B19V antibodies (both B19V IgM and IgG >1:10 positive). In a look-back investigation, no evidence for B19V transmission from donor to recipient either by NAT or IgG or IgM serology was detected at 4 and 9 days post transfusion. Unfortunately no later samples were available.

Discussion

Pathogen reduction methods for blood components (including Intercept) are effective for a large number of viruses; however, it has been reported that they are more efficient against enveloped viruses than against small, non-enveloped viruses such as B19V and hepatitis E virus (HEV). We are not aware of any other case reports of B19V transmission after amotosalen and UVA treatment. There was a recent report of a HEV TTI after Intercept blood system treatment; HEV is a non-enveloped icosahedral virus similar to B19V but with a RNA rather than DNA genome [22]. Here we describe 2 cases of B19V-contaminated pooled PCs which were treated with Intercept blood system and transfused to two recipients before the contamination was detected and the products could be removed from the

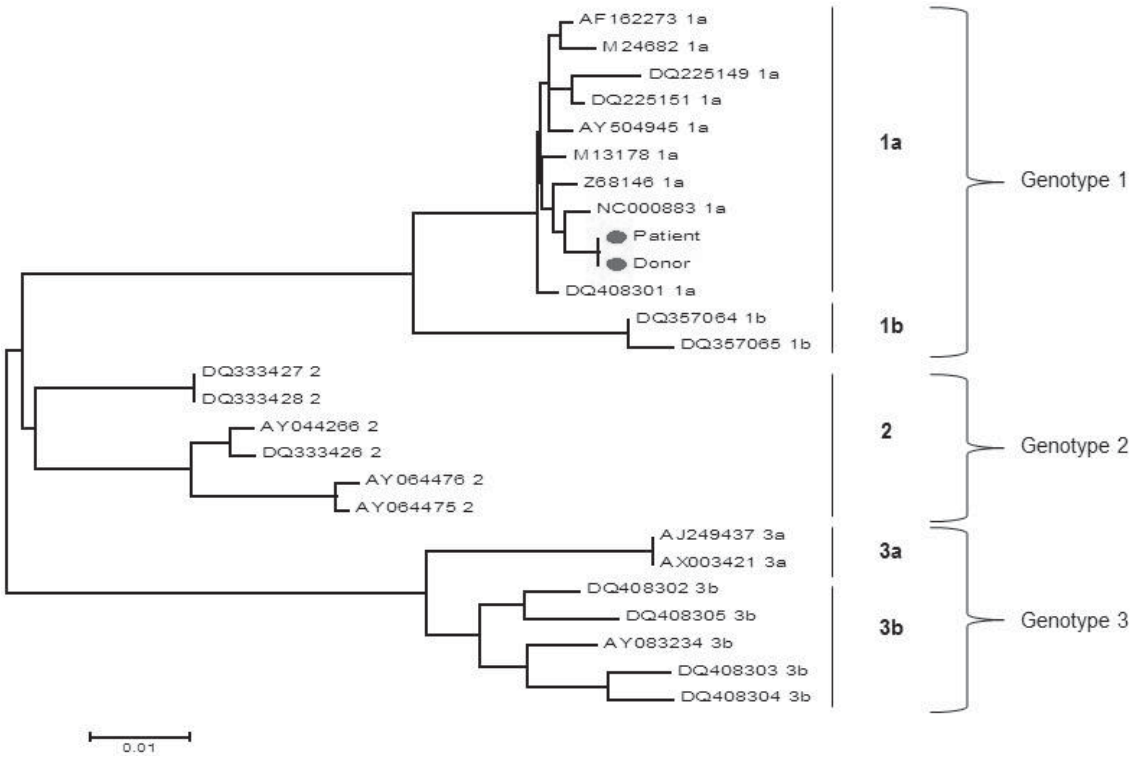

Fig. 1. Case 1. Donor/recipient B19V phylogenetic analysis of a partial NS1-VP1 unique region against known B19V genotype sequences. Nearest-neighbour method from a Kimura 2-parameter distance matrix.

