Screening for \textit{Babesia microti} in the U.S. Blood Supply


**BACKGROUND**

\textit{Babesia microti}, a tickborne intraerythrocytic parasite that can be transmitted by means of blood transfusion, is responsible for the majority of cases of transfusion-transmitted babesiosis in the United States. However, no licensed test exists for screening for \textit{B. microti} in donated blood. We assessed data from a large-scale, investigational product-release screening and donor follow-up program.

**METHODS**

From June 2012 through September 2014, we performed arrayed fluorescence immunoassays (AFIAs) for \textit{B. microti} antibodies and real-time polymerase-chain-reaction (PCR) assays for \textit{B. microti} DNA on blood-donation samples obtained in Connecticut, Massachusetts, Minnesota, and Wisconsin. We determined parasite loads with the use of quantitative PCR testing and assessed infectivity by means of the inoculation of hamsters and the subsequent examination for parasitemia. Donors with test-reactive samples were followed. Using data on cases of transfusion-transmitted babesiosis, we compared the proportions of screened versus unscreened donations that were infectious.

**RESULTS**

Of 89,153 blood-donation samples tested, 335 (0.38%) were confirmed to be positive, of which 67 (20%) were PCR-positive; 9 samples were antibody-negative (i.e., 1 antibody-negative sample per 9906 screened samples), representing 13% of all PCR-positive samples. PCR-positive samples were identified all through the year; antibody-negative infections occurred from June through September. Approximately one third of the red-cell samples from PCR-positive or high-titer AIFA-positive donations infected hamsters. Follow-up showed DNA clearance in 86% of the donors but antibody seroreversion in 8% after 1 year. In Connecticut and Massachusetts, no reported cases of transfusion-transmitted babesiosis were associated with screened donations (i.e., 0 cases per 75,331 screened donations), as compared with 14 cases per 253,031 unscreened donations (i.e., 1 case per 18,074 unscreened donations) (odds ratio, 8.6; 95% confidence interval, 0.51 to 144; \( P = 0.05 \)). Overall, 29 cases of transfusion-transmitted babesiosis were linked to blood from infected donors, including blood obtained from 10 donors whose samples tested positive on the PCR assay 2 to 7 months after the implicated donation.

**CONCLUSIONS**

Blood-donation screening for antibodies to and DNA from \textit{B. microti} was associated with a decrease in the risk of transfusion-transmitted babesiosis. (Funded by the American Red Cross and Imugen; ClinicalTrials.gov number, NCT01528449.)
Babesia microti is an intraerythrocytic parasite that causes babesiosis. The severity of babesia infection ranges from asymptomatic, most commonly in healthy persons, to fatal, most frequently in persons older than 50 years of age, those who have no spleen (or no functional spleen), and those who are immunocompromised. In the United States, B. microti is transmitted to humans primarily by means of the bite of Ixodes scapularis (also called the deer tick).

Babesiosis became a nationally notifiable disease (as defined by the Centers for Disease Control and Prevention [CDC]) in 2011 and was reportable (i.e., reportable to the state, which then notifies the CDC) in 27 states in 2013, with 95% of 1796 cases reported by 7 states: Connecticut, Massachusetts, Minnesota, New Jersey, New York, Rhode Island, and Wisconsin. The occurrence of babesiosis resulting from the bloodborne transmission of parasites from infected donors to recipients through transfusion of red-cell units or platelets that have been contaminated with red cells is of increasing concern. From 1979 through 2009, a total of 162 cases of transfusion-transmitted babesiosis were reported in the United States, 159 of which were caused by B. microti. These numbers probably underestimate the incidence of transfusion-transmitted babesiosis, because infections may be overlooked or misdiagnosed and because investigations to determine the source of babesia infection in the blood recipient may not be initiated. No blood-donation screening tests for B. microti have been licensed by the Food and Drug Administration (FDA). However, donors are asked whether they have ever had babesiosis, and those who respond “yes” are indefinitely deferred; this method is considered to be ineffective.

