Parvovirus B19 infection transmitted by transfusion of red blood cells confirmed by molecular analysis of linked donor and recipient samples

Mei-ying W. Yu, Harvey J. Alter, Maria Luisa A. Virata-Theimer, Yansheng Geng, Li Ma, Cathy A. Schechterly, Camilla A. Colvin, and Naomi L.C. Luban

BACKGROUND: Extremely high viremic levels of parvovirus B19 (B19V) can be found in acutely infected, but asymptomatic donors. However, reports of transmission by single-donor blood components are rare. In this prospective study, paired donor-recipient samples were used to investigate the transfusion risk.

STUDY DESIGN AND METHODS: Posttransfusion plasma or blood samples from recipients were tested for B19V DNA by polymerase chain reaction, generally at 4 and 8 weeks, and for anti-B19V immunoglobulin (Ig)G by enzyme immunoassay, at 12 and 24 weeks. To rule out infection unrelated to transfusion, pretransfusion samples and linked donor's samples for each B19V DNA–positive recipient were assayed for B19V DNA and anti-B19V IgG and IgM. To confirm transmission, sequencing and phylogenetic analysis were performed.

RESULTS: A total of 14 of 869 (1.6%) recipients were B19V DNA positive, but only 1 of 869 (0.12%; 95% confidence interval, 0.0029%-0.6409%) was negative for B19V DNA and anti-B19V IgG before transfusion and seroconverted posttransfusion. This newly infected patient received 5×10^{10} IU B19V DNA in one red blood cell (RBC) unit from an acutely infected anti-B19V–negative donor in addition to RBCs from three other donors that cumulatively contained 1320 IU of anti-B19V IgG. DNA sequencing and phylogenetic analysis showed that sequences from the linked donor and recipient were identical (Genotype 1), thus establishing transfusion transmission.

CONCLUSIONS: The 0.12% transmission rate documented here, although low, could nonetheless result in hundreds or thousands of infections annually in the United States based on calculated confidence limits. Although most would be asymptomatic, some could have severe clinical outcomes, especially in neonates and those with immunocompromised or hemolytic states.

Parvovirus B19 (B19V) is a small, nonenveloped, DNA virus of the *Erythrovirus* genus in the Parvoviridae family. It resists viral inactivation procedures commonly used in the manufacture of pooled plasma products and is the only parvovirus shown to be pathogenic in humans.

When B19V infects erythropoietic progenitors, transient but significant red blood cell (RBC) hypoplasia or aplasia ensues. This is especially common in children, but it also occurs in seronaive adults who have coexisting hemolytic anemias, such as sickle cell disease, hereditary spherocytosis, or RBC enzymopathies (see review[1]). Furthermore, B19V-induced RBC aplasia is seen in patients

ABBREVIATIONS: B19V = parvovirus B19; CBER = Center for Biologics Evaluation and Research; EOS = end of study; TRIPS = Transfusion-Related Infections Prospectively Studied; WB = whole blood.

From the Division of Hematology, Center for Biologics Evaluation and Research (CBER), FDA, and the Department of Transfusion Medicine, Warren Grant Magnuson Clinical Center, NIH, Bethesda, Maryland; and the Division of Laboratory Medicine, Children's National Medical Center, Department of Pediatrics, George Washington University School of Medicine and Health Sciences, Washington, DC.

Address reprint requests to: Mei-ying W. Yu, PhD, Division of Hematology, HFM-345, CBER, FDA, Room 303, 29 Lincoln Drive, Bethesda, MD 20892; e-mail: mei-ying.yu@fda.hhs.gov.

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with congenital and acquired immunodeficiency because high-titer viremia in the absence of an effective humoral immune response can result in prolonged hypoplastic anemia. When B19V infection occurs during pregnancy, hydrops fetalis and fetal loss can result. Arthropathy, neutropenia, and thrombocytopenia have been reported in both children and adults.[1]

B19V transmission occurs through the respiratory route, vertically from mother to fetus, and through transfusion or transplantation—most often during the viremia that precedes clinical presentation. Household, day care, and school transmissions are common. Viral levels as high as 10^{13} genome equivalents (geq)/mL are often found in the blood of asymptomatic individuals during the early phase of acute infection.[2] There are numerous reports of transmission by pooled plasma-derived products, including clotting factor concentrates despite solvent/detergent (S/D) treatment, heat treatment, and/or other viral inactivation methods.[3-8] Infectivity has been correlated with a high concentration of B19V prompting plasma fractionators to implement screening for B19V DNA by nucleic acid testing (NAT) to exclude high-titer donations from entering manufacturing pools.[9-11]

