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Transfusion-Transmitted *Babesia* spp.: Bull's-Eye on *Babesia microti*

David A. Leiby*

Transmissible Diseases Department, American Red Cross Holland Laboratory, Rockville, Maryland 20855

INTRODUCTION

Babesia spp., the etiologic agents of babesiosis in animals and humans, are intraerythrocytic protozoan parasites transmitted to hosts primarily by tick vectors. While long known to cause disease in domestic animals (e.g., cattle tick fever and red water fever), *Babesia* spp. have recently emerged as a growing public health concern for humans, primarily in the United States. The initial U.S. case of human babesiosis was reported from California in 1966, although the species of *Babesia* implicated in this case was never definitively identified (108). Within 3 years (1969), the first documented human infection attributed to *Babesia microti* was reported on Nantucket Island, MA (124). From its initial description on an offshore island of New England, the geographic range where infections with *B. microti* have been reported has expanded to include coastal communities in Connecticut, Massachusetts, New York, and Rhode Island, eventually spreading to the interior of New England (3, 22, 67, 87, 105). Subsequent reports have described *B. microti* infections in patients from New Jersey and the Upper Midwest, specifically Minnesota and Wisconsin (26, 49, 59, 103, 114). Concomitant with an apparent expansion of this parasite's geographic range, cases of human babesiosis have become more frequent in the general U.S. population. Indeed, since the initial description of *B. microti* in 1969, hundreds of human babesiosis cases have been reported in the United States with an increasing frequency each year (76, 122).

With the emergence of human babesiosis as a public health issue in the United States, concern for blood transfusion safety has grown, given the intraerythrocytic location of the parasite (15). Beginning in the early 1980s, cases of transfusion-transmitted *B. microti* began to be reported sporadically, but cases have steadily increased in frequency over the ensuing 30 years. Recent reports documented a dramatic rise in numbers of reported cases, coupled with at least 12 fatalities in transfusion recipients diagnosed with babesiosis (4, 41, 118). Indeed, the U.S. Food and Drug Administration (FDA) noted that in the decade prior to 2005, deaths attributable to transfusion-transmitted *Babesia* (TTB) were virtually absent, but 8 deaths were reported from November 2005 onward. The observed increase

* Mailing address: Transmissible Diseases Department, American Red Cross Holland Laboratory, 15601 Crabbs Branch Way, Rockville, MD 20855. Phone: (301) 738-0608. Fax: (301) 738-0495. E-mail: leibyd@usa.redcross.org.

in the number of deaths was concomitant with an increasing incidence of TTB, as documented by *Babesia*-related biological product deviation reports received by the FDA, rising from 42 reports in the 8-year period from 1997 to 2004 to over 50 reports in the 3-year period from 2005 to 2007 (41). During that same time period, Rhode Island observed 9 cases of TTB from 1999 to 2004 but 12 cases from 2005 to 2007 (4). Similarly, the American Red Cross reported 18 cases of definite or probable TTB from 2005 to 2007 (118), while seven cases were identified in New York City in late 2008, a notable rise over the annual rate in New York City of just 1 to 2 cases per year (91). This well-documented increase in the number of transmission cases has elevated TTB to a key policy issue for blood collection organizations, test manufacturers, and the FDA. To this end, the FDA recently sponsored a workshop addressing TTB in the United States (42). Despite the current recognition that *B. microti* poses a growing blood safety risk, existing options for preventing the transmission of this parasite by blood transfusion are untenable.

Interventions designed to prevent the transmission of pathogenic agents (e.g., HIV, hepatitis B virus [HBV], and West Nile virus [WNV]) by blood transfusion are usually dependent upon risk factor questions in combination with testing for agent-specific antibodies and/or nucleic acids using assays licensed by the FDA for blood screening. For most agents, all blood donors are tested (i.e., universal screening) at every blood donation regardless of previous test results. This approach not only ensures the capture of virtually all infected donors but also is logistically straightforward to administer from an operational perspective. Addressing blood safety issues associated with *B. microti* poses unique challenges to transfusion medicine. As will be discussed below, risk factor questions lack sensitivity and specificity to identify at-risk donors who are almost always asymptomatic. Unlike other agents currently screened for in the U.S. blood supply, *B. microti* has a regional and highly focal distribution, which raises cost-benefit challenges to the universal screening paradigm. Last, there are no licensed blood screening tests available or under development at this time that could be employed to interdict infectious blood units. Taken together, *B. microti* poses new and unique challenges to transfusion medicine that will require creative approaches to reduce the transmission risk for this agent.

Thus, the objectives of this review are to highlight the current challenges that *Babesia* spp. pose for transfusion medicine. While *Babesia* infections occur in many parts of the world, this review will intentionally focus on *B. microti* and the United States, since worldwide this agent is responsible for most human cases of clinical babesiosis and virtually all cases of TTB, both of which predominate in the United States. Initially, this review will cover the specific agents of *Babesia* posing a transfusion risk and their epidemiology, geographic range, transmission routes, and clinical features. Moving to blood-bank-specific topics, we will examine TTB in detail, including case characteristics, types of blood products implicated, and parasite survival under normal storage conditions, followed by a discussion of current management practices for TTB cases. The review will close with an extended discussion of the current and perhaps future options for preventing TTB, highlighting those areas that require specific attention by the blood transfusion community. In the end, this review should lend further credence to the growing consensus viewpoint that *B. microti* poses a significant blood safety threat that warrants our attention and action without further delay.

TABLE 1. Etiologic agents of human babesiosis and associated cases of TTB

Etiologic agent	Geographic distribution (parasite type)	No. of TTB cases
Babesia microti	United States, Northeast and Upper Midwest	70–100
	Europe (not well defined)	1
	Japan	1
	Taiwan (TW-1)	0
Babesia divergens	Europe, primarily England and France	0
	United States, Kentucky and Washington	0
B. divergens-like	Austria, Italy, and Germany (EU-1)	0
	Missouri (MO-1)	0
Babesia duncani	United States, California and Washington	2
Other *Babesia* spp.	United States, California (CA-1–CA-4)	0
	South Korea (KO-1)	0

BIOLOGY AND EPIDEMIOLOGY

Etiologic Agents

Prevailing dogma, perpetuated for many years, has held that human infections with *Babesia* were attributable almost exclusively to *Babesia divergens* and *B. microti*, restricted geographically to focal areas within Europe and the United States, respectively (57). During the past 15 years, however, it has become clear that the phylogeny of *Babesia* is much more complex, with new species and/or types of *Babesia* being identified in the United States, Europe, Asia, and elsewhere, coupled with the recognition of expanded geographic ranges for both *B. divergens* and *B. microti* (Table 1). In 2006, *Babesia duncani* was proposed as a new species, replacing what had previously been designated the WA-1-type parasite, isolated from humans on the U.S. West Coast (21, 104). There has also been a proliferation of a veritable "alphabet soup" of other *Babesia* and *Babesia*-like agents from the United States, Europe, and Asia, including CA-1, MO-1, EU-1, KO-1, and TW-1 (47, 50, 64, 97, 98, 111). *Babesia* phylogeny can be expected to remain highly dynamic, with new agents and expanded geographic ranges being described for existing species due to the increased recognition of the parasite and babesiosis in general.

Geographic Distribution

The United States is an area where *B. microti* is regionally endemic, associated primarily with portions of the Northeast and Upper Midwest. As described above in this review's introduction, historical evidence suggests that *B. microti* initially became established on offshore islands of New England (22, 106, 124) and then moved to neighboring coastal communities of Connecticut, Massachusetts, New York, and Rhode Island

(35). Subsequently, the parasite became firmly established in these states, with widespread endemicity in Connecticut and Rhode Island (2, 3, 105) and focal distributions in Massachusetts (e.g., Nantucket and Cape Cod) and New York (e.g., Long Island and the Lower Hudson Valley) (67, 87). Given New Jersey's proximity to other areas of endemicity, it was perhaps not surprising that *B. microti* was described as endemic to the state in 1999 (26, 49). A second regionalized cluster of *B. microti* has been reported in the Upper Midwest, where the agent seems to be restricted to portions of Minnesota and Wisconsin 59, 103, 114). Autochthonous infections with *B. microti* or *B. microti*-like parasites have also been described in Europe, Japan, and Taiwan (33, 51, 86, 107, 111). The potential emergence of *B. microti* in Europe bears particular attention in the coming years.

B. divergens is limited primarily to Europe, but compared to *B. microti*, its geographic distribution is less well defined. In part, this reflects the fact that relatively few human cases (~30 cases) attributed to infection with *B. divergens* have been described. Virtually all cases reported to date have been identified in highly susceptible asplenic patients from France and England, but cases from Ireland, Spain, Sweden, Switzerland, and what was formerly designated Yugoslavia and the USSR have also been described (38, 56, 129). The EU-1 parasite, which was responsible for the first cases of human babesiosis in Italy and Austria and a subsequent case in Germany, has been described as a distinct agent that is closely related to *B. divergens* (44, 47). Similarly, the MO-1 parasite, isolated in Missouri, is characterized as being distinct but with morphological, antigenic, and molecular characteristics akin to those of *B. divergens* (50). In the neighboring state of Kentucky, an acute babesiosis case was ascribed to *B. divergens*, sharing 98.2 to 99.8% nucleotide homology with three European isolates of *B. divergens* (6), while an isolate from Washington State demonstrated 99.5% homology with *B. divergens* (46). Overall, these reports suggest that *B. divergens* or *B. divergens*-like parasites are present in the United States, but like *B. microti* in Europe, they are not routinely recognized as the cause of clinical disease, and their true geographic distribution continues to evolve.

B. duncani, formerly termed WA-1–WA-2 and CA-5–CA-6, is described as being endemic to the Pacific Northwest, with reports thus far limited to the states of Washington and California (34, 104). The endemicity of *B. duncani*, however, is based on a few human cases, since formal serosurveys for the agent, whether in humans, ticks, or reservoir hosts, are limited. Of note, three cases of human babesiosis were reported in California prior to the initial description of *B. duncani*, which may reflect earlier descriptions of human exposure to *B. duncani* (12, 61, 108). Several other isolates from the Pacific Northwest have also been identified (i.e., CA-1 to CA-4), but they do not align with known species of *Babesia* (97, 98).

A recent report from South Korea described the first case of human babesiosis in that country, which was attributed to a novel type of *Babesia* sp. (64). Sequence analysis of the 18S rRNA gene suggested that this parasite, designated KO-1, was most closely aligned with *Babesia* spp. isolated from sheep in China. Isolated human infections with *Babesia* spp. have also been reported for Egypt, Mexico, and Southern Africa, suggesting that the parasite likely has a broader distribution than currently understood (13, 88, 93). Thus, a better understanding of the geographic distribution of *Babesia* spp. will be forthcoming only through additional and cumulative epidemiological investigations.

Routes of Transmission

Babesia spp. are naturally transmitted to humans and other mammals through the bite of infected ixodid ticks. The primary U.S. vector for *B. microti* is *Ixodes scapularis*, commonly known as the deer or black-legged tick, which also transmits the etiologic agents of Lyme disease (*Borrelia burgdorferi*) and human granulocytic anaplasmosis (*Anaplasma phagocytophilum*). Transmission occurs primarily via questing nymphal ticks, but adult *I. scapularis* ticks can also transmit the parasite. Also critical to the zoonotic life cycle of *B. microti* is the white-footed mouse (*Peromyscus leucopus*), which serves as the reservoir host for the parasite. The white-tailed deer (*Odocoileus virginianus*), while not a competent host for *B. microti*, serves as a maintenance host for adult ticks and thereby transports the infected ticks to previously uninfected areas (53).

