A linked donor-recipient study to evaluate parvovirus B19 transmission by blood component transfusion

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Parvovirus B19V infection can be a serious infection for hematology patients with underlying hemolysis or compromised erythropoiesis syndromes. Although case reports of B19V transmission by blood component transfusion (as contrasted to manufactured plasma derivatives) are rare, no studies have systematically determined a rate of transmission to recipients transfused with B19V DNA–positive components. We used a linked donor and recipient repository and a sensitive, quantitative B19V DNA polymerase chain reaction (PCR) assay to assess such transmission in B19V-susceptible (ie, anti-B19V immunoglobulin G [IgG] negative) recipients. We assessed 112 B19V DNA–positive components from 105 donors (of 12,529 tested donations) transfused into a population of surgical patients with a pretransfusion B19V IgG seroprevalence of 78%. We found no transmission to 24 susceptible recipients from transfusion of components with B19V DNA at concentrations less than 10^4 IU/mL (upper 95% confidence interval, 11.7%). We found an anamnestic IgG response in one pretransfusion seropositive recipient transfused with a component containing greater than 10^10 IU/mL B19V DNA. These findings show either that transmission from components with less than 10^6 IU/mL does not occur, or, if it does, it is an uncommon event. These data do not support the need to routinely screen blood donations with a sensitive B19V DNA nucleic acid assay. (Blood. 2009;114:3677-3683)

Introduction

There have been multiple reports of parvovirus B19 (B19V) transmission by pooled plasma products, including factor VIII concentrate and solvent-detergent–treated pooled plasma, documented by recipient seroconversion in asymptomatic cases or, less frequently, by clinical diagnosis of B19V-related disease in association with positive B19V test results.1-5 These cases, combined with the potential for very high B19V DNA concentrations (up to 10^12 IU/mL) in plasma donations6 and the relative resistance of B19V to inactivation methods,6,6 have led to B19V DNA testing of plasma donations to ensure that manufacturing plasma pools destined for plasma derivatives have a B19V DNA concentration less than or equal to 10^8 IU/mL, a limit proposed by the Food and Drug Administration (FDA).7,8 The same limit for this so-called “in process testing” is a European regulatory requirement for anti-D immunoglobulin (Ig) preparations and plasma treated for virus inactivation.10 To achieve this B19V DNA concentration in the final plasma pool, B19V DNA screening of the plasma donations used to make the pool is performed using assays (applied in minipool format) with the ability to detect approximately 10^9 IU/mL in an input unit of plasma.5

To date, no B19V transmissions from pooled plasma products have been documented when less than 10^7 to 10^8 IU/mL B19V DNA is present in an infused product.3,4,11-13 The reason for this lack of infectivity is not completely understood. It may be due to an inadequate amount of infused infectious virions, a neutralization effect from B19V antibody present in other plasma units in the plasma pool, or a combination of these factors. Recipient factors may also play a role because it has been reported that B19V antibody is protective against B19V reinfection, and most of the adult population is B19V seropositive as a result of previous infection.13

Although concern for transmission of B19V from pooled plasma products has resulted in B19V DNA screening of input plasma donations, less is known about the potential for B19V transmission by transfusion of individual blood components (eg, red cells, platelets, plasma). There are only 4 published clinical cases of B19V transmissions from blood component transfusion (3 from red cells and 1 from platelets).14-17 An additional asymptomatic case has been reported from a recent prospective study of transfusion-transmitted viral infections.18 In contrast, 2 studies have reported a small number of negative results when patients transfused with B19V DNA–positive components were evaluated for laboratory markers of B19V infection.19,20 Nevertheless, given the tropism for21 and potential pathophysiologic effects of B19V infection on erythroid precursor cells,22 concern remains for potential deleterious outcomes in frequently transfused hematology patients with underlying hemolysis or compromised erythropoiesis syndromes.13

Because the sensitivity of B19V DNA assays has improved, B19V DNA prevalence in blood donors has been shown to be
higher than initially documented. B19V DNA is detectable in 0.5% to 0.9% of blood donations, with most showing relatively low DNA concentrations (< 100-1000 IU/mL). In addition, it has become established that B19V infection is often persistent. Thus, some donors may continue to donate for many years with B19V DNA (and potentially infectious virions) in their blood. These observations suggest that the potential for recipients to be exposed to low levels of B19V DNA from blood component transfusion is greater than previously thought.

