



Advancing Transfusion and  
Cellular Therapies Worldwide

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**To:** AABB Members  
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Miriam A. Markowitz – Chief Executive Officer  
**Re:** Babesiosis

### Summary

This bulletin was developed by the AABB Transfusion Transmitted Diseases (TTD) Committee's *Babesia* and Other Tick-Borne Diseases Work Group to provide educational materials and recommendations regarding: 1) the epidemiology of *Babesia* infection and babesiosis case reports, including transfusion-transmitted babesiosis (TTB); 2) clinical manifestations of babesiosis and those patients/recipients at greatest risk; 3) diagnosis and treatment of babesiosis; 4) managing potential TTB including case investigation and look-back; and 5) measures to mitigate the risk of TTB. A listing of critical unresolved issues is provided to emphasize topics that require further discussion. This bulletin supersedes Association Bulletin #09-06, "Transfusion-Transmitted *Babesia*."

Association Bulletins, which are approved for distribution by the AABB Board of Directors, may include announcements of standards or requirements for accreditation, recommendations on emerging trends or best practices, and/or pertinent information. This bulletin contains educational information and recommendations for investigating and decreasing the risk of TTB. These recommendations do not represent requirements for accreditation.

### Background

*Babesia* spp. are obligate intraerythrocytic protozoan parasites that are the etiologic agents of babesiosis, a potentially life-threatening, malaria-like illness in humans.<sup>1</sup> More than 160 cases of TTB have been recognized in the United States, contributing to at least 28 associated deaths in the 30 years from 1979 to 2009.<sup>2</sup> In the US, *B. microti* is the primary agent of human babesiosis, accounting for almost all of the documented cases of transfusion transmission, and is, therefore, the focus of this bulletin.<sup>3</sup> In recent years, documented TTB cases have increased (i.e., three quarters of cases have occurred since 2000), reflecting the fact that *B. microti* is an emerging agent with an expanding geographic distribution and enhanced recognition and reporting.

TTB is the leading infectious cause of mortality (38%) in transfusion recipients as reported to the Food and Drug Administration (FDA).<sup>4</sup> The AABB Emerging Infectious Diseases (EID) subgroup of the TTD Committee categorized *Babesia* as a high priority agent for the development and implementation of an intervention to reduce the incidence of TTB.<sup>5</sup> However, the only approach

currently implemented to reduce the transfusion risk is a largely ineffective donor interview question regarding a history of babesiosis.<sup>6</sup> There are no FDA-licensed donor screening tests, but selective testing with serologic and molecular tests is proceeding under investigational new drug (IND) protocols in some areas. TTB cases continue to be associated with blood products not tested under IND protocols. **Appendix A** contains an updated discussion of the epidemiology of *Babesia* infection.

### **Babesiosis Case Reports**

The expanding geographic range of *B. microti*, increasing incidence of babesiosis, and the threat to the blood supply led the Council of State and Territorial Epidemiologists (CSTE) to designate babesiosis as a nationally notifiable disease in 2011,<sup>7</sup> although not all states have made it a reportable condition as yet. The clinical case definition for babesiosis takes into consideration transfusion for purposes of epidemiologic linkage between a transfusion recipient and a blood donor. A confirmed case of clinical babesiosis, including those observed in blood donors or transfusion recipients, demonstrates clinical signs or symptoms of disease coupled with direct visualization of intraerythrocytic parasites, a positive nucleic acid test, or detection of organisms in an inoculated animal (i.e., confirmatory laboratory evidence). A probable clinical case demonstrates serologic confirmation of babesiosis with objective clinical evidence of disease (i.e., fever, anemia, or thrombocytopenia). In the case of TTB, the evidence may include an implicated blood donor or recipient who has confirmatory laboratory evidence of infection in the absence of signs and symptoms or who has serologic confirmation and may or may not have subjective clinical evidence of infection (i.e., chills, sweats, headache, myalgia, or arthralgia), but does not have any objective clinical evidence of disease.

In states and territories in which babesiosis is reportable, all potential cases should be reported, including reactive test results from donors and recipients identified as part of IND screening or testing implemented after FDA licensure, and suspected TTB cases. The latter should also be reported to the appropriate health department even in states where the condition is not yet reportable, as the health department may be able to assist with the investigation.