In Europe, the primary enzootic vector for *B. microti* is *Ixodes trianguliceps*, a species that does not feed on humans, perhaps explaining why relatively few infections attributable to *B. microti* have been reported from Europe (33). However, the European sheep tick, *Ixodes ricinus*, has been shown to be a competent vector for *B. burgdorferi* as well as *B. microti*; thus, this tick may be responsible for transmission to humans. Indeed, in many geographic locations worldwide, the tick vector implicated in the transmission of *Babesia* is often the ixodid species locally responsible for the transmission of *B. burgdorferi*. Among the so-called *B. microti*-like parasites, transmission details are available only for the parasite previously identified in Japan. In this case, the definitive host has been shown to be a field mouse (*Apodemus speciosus*), which surprisingly had been identified as being infected with a *B. microti*-like parasite more than 25 years ago (112). While the Japanese vector has not been definitively identified, by analogy it is thought to be *Ixodes persulcatus*, the vector for Lyme borreliosis in Japan (107).

In contrast to *B. microti*, the definitive hosts for *B. divergens* are cattle (129). The tick vector for *B. divergens* in Europe has been identified as being *I. ricinus*. This tick has also been shown to be a competent vector for *Babesia* sp. strain EU-1 (7, 9). Roe deer appear to be the wild reservoir host for EU-1, but other reservoir hosts may exist that have not been identified (10).

Considerably less is known about the transmission and life cycle of *B. duncani*. The tick vector has not been definitively verified, but *Ixodes pacificus* has been identified as a potential candidate, since it serves as the principal vector for Lyme borreliosis on the West Coast (34). Similarly, the reservoir host for *B. duncani* is also unknown. For the other remaining *Babesia* species and isolates mentioned in this review, the definitive host and tick vectors have not been identified, clearly suggesting a need for field-based epidemiological studies to delineate the life cycles of these emerging babesial agents.

In addition to vector-borne transmission, other transmission routes include congenital and blood transfusion. While rare, *B. microti* has been shown to be transmitted transplacentally or

perinatally on at least three occasions (25, 90, 109). In all three cases, the pregnant mother was bitten by a tick during her third trimester and developed serological evidence of infection. The infected infants developed babesiosis at 26 days to 5 weeks of age and were successfully treated with clindamycin-quinine and/or atovaquone-azithromycin. As will be described in more detail below, only *B. microti* and *B. duncani* have been implicated in cases of TTB to date.

While transmission via donated organs is biologically feasible, to date no documented cases attributed to organ transplantation have been reported. However, there have been at least three cases of babesiosis in recipients of solid-organ transplants (two renal and one cardiac) that were not directly attributable to the transplanted organ (43, 82, 96, 113). In two of these cases, blood transfusions at the time of transplantation or shortly thereafter were implicated as the source of the *Babesia* infection, while the other organ recipient, who was asplenic, was likely infected naturally after experiencing multiple tick bites during a camping trip in Wisconsin 18 years after receiving a renal transplant (113). These cases highlight the underlying susceptibility of transplant recipients to *Babesia* infection while undergoing long-term immunosuppressive therapy regardless of the transmission route.

CLINICAL FEATURES

Symptoms

Infection with *Babesia* produces a spectrum of disease that can range from asymptomatic to severe, life-threatening illness regardless of the initial mode of transmission. Immunocompetent persons infected with *Babesia* are often asymptomatic or experience only mild symptoms that self-resolve. *B. duncani* has been described as being more virulent than *B. microti*, but this observation is based on studies in hamsters, which may not translate to human infections (127). To date, there have been relatively few documented infections with *B. duncani*, so its virulence for humans in comparison to *B. microti* remains equivocal. Similarly, *B. divergens* is often reported to cause more-severe cases of babesiosis, but this is likely attributable to virtually all known human cases (~30) having occurred in highly susceptible, asplenic patients. It was suggested that in immunocompetent patients with their spleens intact, infection with *B. divergens* may be asymptomatic (38). Thus, the severity of babesiosis symptoms may be more heavily influenced by the immune status of the host than by the infecting species of the parasite.

Symptoms generally appear 1 to 9 weeks postinfection and can include fever, headache, chills, drenching sweats, myalgia, malaise, and hemolytic anemia (57). More complicated cases of babesiosis tend to occur among select patient populations, especially neonates/infants, the elderly, the asplenic, and those who are otherwise immunocompromised (121). In these populations, babesiosis can be life-threatening and is characterized by hemodynamic instability, acute respiratory distress, severe hemolysis, disseminated intravascular coagulation, renal dysfunction, hepatic compromise, myocardial infarction, and death. Parasitemia levels, particularly among asplenic patients, can approach 85%, necessitating immediate treatment (53). It was also suggested that concurrent infections with other tick-borne agents (e.g., *B. burgdorferi*) can result in more severe infections (75). Since *I. scapularis* is known to transmit several tick-borne agents, exposure to more than one agent should be considered whenever an infection with a tick-borne agent is diagnosed. In the United States, case fatality rates associated with clinically apparent *B. microti* infections have been estimated to be approximately 5% (87).

Some patients who apparently resolve infections based on symptoms, via self-cure or chemotherapy, can maintain low-level parasitemia for months to years (71). These chronically infected patients may demonstrate elevated IgG antibody titers, but measurable parasitemia is rarely detected, even by sensitive real-time PCR assays (78, 119). For these chronically infected patients, episodes of immunosuppression can lead to a recrudescence of infection with severe complications (67). Perhaps more importantly for transfusion medicine, these asymptomatic, chronically infected persons likely play primary roles in the transmission of *B. microti* by blood transfusion.

Diagnostic Testing

Symptoms associated with *Babesia* infection are routinely generic in nature, mimicking a number of other infections and clinical conditions, especially influenza. Therefore, the accurate diagnosis of clinical babesiosis is dependent upon the outcome of diagnostic testing in conjunction with observed symptoms, particularly hemolytic anemia. Approaches to diagnostic testing for *Babesia* infections take three primary forms: direct identification of *Babesia*-infected erythrocytes on a peripheral blood smear (45), serological demonstration of antibodies to *Babesia* spp. in patient serum or plasma (18, 72), and/or demonstration of active parasitemia by PCR or animal inoculation (11, 37, 99).

Many acute cases of babesiosis are diagnosed by the direct detection of intraerythrocytic parasites, usually ring-shaped trophozoite forms, in Wright- or Giemsa-stained peripheral blood smears. On rare occasions, extracellular *Babesia* parasites are observed (94, 110). Due to morphological similarities with *Plasmodium* spp. (i.e., malaria parasites), it is critical to distinguish these two parasitic infections; however, only highly trained and experienced microscopists will likely discern a visual difference. The presence of tetrads or "Maltese cross" forms within erythrocytes is diagnostic for *Babesia* infection, although they have been reported to be an uncommon feature of infections with *B. microti* (95). Thus, serological or molecular testing may be needed to differentiate the infection from malaria and to identify the infecting species of *Babesia*. Additionally, a thorough medical history can be helpful in identifying potential exposure risks for *Babesia* spp. and *Plasmodium* spp., including travel, recent blood transfusion, splenectomy, or vector exposure. Unfortunately, most people infected with *Babesia* spp. (or other tick-borne agents) rarely recall an associated tick bite (125). If patients transition from the acute phase to the chronic phase of infection, babesial parasites are rarely observed on blood smears due to low levels of circulating parasites. In these cases, additional testing approaches are required to accurately diagnose babesial infection.

The current serological "gold standard" for detecting *B. microti* antibody is the indirect immunofluorescent antibody (IFA) test, in use since first being described in 1978 (18). While

the IFA test is capable of detecting both IgM and IgG antibodies to *B. microti* (70, 72), it is chiefly used to detect the latter, which may persist for months to years (119). Detection of IgG antibodies is indicative of present or past infections, including those in which parasitemia may have cleared. IFA is generally species specific and non-cross-reactive; thus, the appropriate antigens must be used to detect and distinguish specific *Babesia* species (e.g., *B. microti* antibodies and associated infections can be detected only by using *B. microti* antigens and not those associated with other *Babesia* species [e.g., *B. divergens* or *B. duncani*]). Although the IFA test routinely displays high sensitivity, specificity, and reproducibility (72), it does suffer from subjectivity and resistance to automation. An enzyme immunoassay (EIA) based on recombinant antigens has been described in the literature, but to date, this assay and other high-throughput alternatives have been used largely for research and are not readily available (54).

In contrast to peripheral blood smears, PCR is considered more sensitive for detecting the presence of circulating parasites in both acute infections and, to a lesser extent, chronic *Babesia* infections. Indeed, since parasite levels are extremely low or even intermittent during chronic infections, a negative PCR, particularly in tandem with measurable antibody titers, does not eliminate the potential for an ongoing infection. In addition to low levels of parasitemia in patients, PCR is also limited by the volume of blood that can be reasonably tested and its inability to distinguish between the DNAs of viable and nonviable organisms (57). For many years, *Babesia* PCR was performed primarily by using the nested primer set Bab1 to Bab4 (99), but the development of a more sensitive real-time PCR provides an alternative approach (27).

Although not practical for rapid diagnosis, inoculation of susceptible laboratory animals (e.g., hamsters and gerbils) has proven diagnostically beneficial as an adjunct test for detecting low-level parasitemia and when expanding isolates for identification to the species level and/or further characterization (11, 46, 104, 127). Animals are injected intraperitoneally with 1 to 2 ml of the patient's blood and then monitored at weekly intervals by blood smear for the presence of infected erythrocytes. Since hamsters and/or gerbils are extremely susceptible to *Babesia* infection, they rapidly amplify low levels of infection.

Treatment

Most persons infected with *Babesia* spp. resolve infections spontaneously without the need for antimicrobial therapy. For symptomatic patients with demonstrable parasitemia, treatment may be required to eliminate the parasite, particularly in cases of severe or persistent infection. Historically, the regimen of choice has been a 7- to 10-day course of clindamycin and quinine (126). While highly effective, the tandem use of clindamycin and quinine can produce debilitating side effects, particularly tinnitus, vertigo, and gastroenteritis, that often interfere with the successful completion of drug therapy. An alternative therapeutic approach using atovaquone and azithromycin has been reported to be better tolerated while still offering equal treatment effectiveness (69). For those patients for whom babesial infection persists after treatment (68, 69, 71), additional courses of drug therapy may be required.

In cases of severe babesiosis characterized by high levels of parasitemia (≥10%) with concomitant anemia, exchange transfusion should be considered a treatment option. Similar to procedures for treating malaria, exchange transfusion is designed to rapidly reduce patient parasitemia and related anemia (17, 23, 46, 60, 101). To date, exchange transfusion has been used to treat infections with *B. microti* and *B. divergens*; however, this approach should be considered for other species of *Babesia* as conditions warrant.

SEROPREVALENCE

Defining the incidence, distribution, and prevalence of *Babesia* sp. infections in the U.S. population is critical for formulating appropriate public health policies; however, obtaining the relevant data remains problematic. At this time, babesiosis is not a nationally notifiable disease (41), although public health officials have begun to entertain a change in this policy. Until a change has been implemented, options are limited for developing a clear national picture regarding the impact and distribution of human babesiosis in the United States. Many states do monitor cases of babesiosis, and approximately one-third have explicit reporting requirements based on information available in the Council of State and Territorial Epidemiologists databases (http://www.cste.org/dnn/ProgramsandActivities/PublicHealthInformatics/StateReportableConditionsQueryResults/tabid/261/Default.aspx), but these activities are limited to those states located in regions of the country that have historically been considered areas where *Babesia* spp. are endemic.

