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MINIREVIEW

Babesia microti: from Mice to Ticks to an Increasing Number of Highly Susceptible Humans

Lars F. Westblade,[a,b] Matthew S. Simon,[b] Blaine A. Mathison,[c] Laura A. Kirkman[b,d]

Weill Cornell Medicine, Department of Pathology and Laboratory Medicine, New York, New York, USA[a]; Weill Cornell Medicine, Department of Medicine, Division of Infectious Diseases, New York, New York, USA[b]; ARUP Laboratories, Salt Lake City, Utah, USA[c]; Weill Cornell Medicine, Department of Microbiology and Immunology, New York, New York, USA[d]

ABSTRACT *Babesia microti*, a zoonotic intraerythrocytic parasite, is the primary etiological agent of human babesiosis in the United States. Human infections range from subclinical illness to severe disease resulting in death, with symptoms being related to host immune status. Despite advances in our understanding and management of *B. microti*, the incidence of infection in the United States has increased. Therefore, research focused on eradicating disease and optimizing clinical management is essential. Here we review this remarkable organism, with emphasis on the clinical, diagnostic, and therapeutic aspects of human disease.

KEYWORDS *Babesia microti*, diagnosis, epidemiology, immunosuppression, infection, life cycle, prevention, transmission, treatment, relapse, United States, clinical significance

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Address correspondence to Laura A. Kirkman, lak9015@med.cornell.edu.

L.F.W., M.S.S., B.A.M., and L.A.K. contributed equally to this work.

Human and animal babesiosis is the result of infection with zoonotic tick-borne protozoan parasites of the genus *Babesia*. The prevalence of human disease has increased in the United States and other parts of the world, including Europe, China, and other Asian countries, with the predominant species causing illness in the United States being *Babesia microti* (1–3). In recent years, the incidence of babesiosis in the United States has changed due to *B. microti* transmission beyond the traditional regions in the Northeast and upper Midwest in which the pathogen is endemic and an increasingly immunosuppressed population, for which treatment options can be limited (4). As a result, babesiosis has become more prevalent and difficult to manage, particularly in cases of severe and relapsing disease, where evidence of resistance to the mainstays of prolonged therapy, azithromycin and atovaquone, has been documented (5–8). In this review, we discuss the epidemiology, clinical features, diagnosis, and treatment of disease due to *B. microti*.

EPIDEMIOLOGY

More than 100 different *Babesia* species can infect animals, but significantly fewer are known to cause human disease (9). The primary species that infect humans include *B. microti*, *Babesia divergens*, *Babesia duncani*, and *Babesia venatorum* (9). Compared to the other species, *B. microti* is clearly genetically distinct (10–12). The first human case of babesiosis was reported in 1957, when a splenectomized Yugoslavian herdsmen died of infection with *B. divergens* (13). Babesiosis was first documented in the United States in a splenectomized California resident in 1968, followed by a report in 1970 of a previously healthy woman with an intact spleen who developed a nonspecific febrile illness after a tick bite on Nantucket Island, Massachusetts (14, 15). With additional cases among residents of Nantucket Island, the disease became known as Nantucket fever (16). In the United States, *B. microti* is the most common agent of human babesiosis and

causes disease that remains primarily restricted to the Northeast and upper Midwest (4). Sporadic infections due to *B. duncani* (formerly *Babesia* species WA1) along the Pacific Coast from California to Washington State have been reported, and infrequent infections due to *B. divergens*-like organisms (including the organism currently designated *Babesia* species MO1) have resulted in illness in Kentucky, Missouri, and Washington State (4, 9). Therefore, human disease has emerged as a significant problem in the United States.

Human babesiosis has been nationally reportable to the U.S. Centers for Disease Control and Prevention since 2011. In 2014, a total of 1,744 cases were reported by 22 states, with 94% of cases involving residents of seven states in the Northeast and upper Midwest, namely, Connecticut, Massachusetts, Minnesota, New Jersey, New York, Rhode Island, and Wisconsin (https://www.cdc.gov/parasites/babesiosis/data-statistics/index.html). Surveillance data from health departments in several states have demonstrated increasing incidence over the past decade. In New York State, the incidence of *B. microti* infection increased approximately 3-fold, from 1.7 cases per 100,000 persons in 2006 to 4.5 cases per 100,000 persons in 2015 (https://www.health.ny.gov/statistics/diseases/communicable), and similar patterns have been observed in other states in which the pathogen is endemic (http://www.ct.gov/dph/cwp/view.asp?a=3136&q=388390&dphNav_GID=1601&dphPNavCtr=|#47477). In Pennsylvania, a state not traditionally considered to be at high risk for *B. microti* transmission, the incidence of disease increased 10-fold between 2005 and 2015 (17). The geographic expansion of *B. microti* outside the traditional states in which the pathogen is endemic includes Delaware, Maine, Maryland, New Hampshire, and Virginia (18, 19). Enhanced clinical awareness, coupled with changes in tick, deer, and mouse reservoir populations related to climate change and deforestation, may be responsible, in part, for the increases in disease incidence and geographic expansion (20).