The presence of anti-B19V IgG in the recipient or product may play a role in attenuating transmission. The prevalence of anti-B19V IgG exceeds 50% in some donor populations.[12,13] These antibodies are considered to be neutralizing[14,15] and to confer lifelong immunity.[1] The prevalence of B19 viremia in blood and plasma donors ranges from 0.003% to 0.88%[16-20] depending on the sensitivity of the NAT method employed and whether the testing is performed at the time of an epidemic. Low levels of B19V DNA, ranging from 10 to 10^3 IU or geq/mL, coexist with anti-B19V IgG and may persist for 3 to 5 years in immunocompetent blood donors.[21-24] Infectivity is presumably dependent on the relative balance between viral and neutralizing antibody titers, albeit the minimum infectious dose of B19V DNA, with or without coexisting anti-B19V IgG, is unknown. The IgG antibodies from multiple plasma donors are generally sufficient to render pooled plasma products noninfectious if no donor in the pool has high-level viremia. This provides the rationale for excluding only those plasma donations for further manufacturing that have viral titers exceeding approximately 10^6 IU/mL.[11,25] To date, universal blood donor screening for B19V NAT is not performed in the United States. Case reports of transmission by single-donor blood components are rare. Only four cases of transfusion-transmitted B19V-induced anemia have been documented.[16,26-28] The rarity of such case reports may reflect the fact that most infected individuals are asymptomatic and hence undetected unless enrolled in a prospective study. In this article, we describe a case of transfusion-transmitted B19V infection identified in a prospective study designated TRIPS (Transfusion-Related Infections Prospectively Studied). Pre- and serial posttransfusion samples from recipients and linked donor specimens were collected and placed in frozen storage. This permitted an estimate of the frequency of B19V infection by blood components and the infectious dose relative to the titer of antibody. Further, linked donor-recipient samples allowed for confirmation of causality by DNA sequencing and phylogenetic analysis.

MATERIALS AND METHODS

Patient population and study specimens

The TRIPS repository was initiated in November 2001 and is composed of linked donor-recipient specimens from transfusion recipients enrolled at the NIH Clinical Center (Bethesda, MD) and the Children's National Medical Center (Washington, DC) and from Suburban Hospital (Bethesda, MD). Informed consent was obtained from all donors and recipients in accordance with the Declaration of Helsinki for participation in NIH-sponsored and institutional review board–approved protocols (NIH Protocol 01-CC-0231; Children's National Medical Center, Protocol 2540). Human subjects were assigned a code number, and samples for testing were identified only by that code; the testing laboratories, including the parvovirus testing laboratory at the Center for Biologics Evaluation and Research (CBER), FDA, had no capability of linking the code number to the study participant's name. Thus far, pretransfusion plasma and/or whole blood (WB) samples from 869 enrolled recipients have been collected, generally at 4, 8, 12, and 24 weeks posttransfusion (and/or at the end of study [EOS] for repeatedly transfused subjects followed longer than 24 weeks). WB and/or plasma samples from donors and recipients were stored in 1- to 2-mL aliquots at –80°C in a central repository (SeraCare BioServices, Gaithersburg, MD). Medical records review was performed to obtain details of underlying diseases and clinical circumstances surrounding the transfusion.

Detection and quantitation of B19V DNA by polymerase chain reaction

B19V DNA levels were determined on the first available samples collected after transfusion from each recipient, usually at 4 and 8 weeks, but occasionally also at 2 weeks. DNA was extracted from 0.2 mL of plasma or WB, and B19V DNA was detected and semiquantified by an in-house nested polymerase chain reaction (PCR) method as described previously.[8] A final amplified product of 243 bp was obtained extending from Nucleotide 2951 to Nucleotide 3193, thus covering the junction of the VP1/VP2 region based on the nucleotide numbering of the published B19 Au sequence[29] (GenBank M13178). The first WHO International Standard for B19V DNA[30] (NIBSC

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99/800, 10^6 IU/mL when reconstituted) was diluted 10^3-fold and used as a positive control for extraction and quantification. The level of B19V DNA, expressed as IU/mL, was determined by limiting dilution analysis since the conversion ratio from a geq (or a copy) to IU detected by PCR was 1:1 based on our PCR method. The sensitivity of the PCR assay with an original sample volume of 0.2 mL was 20 IU/mL, as previously described.[8] This B19V PCR procedure detects both Genotypes 1 and 2 of B19V, but not the Genotype 3 variant (see Discussion).

A posttransfusion sample was considered B19V DNA positive only when a separate 0.2-mL sample aliquot was also positive. Only if B19V DNA was positive after transfusion was the pretransfusion sample from that recipient requested from the repository, and a 0.2-mL aliquot was similarly tested by PCR. If the recipient's pretransfusion sample tested B19V DNA negative, suggesting the possibility of transfusion transmission, samples from the linked donor(s) plasma or WB were tested for B19V DNA.