To our knowledge, there have been no large-scale donor/recipient-linked transfusion-transmission studies to evaluate the rate of B19V transfusion transmission. Although it has been assumed by extrapolation from pooled plasma transfusions that single unit blood components with low-level B19V DNA should be noninfectious, this remains speculative because the mechanism of protection in the pooled plasma setting has not been established and may not apply to single unit transfusions.

We undertook this present study to systematically evaluate whether transfusion of blood components with low or moderate levels of B19V DNA (defined as < 10^6 IU/mL) transmits infection to B19V-seronegative susceptible recipients.

**Methods**

**Source of donor and recipient samples**

Tested specimens were from the National Heart, Lung, and Blood Institute (NHLBI) Retrovirus Epidemiology Donor Study Allogeneic Donor and Recipient (RADAR) repository, which was established to investigate possible transfusion-transmitted infections and which has been described in detail in a previous publication. Repository specimens were collected from 2000 through 2003 by blood centers and selected hospitals at 7 geographically dispersed US locations. Repository specimens consisted of 2 frozen 1.8-mL plasma aliquots and a 1.5-mL sample of frozen whole blood.

All enrolled donors and recipients gave informed consent for frozen specimen storage and for subsequent specimen testing for possible transfusion-transmissible infections, in accordance with the Declaration of Helsinki. The study protocol was approved by the institutional review board of each participating institution.

The linked portion of this donor-recipient repository contains pretransfusion and or peritransfusion specimens and follow-up specimens, collected at a 6- to 12-month interval, from 3575 enrolled recipients. It also contains 13 201 donation specimens given by 12 408 distinct donors that were transfused to these recipients. The RADAR enrollment procedure targeted recipients with expected high 1-year survival rates; 88% were cardiac or vascular surgical patients, and the median recipient age was 68 years (range, 59-74 years). Recipients were not evaluated for coexisting immunosuppression, but this is considered unlikely given the primary diagnoses. The mean number of RADAR donation exposures per recipient was 3.9. The distribution of component types transfused was 77% red cells, 13% whole-blood-derived platelet concentrates, and 10% fresh-frozen plasma (FFP). In addition to receiving components with a stored donation specimen in the RADAR repository, these recipients also received a mean of 3.1 components not linked to stored RADAR donations.

The RADAR repository also contains 99 906 specimens from blood donations that were not transfused to enrolled RADAR recipients; this supplementary repository served as a sample source during the assay validation and donor prevalence phase of the study, which has previously been reported.

**Selection and testing of donations**

All RADAR donations transfused to enrolled recipient were tested for B19V DNA, provided there was adequate specimen volume available. Donations found reactive on the B19V DNA assay were subjected to DNA confirmatory and quantitative testing; confirmed positive donations were also tested for B19V IgG and IgM.

**Selection and testing of recipients**

Cases were recipients who were transfused with one or more B19V DNA–positive components. Control recipients were selected to measure the background rate of new infection as a result of factors other than transfusion of a B19V DNA–positive RADAR unit (ie, community-acquired infection in the 6- to 12-month follow-up interval or a transfusion-acquired infection from a B19V DNA–negative RADAR unit in a noninfected, non-RADAR unit). A 1:2 case-control design was used to select control recipients fulfilling the following criteria: all RADAR units received by the recipient were B19V DNA negative, enrollment occurred at the same participating center in approximately the same time frame (to control for community-acquired infection), and age was within 10 years of the case recipient. Using this control selection algorithm, we established that all controls met preestablished age and center criteria, and 94.4% received their transfusion within 11 days of their matched recipient.

Enrollment specimens from all case and control recipients were tested for B19V IgG. Before knowledge of B19V IgG enrollment results, posttransfusion follow-up specimens from all cases and controls were tested for B19V IgG, IgM, and DNA (see “Assay methods”). A positive B19V DNA or IgM result on the follow-up specimen triggered additional testing of the enrollment specimen for these analytes.

For analysis, case and control recipients with negative B19V IgG results before transfusion were subsequently classified as B19V susceptible, and those with positive results were classified as B19V nonsusceptible.