LIFE CYCLE AND TRANSMISSION

Babesiosis is a zoonotic disease with an enzootic cycle between a tick vector and vertebrate hosts (9, 21). *Babesia microti* has a two-host life cycle involving hard-bodied ticks of the genus *Ixodes* as the definitive host and a vertebrate intermediate host (Fig. 1). Humans are accidental hosts and most commonly acquire infection through the bite of an infected tick, with the risk of infection being greatest in late spring and summer, when the population of infected ticks attempting to feed is at its greatest. *Ixodes scapularis* (also known as the deer tick or blacklegged tick) is the predominant vector for *B. microti* in the United States, and the natural reservoir is the white-footed mouse (*Peromyscus leucopus*). *Ixodes scapularis* has a four-stage life cycle (egg, six-legged larva, eight-legged nymph, and adult) that generally lasts 2 years. Each mobile tick stage (larva, nymph, and adult) requires a blood meal on a different vertebrate host. Larval and nymphal ticks leave the vertebrate host to molt between each blood meal, and adult females leave the third and final host in the fall to overwinter before oviposition in the spring. The natural hosts for larvae and nymphs are usually small mammals or birds, while the hosts for the adult ticks are larger mammals, such as deer (although humans can enter the life cycle at any stage). Up to two-thirds of white-footed mice harbor *B. microti* in areas in which the pathogen is endemic, and 9 to 20% of *I. scapularis* nymphs in New England are infested with *B. microti* (22). Most human infections occur when the nymphal form feeds on a human host; however, adult ticks may also transmit *B. microti* (21). Within the human host, *B. microti* invades red blood cells (RBCs), divides approximately every 8 h, exits, and invades other RBCs to establish the erythrocytic cycle that can result in hemolysis and fever in infected individuals.

Babesia microti can be cotransmitted with other pathogens harbored by *I. scapularis*, including *Borrelia burgdorferi* (the agent of Lyme disease), *Anaplasma phagocytophilum* (the cause of human granulocytic anaplasmosis), and several less frequently encountered viral pathogens (e.g., Powassan virus) and bacterial pathogens (4). It has been proposed that coinfection with *B. burgdorferi* increases *B.*