There have been few systematic seroprevalence studies reported for *Babesia* spp. in the general population, and determinations of prevalence in blood donors are rare, except for a few studies from the United States and Europe focused mostly on *B. microti*. In the Northeast United States, the seroprevalence of *B. microti* has been reported to range from 0.3% in Connecticut to 9.5% in Lyme disease patients ($n = 735$) at the University of Connecticut (29, 52, 74, 77, 85). Several reports of *B. microti* in blood donors have reported rates as high as 4.3% (5 of 115 positive by IFA test) on Shelter Island, NY, an area where the parasite is highly endemic (77, 80, 100). A recent, ongoing Connecticut study reported that 1.1% of 21,523 donors tested from 2000 to 2007 were positive for *B. microti* antibodies by IFA testing (62). This study reported the highest rates for New London (1.8%) and Middlesex (1.2%) Counties, but seropositive donors were reported from all counties of Connecticut, suggesting a more widespread distribution.

More than a decade ago, studies of the seroprevalence of *B. duncani*, then identified as WA-1, in residents of Northern California and Washington were reported (98, 104). The observed rates varied widely, from 0.9% (1 of 115) to 17.8% (39 of 219). The only study of blood donors was relatively small ($n = 124$) and identified 20.8% of Sacramento, CA, blood donors with evidence of antibodies to *B. duncani* by IFA testing (34). These relatively high rates have led some to suggest that the serological tests employed in those studies may have lacked specificity (53). Indeed, reliable assays with high sensitivity and specificity for emerging agents such as *B. duncani* are not readily available and, thus, hinder the accurate measurement of parasite distribution and frequency of infection.

Seroprevalence studies in Europe have historically focused

on *B. divergens*, but with a growing interest in the potential emergence of *B. microti* in Europe, several studies have included or focused on this agent. A relatively small study investigating healthy German blood donors identified 8 of 100 (8%) donors with antibodies to *B. microti* (55). A more recent German study tested samples from individuals located in the Rhein-Mein area of Midwestern Germany for antibodies to *B. microti* and *B. divergens* and reported seroprevalence rates of 5.4% (25 of 467) and 3.6% (17 of 467), respectively (58). Within this population of 467 persons, rates for *B. microti* IgG antibody-positive samples were significantly higher ($P < 0.05$) among a group of patients exposed to ticks (21 of 225 [9.3%]) than among a population of healthy blood donors (2 of 120 [1.7%]). A 2002 study of 396 blood donors from Eastern Switzerland identified 5 (1.5%) donors with *B. microti* antibodies (33). Since these samples were collected at blood drives from December through May, outside the normal tick season, these data may represent conservative seroprevalence estimates. Thus, while relatively few studies have been conducted in Europe, there is growing evidence of locally acquired infections with *B. microti* that may pose public health issues in the future (56).

Clearly, the geographic distribution, incidence of new cases, and overall seroprevalence of *Babesia* spp. are not well understood, particularly for emerging subtypes or species (e.g., EU-1 and *B. duncani*). This void of epidemiological data also applies to *B. microti* in the United States, where the true geographic distribution of the parasite has only partially been uncovered. Later in this review, interventions to prevent TTB will be discussed, but in the absence of a clear understanding of the parasite's distribution and prevalence in the United States, any proposed solution will only partially address the problem. Going forward, national surveys of known human babesial parasites in the United States are needed to close the current knowledge gap.

TRANSFUSION TRANSMISSION

Any discussion of transfusion-transmitted pathogenic agents must first consider the parameters used to define cases of transmission. A useful starting point is to consider fundamental requirements for the transmission of a pathogenic agent from a blood donor to a blood recipient that are analogous to Koch's postulates. First, the agent must establish a viable infection in the blood donor. Second, and critical to transmission, the agent must be present and/or circulate in the peripheral blood of the donor. Third, the agent must survive the blood collection process and remain viable under normal blood storage conditions. Last, the agent must be able to infect the blood recipient following blood transfusion. When all four of these requirements are met, as they are for *Babesia* spp., transfusion transmission can and does occur. As discussed above, *Babesia* spp. can infect blood donors and is found in the peripheral blood (i.e., intraerythrocytic). The following discussion serves to address the remaining two requirements needed to establish transfusion transmission.

Survival in Blood Components

As mentioned above, the intraerythrocytic location of *B. microti* provides a suitable niche to facilitate the transmission of this parasite by blood transfusion. The transmissibility of *B. microti* by transfusion is further enhanced by the survival of the parasite in stored blood products. Experimental studies have shown that *B. microti* survives in red cells maintained at 4°C for at least 21 days; however, these study conditions were less than ideal, as the blood was maintained in EDTA tubes (24) and not blood bags designed to enhance cell survival via optimal gas exchange. In contrast, under normal blood bank conditions, a 35-day-old red cell unit was implicated in a TTB case (89). Despite rare reports of extracellular parasites (94, 110) there are no published reports to suggest continued *Babesia* growth and/or replication during red cell storage at 4°C, and anecdotal data suggest that parasite viability gradually declines with time of storage. Similarly, TTB case reports implicating cryopreserved red cell units indicate that *B. microti* can survive indefinitely in the presence of glycerol cryopreservation (39, 128), but in the absence of cryopreservation, the parasite is rapidly killed by freezing (120). Theoretically a single parasite is capable of transmitting infection. Experimental studies, however, have shown that 30 organisms infected about 2/5 inoculated hamsters, and 300 organisms infected all animals (5/5) (28).

Case Characteristics

The first case of TTB is often ascribed to a 1968 report from Ireland that purportedly identified the transmission of *B. divergens* to a 48-year-old asplenic male approximately 4 months after receiving a blood transfusion (31). However, a follow-up report by those same authors attributed this patient's infection with *B. divergens* to a caravan holiday in County Galloway, Ireland, during mid-August (32). Lending further credence to the natural acquisition of infection, three cases of "red water" (babesiosis) were reported in cattle from the same area of County Galloway that August.

Therefore, it appears that the first case of TTB was reported in 1980 from Boston, MA, and involved the transmission of *B. microti* to a 70-year-old patient after the transfusion of 20 platelet units (60). The implicated donor was a summer resident of Nantucket Island who was asymptomatic at the time of blood transfusion. Since that initial report, TTB has been reported with increasing frequency, and current estimates suggest that between 70 and 100 cases have occurred (41, 76, 95). Determining an accurate estimate is difficult, since many cases of TTB have not been reported in an organized way or were not novel enough to warrant consideration for publication (76). More recently, the advent of hemovigilance and governmental reporting systems has allowed more-accurate estimates of current TTB cases. Based on recent data collected over 3 years, the frequency of cases has increased dramatically, with 18 cases reported by the American Red Cross during 2005 to 2007, six cases from Rhode Island in 2007, seven cases in New York City during late 2008, and an extensive review of cases by the FDA from 1997 to 2007 (41, 91, 118). Of particular note and concern, at least 12 fatalities associated with TTB have been reported, 8 of which occurred in the last 4 years.

All reports of TTB cases in the United States have impli-

cated *B. microti*, with the exception of two transmission cases linked to *B. duncani* (formerly known as WA-1) that occurred on the West Coast (Table 1) (48, 65). The index *B. duncani* case occurred in a 76-year-old patient, while the implicated donor resided in Washington State and was generally healthy, although he reported intermittent fatigue. Testing of residual samples from the implicated donation revealed a *B. duncani* titer of 1:65,536, and parasitemia was subsequently confirmed by hamster inoculation. The second *B. duncani* transfusion case was reported from California in a premature infant. The implicated donor was a resident of the San Francisco Bay area, who may have become infected on an outdoor recreational trip. As in the previous report, the implicated donor demonstrated an extremely high *B. duncani* titer, 1:40,960, 2 months after the implicated donation and was demonstrably parasitemic based on xenodiagnosis by hamster inoculation. It is unclear if high IFA titers are common for *B. duncani* infections or if this reflects a peculiarity of this IFA assay, which does not cross-react with *B. microti*. A third, as-yet-unpublished *B. duncani* transmission case reportedly occurred during 2009 in California, but specifics of this case are not yet available. With our current limited understanding of *B. duncani*'s distribution and frequency in blood donors, hospitals and blood transfusion centers on the West Coast need to remain alert to the potential transfusion transmission of this agent.

Outside North America, an autochthonous case of TTB from Japan that involved a *B. microti*-like species of the parasite was reported (84). The only other reported transfusion case outside North America was a 2007 autochthonous case from Germany that involved *B. microti* (Table 1) (51). Although only a single donor demonstrating borderline (IgG titer, 1:32) reactivity to *B. microti* was identifiable, alternative routes of infection in the recipient proved unlikely. In all likelihood, other cases of TTB have occurred outside the United States, but given the general lack of parasite and disease recognition in many areas of the world, one can reasonably infer that transfusion cases outside the United States routinely go undetected.

Clinical features of TTB cases are generally thought to parallel those observed for naturally acquired infections. Incubation times for TTB cases generally mimic what is seen for natural infections; infections usually take 1 to 9 weeks to become apparent. Factors influencing the incubation period are the immune status of the patient, the parasite species and/or strain implicated in transmission, and, although not well defined, infectious dose. Occasionally longer incubation periods have been reported, particularly among patients with sickle cell anemia (4, 19, 118).

For those TTB cases where a specific blood component has been implicated, the vast majority of cases have identified a unit of packed red blood cells (RBCs) as the source of *Babesia* infection (4, 41, 95, 118). Several transmission cases, however, have also implicated platelet products derived from whole blood, which presumably contains either contaminating red cells infected with the parasite or extracellular *B. microti*. In contrast, apheresis platelets have not been implicated in a transfusion case, perhaps since they contain few, if any, contaminating red blood cells. However, the potential for extracellular parasites of *Babesia* suggests that any blood product not frozen may pose a risk of transmitting infection (94, 110).

As observed for the general population, blood recipients at greatest risk for becoming infected with *B. microti* are infants, the elderly, patients without spleens, and those who are immunocompromised. The fact that most blood recipients have underlying health-related issues and tend to be older (i.e., >50 years of age) increases their risk for developing babesiosis following transfusion with a *Babesia*-infected unit. It has also become apparent that sickle cell patients, who are functionally asplenic, are also at an increased risk for infection with *Babesia* (19, 118). However, the primary patient group at risk is the elderly. Previous reports suggested that severe clinical disease and chronic infections are most often observed for healthy patients ≥50 years old (71, 125), a phenomenon reported to be unrelated to an increased risk for the acquisition of natural infections (73). Among blood recipients, a recent report of TTB by the American Red Cross revealed that 13 of 18 (68%) reported cases between 2005 and 2007 involved recipients between the ages of 61 and 84 years old. One explanation for the greater number of cases in elderly patients may be an age-associated decline in resistance to *B. microti* (121). Studies in mice have revealed that resistance to infection with *B. microti* is conferred by the adaptive immune system, which is genetically determined and associated with age.

Cases of TTB are increasingly being reported outside of areas where *Babesia* is normally thought to be endemic. In areas where the disease is not endemic, transmission occurs primarily via two mechanisms. In the first scenario, a blood donor from an area where the parasite is not endemic travels to and becomes infected in an area where *Babesia* is endemic. For example, a transfusion-transmitted case was reported in Canada (where *Babesia* spp. are not endemic), but the implicated donor likely acquired *B. microti* infection during travel to Cape Cod, MA (63). This case also provides instructive lessons on follow-up investigations, as the implicated unit of red cells was collected in February, well outside the known tick season and 6 months after the presumed naturally acquired infection. The donor was asymptomatic but upon follow-up was demonstrably parasitemic by PCR and serologically positive, with a titer of 1:1,024. A similar case occurred in Texas, where a 57-year-old male was identified as having babesial infection 7 weeks posttransfusion (20). The recipient subsequently died due to gastrointestinal hemorrhage with *Babesia*-induced hemolysis identified as a probable complication factor. The implicated donor, identified as being positive by IFA testing and PCR, likely became infected 3 to 5 months earlier while summering in Cape Cod, MA.