**Protocol for evaluating transfusion-transmission**

B19V transmission was defined as seroconversion to IgG or IgM or new detection of B19V DNA. Because our previous experience with B19V antibody testing has shown that specimens near the cutoff could show fluctuating results on different test runs, we required that seroconversion be independently shown by 2 laboratories.

**Assay methods**

**B19V DNA PCR assay.** The B19V DNA polymerase chain reaction (PCR) assay was originally developed by Chiron Corporation and subsequently refined through collaboration between Chiron and Blood Systems Research Institute (BSRI). We previously reported data on assay performance on 5020 plasma samples from the unlinked donor portion of the RADAR repository. The assay had a 50% limit of detection (LOD) of 1.6 IU/mL (95% confidence interval [CI], 1.2-2.1 IU/mL) and a 95% LOD of 16.5 IU/mL (95% CI, 10.6-33.9 IU/mL). We determined that the assay could be used as a quantitative as well as a qualitative assay; because quantitation might not be precise at the lower LOD, we categorized all specimens with quantitative DNA values of greater than 0 but less than 20 IU/mL as having a value of less than 20 IU/mL.

The assay, performed at BSRI, included a magnetic-bead B19V DNA capture step followed by a TaqMan real-time PCR assay targeting the VP1 region of the genotype 1 B19V genome. The assay was subsequently validated as detecting genotype 2 but does not detect genotype 3, which has been identified in Africa but which is very rare outside that continent. An internal control sharing homologous primer region sequences but with a different internal probe binding sequence as the viral target was included in each assay tube. All captured target DNA from 0.5 mL input plasma and the spiked internal control was amplified in a single PCR reaction by using the same primer pair. Amplification and detection occurred in a 96-well optical plate by using dual-plexed TaqMan PCR technology. B19V target and internal control DNA were detected and distinguished by fluorophore-tagged sequence-specific probes. Each plate contained 2 known positive, 2 blinded negative, and 2 blinded positive controls and up to 90 study specimens. A more detailed assay description is provided in the previous publication.

Because the chosen assay cutoff of a cycle threshold (Ct) of less than 40 was designed to maximize assay sensitivity, an algorithm was developed...
for final test interpretation so as to avoid classifying nonspecific reactivity on a single assay run as a confirmed positive result (Figure 1). All initially positive, indeterminate, and invalid specimens were retested in duplicate on plates that included quantitative run standards by using 2 separate 0.5-mL subaliquots subjected to the full extraction, amplification, and detection procedure. This testing served both as confirmation and quantitation. Final interpretation was based on the results of the 3 assays (ie, the initial screening assay and the duplicate repeat assays). Specimens were classified as B19V DNA positive if at least 2 of 3 tests showed reactivity at a CT less than 40.

For determining DNA concentration, duplicate quantitative run standards (containing B19V DNA at 10^6 to 10^8 IU/mL) were placed on each plate, and quantitative results were determined by comparing the specimen CT to the CT of the known standards on the same test run. The assigned quantitative value for each specimen was the average of the duplicate quantitative assays (including zero for a negative test result). Specimens with low CT values (< 30) were diluted 1:10 and 1:100 and then run in triplicate at each dilution. The quantitative result was the average of the 3 test results at the most appropriate dilution adjusted by the dilution factor.

Serologic assays. Testing for B19V IgG and IgM was directed against recombinant VP2 protein and was performed in duplicate by using FDA-cleared test kits (Biotrin) according to the manufacturer’s instructions. Testing was conducted at BSRI and, for a large subset of samples, was repeated at a Center for Biologics Evaluation and Research/Food and Drug Administration (FDA) laboratory (Bethesda, MD). If results fell into the equivocal zone, the assay was repeated in duplicate on a new aliquot, and this repeat result was taken as the final result for the specimen.

Quantitative B19V IgG testing was performed by using a standard curve dilutional analysis method with the World Health Organization First International Standard for B19V serum IgG (93/724) obtained from the National Institute for Biological Standards and Control. This testing was applied to enrollment and follow-up specimens of B19V IgG–positive (“nonsusceptible”) recipients who had been transfused with the 5 highest titer B19V DNA components identified through donor testing.