In June 2012, the American Red Cross began screening blood obtained at selected blood drives using an investigational product-release testing protocol that consisted of an IgG arrayed fluorescence immunoassay (AFIA) to detect B. microti antibodies and a polymerase-chain-reaction (PCR) assay to detect B. microti DNA. We used data collected during screening, donor follow-up, and investigations of cases of transfusion-transmitted babesiosis to assess the natural history of infection in blood donors and the effect of screening on blood safety.
providing informed consent. Whole-blood and plasma samples were collected in 5-ml EDTA tubes and were tested for *B. microti* DNA by PCR assay and for *B. microti* antibodies by AFIA before the release of the product (product release was defined as release for transfusion, on the basis of negative test results). Results were available within 12 hours after receipt of the sample.

Blood donations that were identified by screening as AFIA-reactive or PCR-reactive, which were considered to be the index donations, were removed from the blood supply. Recipient tracing was performed for a 12-month period for donors of reactive samples (see the Supplementary Appendix). Donors were notified of their reactive results and were encouraged to seek medical advice, were indefinitely deferred from donating blood, and were invited to participate in follow-up. Index donation samples that tested reactive by at least one supplemental assay (IgM or IgG Western blot or enhanced-sensitivity PCR assay) or by repeat AFIA or PCR assay on residual samples from the index unit were considered to be confirmed positive samples. Positive predictive values (PPVs) were calculated. Data from this study were combined with data from a repository validation study to assess the results of donor follow-up (see the Supplementary Appendix).8 Donors who participated in follow-up provided additional samples every 6 to 8 weeks until they had AFIA-negative test results (titer of <1:64).

**STUDY OVERSIGHT**

This study was a collaboration between the American Red Cross and Imugen and included a signed confidentiality agreement. The study was supported by internal funds of the American Red Cross. All testing was conducted at Imugen with the use of FDA-approved cost recovery (the FDA allows investigators to recover the actual costs of the investigational testing program). The protocol was approved by the institutional review board of the American Red Cross and the FDA (Investigational New Drug Application number, 14532). All the authors contributed to the study design and data collection and vouch for the integrity and completeness of the data and analyses presented.

**ASSESSMENT OF INFECTIVITY**

Red-cell samples from index donations that were PCR-positive or that were PCR-negative and had a high-titer antibody level (titer of ≥1:512) were injected into hamsters.10-12 For PCR-positive samples, two Syrian hamsters (*Mesocricetus auratus*, Envigo) that had not been previously used for research were injected intraperitoneally with 1.5 ml of red cells (range, 3.9×10⁹ to 5.7×10⁹ red cells per milliliter) on 2 consecutive days. For PCR-negative, high-titer donation samples, three hamsters were injected. Blood was obtained weekly up to 8 weeks after the initial injection and examined for parasites by means of acridine orange staining and microscopy.

To confirm *B. microti* infection, an in-house PCR assay was performed at 4 weeks and 8 weeks after the initial injection. Genomic DNA was extracted from hamster whole blood with the use of the Gentra Puregene Blood Kit (Qiagen). A real-time PCR assay was performed as described previously.13 Animals were killed when the development of parasitemia was observed or after 8 weeks, whichever occurred first. A red-cell donation was considered to be infectious in hamsters if infection developed in at least one animal. Procedures were approved by the institutional animal care and use committee of the American Red Cross.

**CASES OF TRANSFUSION-TRANSMITTED BABESIOSIS**

Suspected cases of transfusion-transmitted babesiosis in patients who had received transfusions were identified by hospitals on the basis of clinician suspicion and were reported to the American Red Cross. Cases in which an implicated donation was confirmed to be positive (i.e., when a donor had an AFIA-positive result at a titer of ≥1:64 or had a PCR-positive result on a follow-up sample that was obtained as part of a transfusion-transmission investigation) were included in this analysis.