### *TTB Cases*

A comprehensive review article from the Centers for Disease Control and Prevention (CDC) compiled data on 159 TTB cases caused by *B. microti* in the US from 1979 (i.e., first reported TTB case) to 2009.<sup>2</sup> These cases were ascertained mostly through passive surveillance, and the authors believe this is an underestimate of the total number of actual cases during this time. Most cases were discovered as a result of the recipient developing clinical symptoms; a minority of cases were detected through recipient tracing from an infected donor (i.e., look-back). Recognized cases included neonates, the elderly, asplenic patients, and those with an attenuated immune response due to cancer and other hematologic disorders.

- All-cause mortality rate was 18% (28 cases).
- Median recipient age was 65 years (range, <1 to 94 years) with 18 (11%) cases occurring in infants and 106 (68%) cases in recipients  $\geq 50$  years of age.
- The most common diagnoses/procedures reflected groups at high risk for receiving Red Blood Cell (RBC) transfusion: hematologic disorders [e.g., hematologic cancer (n=14), sickle cell disease (n=11), thalassemia major (n=7), and other (n=7)]; 22 patients with cardiovascular disease and procedures, and 19 patients with gastrointestinal bleeding or procedures.

- Thirty-two recipients (20%) had undergone surgical splenectomy, either in the past (17 cases), in the peritransfusion period (e.g., posttrauma transfusion and splenectomy) (12 cases), or  $\geq 1$  month after transfusion (3 cases).
- The median interval from transfusion to onset of clinical symptoms was 37 days (range, 11-176 days) and the median interval from symptom onset to diagnosis was 6 days (range, 0-84 days).
- Transfusion of RBC components accounted for 155 of the 159 cases (97%).
- Transmission occurred throughout the RBC storage period (e.g., up to 40 days) and from leukoreduced, irradiated, and frozen-deglycerolized RBC units.
- Four cases were attributed to whole-blood-derived platelets, no cases from apheresis platelets or plasma.
- Cases were linked to donations made in all 12 months, with peak transmission occurring from July to October.
- Nearly 90% of cases were observed in the seven states where *B. microti* is endemic (i.e., CT, MA, MN, NJ, NY, RI, and WI).
- Nineteen cases (13%) occurred in states where *Babesia* is not endemic and were attributed to:
  - RBC units collected in a *Babesia*-endemic area were shipped to a nonendemic area.
  - A donor from a *Babesia*-endemic area donated while visiting a nonendemic area.
  - A donor from a nonendemic area acquired the infection while visiting an area where *Babesia* was endemic.
- Of the total number of cases reported, 77% were reported from 1999 to 2009, suggesting a rapid increase in frequency and/or better surveillance for TTB.

## Information for Clinicians

### *Clinical Manifestations*

Whether transmitted by ticks or blood transfusion, infection with *B. microti* can range from asymptomatic to fulminating disease resulting in death. The incubation period from tick exposure to symptoms can range from 1 week to several months, but usually is 1 to 4 weeks.<sup>1</sup> In contrast, the median interval from transfusion to symptoms in one large case series was 37 days, with a range of 11 to 176 days.<sup>2</sup>

In healthy persons, *B. microti* infection is often asymptomatic or mild. Clinical manifestations commonly include fever (as high as 105.6F), chills, sweats, headache, malaise, myalgia, and other nonspecific flu-like symptoms.<sup>1</sup> Accompanying laboratory findings include hemolysis, hemolytic anemia, and thrombocytopenia. Symptoms generally resolve in a few weeks, but subclinical infection may persist for months to more than a year without treatment. The risk for symptomatic infection and complications is increased among neonates; the elderly; those with an attenuated immune response due to cancer, HIV infection, chronic heart, lung, or liver disease; treatment with immunosuppressive drugs; and especially those with anatomic or functional asplenia (especially as a result of hemoglobinopathy). These risk factors are common among transfusion recipients.