Statistical methods

On the basis of a review of donor B19 viremia and recipient B19V serologic data from phase 1 of this study, we determined that testing of the linked donor and recipient RADAR repository specimens would have sufficient statistical power such that a finding of zero documented transmissions to susceptible recipients would indicate with 95% confidence that the true B19 transfusion-transmission rate was between 0% and 25%. In this current study, StatXact (Cytel) was used to generate upper 95% confidence limits based on zero observed infections. The upper confidence limit for transmission was calculated as a one-sided exact 95% confidence interval for the difference between the infection rate among susceptible cases and susceptible controls, using StatXact (Cytel).

Results

Of the 13,201 linked blood donation repository specimens, 12,529 (95%) had adequate volume for testing. B19V DNA was detectable in 105 donations for a prevalence of 0.84% (95% CI, 0.68%-1.00%). As shown in Table 1, 53%, 71%, and 93% of these donations had B19V DNA concentrations below 20, 100, and 1000 IU/mL, respectively. The 2 donations with DNA concentrations greater than 10^6 IU/mL were negative for B19V-specific IgM and IgG, whereas B19V IgG was detectable in 96% and B19V IgM in 28% of the evaluable remaining B19V DNA–positive donations.

These 105 B19V DNA–positive donations came from 103 donors, 2 of whom gave positive donations on 2 occasions. The 105 positive donations resulted in the transfusion of 112 positive components to enrolled recipients. Four recipients received multiple DNA-positive components such that a total of 107 distinct recipients were transfused with one or more DNA-positive components. Table 2 provides a description of the DNA-positive components transfused to recipients, classified by the DNA concentration of the component, by whether the recipients were susceptible to B19V infection (ie, B19V IgG negative on their enrollment

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Table 1. Quantitative B19V PCR and antibody results on confirmed positive donations

<table>
<thead>
<tr>
<th>B19V DNA concentration, IU/mL, in donation</th>
<th>No. of B19V DNA–positive donations</th>
<th>No. (%) B19V IgM and IgG positive</th>
<th>No. (%) B19V IgM negative, IgG positive</th>
<th>No. (%) B19V IgM negative and IgG negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Less than 20</td>
<td>56</td>
<td>2 (4%)</td>
<td>52 (93%)</td>
<td>2 (4%)</td>
</tr>
<tr>
<td>20 to less than 100</td>
<td>19*</td>
<td>5 (28%)</td>
<td>13 (72%)</td>
<td>0</td>
</tr>
<tr>
<td>10^2 to less than 10^3</td>
<td>23</td>
<td>18 (78%)†</td>
<td>2 (9%)</td>
<td>1 (4%)</td>
</tr>
<tr>
<td>10^3 to less than 10^4</td>
<td>4</td>
<td>4 (100%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>10^4 to less than 10^5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>10^5 to less than 10^6</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1 (100%)</td>
</tr>
<tr>
<td>Subtotal</td>
<td>103*</td>
<td>29 (28%)</td>
<td>67 (66%)</td>
<td>4 (4%)</td>
</tr>
<tr>
<td>More than 10^6</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>105†</td>
<td>29</td>
<td>67</td>
<td>6</td>
</tr>
</tbody>
</table>

The prevalence of B19V DNA–positive donations in 12,529 tested donations was 0.84%.

*One donor was not tested for B19V antibody; percentages have been calculated eliminating that donor from both the numerator and the denominator.

†Two donors were IgM equivocal and IgG positive.

‡The 105 B19V DNA–positive donations came from 103 donors, 2 of whom gave positive donations on 2 occasions.
Table 2. Transfusion of B19V DNA-positive components to recipients

<table>
<thead>
<tr>
<th>B19V DNA concentration, IU/mL, in donation</th>
<th>No. of B19V DNA-positive components transfused to susceptible recipients</th>
<th>Total no. of B19V DNA-positive components transfused</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of B19V DNA-positive donations</td>
<td>Red cells</td>
</tr>
<tr>
<td>Less than 20</td>
<td>56</td>
<td>15</td>
</tr>
<tr>
<td>20 to less than 100</td>
<td>19</td>
<td>3</td>
</tr>
<tr>
<td>10^2 to less than 10^3</td>
<td>23</td>
<td>3</td>
</tr>
<tr>
<td>10^3 to less than 10^4</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>10^4 to less than 10^5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>10^5 to less than 10^6</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Subtotal</td>
<td>103</td>
<td>21</td>
</tr>
<tr>
<td>More than 10^6</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>105</td>
<td>21</td>
</tr>
</tbody>
</table>

*All B19V DNA-positive units transfused to susceptible recipients contained B19V-specific IgG.
†For 7 B19V DNA-positive donations, more than 1 component was transfused; also 4 nonsusceptible recipients received more than 1 positive component.