**STATISTICAL ANALYSIS**

Kaplan–Meier product-limit survival estimates that modeled the time to the first PCR-negative and AFIA-negative result were generated with the use of SAS software, version 9.4 (SAS Institute). Comparisons of red-cell storage age with regard to infectivity in hamsters were made by means of Student’s t-test with a two-sided P value, with the use of InStat software. The proportions of infectious donations among unscreened and screened donations that were collected in Connecticut and Massachusetts over the
study period were compared with the use of Fisher’s exact test.

**RESULTS**

**SCREENING OF DONATIONS**

From June 2012 through September 2014, a total of 89,153 blood donations from 60,512 donors were screened. The test results for 337 donation samples (0.38%; 95% confidence interval [CI], 0.34 to 0.42) showed reactivity for *B. microti* antibodies or DNA. A total of 335 samples, each representing a unique donor, were confirmed to be positive. Of these 335 samples, 67 (20%) were positive on PCR assay, including 9 that were AFIA-negative (titer of <1:64 and IgM-negative), for a yield of 1 antibody-negative sample per 9906 donation samples screened. This yield represents 13% of all PCR-positive results. Of the 9 donors who had PCR-positive, AFIA-negative results, 8 underwent seroconversion; the ninth donor had a repeated PCR-positive result in an independent red-cell sample. One sample that was not confirmed to be positive on AFIA and one that had an inconclusive result on AFIA (because of nonspecific fluorescence) were excluded from further analysis (Fig. 1, and Table S1 in the Supplementary Appendix).

Figure 2 shows the county selection and the results for Connecticut and Massachusetts, which represented 93% of the positive samples in this study. All but one donor resided in the seven states in which babesia is endemic plus New Hampshire (Table S1 in the Supplementary Appendix). PCR-positive donations were identified in all months of the year except April, and PCR-positive, AFIA-negative donations were identified in June through September (Fig. 3). Among the samples that were positive on PCR assay or enhanced-sensitivity PCR assay, the estimated parasite loads ranged from 5 parasites to 3 million parasites per milliliter (median, 480 parasites per milliliter). As compared with donors of nonreactive samples, donors of reactive samples were more likely to be men, white, and at least 45 years old (Table S2 in the Supplementary Appendix; Fig. S1A and S1B in the Supplementary Appendix provide information on donor risk factors and symptom history).

The PPV was 100% among PCR-positive, AFIA-negative samples (9 of 9), among PCR-positive, AFIA-positive samples (67 of 67), and among
PCR-negative, AFIA-positive samples with titers of 1:512 or more (68 of 68). The PPV was 99.5% among PCR-negative, AFIA-positive donations with titers of 1:128 or 1:256 (191 of 192 donations). The overall PPV was 99.7% (335 of 336 donations) (Table S3 in the Supplementary Appendix). High-titer antibody-positive samples were more likely than low-titer ones to be PCR-positive,
but most antibody-positive samples had a low titer and were PCR-negative (Fig. 4).

**FOLLOW-UP SAMPLES**

Follow-up samples were provided by 225 donors, including 25 who were identified in a repository validation study; the overall participation rate through September 15, 2015, was 58% (225 of 386 donors). A total of 56 donors with PCR-positive results and 169 with PCR-negative results participated, providing 1 to 13 samples per donor. The median time to the first follow-up sample was 1.7 months (interquartile range, 1.1 to 2.7), and the median time to the last follow-up sample was 17.2 months (interquartile range, 10.0 to 27.6) after the index donation. Participants were more likely than nonparticipants to be white and at least 25 years old (Table S4 in the Supplementary Appendix). The median time to a PCR-negative sample was 4.7 months (interquartile range, 2.0 to 7.4); 86% of the donors with PCR-positive results (48 of 56 donors) had resolution of reactivity by 1 year (Fig. 5A). The median time to seroreversion, defined as an AFIA titer of less than 1:64, in pooled data from donors with PCR-positive results and those with PCR-negative results, was 17.1 months (interquartile range, 9.9 to 27.6). A total of 19 of 224 donors (8%) had resolution of antibody reactivity by 1 year (Fig. 5B and 5C). One of 9 donors with PCR-positive, AFIA-negative results never had seroconversion (14 parasites per milliliter). The PCR status of samples from 5 donors fluctuated between posi-
Infectivity in Hamsters