Complications of babesiosis may include hemodynamic instability, acute respiratory distress syndrome, severe hemolysis, disseminated intravascular coagulation, renal dysfunction, hepatic compromise, congestive heart failure, and death. Immune dysregulation, including the stimulation of autoantibodies that cross-react with similar antigens on erythrocytes, has been reported from

both community-acquired babesiosis and TTB. These cases of autoimmunity often result in severe hemolysis.<sup>8</sup>

Among hospitalized patients, fatality rates range from 6% to 9%, with rates as high as 21% for immunocompromised patients. Persons who have asymptomatic infection or resolution of symptoms may have low-level parasitemia for weeks to months, sometimes for longer than a year. Asymptomatic parasitemia in potential blood donors presents the opportunity for transfusion transmission, as well as for the appearance or recrudescence of symptoms in chronically infected persons who become immunosuppressed.<sup>9</sup>

Babesiosis cannot be distinguished from other infections solely on the basis of signs and symptoms; diagnostic testing is required. **See Appendix B.** The presence of an unexplained febrile illness, especially when accompanied by hemolytic anemia, should prompt consideration of babesiosis, especially for persons who live or visit *Babesia*-endemic areas or have been recently transfused.

### *Treatment*

Oral atovaquone with azithromycin for  $\geq 7$  to 10 days is an effective, readily available, and well-tolerated treatment.<sup>10</sup> The combination of quinine and clindamycin for a similar duration has been recommended for treatment of symptomatic babesiosis, but can elicit side effects. Illness may persist in severely immunocompromised patients, necessitating additional courses of treatment. Red cell exchange transfusion is used as adjunctive therapy for life-threatening babesial infection (e.g., parasitemia  $\geq 10\%$  or severe hemolysis) with dramatic improvements reported in some cases with severe hemolysis.<sup>8, 11</sup>

## **Managing Potential TTB Cases**

### *Infected Recipient*

- The identification of recipients with TTB requires physicians who are well educated on, and aware of, the risks of TTB. The subsequent investigation is the collaborative responsibility of the hospital transfusion service, collection facilities, and the patient's physician(s). Donors, who are assumed to have been asymptomatic at the time of donation, may exhibit symptoms after donation and receive a subsequent diagnosis of babesiosis (weeks to months following donation) that needs to be reported promptly to the collection facility. Similarly, transfusion services with a suspected TTB case must inform the blood center to facilitate donor evaluation and control of in-date cellular components from suspect donors. Upon suspicion of TTB, confirmation of the diagnosis and infecting species in the recipient is critical (including exclusion of malaria), and whenever possible should be accomplished by direct detection [i.e., smear, polymerase chain reaction (PCR), animal inoculation]. When the patient resides in a *Babesia*-endemic area, it is important to conduct a thorough evaluation of risk factors, and test pretransfusion samples whenever available, to exclude the possibility of preexisting infection. The type of sample and sample storage conditions may influence the suitability of these samples to provide accurate test results, thereby affecting whether these tests can be used to exclude donors as possible sources of infection.

- Generally, the incubation period for reported TTB cases is similar to or longer than that for tick-borne infection. In rare instances (e.g., sickle cell patients) the incubation period can be considerably longer, extending to several months.<sup>1,12</sup>
  - Reporting TTB cases to the authorized state or local health department (see above under “Babesiosis Case Reports”) may assist with the case investigation, as the health department might have access to useful surveillance information that could be of assistance in donor follow-up.
  - The CSTE/CDC surveillance case definition, used for states to notify the CDC of cases, is available as part of the National Notifiable Diseases Surveillance System at: <http://wwwn.cdc.gov/NNDSS/script/casedef.aspx?CondYrID=617&DatePub=1/1/2011>