Discussion

In this study we identified donations that had a potential marker of B19V infectivity (ie, B19V DNA) through retrospective screening of blood donations and subsequently tested recipients of components from these donations for the development of new B19V infection. Our approach was designed to systematically determine a rate of transmission from all units with this potential infectivity marker and to establish either the presence or absence of transmission when it was known that a susceptible (ie, B19V IgG negative) recipient was transfused with a potentially infectious (ie, B19V DNA positive) unit. This study design is in contrast to most other B19V studies in which investigations were structured to prove that transmission occurred in a particular case.

On the basis of our finding of nontransmission in 24 evaluable susceptible (B19V seronegative) recipients of components with a B19V DNA concentration less than 10^6 IU/mL, we conclude that the rate of transmission from such components ranges from 0% to 11.7% (which is the upper 95% confidence bound); thus, either transmission from such components does not occur, or, if it does, it is a relatively uncommon event in comparison to most other transfusion-transmissible viruses in which infection rates exceed 50% (eg, HIV, HCV).31

Table 3. Antibody quantitation studies in recipients transfused with components with the highest B19V DNA concentrations

<table>
<thead>
<tr>
<th>Transfused component results</th>
<th>Recipient results</th>
</tr>
</thead>
<tbody>
<tr>
<td>B19V DNA concentration, IU/mL in donation</td>
<td>B19V IgM/IgG status</td>
</tr>
<tr>
<td>2.9 x 10^10</td>
<td>−/−</td>
</tr>
<tr>
<td>8.2 x 10^7</td>
<td>−/−</td>
</tr>
<tr>
<td>4.3 x 10^4</td>
<td>−/−</td>
</tr>
<tr>
<td>8.6 x 10^3</td>
<td>+/+</td>
</tr>
<tr>
<td>1.8 x 10^3</td>
<td>+/+</td>
</tr>
</tbody>
</table>

*One recipient who received a component with a DNA concentration of 3.1 x 10^9 IU/mL (which was also positive for B19V IgM and IgG) was not included in this table because the enrollment and follow-up specimens were both B19V IgG negative.
Our study is the first to evaluate transmission in multiple recipients who do not have preexisting B19V IgG and hence do not have this mechanism for potential protection against acquiring B19V infection. In a study from Africa, there was a single documented case of lack of B19V transmission to a susceptible pediatric recipient transfused with a red cell unit that had a B19V DNA concentration of $6 \times 10^2$ IU/mL in the presence of B19V IgG. There are somewhat more data about the lack of transmission to recipients with preexisting B19V IgG. In a study conducted in an adult hematology service, 6 adult recipients with hematologic malignancies (5 of whom underwent stem cell transplantation) were identified as transfused with blood components that were retrospectively found to contain B19V DNA at less than $10^6$ geq/mL; in 4 of 5 evaluated cases, the DNA-positive component also contained B19V IgG. Each recipient was B19V DNA negative when tested 3 to 18 days after transfusion, and none showed clinical symptoms of B19V infection on retrospective chart review.

The mechanism to explain lack of transmission to susceptible recipients by B19V DNA–containing units is unknown but could be related to the lack of a large enough inoculating dose of B19 virions to establish infection. This could be due to the ratio between infectious dose and virion number (which is not known), the low levels of transfused intact and/or replication competent virions in units with low DNA concentrations, or neutralization of otherwise infectious virions either by antibody in the transfused unit or by passively transfused antibody from other units. In support of the latter explanations, we note that all DNA-positive units transfused to susceptible recipients in our study contained B19V-specific IgG. In addition, it is highly probable that all recipients of B19V DNA–containing components received some additional blood components with B19V IgG; this is based on our previous findings that 73% of donors who contributed to the RADAR repository had B19V IgG and that RADAR recipients were transfused with an average of 7 blood components.

