Description of 15 DNA-positive and antibody-negative “window-period” blood donations identified during prospective screening for Babesia microti

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BACKGROUND: Blood donation screening detecting only antibodies fails to identify donors in the earliest stage of infection, before a detectable immunologic response, that is, the “window period” (WP). We present data on WP donations identified during prospective screening for Babesia microti, a transfusion-transmissible parasite of increasing concern in the United States.

STUDY DESIGN AND METHODS: Blood donations collected in Connecticut, Massachusetts, Minnesota, and Wisconsin were screened using polymerase chain reaction (PCR) and arrayed fluorescence immunoassay (AFIA) to detect B. microti DNA and antibodies, respectively. Parasite loads were estimated using quantitative PCR. Red blood cell (RBC) samples were inoculated into hamsters to assess infectivity. Donors screening reactive were indefinitely deferred, tested by supplemental methods, and followed to assess DNA and antibody clearance. Demographic data from WP donors (i.e., those screening PCR positive and AFIA negative) were compared to data from other positive donors.

RESULTS: Of 220,479 donations screened from June 2012 to August 2016, a total of 700 were positive, of which 15 (2% of positive donations or 1 per 14,699 screened donations) were confirmed WP donations. The median estimated parasite load in WP donations was 350 parasites/mL, no different than AFIA-positive and PCR-positive donors. Parasite loads in RBC samples from WP units ranged from 14 to 11,022 parasites/mL; RBC samples from three of 10 (30%) WP donations infected hamsters. The mean age of WP donors was 48 years (range, 17-75 years); three (20%) were female. WP donor demographics did not differ significantly from demographics of other donors.

CONCLUSIONS: We report one per 15,000 B. microti WP infections in blood donors in endemic areas, demonstrating the importance of nucleic acid testing to mitigate the risk of transfusion-transmitted babesiosis.

ABBREVIATIONS: AFIA = arrayed fluorescence immunoassay; TTB = transfusion-transmitted babesiosis; WP = window period.

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One such emerging pathogen is *Babesia microti*, an intraerythrocytic parasite that is primarily transmitted to humans by the tick *Ixodes scapularis*, but can also be transmitted through blood transfusion. Babesiosis is most often asymptomatic or mild in healthy individuals, but can be severe or fatal in susceptible individuals, such as asplenic persons (or those lacking a functional spleen), the elderly, and the immunocompromised. However, a recent review highlighted that babesiosis and/or associated death is not limited to the groups mentioned but also occurs in patients of all age groups with a variety of underlying medical conditions. Due to increasing concern, cases of babesiosis have been nationally notifiable since 2011; in 2014, a total of 1760 confirmed or probable cases were reported, with 1736 (99%) occurring in nine states in the northeastern and upper midwestern United States, comprising Connecticut (CT), Massachusetts (MA), Maine (ME), New Hampshire (NH), New Jersey (NJ), New York (NY; includes New York City), Rhode Island (RI), Minnesota (MN), and Wisconsin (WI). However, babesiosis is still not reportable in all states, including some that are emerging as epidemic (e.g., Pennsylvania), so the true number of cases is likely to be higher.

The parasite can be transmitted through red blood cells (RBCs) and platelets (PLTs) contaminated with RBCs collected from asymptomatic blood donors. Transfusion-transmitted babesiosis (TTB) was first reported in 1979 and is of concern in highly endemic areas. The incidence of TTB in the United States appears to be increasing, likely due to enhanced disease awareness, geographical expansion of the disease vector, environmental and behavioral conditions favoring increased association of the parasite and the tick vectors, and increased interaction between infected ticks and humans. There are currently no Food and Drug Administration–licensed blood donation screening tests.

Since 2012, the American Red Cross has been conducting prospective screening for antibodies to and DNA from *B. microti* in donors presenting to donate in select endemic areas of the United States. Fifteen WP donations identified thus far are characterized in this case series.

**MATERIALS AND METHODS**

As part of an investigational new drug study sponsored by IMUGEN, Inc., the Red Cross has been conducting prospective screening on donations collected in selected areas of CT, MA, MN, and WI. Methods have been described elsewhere; briefly, polymerase chain reaction (PCR) and arrayed fluorescence immunoassay (AFIA) were used to detect DNA and antibody, respectively, in 5-mL EDTA whole blood and plasma samples before release of the blood product. Donations testing PCR and/or AFIA-reactive (i.e., positive or inconclusive), considered the index donations, were removed from the blood supply. Residual samples from the reactive index donation underwent supplemental testing (immunoglobulin [Ig]M and IgG Western blot, enhanced [e]-sensitivity PCR for donations that initially screened PCR negative and AFIA reactive, quantitative PCR on PCR-positive donations, and AFIA at a lower titer cutoff (<64) on potential WP donations). Donations that screened and confirmed as PCR positive and AFIA negative (titer <64) were considered WP donations; data from these donations and their donors were extracted for this report.