#### *Case Investigation and Look-Back*

- Typically, babesiosis is diagnosed in a transfused recipient and, if the clinician recognizes a temporal relationship to transfusion, TTB is suspected. Investigations of credible reports of TTB require evaluation of the donors whose cellular products were transfused.
- With confirmation of babesiosis in the recipient, an investigation is initiated by the transfusion service with immediate reporting of the available data (e.g., recipient clinical history and exposure history, other pertinent risk factors, laboratory studies, and the identification of transfused components) to the collection facility.
  - The case report should be evaluated to ascertain the likely window of potential transfusion transmission. All donors associated with cellular products transfused to the recipient within this window should be contacted for an interview regarding potential exposures, signs, and symptoms, and asked to return for follow-up testing. *Babesia* antibody tests are optimal for use in follow-up testing. Some investigational protocols use both antibody and DNA testing by PCR to investigate suspect donors (see below).
    - Reactive serology is consistent with either current or prior infection, but a positive result alone does not provide conclusive evidence that the donor is the cause of the transfusion transmission. Nevertheless, it is necessary to indefinitely defer this donor as presumptively infected. Further, investigation of cases in which large numbers of donors from *Babesia*-endemic areas with high seroprevalence for *B. microti* may result in coincidentally positive results unrelated to the donor’s involvement in the case.
    - In one investigational testing program, PCR has demonstrated that nearly 50% of implicated donors are PCR positive for 2 to 7 months following the implicated donation, including one PCR-positive/seronegative donor. Negative PCR test results do not exclude prior or ongoing infection.
    - In research settings, PCR also may provide a source of nucleic acid for subsequent molecular studies that can be useful for establishing donor/recipient linkage in TTB cases.
  - If archived donor specimens are available for testing from the index or prior donations, they can be useful. However, negative results do not exclude prior or current infection due to potential sample dilution and sub-optimal storage conditions that may not maintain detectable analyte concentrations.
- The medical director should evaluate the number of donors involved, determine and prioritize at-risk donors (e.g., red cell donors, those from *Babesia*-endemic areas), then retrieve and quarantine relevant in-date cellular components from appropriately vetted suspect donors while the investigation is ongoing.

- Implicated donors of cellular products (positive for antibody and/or DNA) are indefinitely deferred and referred to a physician for evaluation. The physician should receive all relevant test results. There is no current protocol for reentry of implicated donors deferred for either a history of babesiosis or reactive test results.
- Donors with negative test results can be cleared and components may be (re)distributed for transfusion, unless there are other reasons to suspect the donor (e.g., recipient with no risk for tick-borne exposure); the donor is either the only donor or the only donor from a *Babesia*-endemic area whose products were transfused.
- At the completion of the investigation, the medical director should determine if there is sufficient evidence to implicate a specific donor or donors in the case. If there is such a donor(s), other donors who have not yet been fully evaluated (for example, because they could not be located or have not complied with requests for blood samples) may be cleared by the medical director from further suspicion, based on available evidence.
- Look-back and recipient notification:
  - Currently there are no requirements for look-back to identify potentially infected recipients of prior donations from donors implicated in TTB; however, some recipients may benefit from this information.
    - The existence of clusters of infection with *Babesia* in the US suggests look-back may be useful for identifying other transmissions.
    - At a minimum, recipients of cellular components from the index or subsequent donations of the implicated donor should be investigated.
    - Current investigational screening methods associated with IND protocols define the period during which prior donations from implicated donors should be investigated.

## **Mitigation Strategies Including Donation Screening Results**

### *Donor History Question and Deferral Due to a History of Babesiosis*

The increasing incidence of TTB has provided impetus for the development of interventions to reduce TTB. The only interventions implemented specifically to prevent TTB include a donor-screening question regarding a “history of babesiosis” and selective avoidance of blood collections in some areas of relatively high *Babesia* endemicity.<sup>3</sup> The high numbers of TTB cases suggest that the use of a babesiosis history question is largely ineffective.

- The 29th edition of *AABB Standards for Blood Banks and Transfusion Services* requires the indefinite deferral of a donor with a history of babesiosis, as noted above [Reference Standard 5.4.1A(11)].<sup>13</sup>
- Similarly, a donor implicated in a TTB case should be considered equivalent to a donor with a history of babesiosis and indefinitely deferred. This is consistent with Reference Standard 5.4.1A(11).
- Investigational testing protocols (see below) require all donors with reactive or inconclusive test results to be indefinitely deferred. There is no reentry process for donors who have been implicated in TTB cases (by virtue of positive research or investigational tests) or for those donors who have been deferred due to a reactive research or reactive/inconclusive investigational test result.