Our negative transmission findings are consistent with previous publications that have shown that high plasma concentrations of B19V DNA are required for transmission in the setting of transfused pooled plasma products. The minimal infectious dose of B19V DNA documented to cause a symptomatic B19V infection in a recipient of factor VIII concentrate devoid of B19V IgG was $2 \times 10^4$ IU based on the infusion of 3 vials of a product with a DNA concentration of $6.5 \times 10^3$ IU/vial (ie, $1.3 \times 10^4$ IU/mL when each vial was reconstituted in a 5-mL volume). Furthermore, we are aware of only one comprehensive quantitative transmission study of pooled plasma products manufactured from multiple donations. That study, conducted approximately 10 years ago, was an open-label phase 4 trial of pooled plasma, solvent detergent-treated (PLAS + SD produced by Vitex, now defunct). One hundred B19V-seronegative volunteers were infused with product from 17 different manufacturing lots. Of 19 subjects who received the product from 3 lots that contained at least $2 \times 10^4$ geq B19V DNA (ie, 200 mL product infused at $> 10^5$ B19V DNA geq/mL), 18 seroconverted and 17 showed B19 viremia. Although the investigators expressed their results in geq/mL, it has subsequently become established that for B19V, an IU and a geq are approximately equivalent. In contrast, there were no seroconversions in 81 subjects who received product from 1 of 14 lots containing less than $10^6$ geq/mL B19V DNA; however, the investigators did not more precisely quantify the amount of B19V DNA in these nontransmitting lots.

In our study, which was designed to systematically study transmissibility from B19V DNA–positive units with less than $10^6$ IU/mL, we transfused only 2 components with high B19V DNA concentrations (>$10^7$ IU/mL) but were unable to directly evaluate their transmissibility in susceptible recipients, because both were transfused to recipients with preexisting B19V IgG. We used quantitative B19V antibody testing to investigate whether exposure to this very high B19V DNA concentration could stimulate the recipient’s immune system to respond. Although not definitive, a 4-fold boost in B19V IgG in the follow-up specimen from one of these recipients suggests that a component with very high B19V DNA concentration (~$5.8 \times 10^4$ IU B19V DNA infused) can result in an anamnestic response (implying transient active viral replication) in a previously exposed recipient when the pretransfusion antibody titer is relatively low (15 IU/mL in this recipient). Our results are consistent with similar 4-fold B19V IgG increases which were reported 1 month after transfusion in 2 of 2 B19V IgG–positive volunteers who remained asymptomatic after transfusion of $200$ mL PLAS + SD at a B19V DNA concentration of $1.6 \times 10^3$ IU/mL. In addition, in the previously described study of adult hematology patients, there was also one B19V IgG–positive recipient of a red blood cell unit containing $2.2 \times 10^6$ geq/mL of B19V DNA; this recipient was positive for B19V DNA at posttransfusion day 5, negative when retested on day 35, and asymptomatic for B19V infection on chart review; B19V IgG titer was not reported.

Despite the large size of our linked donor–recipient repository, the use of a very sensitive B19V DNA assay, and a rigorous testing algorithm, this study was subject to several limitations. The collection of recipient follow-up specimens 6 to 12 months after transfusion limited the laboratory techniques that we could use to diagnose new B19V infection. In addition to our primary assessment of the development of new B19V IgG formation, we also tested for new appearance of B19V IgM and B19V DNA. However, the natural history of acute B19V infection predicts that both of these markers would probably no longer be detectable at the time our follow-up specimens were collected, unless the recipient had developed a persistent infection. Our study was also limited because most recipients (78%) of B19V DNA–positive units were B19V IgG positive before transfusion and thus presumably were partially or totally protected against B19V reinfection. This limited the statistical power of our negative result such that the upper 95% CI could not rule out a transmission rate as high as 11.7%. Furthermore, most of the 24 susceptible recipients received components with very low B19V DNA concentrations ($< 20$ IU/mL). We identified only 5 transfused components with DNA concentrations between $10^3$ and $10^4$ IU/mL; 4 of these were B19V IgM and IgG positive, and one of these (DNA level of $4.3 \times 10^5$ IU/mL) lacked B19V antibody. Furthermore, only one of these components, a plasma unit containing a total infused dose of approximately $7 \times 10^8$ IU in the presence of B19V IgG, was transfused to a susceptible recipient. Similarly, although we identified 45 transfused components with B19V DNA concentrations between 20 and 1000 IU/mL, only 7 were transfused to susceptible recipients. Finally, although we obtained questionnaires from recipients at the time of follow-up (6-12 months after transfusion) and none of the recipients had been diagnosed with B19V disease, we were unable to definitively assess nonspecific symptoms that can occur with B19V infection at such a long interval after transfusion.