Alternatively, recipients in areas where the parasite is not endemic may become infected following the transfusion of blood products imported from an area where *Babesia* is endemic. A recently reported case highlighted the infection of a California resident who received a transfusion of blood products (January 2007) from a donor residing in Maine (92). Reports of clinical babesiosis in Maine are rare (83), but the donor resided in coastal Southern Maine, from which most cases are reported, and the donor's titer was 1:256 approximately 2 months after the implicated donation. The donor acknowledged that he frequented tick-infested areas and may have become infected in late August 2006, when he sought treatment for fever, chills, weight loss, and fatigue—classical symptoms of babesiosis.

With multiple reports of TTB in the last 5 years, it is clear that the number of cases is on the rise, particularly for U.S. cases associated with *B. microti*. The reason for this rise is not readily apparent, but several factors likely contribute to the apparent increase in TTB case frequency. First, recipients of blood transfusions increasingly represent an aged and immunocompromised population, since increasing marrow and solid-organ transplants are performed each year (128). Second, education efforts and published case reports have raised the awareness of TTB, leading to an increased recognition of cases by physicians and hospital transfusion services. Last, and perhaps more importantly, the geographic range of the parasite is expanding, albeit slowly, beyond its historical foci in areas of Southeastern New England that encompass portions of Connecticut, Massachusetts, Rhode Island, and nearby offshore islands where the parasite is highly endemic. Recent serosurveys and clinical case reports suggest a wider distribution, including large portions of Connecticut and New Jersey, the Hudson River Valley, and coastal Maine (26, 49, 62, 83, 87, 92).

Estimates of transfusion risk associated with *B. microti* are limited and vary considerably. A recent report from Rhode Island suggested the mean rate of TTB to be approximately 1 case per 15,000 units of RBCs transfused (4). Estimates from Connecticut ranged from earlier estimates of 1 case per 601 units of transfused RBCs (36) to later estimates of 1 case per 1,800 to 1 case per 100,000 red cell units transfused (5, 14). In most instances, the rate of transmission is likely underestimated due to an ongoing failure to recognize true cases of transmission. With the implementation of a national hemovigilance program in the United States, under the auspices of the National Healthcare Safety Network administered by the CDC, more-accurate estimates of adverse transfusion events and transfusion risk associated with *B. microti* should be forthcoming.

DONOR AND TTB CASE MANAGEMENT

Deferral Criteria

Current standards issued by the American Association of Blood Banks (AABB) require the indefinite deferral of a blood donor with a history of babesiosis (102). At the blood collection site, donors are queried regarding a history of babesiosis, and if they report one, the donor is deferred prior to the blood donation process. Unfortunately, querying donors about a history of babesiosis has been shown to be largely ineffective at reducing transmission risk, as will be discussed below (116). Indeed, most donors who transmit infection are asymptomatic and unaware of an underlying *Babesia* infection, which places them at risk for transmitting the agent. At present, processes are not in place to reenter deferred donors, even if they have cleared the infection based on negative nucleic acid testing (NAT) and serological tests.

TTB Case Management

When babesiosis is confirmed for a patient with a history of blood transfusion, the possibility that the patient acquired the infection from a blood transfusion must be considered, and a case investigation must be initiated. Initially, the investigation must determine if the infection was naturally acquired and preceded recent blood transfusion. Pretransfusion samples, if available, should be evaluated to exclude an underlying infection prior to transfusion. As described above for clinical cases, it is important to differentiate it from malaria because erythrocytes demonstrating ring-form trophozoites of these two agents appear similar on peripheral blood smears. In the absence of evidence or risk factors suggesting a natural infection, the likelihood of transmission by blood transfusion should be considered.

Similar to naturally acquired tick-borne infection, the incubation period in transfusion patients generally ranges from 1 to 9 weeks but can vary considerably in immunocompromised patients (e.g., sickle cell patients) (4, 19, 118). Based on the timing of blood transfusions and observed clinical symptoms, the timing of a potential transmission event can be estimated, and blood donors can be investigated retrospectively. Ideally, segments or retention tubes associated with donations of interest should be tested by serology and PCR to identify the implicated blood product and associated donor. In the absence of materials associated with the donation of interest, all blood donors will need to be contacted to obtain a follow-up sample for testing. Past experience suggests that due to the time elapsed since donation, implicated donors often demonstrate only antibodies to *Babesia*, with PCR assays commonly being negative. This may reflect the clearance of the parasite or parasitemia below PCR detection levels. Thus, a negative PCR result alone does not exonerate a donor. Often, in follow-up investigations, no implicated donor is identified, which in many cases may be due to the resolution of infection, with the concomitant clearance of measurable antibody titers, by an infected donor over 6 to 12 months. If an infected donor is identified, they should be interviewed to determine likely epidemiological risk factors, including travel history, exposure to ticks, and recent symptomatic infections. Implicated donors are deferred indefinitely.

Product Management

Upon the identification of a possible TTB case, all in-date cellular components associated with potentially involved donors should be retrieved and quarantined. If a donor is found to be positive and implicated in a transmission case, the associated blood products are destroyed and the donor is indefinitely deferred. If there are other donors in the investigation, upon the implication of a positive donor, those donors that tested negative should be reinstated, and their associated cellular products should be released.

Look-Back

Research studies using IFA and PCR have demonstrated that "look-back," or recipient tracing, can effectively identify previous transmission events associated with a blood donor identified as being positive for *Babesia* (14, 16). However, presently, neither the AABB nor the FDA provides guidance or requires a follow-up of TTB cases, including specific recommendations for component retrieval or recipient testing. The licensure of a blood screening test in the future may lead

TABLE 2. Strategies for mitigating risk of TTB

Intervention	Current status	Perceived effectiveness
"History of babesiosis"	Included in UDHQ[a]	Largely ineffective; most donors unaware of infection
Risk factor questions	Not currently used	Lack specificity and sensitivity
Blood screening		
Serological testing	Research IFA testing only	Licensed, automated, high-throughput assay unavailable
Nucleic acid testing	Research PCR only	Targets window period infections, but negative result does not preclude infection
Pathogen reduction	Feasibility demonstrated	Technology not licensed in the United States Technology not currently applicable to whole blood or red cells
Leukoreduction	Most blood leukoreduced	Ineffective for intraerythrocytic agents; TTB cases reported
Irradiation	Intermittent use	Parasite survives; TTB cases reported

[a] UDHQ, uniform donor health history questionnaire.

to changes in this policy. The current consensus management approach used by most blood collection agencies is to issue a market withdrawal of the implicated donor's previous and subsequent components. The time frame varies among organizations, from the prior 3 to 12 months to only donations made during the active tick season. Efforts focused on donations during the tick season and the prior 3 months target primarily acute infections but may miss infectious donations made by chronic carriers during the previous 12 months. Any products associated with a subsequent donation should also be withdrawn.

STRATEGIES FOR MITIGATING RISK

As outlined above, TTB has become a critical blood safety issue in the United States, but to date, effective measures for mitigating the risk of transmission have not been implemented. A variety of interventions are available and routinely used by blood collection organizations to prevent the transmission of pathogenic agents by blood transfusion. However, each agent must be evaluated individually, as models designed to prevent the transmission of viral agents do not necessarily translate to parasites, in particular *B. microti*. The following discussion provides an analysis of commonly used interventions and their perceived effectiveness for mitigating the risk associated with TTB (Table 2).

Exclusion Factors

Current approach. At present, the only intervention in place to mitigate the risk of TTB is a question on the uniform donor health history questionnaire asking blood donors if they have had a history of babesiosis. Donors who respond affirmatively are indefinitely deferred from future blood donations based on AABB standards, first published in 1991 (102). Unfortunately, this approach has been proven largely ineffective. A recent report by the American Red Cross indicates that between 2005 and 2007, only 123 out of ~23.5 million presenting Red Cross donors reported a history of babesiosis (118). However, since the same deferral insertion code was used for Chagas' disease, it could not be determined accurately how many deferrals were attributed to babesiosis. Published reports confirmed that most donors implicated in cases of TTB are asymptomatic at the time of donation and are unaware that they are infected. Therefore, the effectiveness of mitigating the risk of TTB based on a history of babesiosis is minimal.

Risk, geographic exposure, and seasonal exposure. Often, the first line of defense in preventing the transmission of agents by blood transfusion is to identify at-risk donors by using screening questions. For example, prospective blood donors are queried regarding recent tattoos or piercings to identify donors potentially exposed to hepatitis B virus. It has been suggested that blood donors at risk for *B. microti* infection may have been exposed to ticks, particularly the deer tick vector. A previous study indicated that blood donors were indeed capable of recalling tick bites in the previous 6 months and were also able to identify the size of the tick (small versus large) (77). This suggested an ability to distinguish deer ticks from larger, more common ticks (e.g., dog ticks). However, that same study compared the seroprevalence rate of *B. microti* in donors who reported tick bites with that in controls and observed no difference in the rates, 0.4% and 0.3%, respectively. It was surmised that donors reporting tick bites may have been more vigilant in looking for ticks and presumably removing them before ticks had a chance to feed and transmit infection. Previous reports of Lyme disease and other tick-borne diseases demonstrated that infected patients rarely recall an associated tick bite with subsequent infection (30, 117, 125). Consequently, querying donors regarding a tick bite appears to provide insufficient specificity and sensitivity to identify infected donors.

As detailed above, *Babesia* spp. have been found to have restricted geographic distributions, with *B. microti* being localized to the Northeast and Upper Midwest. Despite the regional localization of the parasite, geographic criteria have limited usefulness in identifying at-risk donors. Well delineated areas of Connecticut have been identified as locations where *B. microti* is highly endemic, but distally from these areas of high endemicity, the parasite's distribution decreases and is less well defined (62). Similarly, our current understanding of the parasite's distribution is dynamic, with evidence of human infections now being described in Maine and New

Hampshire (83, 92, 123). This may reflect an actual expansion of the parasite's geographic distribution or increased recognition of infection, perhaps acquired elsewhere, by physicians. From the perspective of transfusion transmission, donors from areas where the parasite is not endemic increasingly travel to areas of endemicity where they acquire infections, only to return home and subsequently donate blood that ultimately transmits infection. Risk factor questions focusing on travel to areas of endemicity are likely to have poor specificity. Thus, attempts to identify at-risk donors based on geographic criteria or travel to areas of endemicity are likely to be ineffective.

The transmission of *B. microti* is closely tied to the peak period of tick activity, roughly May through September. It is during these months that ticks, particularly in the nymphal stages, are most active. During these months humans are frequently outdoors pursuing activities (e.g., hiking and gardening, etc.) that increase their likelihood of exposure to ticks and subsequent infection with *B. microti*. In Connecticut, the seropositive rate among blood donors has been shown to be significantly higher during the July-to-September quarter than during the other quarters of the year (62). For this reason, some blood centers have chosen not to collect blood in areas where the parasite is highly endemic during the summer months. For example, the New York Blood Center suspends collections of blood east of the Shinnecock Canal on Long Island from the beginning of June through the end of October. This approach is predicated on most transmissions being confined to the June-through-October period, when ticks are most active and acute infections predominate. While this approach will certainly eliminate exposure during these months, blood donors have been shown to maintain active infections throughout the year (68, 71, 119). Therefore, at no time during the year are donors ensured of being free from infection with *B. microti* and unlikely to transmit infection.