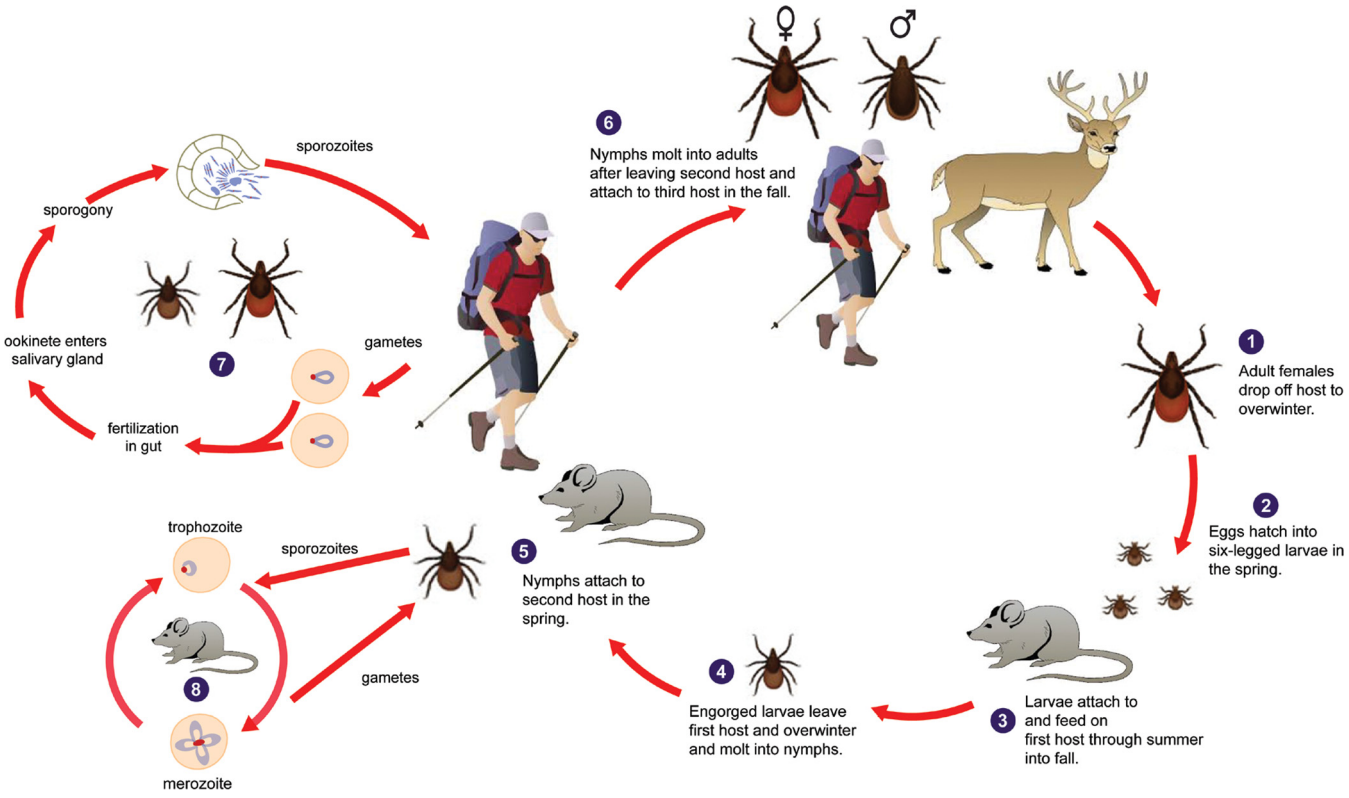

FIG 1 Life cycles of *Ixodes scapularis* and *Babesia microti*. *Ixodes scapularis* has a four-stage life cycle that generally lasts 2 years and includes vertebrate hosts (including rodents, deer, or humans), with each mobile tick stage having a blood meal on a different individual host. During the first year, mated adult female ticks detach from their vertebrate host to overwinter (1), and they lay eggs in the spring. Eggs hatch in the spring (2), and larvae attach to their first vertebrate host (usually small rodents or birds) (3). The six-legged larvae feed on the first host, generally in late summer, and may become infected with *B. microti* while taking a blood meal. Engorged larvae leave the host, overwinter, and molt into nymphs (4). In the spring of the second year, nymphs attach to a second vertebrate host (e.g., rodents, deer, or humans) (5); in the fall, they leave the second host to molt into adults and attach to a third vertebrate host (such as deer or humans) (6). *Babesia microti* has a two-host life cycle, including *I. scapularis* as the definitive host and a vertebrate intermediate host. In the tick (7), gametes ingested during a blood meal undergo sexual reproduction, leading to the eventual formation of infectious sporozoites. In the mammalian host (e.g., rodents or humans) (8), sporozoites initiate the erythrocytic cycle, resulting in the formation of trophozoites that divide asexually by budding. Subsequently, trophozoites transform into merozoites and perpetuate the erythrocytic cycle or gametes are ingested by ticks during a blood meal to initiate the sexual cycle. All mobile stages of *I. scapularis* may acquire *B. microti* parasites from infected first or second vertebrate hosts, but only nymphs and adults transmit parasites.

microti transmission, and it is thought to increase disease severity and duration in the rodent and human hosts (2). The geographic spread of *B. microti* appears to lag behind areas in which Lyme disease is endemic (2), potentially allowing prediction of future *B. microti* expansion and implementation of public health resources to mitigate disease, particularly among highly susceptible immunocompromised persons.

Babesia microti is currently the leading cause of RBC-transfusion-transmitted infections reported to the U.S. Food and Drug Administration (FDA) and the leading infectious cause of transfusion-related deaths reported to the FDA (https://www.fda.gov/BiologicsBloodVaccines/SafetyAvailability/ReportaProblem/TransfusionDonationFatalities/ucm346639.htm#D). A case series of transfusion-associated babesiosis documented 162 cases between 1979 and 2010, with the majority of cases occurring since 2000 (23). Of the 162 cases, 159 were due to *B. microti* and 3 to *B. duncani*. However, the true incidence during this time period was undoubtedly higher, because of underrecognition and underreporting. Thirteen percent of these transfusion-associated cases occurred in states outside traditional areas of transmission, due to donor travel or shipment of blood products, which raises challenges regarding screening. At present, prospective blood donors are asked to report a history of babesiosis, but no FDA-licensed screening assay for blood donors has been approved. Several tests

for screening blood donations, including immunologically based and nucleic acid-based platforms, have been evaluated in large clinical trials and exhibit high sensitivity and specificity (24). Serological prevalence in the donor population in regions in which the pathogen is endemic can be as high as 2%, with 12 to 20% of antibody-positive donors also being positive by PCR (25–27).