We expressed our findings as the rate of transmission in susceptible recipients because this allowed us to extrapolate our findings to other transfused recipient populations; i.e., it allowed us to calculate a per unit risk. This per unit risk in our older surgical recipients can then be applied to populations with a higher susceptibility rate (eg, fetuses undergoing intrauterine transfusion, young patients with sickle cell anemia or thalassemia, patients with...
congenital or acquired hypogammaglobulinemia), based on the assumption that the equivalent dose of B19V transfused into a B19V IgG-negative hematolymph or surgical patient will result in productive infection (ie, viral replication) at the same rate. In our opinion, it is unlikely that the infectivity of a B19V DNA–positive transfused unit will be related either to the underlying disease or to the overall immune status of a B19V seronegative recipient, even though it is well accepted that the clinical manifestations of a B19V infection will be influenced by such host factors (ie, if infected with B19V, an immunosuppressed patient or one with an underlying hemolytic syndrome might have a worse clinical outcome). 7

We can also analyze our data on a population-wide basis; looked at in this way, we did not detect any cases of definite B19V transmission (with the exception of the one possible case of an anamnestic immune response) after the transfusion of blood components from 12,529 B19V DNA–tested donations into a recipient population with a pretransfusion B19V IgG prevalence of 78%.

As part of this study, we also generated a large body of blood donor data. We found that B19V DNA prevalence in 12,529 tested donations was 0.84%, consistent with our previous report of 0.88% in 5020 donation samples from the same RADAR repository and with higher end estimates in literature. 23-25 The large majority of our DNA-positive donations had low or very low DNA concentrations (53%, 71%, and 93% below 20, 100, and 1000 IU/mL, respectively), consistent with the interpretation that the increased DNA prevalence found in recent donor studies is due to the use of more sensitive nucleic acid testing assays. In contrast to the high rate of overall DNA detection, our rate of detection of high-titer DNA positives (>10^6 IU/mL) was approximately 1 in 6000, consistent with both the newer and older literature. 7, 34, 35 These high-titer units are known to occur in the acute phase of B19V infection; thus, they lack both B19V IgG and IgM antibody as was the case in this study. 31 In contrast, 96% of the remaining DNA-positive donations were B19V IgG positive, which is the expected result in resolved or persistent infection. 35, 36

Current practices for blood donor screening for B19V in developed countries are almost exclusively confined to testing plasma designated for fractionation for the presence of high B19V DNA concentrations. 8, 13 There has been recent debate about whether such screening should also be applied to transfused blood components; this is currently not done because of the lack of demonstrated adverse clinical outcomes from B19V infection in blood component recipients and the considerable expense of such testing. We are aware of only one country, Germany (which also performs blood testing for Austria), in which some blood banks currently conduct B19V DNA screening of blood donations and use the results to release blood components for transfusion. 35 Their testing is conducted in pools of 96 samples with an assay that can reliably detect units with B19V DNA greater than 10^5 IU/mL. Other German blood banks conduct B19V DNA testing retrospectively after the red cell component has been transfused. 35 In a recent abstract, preliminary data indicate that B19V transmission (documented by a positive B19V DNA test in the transfused recipient) from retrospectively tested red cell components occurred when the B19V DNA concentration was greater than 10^5 IU/mL but not when the concentration was below this threshold. 37

Our study results confirm that, if prospective, real-time B19V DNA blood donor screening were to be performed, the assay sensitivity used in Germany (ie, detection limit < 10^4 IU/mL) is reasonable in that it ensures recipient safety while preventing unnecessary discard of a much larger number of blood components. Our findings do not support the need to use more-sensitive B19V DNA nucleic acid screening assays. In conclusion, our data indicate that blood components with B19V DNA less than 10^6 IU/mL (almost all of which contain B19V-specific antibody) are unlikely to transmit B19V infection.

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Authorship


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A complete list of the members of the NHLBI REDS-II appears in the supplemental Appendix (available on the Blood website; see the Supplemental Materials link at the top of the online article).

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A linked donor-recipient study to evaluate parvovirus B19 transmission by blood component transfusion

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