Blood Screening

Serological screening. Blood screening based on the serological measurement of donor antibodies to an infecting agent is commonly used in transfusion medicine to detect donors exposed to and at risk for transmitting HIV, HBV, hepatitis C virus (HCV), and *Trypanosoma cruzi*. As indicated above, infections with *B. microti* elicit both IgM and IgG antibody responses in humans. IgM antibodies are characteristic of early, acute infections and are measurable for only a few weeks. IgG antibodies appear shortly thereafter, and levels may remain elevated for 6 to 12 months until the infection clears but in some cases may even persist for years after the resolution of clinical symptoms. Recent evidence suggests that the latter observations of IgG antibodies may be indicative of active, chronic infections characterized by elevated antibody titers with low levels of parasitemia that may or may not be measurable by nucleic acid testing (119).

While the serological screening of blood donors is an attractive option for mitigating risk, at present there are no assays that have been licensed by the FDA for this purpose. In the absence of a licensed test, screening based on IFA testing under an investigational new drug (IND) application has been proposed, but this should be viewed as a short-term solution. While the IFA test is considered the current gold standard, it is subjective in nature and not readily amenable to the high throughput needed for the testing of blood donors. An assay based on an EIA/enzyme-linked immunosorbent assay (ELISA) format that could be used with existing testing platforms would provide for high throughput and greatly enhance the consistency of the results. ELISAs using recombinant antigens have been developed that show promise, but thus far, these tests have been used as research assays only (54, 81). A primary hurdle for assay development remains convincing manufacturers that a sufficient market will be available to allow the recovery of assay development and licensure costs. This is particularly problematic, as proposed testing algorithms for *B. microti* often envision regionalized testing as opposed to nationwide, universal screening.

If and when serological screening is implemented, consideration should be given to testing year round as opposed to only during the portion of the year when ticks are most active (May through September). As discussed above, recent evidence suggests that infected donors can harbor *B. microti* infections for months to years and are capable of transmitting the parasite outside the recognized "tick season." Additionally, yearly environmental fluctuations may allow ticks to appear earlier in the year or survive later than normally observed, thereby extending the primary exposure period for humans.

Nucleic acid testing. An alternative, and at times complementary, approach to serological screening is NAT currently in place for HIV, HBV, HCV, and WNV (8). With the exception of WNV, NAT was implemented to detect window period infections, that is, the early acute stage of infection before antibodies have developed but when nucleic acids of the infecting agent are present and detectable. During the early acute stages of babesial infection, parasitemia levels are highest and often detectable only by PCR (53, 122). Parasitemia wanes with time and is difficult to detect after about 2 months (71). Thereafter, parasitemia levels are very low and intermittent, making detection by even the most sensitive PCR assays difficult. For this reason, a negative PCR result for a donor or recipient does not rule out an active infection.

With regard to *B. microti*, a potential role for NAT would be to identify *Babesia* window period infections not detectable by serological screening. Perhaps similar to the initial implementation phase of WNV NAT (115), *Babesia* NAT could be seasonal in nature, limited to those portions of the year when ticks are actively feeding and transmitting *B. microti* (e.g., May through September). Presumably, during the remainder of the year, *Babesia* infections would be detected by year-round serological screening. After the acute stage, parasitemia is low and intermittent, rendering additional NAT of little value given the presumed yield and concurrent detection by serological assays. However, technical hurdles to implementing NAT for *Babesia* remain. Unlike the viral agents tested to date, *B. microti* is an intraerythrocytic parasite present in comparatively low numbers. NAT would require whole-blood (i.e., red cells) samples that would likely need to be concentrated and then lysed to increase assay sensitivity. While these requirements do not represent technologically insurmountable hurdles, to date NAT for blood screening has not been approached in this fashion.

Pathogen Reduction

In March 2007, the Canadian Blood Services and Hema-Quebec organized a consensus development conference to discuss the current status of pathogen inactivation (66). The consensus panel acknowledged that although the present risk of transfusion-transmitted disease is extremely low, emerging transfusion-transmitted pathogens (e.g., *Babesia*) represent an increasing concern. At the close of the conference, the panel endorsed a proactive approach, citing the need for an implementation of pathogen inactivation technologies, particularly for emerging agents that pose blood safety issues in that absence of blood screening tests.

A recent study demonstrated the efficacy of the photochemical inactivation of *B. microti* in platelet and plasma components using amotosalen and long-wavelength UV light (40). For both components, a mean inactivation of greater than 5.3 logs was reported, with no viable *B. microti* parasites observed. Similar results have been reported for riboflavin and UV light: the absence of measurable parasitemia in hamsters receiving treated blood and an observed inactivation of 4 to 5 logs in both platelet and plasma components (120). While both methods demonstrate feasibility and are presently used in portions of Europe for platelet and plasma products, two major obstacles to implementation in the United States exist. First, neither approach has been licensed by the FDA for use in the United States. Second, and perhaps more important, neither approach has been evaluated with whole-blood or red cell components, the source of the vast majority of transfusion-transmitted cases of *B. microti*. Unlike platelets and plasma, whole-blood or red cell units may pose unique challenges for the transmission of light energy necessary for photochemical inactivation, particularly for an intracellular pathogen.

Other Approaches

Two additional approaches to product manipulation are currently in use, but neither appears to prevent TTB. Presently, a significant portion of the U.S. blood supply undergoes leukoreduction via filtration. Leukoreduction removes donor leukocytes from blood products, thereby reducing the likelihood of febrile nonhemolytic reactions, HLA alloimmunization, and the transmission of viruses and bacteria that may be associated with leukocytes. However, the intraerythrocytic niche occupied by *Babesia* parasites suggests that leukoreduction will have little to no impact on preventing this parasite's transmission. Infact, transfusion cases that involved leukoreduced blood products have been reported routinely (95, 118). Similarly, gamma irradiation likely has a minimal impact on the infectivity of *Babesia*, as transfusion cases implicating irradiated blood products were reported previously (82).

Implementation and Cost-Benefit

Strategies for the implementation of an intervention to mitigate the risk of transmitting *B. microti* and cost-benefit considerations are inexorably linked. Universal screening would ensure that virtually all at-risk donors would be identified. However, given that areas of endemicity associated with *B. microti* are geographically limited to the Northeast and Upper Midwest, universal screening would probably not be cost-effective. A more cost-effective approach would be selective screening based on regionally or locally identified areas of endemicity. To date, selective screening strategies have rarely been used in the United States to address transfusion-transmitted agents, but several blood organizations recently implemented selective screening strategies for *Trypanosoma cruzi* (Chagas' disease) testing (116). These organizations now test most blood donors only once for *T. cruzi* antibodies; donors who test negative are not retested following future donations, while positive donors are permanently deferred. In the case of *T. cruzi*, selective testing strategies are reasonable, since an active transmission of the agent in the United States is an extremely rare event. The adoption of a selective screening strategy for Chagas' disease testing may allow other agents to be considered for selective screening in the future.

For *B. microti*, the most cost-effective approach would be to target well-defined areas where the disease is highly endemic (e.g., New London and Middlesex Counties, CT) (62). These foci of high endemicity are likely responsible for the majority of infections in blood donors, and they could be identified through targeted screening. Unfortunately, the geographic range of *B. microti* is not well defined but is known to be expanding. For example, as one moves distally from New London and Middlesex Counties in Connecticut, the seroprevalence rate among donors decreases, but significant numbers of positive donors are still identifiable (62). Thus, consideration should perhaps be given to regional testing in which large areas of the Northeast and Upper Midwest are targeted for testing. In this scenario, donors with no risk factors or exposure would certainly be tested, but almost all at-risk donors would be identified. This viewpoint is gaining traction in transfusion medicine. At the 26 July 2010 meeting of the FDA's Blood Product Advisory Committee, the committee voted unanimously to recommend that if the screening of blood donors for *B. microti* is implemented, it would be appropriate to screen regionally in areas of high endemicity, but the committee did not provide guidance on which regions should be included or the format of a screening test (1).

Donors who visit these regions and become infected by *B. microti*, only to return to areas where the parasite is not endemic, would be missed, and without universal screening, there is no way to identify these donors short of obtaining a travel history, which is not desirable based on the current blood bank experience with malaria deferral criteria (79). Thus, regionalized testing appears to be the most cost-effective approach from a blood safety, blood availability, and cost structure standpoint; however, final analysis will be dependent upon the cost of the test and licensure by the FDA. Any discussion of the anticipated cost of a screening program would be premature at this time. The costs of screening tests vary considerably and are dictated by the size of the market, cost recovery, vendor pricing, and corporate contracts with blood banks that often "bundle" tests for pathogen markers. Furthermore, since there is no consensus on which population should be screened, reasonable estimates of cost cannot be made at this time.

An unrelated option proposed for *B. microti* is to provide immunocompromised blood recipients with blood products that have been certified as being *B. microti* negative. (i.e., cytomegalovirus model). This approach has several drawbacks. First, this does not protect the immunocompetent recipient

who may experience severe consequences from a *B. microti* infection acquired via blood transfusion. Second, it is difficult to define what proportion of the population should be viewed as immunocompromised and at risk for acquiring *Babesia* from a blood transfusion. As mentioned above, it has been suggested that blood recipients over 50 years of age are at an increased risk of *Babesia* infection (71, 73). Since this population would represent a majority of blood recipients, this appears to be an untenable option. Finally, from a logistics and cost standpoint, maintaining a separate and specialized inventory of products that will also require specific marketing is a costly approach.

SUMMARY AND FUTURE DIRECTIONS

Despite the growing consensus that mitigation efforts are needed to prevent the transmission of *B. microti* in portions of the United States, future progress will likely hinge upon the resolution of two key issues in the near future. First, the prolonged absence of an automated, high-throughput assay for *B. microti* blood screening will continue to hinder mitigation efforts going forward. While the high financial cost and reduced expected return on investment for an FDA-licensed test that may be introduced regionally are well known, flexible approaches to test development and eventual licensure are needed. The FDA has recently signaled a willingness to consider creative approaches to mitigating the risk for emerging transfusion-transmitted agents. Given the inordinate cost associated with developing and licensing a blood screening assay, thought should perhaps be given to approaches that reduce the financial burden of test manufacturers while still ensuring current levels of blood safety. Changes to the licensing process may allow new "players" in the field, eventually leading to new and better products through competition and lower overall costs.

Also of importance is an acceptance that interventions designed to prevent *Babesia* transmission are not going to achieve zero risk, unless we screen the blood supply universally. For a parasite that has shown regional endemicity, the universal screening paradigm may be difficult to support based on cost-benefit analyses. Therefore, we must focus our efforts on regions of the United States where the vast majority of infections occur. Admittedly, this does not provide a solution for donors from areas where the parasite is not endemic who travel to areas of endemicity, become infected, and later transmit the parasite through donation in an area where the parasite is not endemic. While we may one day need to address travel-related infections, we first need to address transmission in regions of the country where the parasite is highly endemic. If we wait for the 100% solution, blood recipients will continue to be infected, and in some cases die, at ever-increasing rates.