Anti-B19V antibody assays

Anti-B19V IgG testing was performed qualitatively according to the manufacturer's instructions on 12- and 24-week (or EOS) samples with an FDA-cleared enzyme immunoassay (EIA) kit (Biotrin International Ltd, Dublin, Ireland) consisting of wells coated with recombinant B19 capsid protein (VP2). Index values of less than 0.9 are deemed negative and more than 1.1 are positive, while those between 0.9 and 1.1 are equivocal. The index value is obtained from the mean absorbance value for the test sample divided by the cutoff value, which is, as instructed by the kit, computed by multiplying the mean absorbance of the calibrator by the lot-specific constant. Further, we developed a semiquantitative assay to measure anti-B19V IgG levels that permitted calculating an infectious dose when B19V DNA and anti-B19V IgG were both present. This assay made use of a working standard solution consisting of 1 IU/mL anti-B19V IgG prepared by diluting the first WHO anti-B19 serum IgG standard[31] (NIBSC 93/724, 100 IU of anti-B19V IgG/mL when reconstituted) with sample diluent from the kit. A six-point standard curve was set up by twofold serial dilutions of the WHO working solution, providing a range from 0.031 to 1 IU/mL. Each plasma sample was diluted 30-fold or more, and then twofold serial dilutions were made. Aliquots of 100-µL of diluted standard or plasma solutions were incubated with the coated wells according to the manufacturer's instructions. Anti-B19V IgG levels in IU/mL were calculated by using a parallel-line model in statistical analysis software (CombiStats, Version 4.0) provided by the European Directorate for the Quality of Medicines & HealthCare (EDQM), Council of Europe (Strasbourg, France).

If recipients were confirmed B19V DNA positive (i.e., ≥20 IU/mL) from the first available sample collected after transfusion, anti-B19V IgM assays were performed on all plasma samples collected from the recipient, along with the associated donor samples. A B19V IgM EIA kit (Biotrin) was used to detect the presence of captured antibodies in human serum or plasma by means of biotinylated B19V VP2 protein according to the kit's instructions with similar computation of index values mentioned above for anti-B19V IgG testing. When a sample was scored as anti-B19V IgM equivocal, the same sample was retested for confirmation since plasma collected within 1 to 2 weeks of the initial reactive result was not available for retesting as recommended by the kit manufacturer.

DNA sequencing and phylogenetic analysis

Extracted DNA samples were amplified by a seminested B19V PCR procedure described previously[8] so that a longer final amplified product of 786 bp (Nucleotides 2408-3193), covering the entire VP1-unique region and a portion of the VP2 region, could be obtained. The amplified product was further purified by a PCR purification kit (QIAquick, Qiagen, Inc., Valencia, CA) and directly sequenced without the need for cloning since the samples sequenced contained relatively high levels of B19 DNA. The sequencing primers were the same as those used for the seminested PCR procedure. The WHO B19V DNA standard was similarly extracted and amplified, and the amplified product was sequenced for comparison. Sequences, each 710 nucleotides in length corresponding to Nucleotides 2465 to 3124 of the VP1-unique region plus Nucleotides 3125 to 3174 of the VP2 region, were aligned against other corresponding published sequences from GenBank by using ClustalW2, a general-purpose multiple alignment program. Phylogenetic analysis was performed with a neighbor-joining algorithm in computer software (Molecular Evolutionary Genetics Analysis, v.4, MEGA4, Center of Evolutionary Functional Genomics, Arizona State University, Tempe, AZ).

Statistical analysis

Statistical software (StatXact, v.8, Cytel, Inc., Cambridge, MA) was used to calculate 95% confidence interval (CI) based on one observed B19V infection in this study.

RESULTS

Fourteen of the 869 (1.6%) recipients were found to be B19V DNA positive in their early posttransfusion specimens; of these, seven were children and seven (Recipients 3-6, 8, 12, and 14 in Table 1) were adults. Pretransfusion and serial posttransfusion plasma samples from these 14 B19V DNA–positive recipients were tested for viral levels and IgM/IgG anti-B19V antibodies, and the results are shown in Table 1. Six

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TABLE 1. Analysis of 14 recipients positive for B19V DNA after transfusion*

	Before transfusion			After transfusion		
	B19V DNA	B19V antibodies		B19V DNA†	B19V antibodies‡	
Recipient	(IU/mL)	IgG	IgM	(IU/mL)	IgG	IgM
1	63	Positive	Negative	20	Positive	Negative
2	20	Positive	Negative	40	Positive	Negative
3	20	Positive	Negative	58	Positive	Negative
4	60	Positive	Negative	40	Positive	Negative
5	20	Positive	Negative	630	Positive	Negative
6	40	Positive	Negative	63	Positive	Negative
7	Negative	Positive	Negative	20	Positive	Negative
8	632	Positive	Positive	1.4×10^3	Positive	Negative
9	6.3×10^6	Positive	Positive	6.3×10^3	Positive	Positive
10	2×10^7	Positive	Positive	630	Positive	Positive
11	$>2 \times 10^{10}$	Negative	Positive	1.4×10^4	Positive	Positive
12	63	Negative	Negative	200	Positive	Negative
13	Negative	Negative	Negative	200	Negative	Negative
14	Negative	Negative	Negative	6×10^6	Positive	Positive (12 week)

* Posttransfusion samples were tested first. Only 14 recipients tested positive and hence their pretransfusion samples were obtained from the repository and tested for all B19V markers. All other recipients whose posttransfusion samples were negative for B19V DNA (i.e., <20 IU/mL) were not further investigated.

† B19V DNA levels listed were determined from the first available samples collected after transfusion, mostly at 4 weeks, except at 8 weeks for Recipients 5, 11, and 12 and at 2 weeks for Recipients 6 and 14.