If available, RBC samples from WP donations were inoculated (1.5 mL injected intraperitoneally on 2 consecutive days) into naive Syrian hamsters (*Mesocricetus auratus*; Envigo; duplicate for PCR-positive and triplicate for PCR-negative samples) to determine infectivity. Infection was identified and confirmed by acridine orange staining and microscopy and in-house real-time PCR as previously described. Animals were euthanized upon development of parasitemia or after 8 weeks, whichever occurred first. As previously documented, high-titer AFIA-positive samples were more likely to be PCR positive, and presumed likely infectious, than low-titer samples. Thus, those selected for hamster challenge included any PCR-positive sample and those PCR-negative samples that had a high AFIA titer (≥512).

Donors of AFIA- and/or PCR-reactive donations were deferred indefinitely, encouraged to seek medical advice, and invited to participate in a follow-up study. During follow-up, donors were invited to return approximately every 6 to 8 weeks to provide follow-up samples, which were tested using the screening PCR assay to determine DNA persistence. The earliest PCR-negative sample identified in a donor during follow-up was confirmed as negative by enhanced-sensitivity PCR. Follow-up samples were also tested using AFIA to measure seroconversion (defined as an antibody titer of ≥64 on one or more follow-up samples) and seroreversion (defined as antibody <64 after seroconversion during follow-up).

Statistical analyses were performed using computer software (SAS 9.4, SAS Institute). Means were reported for normally distributed continuous variables, as identified by the Shapiro-Wilk normality test; medians were otherwise reported. Descriptive data from other positive donations identified during the investigational new drug study (i.e., PCR positive/AFIA positive and PCR negative/AFIA positive) were compiled for comparison with WP donation data. Count data were compared using chi-square or Fisher’s exact test, depending on cell size. Mean age in WP donors versus other donors was compared using t test. The Kolmogorov-Smirnov test was used to compare parasite loads in WP versus other donations. Study procedures were approved by the American Red Cross Institutional Review Board and Institutional Animal Care and Use Committee.
RESULTS

Between June 4, 2012, and August 31, 2016, the American Red Cross screened 220,479 donations and identified 704 reactive donations, including 700 confirmed-positive donations. Of these, 114 (16%) were PCR-positive donations, of which 15 were WP donations (i.e., one per 14,699 screened donations, 2% of all positive donations, and 13% of PCR-positive donations). Samples from all 15 WP donations were confirmed as PCR positive by quantitative PCR and confirmed as IgM negative and AFIA negative at a titer of less than 64; a sample from one WP donation tested inconclusive on IgG Western blot due to background staining, while the rest confirmed negative for IgG antibodies (Table 1).

The median parasite load in WP donations was 350 parasites/mL (interquartile range [IQR], 998.5; n = 15), while the median parasite load in PCR-positive and AFIA-positive donations was 366 parasites/mL (IQR, 3584; n = 77; p = 0.65; Fig. 1). Higher maximum parasite loads were observed for PCR-positive and AFIA-positive donations (range, 5-2,990,624 parasites/mL) versus those of WP donations (range, 14-11,020 parasites/mL). RBC samples from three of 10 (30%) WP donations inoculated into hamsters resulted in infections, compared to 26 of 44 (59%) from PCR-positive, AFIA-positive donations (p = 0.10 using Fisher’s exact test to compare proportions; Table 1). In contrast, PCR-negative, high-titer (≥512) samples were rarely infectious (two of 53; 4%) and less likely to infect hamsters than samples from WP donations (p = 0.025).

Donors of WP donations resided in CT (n = 9), MA (n = 3), MN, WI, and NY (n = 1 each). The mean age was 48 years (range, 17-75 years) and 20% (3/15) were female; the mean age of donors of other positive donations was 48 years (range, 17-91 years, p = 0.94) and 32% (216/685) were female (p = 0.41). WP donations were detected in June (n = 6), July (n = 3), August (n = 4), and September (n = 2); other positive donations were identified year-round. All WP donors were repeat donors, compared to 88% (606/685) among other donors with positive donations (p = 0.16).

Thirteen donors (87%) participated in follow-up; the median number of follow-up samples collected was 4.5 (IQR, 7) samples, and median time to first follow-up sample collection was 1.6 (IQR, 3.4) months. Five (38%) donors retained PCR positivity at first follow-up; median time to first PCR-negative follow-up sample was 1.4 (IQR, 2.1) months (Table 1).