REFERENCES

1. **ABC Newsletter.** 30 July 2010. BPAC eyes regional testing for *Babesia* but seeks more data. ABC Newsl. **29:**1, 8–10.
2. **Anderson, J. F., and L. A. Magnarelli.** 2004. Babesiosis in Fairfield County, Connecticut. Emerg. Infect. Dis. **3:**545–546.
3. **Anderson, J. F., E. D. Mintz, J. J. Gadbaw, and L. A. Magnarelli.** 1991. *Babesia microti*, human babesiosis, and *Borrelia burgdorferi* in Connecticut. J. Clin. Microbiol. **29:**2779–2783.
4. **Asad, S., J. Sweeney, and L. A. Mermel.** 2009. Transfusion-transmitted babesiosis in Rhode Island. Transfusion **49:**2564–2573.
5. **Badon, S. J., J. Trouern-Trend, and R. G. Cable.** 2003. Eleven years of experience investigating suspected post-transfusion babesiosis. Transfusion **43**(Suppl.)**:**78A.
6. **Beattie, J. F., M. L. Michelson, and P. J. Holman.** 2002. Acute babesiosis caused by *Babesia divergens* in a resident of Kentucky. N. Engl. J. Med. **347:**697–698.
7. **Becker, C. A. M., A. Bouju-Albert, M. Jouglin, A. Chauvin, and L. Malandrin.** 2009. Natural transmission of zoonotic *Babesia* spp. by *Ixodes ricinus* ticks. Emerg. Infect. Dis. **15:**320–322.
8. **Bihl, F., D. Castelli, F. Marincola, R. Y. Dodd, and C. Brander.** 2007. Transfusion-transmitted infections. J. Transl. Med. **5:**25. doi:10.1186/1479-5876-5-25.
9. **Bonnet, S., N. Brisseau, A. Hermouet, M. Jouglin, and A. Chauvin.** 2009. Experimental in vitro transmission of *Babesia* sp. (EU1) by *Ixodes ricinus*. Vet. Res. **40:**21.
10. **Bonnet, S., M. Jouglin, M. L'Hostis, and A. Chauvin.** 2007. *Babesia* sp. EU1 from roe deer and transmission within *Ixodes ricinus*. Emerg. Infect. Dis. **13:**1208–1210.
11. **Brandt, F., G. R. Healy, and M. Welch.** 1977. Human babesiosis: the isolation of *Babesia microti* in golden hamsters. J. Parasitol. **63:**934–937.
12. **Bredt, A. B., and W. M. Weinstein.** 1981. Treatment of babesiosis in asplenic patients. JAMA **245:**1938–1939.
13. **Bush, J. B., M. Isaäcson, A. S. Mohamed, F. T. Potgieter, and D. T. de Waal.** 1990. Human babesiosis—a preliminary report of 2 suspected cases in southern Africa. S. Afr. Med. J. **78:**699.
14. **Cable, R. G., S. Badon, J. Trouern-Trend, J. E. Militscher, R. L. Houghton, M. J. Lodes, D. H. Persing, M. L. Eberhard, N. J. Pieniazek, B. L. Herwaldt, and D. A. Leiby.** 2001. Evidence for transmission of *Babesia microti* from Connecticut blood donors to recipients. Transfusion **41**(Suppl.)**:**12S–13S.
15. **Cable, R. G., and D. A. Leiby.** 2003. Risk and prevention of transfusion-transmitted babesiosis and other tick-borne diseases. Curr. Opin. Hematol. **10:**405–411.
16. **Cable, R. G., J. Trouern-Trend, S. J. Badon, and D. A. Leiby.** 2003. Look-back on donors found seropositive for *B. microti*: an 11 year experience in an endemic area for babesiosis. Transfusion **43**(Suppl.)**:**13A.
17. **Cahill, K. M., J. L. Benach, L. M. Reich, E. Bilmes, J. H. Zins, F. P. Siegel, and S. Hochweis.** 1981. Red cell exchange: treatment of babesiosis in a splenectomized patient. Transfusion **21:**193–198.
18. **Chisholm, E. S., T. K. Ruebush, A. J. Sulzer, and G. R. Healy.** 1978. *Babesia microti* infection in man: evaluation of an indirect immunofluorescent antibody test. Am. J. Trop. Med. Hyg. **27:**14–19.
19. **Cirino, C. M., S. F. Leitman, E. Williams, D. Fedorko, T. N. Palmore, A. Klion, C. Ockenhouse, C. Fitzhugh, J. F. Tisdale, and M. M. Hsieh.** 2008. Transfusion-associated babesiosis with an atypical time course after non-myeloablative transplantation for sickle cell disease. Ann. Intern. Med. **148:**794–795.
20. **Congelosi, J. J., B. Sarvat, J. C. Sarria, B. L. Herwaldt, and A. J. Indrikovs.** 2008. Transmission of *Babesia microti* by blood transfusion in Texas. Vox Sang. **95:**331–334.
21. **Conrad, P. A., A. M. Kjemtrup, R. A. Carreno, J. Thomford, K. Wainright, M. Eberhard, R. Quick, S. R. Telford, and B. L. Herwaldt.** 2006. Description of *Babesia duncani* n. sp. (Apicomplixa: Babesiidae) from humans and its differentiation from other piroplasms. Int. J. Parasitol. **36:**779–789.
22. **Dammin, G. J., A. Spielman, J. L. Benach, and J. Piesman.** 1981. The rising incidence of clinical *Babesia microti* infection. Hum. Pathol. **12:**398–400.
23. **Dorman, S. E., M. E. Cannon, S. R. Telford, K. M. Frank, and W. H. Churchill.** 2000. Fulminant babesiosis treated with clindamycin, quinine, and whole-blood exchange transfusion. Transfusion **40:**375–380.
24. **Eberhard, M. L., E. M. Walker, and F. J. Steurer.** 1995. Survival and infectivity of *Babesia* in blood maintained at 25 C and 2-4 C. J. Parasitol. **81:**790–792.
25. **Esernio-Jenssen, D., P. G. Scimeca, J. L. Benach, and M. J. Tenenbaum.** 1987. Transplacental/perinatal babesiosis. J. Pediatr. **110:**570–572.
26. **Eskow, E. S., P. J. Krause, A. Spielman, K. Freeman, and J. Aslanzadeh.** 1999. Southern extension of the range of human babesiosis in the eastern United States. J. Clin. Microbiol. **37:**2051–2052.
27. **Espy, M. J., J. R. Uhl, L. M. Sloan, S. P. Buckwalter, M. F. Jones, E. A. Vetter, J. D. C. Yao, N. L. Wengenack, J. E. Rosenblatt, F. R. Cockerill, and T. F. Smith.** 2006. Real-time PCR in clinical microbiology: applications for routine laboratory testing. Clin. Microbiol. Rev. **19:**165–256.
28. **Etkind, P., J. Piesman, T. K. Ruebush, A. Spielman, and D. D. Juranek.** 1980. Methods for detecting *Babesia microti* infection in wild rodents. J. Parasitol. **66:**107–110.
29. **Filstein, M. R., J. L. Benach, D. J. White, B. A. Brody, W. D. Goldman, C. W. Bakal, and R. S. Schwartz.** 1980. Serosurvey or human babesiosis in New York. J. Infect. Dis. **141:**518–521.
30. **Fishbein, D. B., J. E. Dawson, and L. E. Robinson.** 1994. Human ehrlichiosis in the United States, 1985-90. Ann. Intern. Med. **120:**736–743.
31. **Fitzpatrick, J. E. P., C. C. Kennedy, M. G. McGeown, D. G. Oreopoulos, J. H. Roberston, and M. A. O. Soyannwo.** 1968. Human case of piroplasmosis (babesiosis). Nature **217:**861–862.
32. **Fitzpatrick, J. E. P., C. C. Kennedy, M. G. McGeown, D. G. Oreopoulos, J. H. Roberston, and M. A. O. Soyannwo.** 1969. Further details of third recorded case of redwater (babesiosis) in man. Br. Med. J. **4:**770–772.
33. **Foppa, I. M., P. J. Krause, A. Spielman, H. Goethert, L. Gern, B. Brand, and S. R. Telford.** 2002. Entomologic and serologic evidence of zoonotic