*Donation Screening Under Research or IND Protocols*

Several centers in *Babesia*-endemic areas of the US have evaluated research tests or implemented testing performed under IND protocols. Research studies have established baseline seroprevalence data, the impact of look-back (i.e., recipient tracing) investigations, and potential TTB mitigation strategies.<sup>14-17</sup> However, published research studies have been relatively small and lack methods to differentiate true-positive from false-positive results. IND-related protocols were designed to evaluate the performance characteristics of nucleic acid testing (NAT) and serology-based assays, to assess their ability to prevent or reduce TTB, and to determine the prevalence and incidence of infection in selected *Babesia*-endemic areas. Results of these studies will enable the development of policy assessing the value of each test, the level of sensitivity required for each test, as well as if, when, and where to apply them. Additional considerations will include cost-effectiveness of testing algorithms and the willingness of hospitals to pay for testing.<sup>18</sup> Results of investigational testing have been published, summarized in the latest version of the *Babesia* EID Fact Sheet, and presented at the 2013 AABB Annual Meeting. Updated highlights are presented below.

*1. Results of repository testing using investigational tests*

- A recent American Red Cross study of 13,269 linked repository donation samples tested by investigational real-time PCR and arrayed fluorescence immunoassay (AFIA) using a cutoff of 1:64 found rates of seropositivity of 0.025% in low-risk areas (AZ, OK), 0.12% in moderate-risk areas (MN, WI) with 2/4,167 (0.05%) antibody and DNA positive, and 0.75% in high-risk areas (highly endemic counties in CT, MA) with 5/5,080 (0.1%) antibody and DNA positive. All positive samples were confirmed by PCR and/or Western blot in index or follow-up samples.<sup>19</sup>
- A peptide-based enzyme immunoassay (EIA) under IND protocol (Immunetics, Boston, MA) was assessed by New York Blood Center and Blood Systems, Inc., in an unlinked study of 15,000 donors from three geographic areas: Suffolk County (NY) representing a high *Babesia*-endemic area, New York City serving as a low *Babesia*-endemic area, and AZ designated as the nonendemic area.<sup>20</sup> Donor results and those from 72 clinical cases of laboratory-confirmed babesiosis were compared by the investigational EIA (see table below). Results of EIA testing were compared to the results of a research-based immunofluorescence assay (IFA) and a research-based, real-time PCR. Only one EIA repeat-reactive sample was PCR positive; this sample was from the high *Babesia*-endemic area. No additional results were provided to clarify the higher reactivity rates observed in EIA versus IFA testing.

<b>Study samples</b>	<b>EIA repeat reactive (%)</b>	<b>IFA+ at a 1:64 cutoff (%)</b>
High <i>Babesia</i> -endemic, Suffolk Co., NY	54 (1.08)	18 (0.36)
Low-endemic, NYC	37 (0.74)	3 (0.06)
Non-endemic, AZ	21 (0.42)	1 (0.02)
Clinical cases	71 (95.9)	71 95.9)

## 2. Prospective investigational screening

- Rhode Island Blood Center is using a selective testing algorithm under an IND protocol with AFIA (1:128 cutoff) and real-time PCR assays (IMUGEN, Norwood, MA) to maintain an inventory of test-negative blood components for transfusion to high-risk patients (i.e., neonates, pediatric patients with hemoglobinopathies). A preliminary published report from this IND study revealed 26/2113 (1.23%) antibody positive; one donor had an indeterminate PCR result (0.05%). No reported cases of transmission occurred with any *B. microti*-screened units transfused to the targeted patients (0/787 units).<sup>21</sup> Unpublished data from July 10 through February 2014 for 9,134 tested units show 67 (0.73%) were antibody positive, 1 (0.01%) was PCR and antibody positive, 2 (0.02%) were PCR indeterminate, and 2 (0.02%) showed nonspecific fluorescence. No reported cases of TTB occurred with any *B. microti*-screened units transfused to the targeted patients (0/4,347) or to any patient who received tested units (0/9,058). In contrast, during this same period there have been 11 cases of TTB associated with units not screened for *B. microti* from 351,796 allogeneic donations, yielding an observed TTB risk of 1:32,000.
- Another IND study from the American Red Cross reported testing 84,209 donations as part of product release during 2012-2014 (through April 30) in endemic counties of CT, MA, MN, and WI using the investigational AFIA (1:128 cutoff) and real-time PCR assays (IMUGEN).<sup>22</sup> All positives were confirmed by PCR and/or Western blot positivity in index or follow-up samples. Preliminary data include 331 (0.39%) reactive donations with 46 reactive by both assays (including one antibody positive/PCR-inconclusive donation that was PCR positive by a more sensitive confirmatory method), 277 with antibody reactivity only (including 10 antibody-positive/PCR-negative donations that were PCR positive by a more sensitive confirmatory method), and eight that were DNA-positive only (window-period rate of 1:10,500). PCR-positive donations had parasite loads of 9 to 3 million piroplasms/mL with 19/31 (61%) infectious in hamsters; an additional 2/33 antibody-only positive donations were infectious in hamsters. Since the initiation of screening in June-July 2012 through April 30, 2014, no cases of TTB from screened units have occurred whereas 18 cases of TTB associated with unscreened units have occurred throughout the American Red Cross system. Positive donors associated with TTB from unscreened units were identified in CA (donor exposure in ME), CT, MA, MN, NH, and NJ. When restricted to the 10 counties involved in the IND protocol, a comparison of TTB cases attributable to unscreened vs screened units during the IND period approached significance (10 TTB cases/200,488 unscreened units vs 0/59,848; odds ratio 6.3; p=0.07) and demonstrates an observed residual TTB risk of 1:20,000 transfused units in *Babesia*-endemic areas.