transfusion process. The B19V viral burden of the original donations differed by approximately 2.5 logs (4.87×10^{10} IU/ml vs. 1.46×10^{8} IU/ml). A B19V transmission (52 IU/ml in recipient 1, 10 days post transfusion) was documented for the donation with the high B19V viral load (>4.87×10^{10} IU/ml) and confirmed by identical sequences identified in donor and patient. No transmission was detected, however, in case of the PC produced from the donation contaminated with 1.46×10^{8} IU/ml B19V, suggesting that there is a B19V viral load 'threshold value' at which pathogen inactivation is exceeded. The data presented in case 1 is consistent with a passive transmission of B19V to a B19V seropositive patient which has caused a reactivation of the immune response. An infection may have transiently occurred; however, due to the patient's presumed seropositivity and likely B19V IgG antibodies from the other donors in the pooled PC a transmission, but not an infection, is the most likely explanation. Further serological B19V assays (IgM and IgG Immunoblot) on the samples available revealed a B19V IgM response in the sample 4 days after transfusion and a high avidity in the B19V IgG response; both results are consistent with a B19V transmission to a seropositive individual.

Asymptomatic B19V primary infected individuals often present with extremely high viral burden of 10^{8} to 10^{14} IU/ml which are often identified in unsuspected blood donors [7, 23]. Our routine B19V NAT screen of pooled blood donations (up to 480 donations) identifies those pools harbouring a B19V viral load >1.00×10^{4} IU/ml to be resolved to the single positive donation. From 2008 to 2015, 973,796 blood donations were screened. 68 donations were identified with a viral burden >1.00×10^{6} IU/ml (0.007% of the total donations tested) after resolving the pools to the single donation. Similar levels of B19V DNA-positive donations have been reported in other middle European countries (The Netherlands: 0.006% >1.00×10^{6} IU/ml and Germany/Austria: 0.0018% >1.00×10^{5} IU/ml) [24, 25]. Interestingly nearly 40% of the B19V-positive samples harboured high B19V burdens (26 donations, >1.00×10^{10} IU/ml, 0.003% donations tested), similar to the donation implicated in case 1 (4.87×10^{10} IU/ml). Such very high B19V DNA-positive blood levels occur during the short highly viraemic phase after primary infection before the B19V IgM and IgG immune response starts around 10 days after infection [2, 7]. In both cases described, the plasma of the B19V-contaminated donation was devoid of both B19V-specific IgM and IgG antibodies. Unfortunately, the B19V anti-IgG and -IgM status of the 8 other donors involved in the pooled platelet concentrates could not be determined as no sample was available. However, since the seroprevalence of B19V IgG antibodies in adults is known to be high (>60% at 20 years up to >75% at 65 years), it is highly likely that both concentrates contain at least one seropositive donor and thus similar levels of neutralising antibodies [26]. Differences in the levels of B19V IgG antibodies in the two PCs may have played a role in the outcome of the two cases; however, the distinct difference between the rest viral concentrations is in our opinion the major distinction and therefore the probable essential difference.

Amotosalen and UVA treatment is clearly an effective procedure to inactivate or reduce viral, bacterial and parasitic contamination of platelets and (in the future) other blood components [14, 15]. Due to its often extremely high B19V blood concentrations and the reported less effectively of the amotosalen and UVA treatment against the tightly formed protein B19V capsids, the potential for a B19V transmission and perhaps infection from B19V-contaminated blood products has not been eliminated. The PCR inhibition assay reported by Sawyer and colleagues [17] demonstrated

Table 1. Case studies: B19V burden estimations after pathogen reduction under 2 different assumptions: log reduction of the viral burden of 5 and 3.5[a]

Range log reduction	B19V after reduction, IU/ml	B19V in 25ml rest plasma in PC before reduction, IU	B19V in 25ml rest plasma in PC after reduction, IU
Case 1 (B19V burden: 4.87×10^{10})			
5 log	4.87×10^{5}	1.22×10^{12}	1.22×10^{7}
3.5 log	1.54×10^{7}	1.22×10^{12}	3.85×10^{8}
Case 2 (B19V burden: 1.46×10^{8})			
5 log	1.46×10^{3}	3.65×10^{9}	3.65×10^{4}
3.5 log	4.62×10^{4}	3.65×10^{9}	1.15×10^{6}

[a]The range of B19V reduction is reported to range from 3.5–5 log [12, 13].

a 6-log inactivation of full-length B19V DNA templates, but only a 2-log inhibition of 1kb templates. There thus appears to be enough adducts to inactivate 10^6 IU of B19V. In case 1, the contaminated pooled PC transfused contained 1.22×10^{12} IU of B19V prior to Intercept treatment. Even a 6-log reduction would leave a substantial number of virus particles without adducts and thus potentially infectious. In case 2 the viral load was >2 logs lower than in case 1, and thus less viral particles were potentially free from adducts after Intercept treatment.