Among 138 donation samples that were either PCR-positive or PCR-negative with a high titer (≥1:512) on the screening AFIA, red-cell samples for injection into hamsters were available from 93 (67%) — 46 PCR-positive samples and 47 PCR-negative, AFIA high-titer samples. Overall, 27 samples (29%) were infectious. Of the 46 PCR-positive samples that were injected into hamsters, 25 (54%) were infectious. Samples from 2 of the 47 PCR-negative, AFIA high-titer units (4%) were infectious, 1 of which was positive on enhanced-

Figure 5. Kaplan–Meier Estimates of the Survival Function.

Panel A shows the Kaplan–Meier estimate of the survival function modeling the time to the first PCR-negative follow-up sample in blood donors whose samples were PCR-positive for *B. microti* on the index donation sample, stratified according to the presence or absence of antibodies (according to the AFIA result on the index donation sample). Circles indicate the last follow-up for donors who did not have reversion to PCR-negative status during the study period (0 donors whose index donation sample was PCR-positive, AFIA-negative and 2 whose index donation sample was PCR-positive, AFIA-positive). For example, the first circle on the AFIA-positive plot represents a donor whose last follow-up occurred 2.7 months after the index donation, at which point the sample remained PCR-positive. Dashed lines mark 1 year and 2 years since the index donation. Panel B shows the Kaplan–Meier estimate of the survival function modeling the time to the first antibody-negative (titer of <1:64) follow-up sample in blood donors whose index donation samples were PCR-positive for *B. microti*, stratified according to index titer. One donor with a PCR-positive, AFIA-negative result who did not undergo seroconversion was excluded from the analysis. Circles indicate the time of last follow-up for 36 donors (65% of those followed) who did not undergo seroreversion to AFIA-negative status during the study (3 donors whose index donation sample had a titer of <1:64, 9 with an index titer of 1:256, 6 with an index titer of 1:512, and 20 with an index titer of ≥1:1024). Dashed lines indicate the number of years since the index donation. Panel C shows the Kaplan–Meier estimate of the survival function modeling time to first antibody-negative follow-up sample in donors whose index donation samples were PCR-negative for *B. microti*, stratified according to index titer. Circles indicate the time of last follow-up for 135 donors (80% of those followed) who did not undergo seroreversion to AFIA-negative status during the study (61 donors whose index donation samples had a titer of 1:128, 36 whose index samples had a titer of 1:256, 23 whose index samples had a titer of 1:512, and 15 whose index samples had a titer ≥1:1024).
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sensitivity PCR assay (AFIA titer of ≥1:1024; 40 parasites per milliliter), leaving 1 infectious red-cell sample (AFIA titer of 1:512) that had PCR-negative test results according to the methods used in this study. Infectious red-cell samples were generally held for shorter periods of time before injection than were noninfectious red-cell samples (mean, 17 days [range, 7 to 34] vs. 24 days [range, 11 to 55]; P = 0.01), which indicates that infectivity was probably underestimated.