‡ For each sample, the qualitative method for detecting anti-B19V (either IgG or IgM) has been described in detail under Materials and Methods. However, a designation of positive or negative in this table refers to the results obtained for testing both 12- and 24-week (or EOS) samples with the following exceptions: anti-B19V IgM was positive only in the 12-week sample for Recipient 14; anti-B19V testing was performed only on the 12-week sample for Recipients 5, 10, and 12; only on the 24-week (or EOS) sample for Recipients 1, 6, and 13; and only on the 4-week sample for Recipient 9 because of sample availability.

recipients (Recipients 1-6) had low-level viremia and anti-B19V IgG in their pretransfusion sample, indicating prior chronic infection unrelated to the index transfusion. One recipient (Recipient 7) was anti-B19V IgG positive and B19V DNA negative before transfusion and then, in the 4-week posttransfusion sample, displayed very low-level, transient viremia that coexisted with anti-B19V IgG. This case was considered to have existing B19V infection with fluctuating low-level viremia. Three recipients (Recipients 8-10) were found positive for both B19V DNA ($\leq 2 \times 10^7$ IU/mL) and anti-B19V (both IgM and IgG) before transfusion, two (Recipients 9-10) showing diminishing titers after transfusion and one (Recipient 8) showing a slight increase in titer. These three patients appear to have had an acute B19V infection that predated the transfusion. Recipient 11 clearly had an acute B19 infection that predated the transfusion since the pretransfusion sample had a very high B19V DNA level ($>2 \times 10^{10}$ IU/mL) associated with IgM antibody in the absence of IgG; after transfusion the viral level diminished and the patient seroconverted for anti-B19V IgG. Recipient 12 appeared to be in the seronegative window period of infection before transfusion since only low-level B19V DNA was detected before transfusion, and seroconversion for anti-B19V IgG was demonstrated after transfusion. Recipient 13 was negative for all B19V markers before transfusion; a single posttransfusion sample at 4 weeks had a B19V DNA level of 200 IU/mL, but later samples were negative and there was no evidence of antibody seroconversion. We considered this more likely a false-positive DNA determination than a transfusion-associated infection. Thus, only Recipient 14 fulfilled the criterion for a transfusion-related infection in that the recipient was negative for all B19V markers before transfusion and then developed high-level B19V DNA (6×10^6 IU/mL) and underwent seroconversion for IgM and IgG antibodies after transfusion. This patient is the subject of the following case report.

CASE REPORT

The single recipient infected with B19V through transfusion during the course of this study was a 35-year-old white female with a primary diagnosis of low-grade adenocarcinoma of the appendix with peritoneal carcinomatosis. She was married, had no children, and had no prior history of receiving a blood transfusion. As part of her surgical protocol, the patient received irradiated, leukoreduced RBCs from a total of six donors, consisting of two different units given every other day over a period of 5 days. A pretransfusion sample was collected on May 23, 2005, before her surgery.

As shown in Table 2, this seronegative recipient became strongly B19V DNA positive 2 weeks posttransfusion and remained positive at 4, 8. and 12 weeks, but was negative by Week 24. Anti-B19V (IgM and IgG) were absent before transfusion and then detected in both the 8- and the 12-week posttransfusion samples. IgG antibody persisted through the 24-week sample, but IgM antibody was no longer detectable at that time.

TABLE 2. B19V DNA and anti-B19V in a recipient*

Plasma sample	B19V DNA (IU/mL)	Anti-B19V	
		IgG	IgM
Before transfusion	Negative†	Negative	Negative
After transfusion			
2 weeks	6×10^6	Negative	Equivocal‡
4 weeks	20	Negative	Negative
8 weeks	630	Positive	Positive
12 weeks	140	Positive	Positive
24 weeks/EOS	Negative	Positive	Negative

* Recipient 14 in Table 1.
† Less than 20 IU/mL B19V DNA.
‡ Retested sample was also scored as "equivocal" according to the kit's instructions.

TABLE 3. B19V marker testing of donations received by the B19V-infected recipient

Donor number	Transfusion day	B19V DNA (IU/mL)	Anti-B19V	
			IgG (IU/mL)	IgM
1	0*	NT†	NT	NT
2	0	Negative	Negative	Negative
3	2	Negative	Positive (22‡)	Negative
4	2	5×10^9‡	Negative	Negative
5	4	Negative	Positive (77‡)	Negative
6	4	Negative	Positive (33‡)	Negative

* RBC units from two donors were transfused every other day starting on Day 0, the date of surgery, for a total of 6 RBC units.
† NT = not tested; sample was not available for testing.
‡ Geometric mean titer from three independent assays.

In the complex setting of surgical blood loss and transfusion replacement, measurement of hematocrit and hemoglobin could not discern whether the parvovirus infection had a suppressive effect on RBC production. Because the infection was not recognized until stored samples were retrospectively tested, reticulocyte counts were not performed. Platelet (PLT) and white blood cell counts remained normal and hospital chart review showed no record of the temporal occurrence of fever, rash, arthritis, or cardiopulmonary dysfunction.

The recipient was transfused with 6 RBC units: 2 units during exploratory surgery and 2 units on Postoperative Days 2 and 4, respectively. Of the six donors, one had no stored sample available for testing; one was negative for all B19V markers; three donors were positive for anti-B19V IgG (22, 77, and 33 IU/mL, respectively), but negative for IgM antibody and B19V DNA; and one donor (Donor 4) was acutely infected and had high-level B19V DNA (5×10^9 IU/mL) without any detectable anti-B19V (Table 3).