transmission of *Babesia microti*, Eastern Switzerland. Emerg. Infect. Dis. **8:**722–726.
34. **Fritz, C. L., A. M. Kjemtrup, P. A. Conrad, G. R. Flores, G. L. Campbell, M. E. Schriefer, D. Gallo, and D. J. Vugia.** 1997. Seroepidemiology of emerging tickborne infectious diseases in a Northern California community. J. Infect. Dis. **175:**1432–1439.
35. **Gadbaw, J. J., J. F. Anderson, M. L. Carter, and J. L. Hadler.** 1989. Babesiosis—Connecticut. MMWR Morb. Mortal. Wkly. Rep. **38:**649–650.
36. **Gerber, M. A., E. D. Shapiro, P. J. Krause, R. G. Cable, S. J. Badon, and R. W. Ryan.** 1994. The risk of acquiring Lyme disease or babesiosis from a blood transfusion. J. Infect. Dis. **170:**231–234.
37. **Gleason, N. N., G. R. Healy, K. A. Western, G. D. Benson, and M. G. Shultz.** 1970. The "Gray" strain of *Babesia microti* from a human case established in laboratory animals. J. Parasitol. **56:**1256–1257.
38. **Gorenflot, A., K. Moubri, E. Precigout, B. Carcy, and T. P. M. Schetters.** 1998. Human babesiosis. Ann. Trop. Med. Parasitol. **92:**489–501.
39. **Grabowski, E. F., P. J. V. Giardina, D. Goldberg, H. Masur, S. E. Read, R. L. Hirsch, and J. L. Benach.** 1982. Babesiosis transmitted by a transfusion of frozen-thawed blood. Ann. Intern. Med. **96:**466–467.
40. **Grellier, P., J. Benach, M. Labaied, S. Charneau, H. Gil, G. Monsalve, R. Alfonso, L. Sawyer, L. Lin, M. Steiert, and K. Dupuis.** 2008. Photochemical inactivation with amotosalen and long-wavelength ultraviolet light of *Plasmodium* and *Babesia* in platelet and plasma components. Transfusion **48:** 1676–1684.
41. **Gubernot, D. M., C. T. Lucey, K. C. Lee, G. B. Conley, L. G. Holness, and R. P. Wise.** 2009. *Babesia* infection through blood transfusions: reports received by the US Food and Drug Administration, 1997-2007. Clin. Infect. Dis. **48:**25–30.
42. **Gubernot, D. M., H. L. Nakhasi, P. A. Mied, D. M. Asher, J. S. Epstein, and S. Kumar.** 2009. Transfusion-transmitted babesiosis in the United States: summary of a workshop. Transfusion **49:**2759–2771.
43. **Gupta, P., R. W. Hurley, P. H. Helseth, J. L. Goodman, and D. E. Hammerschmidt.** 1995. Pancytopenia due to hemophagocytic syndrome as the presenting manifestation of babesiosis. Am. J. Hematol. **50:**60–62.
44. **Haselbarth, K., A. M. Tenter, V. Brade, G. Krieger, and K. P. Hunfeld.** 2007. First case of human babesiosis in Germany—clinical presentation and molecular characterization of the pathogen. Int. J. Med. Microbiol. **297:** 197–204.
45. **Healy, G. R., and T. K. Ruebush.** 1980. Morphology of *Babesia microti* in human blood smears. Am. Soc. Clin. Pathol. **73:**107–109.
46. **Herwaldt, B. L., G. Bruyn, N. J. Pieniazek, M. Homer, K. H. Lofy, S. B. Slemenda, T. R. Fritsche, D. H. Persing, and A. P. Limaye.** 2004. *Babesia divergens*-like infection, Washington State. Emerg. Infect. Dis. **10:**622–629.
47. **Herwaldt, B. L., S. Cacciò, F. Gherlinzoni, H. Aspöck, S. B. Slemenda, P. Piccaluga, G. Martinelli, R. Edelhofer, U. Hollenstein, G. Poletti, S. Pampiglione, K. Löschenberger, S. Tura, and N. J. Pieniazek.** 2003. Molecular characterization of a non-*Babesia divergens* organism causing zoonotic babesiosis in Europe. Emerg. Infect. Dis. **9:**942–948.
48. **Herwaldt, B. L., A. M. Kjemtrup, P. A. Conrad, R. C. Barnes, M. Wilson, M. G. McCarthy, M. H. Sayers, and M. L. Eberhard.** 1997. Transfusion-transmitted babesiosis in Washington State: first reported case cause by a WA1-type parasite. J. Infect. Dis. **175:**1259–1262.
49. **Herwaldt, B. L., P. C. McGovern, M. P. Gerwel, R. M. Easton, and R. R. MacGregor.** 2003. Endemic babesiosis in another Eastern state: New Jersey. Emerg. Infect. Dis. **9:**184–188.
50. **Herwaldt, B. L., D. H. Persing, E. A. Precigout, W. L. Goff, D. A. Mathiesen, P. W. Taylor, M. L. Eberhard, and A. F. Gorenflot.** 1996. A fatal case of babesiosis in Missouri: identification of another piroplasm that infects humans. Ann. Intern. Med. **124:**643–650.
51. **Hildebrandt, A., K.-P. Hunfeld, M. Baier, A. Krumbholz, S. Sachse, T. Lorenzen, M. Kiehntopf, H.-J. Fricke, and E. Straube.** 2007. First confirmed autochthonous case of human *Babesia microti* infection in Europe. Eur. J. Clin. Microbiol. Infect. Dis. **26:**595–601.
52. **Hilton, E., J. DeVoti, J. L. Benach, M. L. Halluska, D. J. White, H. Paxton, and S. J. Dumler.** 1999. Seroprevalence and seroconversion for tick-borne diseases in a high-risk population in the Northeast United States. Am. J. Med. **106:**404–409.
53. **Homer, M. J., I. Aguilar-Delfin, S. R. Telford, P. J. Krause, and D. H. Persing.** 2000. Babesiosis. Clin. Microbiol. Rev. **13:**451–469.
54. **Houghton, R. L., M. J. Homer, L. D. Reynolds, P. R. Sleath, M. J. Lodes, V. Berardi, D. A. Leiby, and D. H. Persing.** 2002. Identification of *Babesia microti*-specific immunodominant epitopes and development of a peptide EIA for detection of antibodies in serum. Transfusion **42:**1488–1496.
55. **Hunfeld, K. P., R. Allwinn, S. Peters, P. Kraiczy, and V. Brade.** 1998. Serologic evidence for tick-borne pathogens other than *Borrelia burgdorferi* (TOBB) in Lyme borreliosis patients from Midwestern Germany. Wien. Klin. Wochenschr. **119:**901–908. (In German.)
56. **Hunfeld, K.-P., and V. Brade.** 2004. Zoonotic *Babesia*: possibly emerging pathogens to be considered for tick-infested humans in central Europe. Int. J. Med. Microbiol. **293**(Suppl. 37)**:**93–103.
57. **Hunfeld, K.-P., A. Hildebrandt, and J. S. Gray.** 2008. Babesiosis: recent insights into an ancient disease. Int. J. Parasitol. **38:**1219–1237.
58. **Hunfeld, K. P., A. Lambert, H. Kampen, S. Albert, C. Epe, V. Brade, and A. M. Tenter.** 2002. Seroprevalence of *Babesia* infections in humans exposed to ticks in Midwestern Germany. J. Clin. Microbiol. **40:**2431–2436.
59. **Iacopino, V., and T. Earnhart.** 1990. Life-threatening babesiosis in a woman from Wisconsin. Arch. Intern. Med. **150:**1527–1528.
60. **Jacoby, G. A., J. V. Hunt, K. S. Kosinski, Z. N. Demirjian, C. Huggins, P. Etkind, L. C. Marcus, and A. Spielman.** 1980. Treatment of transfusion-transmitted babesiosis by exchange transfusion. N. Engl. J. Med. **303:**1098–1100.
61. **Jerant, A. F., and A. D. Arline.** 1993. Babesiosis in California. West. J. Med. **158:**622–625.
62. **Johnson, S. T., R. G. Cable, L. Tonnetti, B. Spencer, J. Rios, and D. A. Leiby.** 2009. Seroprevalence of *Babesia microti* in blood donors from *Babesia*-endemic areas of the northeastern United States: 2000 through 2007. Transfusion **49:**2574–2582.
63. **Kain, K. C., S. B. Jassoum, I. W. Fong, and B. Hannach.** 2001. Transfusion-transmitted babesiosis in Ontario: first reported case in Canada. CMAJ **164:**1721–1723.
64. **Kim, J.-Y., S.-H. Cho, H.-N. Joo, M. Tsuji, S.-R. Cho, I.-J. Park, G.-T. Chung, J.-W. Ju, H.-I. Cheun, H.-W. Lee, Y.-H. Lee, and T.-S. Kim.** 2007. First case of human babesiosis in Korea: detection and characterization of a novel type of *Babesia* sp. (KO1) similar to ovine *Babesia*. J. Clin. Microbiol. **45:**2084–2087.
65. **Kjemtrup, A. M., B. Lee, C. L. Fritz, C. Evans, M. Chervenak, and P. A. Conrad.** 2002. Investigation of transfusion transmission of a WA1-type babesial parasite to a premature infant in California. Transfusion **42:**1482–1487.
66. **Klein, H. G., D. Anderson, M. J. Bernardi, R. Cable, W. Carey, J. S. Hoch, N. Robitaille, M. L. Silviotti, and F. Smaill.** 2007. Pathogen inactivation: making decisions about new technologies. Report of a consensus conference. Transfusion **47:**2338–2346.
67. **Kogut, S. J., C. D. Thill, M. A. Prusinski, J.-H. Lee, P. B. Backenson, J. L. Coleman, M. Anand, and D. J. White.** 2005. *Babesia microti*, upstate New York. Emerg. Infect. Dis. **11:**476–478.
68. **Krause, P. J., B. E. Gewurz, D. Hill, F. M. Marty, E. Vannier, I. M. Foppa, R. R. Furman, E. Neuhaus, G. Skowron, S. Gupta, C. McCalla, E. L. Pesanti, M. Young, D. Heiman, G. Hsue, J. A. Gelfand, G. P. Wormser, J. Dickason, F. J. Bia, B. Hartman, S. R. Telford, D. Christanson, K. Dardick, M. Coleman, J. E. Girotto, and A. Spielman.** 2008. Persistent and relapsing babesiosis in immunocompromised patients. Clin. Infect. Dis. **46:**370–376.
69. **Krause, P. J., T. Lepore, V. K. Sikand, J. Gadbaw, Jr., G. Burke, S. R. Telford, P. Brassard, D. Pearl, J. Azlanzadeh, D. Christianson, D. McGrath, and A. Spielman.** 2000. Atovaquone and azithromycin for treatment of babesiosis. N. Engl. J. Med. **343:**1454–1458.
70. **Krause, P. J., R. Ryan, S. Telford, D. Persing, and A. Spielman.** 1996. Efficacy of immunoglobulin M serodiagnostic test for rapid diagnosis of acute babesiosis. J. Clin. Microbiol. **34:**2014–2016.
71. **Krause, P. J., A. Spielman, S. R. Telford, V. K. Sikand, K. McKay, D. Christianson, R. J. Pollack, P. Brassard, J. Magera, R. Ryan, and D. H. Persing.** 1998. Persistent parasitemia after acute babesiosis. N. Engl. J. Med. **339:**160–164.
72. **Krause, P. J., S. R. Telford, R. Ryan, P. A. Conrad, M. Wilson, J. W. Thomford, and A. Spielman.** 1994. Diagnosis of babesiosis: evaluation of a serologic test for the detection of *Babesia microti* antibody. J. Infect. Dis. **169:**923–926.
73. **Krause, P. J., S. R. Telford, R. J. Pollack, R. Ryan, P. Brassard, L. Zemel, and A. Spielman.** 1992. Babesiosis: an underdiagnosed disease of children. Pediatrics **89:**1045–1048.
74. **Krause, P. J., S. R. Telford, R. Ryan, A. B. Hurta, I. Kwasnik, S. Luger, J. Niederman, M. Gerber, and A. Spielman.** 1991. Geographical and temporal distribution of babesial infection in Connecticut. J. Clin. Microbiol. **29:**1–4.
75. **Krause, P. J., S. R. Telford, A. Spielman, V. Sikand, R. Ryan, D. Christianson, G. Burke, P. Brassard, R. Pollack, J. Peck, and D. H. Persing.** 1996. Concurrent Lyme disease and babesiosis. Evidence for increased severity and duration of illness. JAMA **275:**1657–1660.
76. **Leiby, D. A.** 2006. Babesiosis and blood transfusion: flying under the radar. Transfusion **90:**157–165.
77. **Leiby, D. A., A. P. Chung, R. G. Cable, J. Trouern-Trend, J. McCullough, M. J. Homer, L. D. Reynolds, R. L. Houghton, M. J. Lodes, and D. H. Persing.** 2002. Relationship between tick bites and the seroprevalence of *Babesia microti* and *Anaplasma phagocytophila* (previously *Ehrlichia* sp.) in blood donors. Transfusion **42:**1585–1591.
78. **Leiby, D. A., A. P. S. Chung, J. E. Gill, R. L. Houghton, D. H. Persing, S. Badon, and R. G. Cable.** 2005. Demonstrable parasitemia among Connecticut blood donors with antibodies to *Babesia microti*. Transfusion **45:**1804–1810.
79. **Leiby, D. A., M. L. Nguyen, and E. P. Notari.** 2008. Impact of donor deferrals for malaria on blood availability in the United States. Transfusion **48:**2222–2228.
80. **Linden, J. V., S. J. Wong, F. K. Chu, G. B. Schmidt, and C. Bianco.** 2000. Transfusion-associated transmission of babesiosis in New York State. Transfusion **40:**285–289.
81. **Lodes, M. J., R. L. Houghton, E. S. Bruinsma, R. Mohamath, L. D. Reyn-**