B19V infectivity reduction after treatment with the Intercept blood system has been reported to range between 3.5 and 5 logs [14, 15]. After Intercept treatment of the transfused PCs produced from the pooled buffy coats, there still remains 125–130 ml of the original plasma or about 25 ml from each donation of a pool of 5 donations. Extrapolated from these calculations it can be assumed that in the first case 1.22×10^{12} IU of B19V were treated with Intercept blood system (table 1) and thus between 1.22×10^{7} and 3.85×10^{8} IU remained in the PC, depending on the expected range of pathogen reduction, and this was transfused to the recipient. Thus the presumed underlying B19V immunity was not sufficient to neutralise and eliminate the passive transfer of the virus after 10 days, or perhaps the virus was able to transiently infect the recipient and produce new virus. This observation was consistent with B19V IgG increases after transfusion of two B19V IgG-positive volunteers with 200 ml of PLAS+SD at a DNA concentration of 1.6×10^{8} IU/ml [5] as well as with a further report of a four-fold boost in the B19V IgG in a follow-up sample from a recipient of a blood component with a very high B19V content (approximately 5.8×10^{11} IU B19V DNA) [27].

This report brings up several points which need to be addressed by the blood transfusion community in the future. High-titre B19V blood donations are often detected by blood transfusion services especially during the periodic waves of infection which are known to occur. The current use of pathogen reduction technologies has certainly been successful in reducing the risk of viral transmission via transfusion. However, it does have limitations with regard to high titre non-enveloped viruses as highlighted in the present report and is at present only used routinely for PCs. B19V NAT is at present not required for the release of blood products, and in addition only those donations with $>10^6$ IU/ml are identified. In the future it is not prudent to suggest for high-risk patients (i.e. pregnant women and immunocompromised patients) a selection of blood products which have been screened with single-unit NAT for B19V and perhaps other non-enveloped viruses. Alternatively B19V NAT could be declared mandatory for the release of all blood products with the already known consequences. This in turn poses the pertinent question whether national health services are prepared to bear the extra cost this will bring about.

Author Contributions

Peter Gowland collected and analysed the data, performed the phylogenetic analysis and wrote the paper. Stefano Fontana analysed the data and supervised the blood collection. Martin Stolz supervised the molecular analysis. Nicola Andina supervised the blood collection and collected the patient data. Christoph Niederhauser supervised the laboratory analysis, analysed the data and wrote the paper.

Disclosure Statement

The authors declare no conflicts of interest.

References

1 Young NS, Brown KE: Parvovirus B19. N Engl J Med 2004;350:586–597.
2 Mitteilungen des Arbeitskreises Blut des Bundesministeriums für Gesundheit. Bundesgesundheitsblatt 2010; 53:944–956
3 Blümel J, Schmidt I, Effenberger W, Seitz H, Willkommen H, Brackmann HH, et al: Parvovirus B19 transmission by heat-treated clotting factor concentrates. Transfusion 2002;42:1473–1481.
4 Satake M, Hoshi Y, Taira R, Momose SY, Hino S, Tadokoro K: Symptomatic parvovirus B19 infection caused by blood component transfusion. Transfusion 2011;51:1887–1895.
5 Kleinman SH, Glynn SA, Lee TH, Tobler LH, Schlumpf KS, Todd DS, et al: A linked donor-recipient study to evaluate parvovirus B19 transmission by blood component transfusion. Blood 2009;114:3677–3683.
6 Hourfar MK, Mayr-Wohlfart U, Themann A, Sireis W, Seifried E, Schrezenmeier H, et al: Recipients potentially infected with parvovirus B19 by red blood cell products. Transfusion 2011;51:129–136.
7 Corcoran A, Doyle S: Advances in the biology, diagnosis and host-pathogen interactions of parvovirus B19. J Med Microbiol 2004;53:459–475.