We calculated the total infectious dose by making two assumptions, namely, that there was 10 mL of residual plasma per RBC unit and that the unit unavailable for testing was negative for all B19V markers. With these assumptions, we estimate that the recipient received 5×10^{10} IU of B19V DNA from the acutely infected donor's RBC unit and 1320 IU of anti-B19 IgG from three donors' units (220, 770, and 330 IU).

To confirm that the B19V-contaminated RBC unit was the source of infection, direct sequencing of PCR-amplified products from the linked, implicated donor and the recipient was performed. We chose to sequence primarily the VP1-unique region, which exhibits the most variation in both DNA sequences and protein expression.[8,32] Plasma and WB from the implicated B19V DNA-positive donor (Donor 4 in Table 3) and plasma from the 2- and 8-week posttransfusion recipient samples were extracted for DNA and amplified by PCR using primers encompassing the entire VP1-unique region and a portion of the VP2 region. The amplified regions were sequenced and subjected to sequence alignment analysis. For comparison, a positive control (B19V DNA present in the WHO B19V DNA standard) was similarly PCR amplified and the purified-amplified product was sequenced. Identical 710 nucleotides covering the 650-nucleotide VP1-unique region and the 60-nucleotide VP2 region were obtained from the recipient's 2- and 8-week plasma samples and the donor's plasma and WB samples (Fig. 1A). In contrast, sequences similarly obtained from the WHO International Standard for B19V DNA had four nucleotide differences (C2531G, C2578T, A2736G, and T2786C) within the VP1-unique region while the published sequences of a well-known B19V strain, designated as Au,[29] differed by three nucleotide substitutions (the first three). These nucleotide differences would yield only two amino acid residue changes between the infecting strain in our patient and the WHO standard or the Au strain. By phylogenetic analysis, both the recipient and the donor were closely related on the same branch of the Genotype 1 phylogenetic tree and were distinct from the WHO standard and other known B19 isolates (Fig. 1B). Thus, in this prospective study, although 14 of 869 (1.6%) recipients were found to be B19V DNA positive, the observed transmission rate by transfusion was 1 in 869 (0.12%) with a 95% CI of 0.0029% to 0.6409%.

DISCUSSION

Of four previously reported cases of B19V transmission by single-donor blood components, three[16,26,27] were by RBC transfusions and one[28] was by PLTs. All these cases were identified by retrospective studies triggered by clinical symptoms or unexplained anemia observed in recipients who were immunocompromised and negative for all B19V markers before transfusion (pretransfusion sample not

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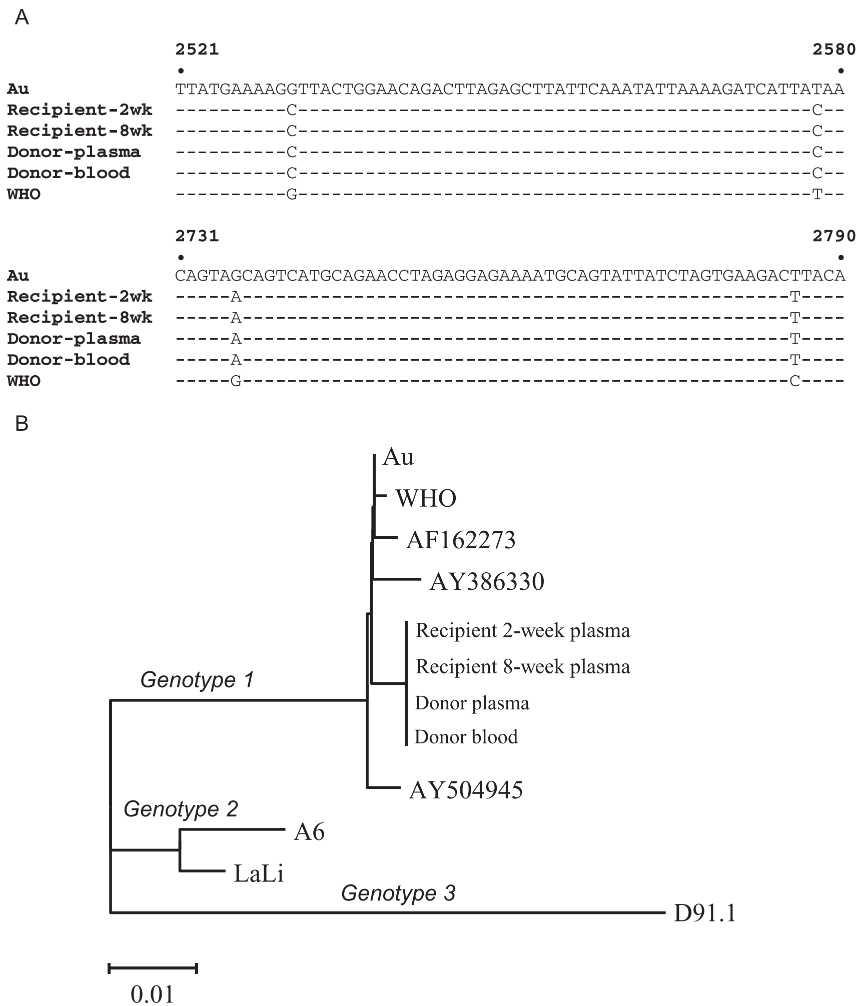