Twelve of the 13 WP donors participating in follow-up (92%) tested AFIA positive on first follow-up, suggesting that seroconversion had occurred. The 13th donor (Donor...
M) provided two follow-up samples, one at 1.5 and another at 17.5 months post index, both of which were antibody-negative at a <64 titer on AFIA and negative on IgM and IgG Western blot, suggesting failure to seroconvert in this donor. *B. microti* DNA in the index donation was confirmed by repeat PCR testing of the donor’s PCR-positive RBC component (14 parasites/mL) but samples from this donation were not infectious in hamsters (Table 1). Five donors seroreverted during follow-up; mean time to seroreversion was 28.4 months from the time of the index donation (range, 10-37.3 months; Fig. 2).

**DISCUSSION**

During the Red Cross’ ongoing investigational study assessing the efficacy of a *B. microti* blood donation screening system, we identified 15 WP donations collected in endemic areas in which screening occurred, for a WP rate of one per 14,699 donations screened. For comparison, the current yields for HIV, HCV, and hepatitis B virus WP units through August 31, 2016, using Red Cross donations to determine national rates are one per 1,020,000, one per 218,000, and one per 681,000, respectively (American Red Cross, unpublished). Thus, the WP yield rates for *B. microti* are 15- to 70-fold higher than for the three viral pathogens for which NAT was initially introduced. We also estimated the residual risk of TTB to be one per 101,000 donations in nine endemic states in the northeastern and midwestern United States (CT, MA, ME, NH, NJ, NY, RI, MN, and WI) and one per 18,000 donations in 10 counties of two highly endemic states (CT and MA). In contrast, the estimated residual risks for sepsis from contaminated apheresis PLTs and transfusion-associated acute lung injury from apheresis PLTs infection are one per 107,000 and one per 138,000 donations, respectively, these are still considered unacceptably high and further interventions are being implemented.

Although the only case series report of WP units for *B. microti*, this report has some limitations. The screening program is being conducted in endemic areas of the country, so rates do not translate to nonendemic blood collection areas; however, this does not impact the relevance of the findings. Fifteen WP donations were identified and clinical data were not uniformly available; however, nearly 90% (13/15) of WP donors participated in follow-up. Of these, some donors had wide intervals between follow-up visits, potentially limiting our precision to detect changes in donors’ parasite loads and the time point of seroconversion. Nevertheless, this case series clearly demonstrates the frequency and characteristics of early *B. microti* infections in blood donors and their relevance to developing blood safety policy.

Based on two follow-up samples (one collected at 1.5 and one at 17.5 months post index), one donor, a 55-year-old woman who lived in rural CT, did not demonstrate seroconversion; the reason for this is unclear. This could have been due to very low parasitemia (estimated 14 parasites/mL on index donation—the lowest identified among WP donations) or donor-related factors, such as unreported immunosuppression. The donor’s medical history was unremarkable upon presenting to donate; however, she did not consent to a *Babesia*-specific risk factor questionnaire and additional clinical information is unavailable. It is also possible that during the 16 months between follow-
TABLE 1. Donor characteristics and supplemental assay results of WP (PCR-positive and AFIA-negative) donations identified during investigational prospective screening for B. microti*

<table>
<thead>
<tr>
<th>Donor</th>
<th>Month of collection</th>
<th>Sex</th>
<th>Age (years)</th>
<th>State of residence</th>
<th>FT/RPT</th>
<th>IgM Western blot</th>
<th>IgG Western blot</th>
<th>Estimated parasites/mL</th>
<th>Hamster infectivity</th>
<th>First F/U sample (days)†</th>
<th>First F/U sample (days)‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>June</td>
<td>Male</td>
<td>41</td>
<td>CT</td>
<td>Repeat</td>
<td>Negative</td>
<td>Negative</td>
<td>480</td>
<td>Negative</td>
<td>39</td>
<td>39</td>
</tr>
<tr>
<td>B</td>
<td>June</td>
<td>Male</td>
<td>39</td>
<td>CT</td>
<td>Repeat</td>
<td>Negative</td>
<td>Negative</td>
<td>1,100</td>
<td>Positive</td>
<td>30</td>
<td>86‡</td>
</tr>
<tr>
<td>C</td>
<td>July</td>
<td>Male</td>
<td>45</td>
<td>CT</td>
<td>Repeat</td>
<td>Negative</td>
<td>Negative</td>
<td>350</td>
<td>Negative</td>
<td>453</td>
<td>453</td>
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<tr>
<td>D</td>
<td>August</td>
<td>Female</td>
<td>54</td>
<td>MA</td>
<td>Repeat</td>
<td>Negative</td>
<td>Inconclusive§</td>
<td>1,000</td>
<td>Negative</td>
<td>26</td>
<td>219</td>
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<tr>
<td>E</td>
<td>August</td>
<td>Male</td>
<td>19</td>
<td>MA</td>
<td>Repeat</td>
<td>Negative</td>
<td>Negative</td>
<td>400</td>
<td>Positive</td>
<td>43</td>
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<tr>
<td>F</td>
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<td>71</td>
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<td>Repeat</td>
<td>Negative</td>
<td>Negative</td>
<td>40</td>
<td>NA‖</td>
<td>97</td>
<td>97</td>
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<td>G</td>
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<td>17</td>
<td>WI</td>
<td>Repeat</td>
<td>Negative</td>
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<td>112</td>
<td>Negative</td>
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<td>H</td>
<td>September</td>
<td>Female</td>
<td>55</td>
<td>CT</td>
<td>Repeat</td>
<td>Negative</td>
<td>Negative</td>
<td>110</td>
<td>Negative</td>
<td>182</td>
<td>182</td>
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<tr>
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<td>Positive</td>
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<td>J</td>
<td>September</td>
<td>Male</td>
<td>35</td>
<td>WI**</td>
<td>Repeat</td>
<td>Negative</td>
<td>Negative</td>
<td>32</td>
<td>Negative</td>
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<td>K</td>
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<td>L</td>
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<td>Negative</td>
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<td>55</td>
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<td>Negative</td>
<td>14</td>
<td>Negative</td>
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<tr>
<td>N</td>
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<td>63</td>
<td>WI</td>
<td>Repeat</td>
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<td>Negative</td>
<td>20</td>
<td>Negative</td>
<td>NA§§</td>
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<td>O</td>
<td>August</td>
<td>Male</td>
<td>29</td>
<td>CT</td>
<td>Repeat</td>
<td>Negative</td>
<td>Negative</td>
<td>2,093</td>
<td>NA‖</td>
<td>NA§§</td>
<td>NA</td>
</tr>
</tbody>
</table>