See **Appendix C** for additional data from other prevalence studies and from look-back investigations.

### *Other Considerations*

Several other approaches to mitigating risk have been investigated with varying degrees of success. Some approaches such as leukoreduction are largely ineffective, while newer technologies including pathogen inactivation/reduction have demonstrated feasibility, but remain unlicensed in the US.



1. *Donor screening risk-factor questions*

Several follow-up studies of positive donors to identify risk factors associated with infection have been performed.<sup>17</sup> Common risk factors include tick exposure, proximity to woods and deer, and outdoor activities (e.g., hiking, gardening). The potential use of a tick exposure question was previously explored in a research study using IFA and EIA. No differences in the seroprevalence of *B. microti* were observed for donors reporting tick bites compared to control donors.<sup>23</sup> On occasion, donors may provide postdonation information on tick exposure, recent diagnosis of Lyme disease, etc. In these instances, appropriate actions are left to local policies and/or the discretion of the medical director.

2. *Leukoreduction/gamma irradiation*

Given that babesial parasites are intraerythrocytic, leukoreduction will likely have minimal to no impact on transmission. This conclusion is supported by reports of TTB cases associated with leukoreduced blood products.<sup>6</sup> Similarly, the occurrence of TTB cases associated with gamma irradiated products suggests this approach is also ineffective.<sup>24</sup>

3. *Pathogen inactivation/reduction*

Pathogen inactivation (amotosalen + ultraviolet light) and pathogen reduction (riboflavin + ultraviolet light) have been shown to be effective at inactivating/reducing *B. microti* parasites in platelet and plasma products inoculated with infected hamster erythrocytes.<sup>25,26</sup> Recent studies have also demonstrated the efficacy of pathogen reduction in whole blood (including RBC units), but at lower levels compared to platelet and plasma products.<sup>27</sup> However, to date neither approach has been licensed by the FDA for use in the US.

### **Critical Issues That Require Further Consideration**

- If implemented, in what geographic regions will testing occur?
  - In July 2010, the FDA Blood Products Advisory Committee supported the concept of regional testing, but it is not clear if testing would be limited to the seven states where *Babesia* is endemic or if it will include other states judged to be at lower risk.
- What tests should be used and when?
  - Current options used under IND protocols include: antibody only, NAT only, or antibody and NAT.
  - If NAT is implemented, should it be used seasonally or year-round?
- Which patients should receive tested blood products: high-risk patients only (e.g., neonates, pediatric patients with hemoglobinopathies) or all patients?
- If the FDA licenses an assay, will it also mandate testing?
  - Given constrained reimbursement policies, will hospitals be able to pay for blood products that have been tested before such tests are mandated?
- During the IND phase, will testing capacity meet demands?