Fig. 1. (A) B19 nucleotide sequence alignments within the VP1-unique region (Nucleotides 2465-3124) and a portion of the N-terminal VP2 region (Nucleotides 3125-3174). All sequences obtained for Nucleotides 2465 to 2520, 2581 to 2730, and 2791 to 3174 were identical and therefore are not shown. Nucleotide numbering of the sequence is based on the published B19 Au strain (M13178). Sequences were determined directly from purified PCR-amplified products derived from the patient's 2- and 8-week plasma samples and from plasma and WB samples from Donor 4 (Table 3). As the positive control, the WHO B19V DNA standard was similarly sequenced. (B) Phylogenetic comparison of the above-mentioned B19 sequences (Nucleotides 2465-3174; 710 nucleotides) along with other corresponding published B19 sequences in the GenBank, that is, Genotype 1 = M13178 (Au), AF162273, AY386330, and AY504945; Genotype 2 = AY064476 (A6) and AY044266 (Lali); and a Genotype 3 = AY083234 (D91.1). Evolutionary distances are in units of the number of base substitutions per site (0.01 unit shown).

available in one case[16]). In two studies,[26,28] the implicated donors were positive for both B19V DNA and IgM antibody, while in the third study,[16] the implicated RBC unit was B19V DNA positive in the absence of antibody. In the fourth reported case,[27]donor samples were not available. In only one study[28] was DNA sequencing and phylogenetic analysis performed to confirm that the donor was the source of infection. Furthermore, in all these case reports, detection of B19V DNA by PCR was qualitative, and hence the amount of B19V DNA infused is unknown.

Two other studies[33,34] assessed the safety of single-donor blood products containing a known amount of B19V DNA. In one,[33] 200 mL of a WB unit containing a low level (5.8×10^2 IU/mL) of B19V DNA was transfused to a seronegative (hence susceptible) pediatric recipient. The other study[34] retrospectively evaluated several adult hematologic patients who received blood components with B19V DNA levels ranging from less than 600 to 2.2×10^6 geq/mL. However, neither of the recipient populations had any clinical or laboratory evidence of B19V infection, possibly because anti-B19V IgG was present in either the transfused blood components or the recipients. Although quantitative titers of B19V IgG were not provided, the antibodies likely played a protective role in attenuating transmission.[14,15]

One recent study[35] evaluated retrospectively the rate of transmission in susceptible recipients, that is, those B19V IgG–negative individuals who received B19V DNA–positive blood components, by using linked donor and recipient repository samples established in 2000 to 2003 within the United States. A B19V DNA prevalence of 0.84% (105/12,529) was detected in those linked donations, consistent with the 0.88% prevalence[20] found earlier in unlinked donations from the same repository. Of the 105 recipients of B19V DNA–positive donations, 78% had anti-B19V IgG in their pretransfusion samples leaving only 24 susceptible recipients eligible for evaluation. No susceptible recipient was infected by transfusion; however, all received blood products containing only low-level ($<10^4$ IU/mL) B19V DNA that coexisted with anti-B19V IgG. In addition, the study design did not include early posttransfusion samples to detect transient viremia or the appearance of anti-B19V IgM. Three blood components derived from donations with higher titers of B19V DNA ($>10^5$ IU/mL) and devoid of anti-B19 IgG were transfused but their infectivity could not be

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evaluated since each was infused into a nonsusceptible recipient.

The TRIPS study described herein is the first to investigate prospectively B19V transmission associated with transfusion of blood and blood components to susceptible immunocompromised or immunocompetent recipients. After the initial demonstration that 1.6% (14/869) of recipients had detectable B19V DNA after transfusion, the availability of pretransfusion samples allowed identification of those who were already infected with B19V. The majority had clear evidence of B19V infection existing before entry into the study and, in one case, evidence of a very recent infection that might have been attributed to transfusion if the appropriate pre- and posttransfusion samples had not been available.

Of the 14 recipients who were viremic after transfusion, only one was seronegative before transfusion and subsequently exhibited a seroconversion profile indicative of B19V transmission. This occurred after receiving a unit of RBC that had a minimum volume of plasma associated with it. By assuming that approximately 10 mL of plasma was present, we estimated that the patient received 5×10^{10} IU of B19V DNA. In reports of seronegative persons with hemophilia infused with contaminated coagulation concentrates[4] or seronegative volunteers experimentally exposed to B19V,[36] viral DNA was usually detected within 1 week after exposure. In the latter study,[36] peak viremia and seroconversion to anti-B19V IgM occurred within 2 weeks. The recipient infected in our study exhibited maximal viremia (6×10^6 IU/mL) 2 weeks after transfusion, but anti-B19V IgM was not detected until Week 8, simultaneous with the appearance of anti-B19V IgG. This delay might have been due to the modulating effect of B19V IgG–neutralizing antibodies present in three other RBC units transfused in close proximity to the implicated unit.[1,14,15]