Cases Associated with Unscreened Donations

During the study period, 29 cases of transfusion-transmitted babesiosis from unscreened donations were confirmed by linkage to a transfused cellular component from donors who later had a positive test result. Donors of implicated infectious samples resided in eight states: Connecticut (eight donors), Massachusetts (seven), New Jersey (five), New Hampshire (three), Maine (three), Minnesota (one), New York (one), and California (one travel-associated case linked to a donor infected in Rhode Island). Table S6 in the Supplementary Appendix provides data on the reported cases of transfusion-transmitted babesiosis to the American Red Cross each year during the period from January 1, 2010, through August 31, 2016. The estimated risk of transfusion-transmitted babesiosis in these states in which babesia is endemic, including New Hampshire and Maine, was 28 cases per 2,825,414 donations or 1 case per 101,000 donations, whereas the incidence outside these states that was attributable to travel was 1 case per 10,359,000 donations.

The proportions of infectious donations among the unscreened and screened donations were compared. Among 75,331 screened donations, zero cases of transfusion-transmitted babesiosis were identified from screened donation samples, which implied that none of the donations were infectious. Among 253,031 unscreened donations, 14 (i.e., 1 in 18,074) were implicated in cases of transfusion-transmitted babesiosis, which suggests that 14 donations were infectious and 253,017 were not infectious, although we cannot exclude the possibility that the disease was not associated with transfusion. These proportions (0 in 75,331 and 14 in 253,031) were compared, and the odds of an unscreened donation being infectious were 8.6 times (95% CI, 0.51 to 144) as high as the odds of a screened donation being infectious (P = 0.05).

Among implicated donors who had follow-up results that were found to be positive for babesia, 1 had PCR-positive, AFIA-negative results 89 days after the implicated unit was obtained, 9 had PCR-positive, AFIA-positive results (median, 94 days; interquartile range, 76 to 98), and 19 had PCR-negative, AFIA-positive results (median, 111 days; interquartile range, 88 to 157). A total of 10 donors with PCR-positive results were identified at 2 to 7 months after their implicated donation. All 29 presumed cases of transfusion-transmitted babesiosis were attributed to red cells; the mean time from donation to transfusion was 20 days (range, 8 to 42).

Discussion

From June 2012 through September 2014, the American Red Cross screened 89,153 blood donations for B. microti DNA and antibodies to B. microti and removed 335 positive donations (0.38%) from the blood supply, including 9 that were positive only on PCR testing. Hamster-infectivity data, which probably underestimated risk, and the continuing occurrence of cases of transfusion-transmitted babesiosis from unscreened donations, for which the risk is also likely to be underestimated, suggest that this investigational screening protocol removed infectious donations from the blood supply. Most asymptomatic donors who are deferred from donating blood retain PCR-positive status for less than 1 year, whereas antibody reactivity (titer of >1:64) may be retained for several years (Fig. 5A, 5B, and 5C). Screening has continued for hospitals that have specifically requested tested blood. An additional 367 confirmed positive donation samples from 131,326 screened samples (0.28%) were identified by the American Red Cross between October 1, 2014, and August 31, 2016.

This study has limitations. The screening protocol is specific to B. microti and does not detect other babesia species, including B. duncani, which was implicated in 3 of 162 cases of transfusion-transmitted babesiosis from 1979 through 2009. Moreover, 14 index donation samples had screening results that were PCR-negative but had detectable levels of DNA when tested by means of enhanced-sensitivity PCR assay, which shows the need for continued improvements in the sensitivity of the PCR assay. Despite the size of the study, these data may not be representative of
B. microti infection in the general population and should be interpreted with caution. Blood donors are healthy persons whose clinical course probably differs from persons in whom severe disease develops. Furthermore, we cannot exclude some degree of bias related to the decision not to participate in screening or follow-up (Tables S2 and S4 in the Supplementary Appendix). Continual donor follow-up and additional well-controlled studies are necessary to assess further the natural history of infection in healthy and susceptible persons. Data on infection and disease in donors (e.g., the number of donors who opted out of screening or who sought treatment after a positive screening test) and recipients are limited and are subject to the constraints of passive reporting. Our data were collected in areas that were geographically limited (Connecticut, Massachusetts, Minnesota, and Wisconsin), and any discussion of risk estimates, effect of screening, and interpretation of the data should take this into consideration. Finally, although we report a marginally significant association between the odds of infectious samples in unscreened versus screened donations, the statistical inference, which is based on zero counts, small proportions, and absent covariates, must be viewed with caution.