*Screening occurred between June 2012 and August 2016 in counties in CT, MA, MN, and WI identified as high risk based on the number of babesiosis cases reported to health departments and a previously published prevalence study.18 Unless otherwise noted, supplemental assays were performed on surplus samples collected directly from the index donation.

†Time since index donation. All donors participating in F/U tested AFIA positive on the first F/U sample, with the exception of Donor M.
‡F/U sample collected 30 days post index was unsuitable for PCR testing. A F/U sample collected 86 days post index tested PCR inconclusive and negative by enhanced-sensitivity PCR.
§Western blot result inconclusive due to background staining.
‖RBC samples not available for hamster inoculation.
¶Follow-up; RPT
§§Donors N and O did not agree to participate in follow-up.
††Most recent F/U sample, collected 41 days post index, tested PCR positive.
‡‡No evidence of seroconversion in donor. Follow-up samples were collected at 40 and 487 days post index; both samples tested negative on AFIA; PCR, and enhanced-sensitivity PCR.
§§§Donors N and O did not agree to participate in follow-up.
FT = first time; F/U = follow-up; RPT = repeat.

up samples, the donor seroconverted with a low-level antibody response but then seroreverted to antibody negative before the next sampling. Continued identification and follow-up of WP donors will be necessary to determine the frequency of these “nonconverters” or “low-level converters” in the donor population and the associated clinical implications.

The risk that WP donations pose to recipients has not been determined. While one other study identified a possible WP infection during surveillance of the blood donor population,21 the number of donations screened in that study was small and no index confirmation or follow-up samples from the WP donor were obtained. In our study, the parasite loads in WP donations did not differ significantly compared to the parasite loads from the other PCR-positive donations, nor was the rate of hamster infectivity significantly different between groups. While the small number of WP donations may have restricted our ability to detect significant differences between groups, and there are limitations to extrapolating animal study findings to humans, this suggests that WP donations pose a risk similar to that from PCR-positive and AFIA-positive donations (p = 0.10) and a risk higher than that from PCR-negative donations (p = 0.025).

WP donations have also been associated with TTB cases. Among the 62 TTB cases reported to the Red Cross from January 1, 2010, through August 31, 2016, one WP donor was implicated in a case in which she tested PCR positive and AFIA negative (titer < 64 with an estimated parasite load of 205 parasites/mL) 89 days after the implicated unit was collected (American Red Cross, unpublished).11 Among 20 TTB cases identified in Rhode Island in which retained RBC segments from the implicated unit were available for testing, three (15%) WP donations were identified.22 Additional recipient complication data will be necessary to further characterize the risk that WP donations pose to the blood supply.

This case series establishes the presence and rate (one per 15,000 donations screened) of B. microti WP infections in the blood donor population in highly endemic areas of the United States. Pathogen reduction and inactivation in RBC or whole blood units has demonstrated the potential to decrease the risk of many transfusion-transmitted infections, including B. microti.23-25 However, until such technologies become widely available and utilized, NAT will play an important role in conjunction with antibody detection in mitigating TTB risk.
CONFLICT OF INTEREST

MEH and VPB are employees of IMUGEN, Inc. The other authors have disclosed no conflicts of interest.

REFERENCES