Sequencing/phylogenetic analysis established that both the donor and the recipient were infected with B19V Genotype 1. Although B19V strains are genetically more diverse than previously thought and have been classified into three genotypes,[37] Genotype 1 is still the most prevalent in western countries while Genotype 2, though less common, has been detected in plasma and coagulation factor concentrates.[38-40] Genotype 3 is found predominantly in West Africa[41] and rarely in other areas of the world. Because some B19V NAT assays detect only Genotype 1,[40,42] some earlier investigations may have underestimated the diversity of B19V in the specimens examined. Recently a source plasma donor infected with a Genotype 3 strain was identified in the United States by a plasma fractionator performing a B19V NAT screening procedure capable of detecting all three genotypes in a minipool format.[43] The NAT procedure used in our study detects both Genotypes 1 and 2, but not Genotype 3.[11] Interestingly, B19V genotype variants have been shown to be very similar in functional and immunologic studies, and current data suggest that only one serotype exists for B19V.[38,41,44]

In our study, the infectious dose received by the susceptible (seronegative) recipient was 5×10^{10} IU of B19V DNA from 1 RBC unit (derived from an acutely infected donor) given in temporal proximity to a total of 1320 IU of anti-B19V IgG antibodies from 3 other RBC units. In the B19V transmission incident associated with pooled plasma, S/D treated, a pooled plasma product known to contain anti-B19V IgG,[9,45] the infectious dose received by susceptible immunocompetent volunteers was more than 2×10^9 geq or IU of B19V DNA, that is, 200 mL per implicated lot containing more than 10^7 geq/mL. In contrast, those same susceptible individuals were not infected when each received a dose of less than 2×10^6 IU of B19V DNA from product lots containing less than 10^4 IU/mL, indicating that infectivity is dependent on the balance between viral and neutralizing antibody titers. Moreover, when recipients were seropositive before transfusion, with anti-B19V IgG levels ranging from 19 to 39 IU/mL, the high-titer implicated product (i.e., that with $>10^7$ IU of B19V DNA/mL) was not infectious. Thus, there must be a specific level of anti-B19V IgG that confers protection against infection.[45] The lowest infectious dose reported was 2×10^4 IU of B19V DNA administered to a susceptible immunocompetent individual who received a Factor (F)VIII coagulation product devoid of any detectable anti-B19 IgG.[8]

The case found in our study represents the fifth documented B19V infection transmitted by cellular blood products and the fourth transmitted by RBCs. Although the observed incidence rate of 0.12% (1/869) is relatively low, it could translate to 4800 transfusion-transmitted B19V infections annually among the approximately 4 million blood recipients in the United States. However, because only one definite B19V transmission was observed in this population, the 95% CI for the incidence rate is very broad (0.0029%-0.6409%) and the number of projected cases could be as low as 116 or as high as 25,636. The proportion of such infections that would have serious clinical consequences is unknown, but clearly neonates, persons with congenital and acquired hemolytic anemias, and immunocompromised patients would be at increased risk of untoward clinical events.

Currently, most plasma fractionators in the United States perform minipool B19V NAT screening as an in-process control to detect and exclude donations with B19V DNA levels of approximately 10^6 IU/mL or higher so that the level of B19V DNA in manufacturing plasma pools destined for the production of plasma derivatives will not exceed the limit of 10^4 IU/mL.[11,46] In Europe, a similar limit has been in place for plasma pools used for manufacturing anti-D immunoglobulins and plasma treated for virus inactivation.[40,47-49] It is recommended that all B19V NAT

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procedures detect all three B19V genotypes.[46,50] In addition to screening, viral inactivation and removal procedures have been incorporated into the manufacture of plasma derivatives. The net effect of screening and virus inactivation when combined with complexing and/or neutralizing anti-B19 IgG antibodies that are invariably present in large plasma pools is that the final products contain little or no infectious virus. This has been confirmed in a recent survey of FVIII concentrates.[11]

In contrast to pooled plasma products, WB donations are not tested for B19V DNA, and no viral inactivation procedure is in place for blood components in the United States. The fact that screening for B19V DNA is not performed is due both to the lack of licensed commercial assays and to the absence of compelling evidence for significant clinical risk. Nonetheless, it is legitimate to ask whether the small, but finite risk could be significantly reduced by testing WB donations and withholding units that exceed a threshold level of B19V DNA (e.g., that applied to units of plasma used for manufacturing pooled products) without compromising the supply of blood components. Some blood centers in Germany and Austria have screened WB units by a B19V minipool real-time NAT procedure for several years. Blood components associated with donations having 10^5 IU/mL or more, regardless of whether they contain anti-B19V IgG antibodies, are discarded to protect at-risk individuals, whereas units with less than 10^5 IU/mL are released because of the apparently universal coexistence of anti-B19V IgG.[19] In a subsequent retrospective, linked donor-recipient infectivity study,[51] preliminary data indicate that nearly 50% (7/15) of recipients transfused with RBC units from WB donations having more than 10^5 IU/mL of B19V DNA were B19V infected, and the link between the B19V donor and recipient was demonstrated by sequence analysis. In contrast, none of 16 recipients transfused with RBC units containing less than 10^5 IU/mL B19V DNA was infected. Thus, in that study, WB screening with a threshold level of 10^5 IU/mL seems to have been warranted.