## Key Points

1. Transfusion-transmitted babesiosis (*B. microti*) poses a significant threat to patients. There is significant risk at varying levels in at least seven states (CT, MA, MN, NJ, NY, RI, and WI), but some risk is also present in other states.
2. Transfusion-transmission has been documented in nonendemic areas due to several factors including donor travel to high-risk areas and widespread distribution of blood collected in *Babesia*-endemic areas across the US.
3. Although seasonal variation in incidence and TTB occur, infectious donors and TTB have been documented year-round.
4. Babesiosis, including TTB, is now nationally notifiable, but is not reportable in every state.
5. TTB is responsible for 38% of transfusion-related infectious fatalities reported to the FDA in transfusion recipients; this is the highest percentage among all infectious agents.
6. Patient susceptibility varies and cannot be predicted accurately, but the young, old (>50 years old), asplenic, and those with attenuated immune responses are at greatest risk for severe complications from TTB.
7. *Babesia* has been prioritized as an agent with a critical need for the development and implementation of an intervention to reduce the incidence of TTB.
8. Intervention methods to reduce TTB incidence currently in place, including donor questioning or restricted collection areas, are ineffective.
9. No FDA-licensed testing method to reduce TTB incidence is available.
10. IND testing methods, including both antibody and nucleic acid tests, have demonstrated encouraging results.

## Recommendations

1. Hospitals and blood centers in *Babesia*-endemic areas should consider what interventions are available and may be appropriate to reduce the risk of TTB.
2. Hospitals and/or blood centers interested in testing should contact an IND sponsor.
3. Hospitals and blood centers should fully investigate and report all cases of TTB.

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## Appendix A

### Epidemiology of Human Babesiosis

Human babesiosis is a tick-borne zoonosis caused by intraerythrocytic protozoan parasites of the genus *Babesia*. *Babesia* parasites also can be transmitted by blood transfusion, which has been documented for *B. microti* and *B. duncani*.<sup>1,2</sup>

In the eastern US, *B. microti* is transmitted by *Ixodes scapularis*, commonly known as the black-legged or deer tick, which also is the vector for Lyme disease (*Borrelia burgdorferi*), human granulocytic anaplasmosis (*Anaplasma phagocytophilum*), and several other pathogens.<sup>3</sup> Tick-borne transmission of *B. microti* to humans is regional and seasonal. It is most common in the Northeast and is widespread in coastal areas of CT, MA, NJ, NY, and RI, and is expanding into coastal ME and NH. A region of significant but lower risk is in the upper Midwest (MN and WI). Although *I. scapularis* is common throughout the eastern US, there have been no documented *B. microti* cases south of MD.

The primary reservoir host for *B. microti* is the white-footed mouse (*Peromyscus leucopus*). Although white-tailed deer (*Odocoileus virginianus*) are not competent hosts for *B. microti*, they play important roles in amplifying tick populations by providing a blood meal and transporting ticks, thereby expanding their geographic distribution.

Tick-borne transmission occurs during late spring through summer (May through September) because of the coincidence of tick and human activity; the parasite typically is spread by the nymphal stage of *I. scapularis*, which is most active during warm months in brushy, wooded, or grassy habitats. Most *B. microti* patients seek treatment during late June, July, and early- to mid-August, but new cases have been identified even in winter months due to exacerbation of a subclinical infection that may have been acquired months earlier. The adult deer tick is a poor vector of *B. microti* and thus people are rarely exposed during the fall and winter months.<sup>4</sup>

In 2012, the Centers for Disease Control and Prevention (CDC) was notified of 937 confirmed and probable cases of babesiosis, with 96% (895) reported from the seven highest incidence states.<sup>5</sup> In 2011, among reported cases, 62% were males, median age was 62 years (range <1-98 years), and 82% had symptom onset in June through August.<sup>6</sup> Ten cases were reported to the CDC as transfusion-transmitted babesiosis (TTB), but implicated donors were identified only in two of these cases. As of January 10, 2014, 1,446 confirmed and probable cases were reported in 2013,<sup>7</sup> but there is substantial lag in CDC notification due to the time it takes for case investigation and classification.