olds, D. R. Benson, P. J. Krause, S. G. Reed, and D. H. Persing. 2000. Serological expression cloning of novel immunoreactive antigens of *Babesia microti*. Infect. Immun. **68:**2783–2790.
82. **Lux, J. Z., D. Weiss, J. V. Linden, D. Kessler, B. L. Herwaldt, S. J. Wong, J. Keithly, P. Della-Latta, and B. E. Scully.** 2003. Transfusion-associated babesiosis after heart transplant. Emerg. Infect. Dis. **9:**116–119.
83. **Maine Center for Disease Control and Prevention.** 5 May 2010, accession date. Reportable infectious diseases in Maine, 2008 summary. Maine Center for Disease Control and Prevention, Augusta, ME. http://www.maine.gov/dhhs/boh/ddc/epi/publications/2008AnnualReport.pdf.
84. **Matsui, T., R. Inoue, K. Kajimoto, A. Tamekane, A. Okamura, Y. Katayama, M. Shimoyama, K. Chihiara, A. Saito-Ito, and M. Tsuji.** 2000. First documentation of transfusion-associated babesiosis in Japan. Rinsho Ketsueki **41:**628–634. (In Japanese.)
85. **McQuiston, J. H., J. E. Childs, M. E. Chamberland, and E. Tabor.** 2000. Transmission of tick-borne agents of disease by blood transfusion: a review of known and potential risks in the United States. Transfusion **40:**274–284.
86. **Meer-Scherrer, L., M. Adelson, R. Mordechai, B. Lattaz, and R. Tilton.** 2004. *Babesia microti* infection in Europe. Curr. Microbiol. **48:**435–437.
87. **Meldrum, S. C., G. S. Birkhead, D. J. White, J. L. Benach, and D. L. Morse.** 1992. Human babesiosis in New York State: an epidemiological description of 136 cases. Clin. Infect. Dis. **15:**1019–1023.
88. **Michael, S. A., T. A. Morsy, and M. F. Montasser.** 1987. A case of human babesiosis (preliminary case report in Egypt). J. Egypt. Soc. Parasitol. **17:**409–410.
89. **Mintz, E. D., J. F. Anderson, R. G. Cable, and J. L. Hadler.** 1991. Transfusion-transmitted babesiosis: a case report from a new endemic area. Transfusion **31:**365–368.
90. **New, D. L., J. B. Quinn, M. Z. Qureshi, and S. J. Sigler.** 1997. Vertically transmitted babesiosis. J. Pediatr. **131:**163–164.
91. **New York City Department of Health and Mental Hygiene.** 2008. Health advisory #5: increase in transfusion-associated babesiosis in NYC. New York City Department of Health and Mental Hygiene, New York, NY. http://www.nyc.gov/html/doh/downloads/pdf/cd/2009/09md05.pdf.
92. **Ngo, V., and R. Civen.** 2009. Babesiosis acquired through blood transfusion, California, USA. Emerg. Infect. Dis. **15:**785–787.
93. **Osorno, B. M., C. Vega, M. Ristic, C. Robles, and S. Ibarra.** 1976. Isolation of *Babesia* spp. from asymptomatic human beings. Vet. Parasitol. **2:**111–120.
94. **Pantanowitz, L., and M. E. Cannon.** 2001. Extracellular *Babesia microti* parasites. Transfusion **41:**440.
95. **Pantanowitz, L., S. R. Telford, and M. E. Cannon.** 2002. The impact of babesiosis on transfusion medicine. Transfus. Med. Rev. **16:**131–143.
96. **Perdrizet, G. A., N. H. Olson, P. J. Krause, G. T. Banever, A. Spielman, and R. G. Cable.** 2000. Babesiosis in a renal transplant recipient acquired through blood transfusion. Transplantation **70:**205–208.
97. **Persing, D. H., and P. A. Conrad.** 1995. Babesiosis: new insights from phylogenetic analysis. Infect. Agents Dis. **4:**182–195.
98. **Persing, D. H., B. L. Herwaldt, C. Glaser, R. S. Lane, J. W. Thomford, D. Mathiesen, P. J. Krause, D. F. Phillip, and P. A. Conrad.** 1995. Infection with a *Babesia*-like organism in northern California. N. Engl. J. Med. **332:**298–303.
99. **Persing, D. H., D. Mathiesen, W. F. Marshall, S. R. Telford, A. Spielman, J. W. Thomford, and P. A. Conrad.** 1992. Detection of *Babesia microti* by polymerase chain reaction. J. Clin. Microbiol. **30:**2097–2103.
100. **Popovsky, M. A., L. E. Lindberg, A. L. Syrek, and P. L. Page.** 1988. Prevalence of *Babesia* antibody in a selected blood donor population. Transfusion **28:**59–61.
101. **Powell, V. I., and K. Grima.** 2002. Exchange transfusion for malaria and Babesia infection. Transfus. Med. Rev. **16:**239–250.
102. **Price, T. H. (ed.).** 2009. Standards for blood banks and transfusion services, 26th ed. American Association of Blood Banks, Bethesda, MD.
103. **Pruthi, R. K., W. F. Marshall, J. C. Wiltsie, and D. H. Persing.** 1995. Human babesiosis. Mayo Clin. Proc. **70:**853–862.
104. **Quick, R. E., B. L. Herwaldt, J. W. Thomford, M. E. Garnett, M. L. Eberhard, M. Wilson, D. H. Spach, J. W. Dickerson, S. R. Telford, K. R. Steingart, R. Pollock, D. H. Persing, J. M. Kobayashi, D. D. Juranek, and P. A. Conrad.** 1993. Babesiosis in Washington State: a new species of *Babesia*? Ann. Intern. Med. **119:**284–290.
105. **Rodgers, S. E., and T. N. Mather.** 2007. Human *Babesia microti* incidence and *Ixodes scapularis* distribution, Rhode Island, 1998-2004. Emerg. Infect. Dis. **13:**633–635.
106. **Ruebush, T. K., P. B. Cassaday, H. J. Marsh, S. A. Lisker, D. B. Voorhees, E. B. Mahoney, and G. R. Healy.** 1977. Human babesiosis on Nantucket Island. Clinical features. Ann. Intern. Med. **86:**6–9.
107. **Saito-Ito, A., M. Tsuji, Q. Wei, S. He, T. Matsui, M. Kohsaki, S. Arai, T. Kamiyama, K. Hioki, and C. Ishihara.** 2000. Transfusion-acquired, autochthonous human babesiosis in Japan: isolation of *Babesia microti*-like parasite with hu-RBC-SCID mice. J. Clin. Microbiol. **38:**4511–4516.
108. **Scholtens, R. G., E. H. Braff, G. R. Healy, and N. Gleason.** 1968. A case of babesiosis in man in the United States. Am. J. Trop. Med. Hyg. **17:**810–813.
109. **Sethi, S., D. Alcid, H. Kesarwala, and R. W. Tolan.** 2009. Probable congenital babesiosis in infant, New Jersey, USA. Emerg. Infect. Dis. **15:**788–791.
110. **Setty, S., Z. Khalil, P. Schori, M. Azar, and P. Ferrieri.** 2003. Babesiosis. Two atypical cases from Minnesota and a review. Am. J. Clin. Pathol. **120:**554–559.
111. **Shih, C. M., L. P. Liu, W. C. Chung, S. J. Ong, and C. C. Wang.** 1997. Human babesiosis in Taiwan: asymptomatic infection with a *Babesia microti*-like organism in a Taiwanese women. J. Clin. Microbiol. **35:**450–454.
112. **Shiota, T., H. Kurimoto, N. Haguma, and Y. Yoshida.** 1984. Studies on *Babesia* first found in murine in Japan: epidemiology, morphology and experimental infection. Zentralbl. Bakteriol. Mikrobiol. Hyg. A **256:**347–355. (In German.)
113. **Slovut, D. P., E. Benedetti, and A. J. Matas.** 1996. Babesiosis and hemophagocytic syndrome in an asplenic renal transplant recipient. Transplantation **62:**537–539.
114. **Steketee, R. W., M. R. Eckman, E. C. Burgess, J. N. Kuritsky, J. Dickerson, W. L. Schell, M. S. Godsey, and J. P. Davis.** 1985. Babesiosis in Wisconsin. JAMA **253:**2675–2678.
115. **Stramer, S. L., C. T. Fang, G. A. Foster, A. G. Wagner, J. P. Brodsky, and R. Y. Dodd.** 2005. West Nile virus among blood donors in the United States, 2003 and 2004. N. Engl. J. Med. **353:**451–459.
116. **Stramer, S. L., R. L. Townsend, G. A. Foster, D. E. Krysztof, D. A. Leiby, J. P. Brodsky, C. Rouault, B. A. Lenes, and R. Y. Dodd.** 2010. Experience with selective testing for antibody to *Trypanosoma cruzi* following validation using universal testing. Vox Sang. **99**(Suppl. 1)**:**313.
117. **Strle, F., R. B. Nadelman, J. Cimperman, J. Nowakowski, R. N. Picken, I. Schwartz, V. Maraspin, M. E. Aguero-Rosenfeld, S. Varde, S. Lotric-Furlan, and G. P. Wormser.** 1997. Comparison of culture-confirmed erythema migrans caused by *Borrelia burgdorferi* sensu stricto in New York State and by *Borrelia afzelii* in Slovenia. Ann. Intern. Med. **130:**32–36.
118. **Tonnetti, L., A. F. Eder, B. Dy, J. Kennedy, P. Pisciotto, R. J. Benjamin, and D. A. Leiby.** 2009. Transfusion-transmitted *Babesia microti* identified through hemovigilance. Transfusion **49:**2557–2563.
119. **Tonnetti, L., S. T. Johnson, R. G. Cable, J. Rios, B. R. Spencer, and D. A. Leiby.** 2009. Natural history study (NHS) of *Babesia microti* in Connecticut blood donors. Transfusion **49**(Suppl.)**:**35A.
120. **Tonnetti, L., M. C. Proctor, H. L. Reddy, R. P. Goodrich, and D. A. Leiby.** 2010. Evaluation of the Mirasol PRT system against *Babesia microti* in apheresis platelets and plasma. Transfusion **50:**1019–1027.
121. **Vannier, E., I. Borggraefe, S. R. Telford, S. Memon, T. Brauns, A. Spielman, J. A. Gelfand, and H. W. Wortis.** 2004. Age-associated decline in resistance to *Babesia microti* is genetically determined. J. Infect. Dis. **189:**1721–1728.
122. **Vannier, E., B. E. Gewurz, and P. J. Krause.** 2008. Human babesiosis. Infect. Dis. Clin. North Am. **22:**469–488.
123. **Walk, S. T., G. Xu, J. W. Stull, and S. M. Rich.** 2009. Correlation between tick density and pathogen endemicity, New Hampshire. Emerg. Infect. Dis. **15:**585–587.
124. **Western, K. A., G. D. Benson, N. N. Gleason, G. R. Healy, and M. G. Schultz.** 1970. Babesiosis in a Massachusetts resident. N. Engl. J. Med. **283:**854–856.
125. **White, D. J., J. Talarico, H. G. Chang, G. S. Birkhead, T. Heimberger, and D. L. Morse.** 1998. Human babesiosis in New York State. Review of 139 hospitalized cases and analysis of prognostic factors. Arch. Intern. Med. **158:**2149–2154.
126. **Wittner, M., K. S. Rowin, H. B. Tanowitz, J. F. Hobbs, S. Saltzman, B. Wenz, R. Hirsch, E. Chisholm, and G. R. Healy.** 1982. Successful chemotherapy of transfusion babesiosis. Ann. Intern. Med. **96:**601–604.
127. **Wozniak, E. J., L. J. Lowenstine, R. Hemmer, T. Robinson, and P. A. Conrad.** 1996. Comparative pathogenesis of human WA1 and Babesia microti isolates in Syrian hamster model. Lab. Anim. Sci. **46:**507–515.
128. **Zhao, Y., K. R. Love, S. W. Hall, and F. V. Beardell.** 2009. A fatal case of transfusion-transmitted babesiosis in the State of Delaware. Transfusion **49:**2583–2587.
129. **Zintl, A., G. Mulcahy, H. E. Skerrett, S. M. Taylor, and J. S. Gray.** 2003. *Babesia divergens*, a bovine blood parasite of veterinary and zoonotic importance. Clin. Microbiol. Rev. **16:**622–636.

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David A. Leiby, Ph.D., received a B.S. in Biology from Lafayette College, Easton, PA; an M.S. in Biology from Rutgers University, Camden, NJ; and an M.S. and a Ph.D. in Zoology from the Ohio State University, Columbus, OH. He was a National Research Council Postdoctoral Resident Research Associate in the Cellular Immunology Department at the Walter Reed Army Institute of Research, Washington, DC. For the past 17 years, Dr. Leiby has been affiliated with the American Red Cross, where he is the Head of the Transmissible Diseases Department at the Jerome H. Holland Laboratory for the Biomedical Sciences in Rockville, MD. He is the principal investigator for comprehensive, multicenter, epidemiological studies of Chagas' disease, tick-borne pathogens, and malaria in blood donors. Dr. Leiby has published over 70 refereed papers and book chapters and is frequently invited both nationally and internationally to speak at meetings and institutions. Dr. Leiby is also an Associate Professor of Microbiology and Tropical Medicine at the George Washington University, Washington, DC.