Babesiosis is currently an infectious risk to the U.S. blood supply. It was responsible for 27% of the deaths (4 of 15 deaths) in blood-transfusion recipients that were reported to the FDA from 2010 through 2014. The American Red Cross identified 62 probable cases of transfusion-transmitted babesiosis in which a B. microti–positive donor was identified in the period from January 1, 2010, through August 31, 2016, and 29 of these occurred during this study period (Table S6 in the Supplementary Appendix). A previous pilot study that used similar assays also showed that screening may be efficacious.

Our data imply that screening with the use of a PCR-based and AFIA-based protocol removes infectious donation samples from the blood supply and reduces the incidence of transfusion-transmitted babesiosis. Our geographically specific per-donation risk of transfusion-transmitted babesiosis with unscreened samples of approximately 1 case per 18,000 donations is higher than the current residual risks of other serious transfusion-related adverse events, including sepsis from contaminated platelets (1 case per 107,000 donations), transfusion-associated acute lung injury (1 case per 138,000 donations), and infection with human immunodeficiency virus or hepatitis B or C virus (1 case per 1,000,000 donations for each type of infection).

Although reducing the frequency of transfusion-transmitted babesiosis is important, there are a number of issues that need to be resolved. Our data suggest that the greatest efficacy would be achieved by testing for both antibodies and DNA, because studies in animals have shown infectivity from single-marker–positive samples, particularly those that test positive with the use of the PCR assay. Furthermore, the geographic areas affected by B. microti are restricted but expanding. Our data, combined with reports of cases of babesiosis nationwide, suggest that a screening strategy that is limited to states in which babesia is endemic, including antibody and nucleic acid testing year-round, would balance risk reduction and the judicious use of resources. Periodic reevaluation will be necessary to address the identification of cases in states, such as Pennsylvania, that were previously considered to be at low risk for babesiosis (Table S6 in the Supplementary Appendix). Risk-targeted screening (i.e., transfusing units screened for babesia only into high-risk patients) is initially appealing but introduces many potential challenges and has not been favorable in cost-effectiveness modeling. Seasonal testing has likewise been proposed as a means of decreasing the financial burden; however, our data show that PCR-positive donation samples have been identified year-round (Fig. 3).

In May 2015, the Blood Products Advisory Committee of the FDA voted in favor of antibody screening in all 50 U.S. states. Although the testing algorithm that was used in this study performed better than other individual assays that have been used to ensure a safe blood supply, with a combined specificity of 99.98% in retrospective studies and more than 99.999% in prospective studies and an overall PPV of 99.7% in prospective studies (Table S3 in the Supplementary Appendix), it is possible that when the antibody test is implemented widely in areas of low prevalence, the PPV will decrease (the lowest PPVs occurred in the category of AFIA-positive low-
titer, PCR-negative samples). If universal screening is adopted, it may result in the unnecessary deferral of donors with false reactive results and of those with resolved infection, while also imposing a substantial financial burden on hospitals and the blood industry. Of the 220,479 total donations that were prospectively screened from June 4, 2012, through August 31, 2016, only four false antibody-reactive results occurred (one was described in this study). The high degree of specificity ensures the ongoing availability of donors and minimizes incorrect messages to donors. Moreover, our data show that, although PCR-reactivity resolves within 1 year in most donors, antibodies are retained much longer. Algorithms for the reentry of persons into the pool of eligible donors are crucial for maintaining the engagement of donors who have reactive results, particularly those with false reactive results and those with remote previous infections.

In conclusion, transfusion-transmitted babesiosis is of increasing concern and requires effective interventions. Our experience with prospective blood-donation screening provides a potential testing strategy.

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REFERENCES