In conclusion, this study revealed a new B19V infection related to single-donor blood products, only the fifth such case in the literature. In the absence of an obvious clinical syndrome, detection of B19V infection depended on the availability of pre- and posttransfusion samples to differentiate new from existing infection. The transmission was confirmed by sequencing and phylogenetic analysis of linked donor-recipient samples and demonstrated an identical Genotype 1 sequence over the 710-nucleotide skein analyzed. Despite the low rate of transmission documented in this study, this incidence of new transfusion-transmitted B19V infections could result in numerous infections annually, some of which would have clinical consequences in susceptible populations. The introduction of B19V NAT screening of WB with a threshold level of 10^5 or more or 10^6 IU/mL or more would have interdicted transfusion of the product from this donor acutely infected with B19V and avoided this proven transmission. However, the decision to introduce universal B19V donor testing is complex and needs to be guided by additional prospective studies or further retrospective analyses of repository samples from prior studies with appropriate donor-recipient linkage.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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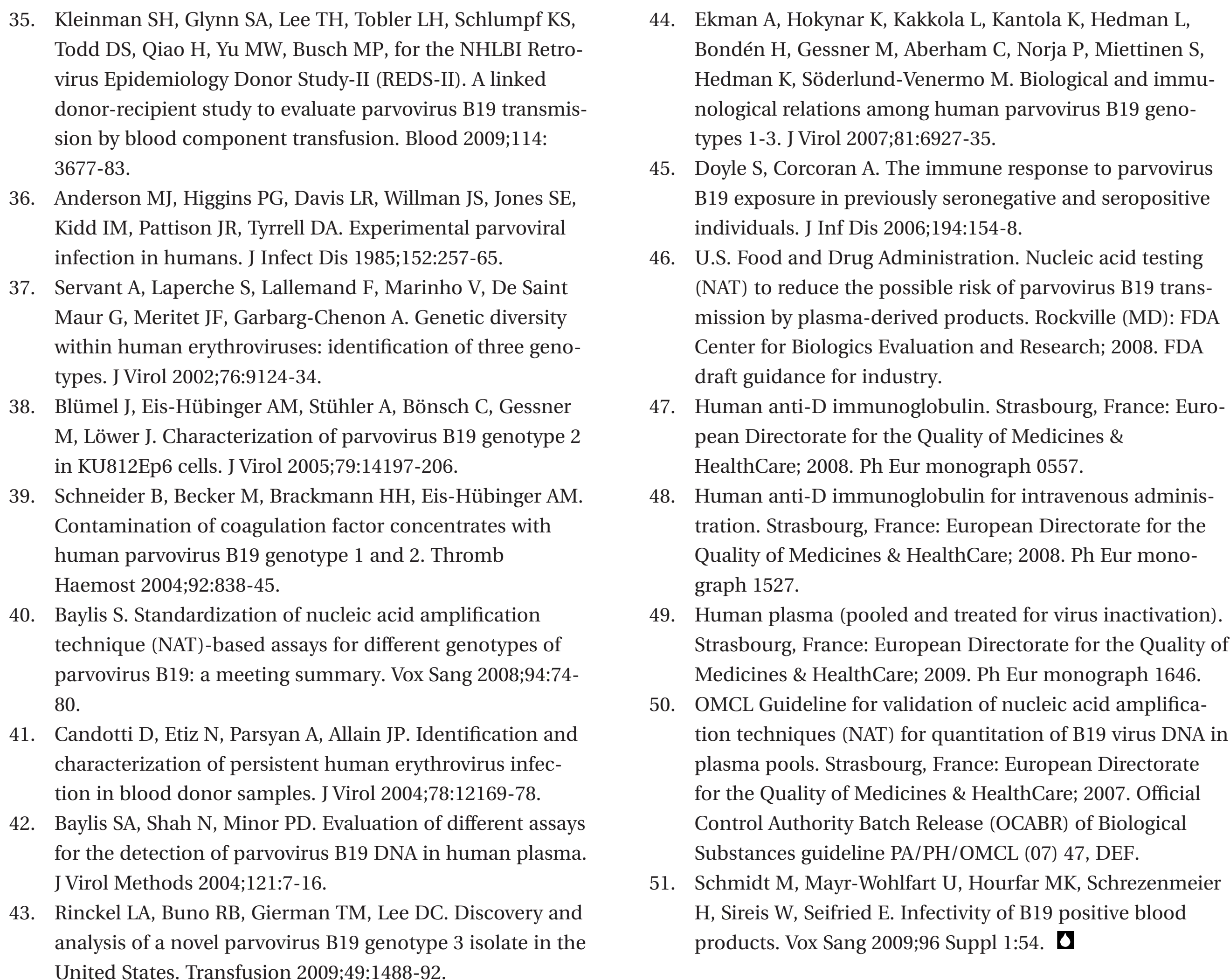

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