Three cases of TTB due to *B. duncani* infection have been documented in CA and WA; however, this parasite's geographic range, tick vector, and reservoir host(s) are not known in the US.<sup>2</sup>

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## Appendix B

### Diagnostic Testing Options for Babesiosis

- Commercial options for diagnostic testing are limited. No Food and Drug Administration (FDA)-licensed blood donor screening assay or FDA-approved diagnostic test exists. Diagnosis is based on direct examination of Giemsa- or Wright-stained blood smears for intraerythrocytic parasites; IgM and IgG antibody detection by standard or automated IFA [arrayed fluorescence immunoassay (AFIA); IMUGEN, Inc., Norwood, MA], (cut-off titers vary with assay and lab), enzyme immunoassay (EIA) and Western blot; molecular methods [polymerase chain reaction (PCR) tests have been developed using primers from conserved regions of the *Babesia* 18S rRNA gene], and lastly animal inoculation. The sensitivity and specificity of these techniques are highly variable.
- Acute, symptomatic cases of babesiosis usually are diagnosed by detection of *Babesia* parasites during review of blood smears.
  - Differentiation of *Babesia* from *Plasmodium* (malarial) parasites requires an experienced microscopist. Digital images of blood films may be submitted to DPDx, a telediagnostic service provided by the Centers for Disease Control and Prevention (CDC) Division of Parasitic Diseases and Malaria (<http://www.dpd.cdc.gov/dpdx>).
  - Some *Babesia* species—including *B. microti* and *B. duncani*—cannot be distinguished from each other by microscopy. If *Babesia* parasites are evident on a blood smear, but *B. microti* PCR results are negative, additional molecular and/or serologic testing may be required to identify the infecting *Babesia*.
- During subsequent stages of infection, parasitemia levels may be below the threshold for visual detection, but might be detectable by PCR and/or animal inoculation.
  - Detection of DNA by PCR in the absence of antibody may be indicative of a window-period infection, but should be confirmed by donor follow-up and repeat serology.
  - Although PCR is more sensitive than blood-smear examination, negative results do not preclude infection or infectivity.
  - Molecular detection has been limited by low concentrations of circulating parasites in some cases coupled with the relatively low input volume of blood tested.
- Except for the early acute stage of infection (i.e., window period), serologic techniques provide evidence of current or past infection. Seropositivity can persist for many months, sometimes years, even after apparent resolution of infection.
  - The gold-standard methodology is the IFA antibody test, which generally uses *Babesia*-infected hamster erythrocytes as the antigen source.<sup>1</sup>
  - Because serologic testing is species specific, the pertinent antigen source must be used (e.g., *B. microti* or *B. duncani*).

### Appendix B Reference

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## Appendix C

### Donation Screening Under Research Protocols

#### Prevalence Studies Using Research Tests

- Seroprevalence by a research-based indirect immunofluorescence assay (IFA) in healthy blood donors was reported as high as 2% in the *Babesia*-endemic areas of the Northeast and Upper Midwest, areas that encompass approximately 16% of the US population. Areas of hyperendemicity (up to 10% infected) in the general population have been reported in portions of CT, MA, NY, and RI, particularly on offshore islands (e.g., Martha's Vineyard and Nantucket Island, MA; Shelter Island, NY).<sup>1</sup>
- One study of blood donors in MN reported 42 of 2150 (2%) positive for *B. microti* by research-based IFA; one sample was also positive by research-based real-time polymerase chain reaction (PCR). The study focused on donors residing in areas considered endemic for *B. microti*. All positive donors reported extensive outdoor activities, many with tick exposure.<sup>2</sup>
- A limited study in the highly endemic areas of CT tested 1002 donors during the tick season by research-based IFA and real-time PCR assays. Twenty-five (2.5%) donors were positive by IFA and 3 (0.3%) positive by PCR. Among the PCR-positive donors, two were IFA positive, while one was IFA negative and suggestive of a window-period infection.<sup>3</sup>
- The prevalence rates in the above studies are likely overestimates as false-positive results could not be excluded.

#### Results from Look-Back

The infectivity of blood products from regions of high *Babesia* endemicity in CT during 1999 to 2005 was assessed by research-based IFA and real-time PCR screening of donors (i.e., before investigational new drug studies).<sup>4</sup> Recipient tracing and testing demonstrated that 12.7% of recipients who received a seropositive index or prior donation from a seropositive donor were *Babesia* positive by IFA and/or PCR. Recipient positivity was primarily associated with transfusion of a positive index component (50%) vs a prior donation from an infected donor (7.3%), and donors who were PCR positive (33.3%) vs negative (2.9%). Seven of eight positive recipients received RBC units while one received whole-blood-derived platelets; RBC age at the time of transfusion ranged from 7 to 42 days, and the platelets at transfusion were 5 days old.

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