8 Parsyan A, Candotti D: Human erythrovirus B19 and blood transfusion – an update. Transfus Med 2007;4: 263–278.
9 Yu MY, Alter HJ, Virata-Theimer ML, Geng Y, Ma L, Schechterly CA, et al: Parvovirus B19 infection transmitted by transfusion of red blood cells confirmed by molecular analysis of linked donor and recipient samples. Transfusion 2010;50:1712–1721.
10 Juhl D, Özdemir M, Dreier J, Görg S, Hennig H: Lookback study on recipients of Parvovirus B19 (B19V) DNA-positive blood components. Vox Sang 2015;109: 305–11
11 Brown KE, Simmonds P: Parvoviruses and blood transfusion. Transfusion 2007;47:1745–1750.
12 Wu CG, Mason B, Jong J, Erdmann D, McKernan L, Oakley M, et al: Parvovirus B19 transmission by a high-purity factor VIII concentrate. Transfusion 2005; 45:1003–1010.
13 Brown KE, Young NS, Alving BM, Barbosa LH: Parvovirus B19: implications for transfusion medicine. Summary of a workshop. Transfusion 2001;41:130–135.
14 Prowse CV: Component pathogen inactivation: a critical review. Vox Sang 2013;104:183–199.
15 Irsch J, Lin L: pathogen inactivation of platelet and plasma blood components for transfusion using the INTERCEPT Blood System™. Transfus Med Hemother 2011;38:19–31.
16 Blutspende SRK Schweiz: Vorschriften / Empfehlungen / mitgeltende Dokumente. Kapitel 18 B: Spezifikationen: Thrombozytenkonzentrate. *https://sbsc-bsd.ch/dokuman/DokumenteBSD/Allgemeines/tabid/107/Default.aspx* (last accessed April 6, 2016).
17 Sawyer L, Hanson D, Castro G, Luckett W, Dubensky TW Jr, Stassinopoulos A: Inactivation of parvovirus B19 in human platelet concentrates by treatment with amotosalen and ultraviolet A illumination. Transfusion 2007;47:1062–70.
18 Lin L, Hanson CV, Alter HJ, Jauvin V, Bernard KA, Murthy KK, et al: Inactivation of viruses in platelet concentrates by photochemical treatment with amotosalen and long-wavelength ultraviolet light. Transfusion 2005;45:580–590.
19 Candotti D, Etiz N, Parsyan A, Allain JP: Identification and characterization of persistent human erythrovirus infection in blood donor samples. J Virol 2004;78: 12169–12178.
20 Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, et al: Clustal W and Clustal X version 2.0. Bioinformatics 2007;23:2947–2948.
21 Tamura K, Stecher G, Peterson D, Filipski A, Kumar S: MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Mol Biol Evol 2013;30:2725–2729.
22 Hauser L, Roque-Afonso AM, Beylouné A, Simmonet M, Fischer BD, des Roziers NB, et al: Hepatitis E transmission by transfusion of Intercept blood system-treated plasma. Blood 2014 30;123:796–797.
23 Prowse C, Ludlam CA, Yap PL: Human parvovirus B19 and blood products. Vox Sang 1997;72:1–10.
24 Kooistra K, Mesman HJ, de Waal M, Koppelman MH, Zaaijer HL: Epidemiology of high-level parvovirus B19 viraemia among Dutch blood donors, 2003–2009. Vox Sang 2011;100:261–266.
25 Schmidt M, Themann A, Drexler C, Bayer M, Lanzer G, Menichetti E, et al: Blood donor screening for parvovirus B19 in Germany and Austria. Transfusion 2007;47:1775–1782
26 Röhrer C, Gärtner B, Sauerbrei A, Böhm S, Hottenträger B, Raab U, et al: Seroprevalence of parvovirus B19 in the German population. Epidemiol Infect 2008; 136:1564–1575.
27 Doyle S, Corcoran A: The immune response to parvovirus B19 exposure in previously seronegative and seropositive individuals. J Infect Dis 2006;194:154–158.