A recent trial evaluated the effectiveness of antibody- and nucleic acid-based screening of blood donations for the prevention of transfusion-transmitted *B. microti* in areas with high rates of transmission, between June 2012 and September 2014 (27). During this period, donated blood was screened with both antibody- and nucleic acid-based methods for a portion of each week, while blood was not screened for the remaining part of the week. No cases of transfusion-acquired babesiosis were reported from the screened donations (0 cases per 75,331 screened donations), while 14 cases occurred from 253,031 unscreened donations (1 case per 18,074 unscreened donations). The authors concluded that screening was highly effective in preventing transfusion-acquired babesiosis, but neither the serologically based nor nucleic acid-based tests alone detected all potentially transmissible cases. Also notable was the persistence of test positivity even in healthy asymptomatic donors, with PCR positivity persisting for a mean of 4.7 months and antibody reactivity persisting for several years. These findings have implications for defining the criteria for reentry of *B. microti*-positive individuals into the U.S. blood supply donor pool.

Several health economics modeling studies have evaluated the cost-effectiveness of blood donor screening for *B. microti*, with conflicting results (28–30). One study determined antibody screening in areas in which the pathogen is endemic to cost $760,000 per quality-adjusted life year (QALY) gained, a general measure of disease-related morbidity and death (28). This finding is significantly greater, i.e., less favorable, than traditional health care cost-effectiveness thresholds of $50,000/QALY to $100,000/QALY. However, this finding is consistent with the cost-effectiveness of screening for other transfusion-transmitted infections, such as West Nile Virus, and could be economically justified by a greater societal willingness to prevent transfusion-transmitted diseases (31). In 2015, the FDA Blood Products Advisory Committee recommended adoption of year-round national serological testing, with additional PCR-based testing only in states in which the pathogen is endemic. At present, several blood collection agencies in states with high transmission rates, including New York, New Jersey, Rhode Island, Minnesota, and Wisconsin, are employing laboratory-based screening through investigational protocols, but many other blood collection agencies have not yet adopted laboratory-based screening, since final FDA recommendations and assay approval are still pending (Beth Shaz, New York Blood Center, personal communication).

Alternative modes of transmission remain rare. However, several cases of congenital babesiosis with *B. microti*, in which asymptomatic maternal infection resulted in symptomatic infection of infants between 19 and 41 days after birth, have been reported (32–34). One reported case was confirmed through detection of *B. microti* DNA in placental tissue and positive *B. microti* serological findings for a heel-stick blood sample on the infant's third day of life. Recently, *B. microti* transmission through organ transplantation was reported; two renal transplant recipients who received organs from the same donor developed babesiosis following transplantation (35).

CLINICAL FINDINGS

The hallmarks of babesiosis are fever and fatigue. Infections can be asymptomatic or range from an influenza-like illness to severe disease with end-organ compromise (renal failure, acute respiratory distress syndrome, disseminated intravascular coagulation, or splenic infarction or rupture). Relapsing disease and treatment failures are primarily observed among patients with asplenia and/or other immune deficits (17, 21, 36).

Hemolysis underlies the central pathogenic trait associated with *Babesia* infection; this is primarily a result of egress of the parasites from infected RBCs and subsequent

irreparable damage to the RBC membranes (9, 37). Typical laboratory findings include anemia, thrombocytopenia, elevated ferritin levels, low haptoglobin levels, and other indicators of hemolysis (4, 21). Markedly reduced levels of high-density lipoprotein cholesterol (HDL-C) in patients with babesiosis have been observed, with extremely low HDL-C levels and very high ferritin levels being strongly associated with disease (38, 39). Both HDL-C and ferritin levels appear to normalize posttreatment, potentially permitting their use as biomarkers for diagnosis and treatment efficacy.

In addition to hemolytic activity directly related to the life cycle of the parasites, there are reports of *Babesia* precipitating autoimmune hemolysis through several mechanisms, including deposition of cross-reacting microbial antigens, adsorption of immune complexes and complement, and deregulation of immune tolerance provoked by active infection (37, 40). Rarely, patients exhibiting warm autoimmune hemolytic anemia postinfection have been described (41).

Risk factors for severe disease are associated with immune compromise and include advanced age, asplenia, HIV/AIDS, malignancy, organ transplantation, and a history of immunomodulating agents (such as rituximab, etanercept, or corticosteroids) (5, 21, 36). Mortality rates of 6 to 21% have been reported, and severe infection and end-organ complications may develop in up to 57% of immunocompromised patients (36, 42). Besides prompt anti-*Babesia* treatment, RBC exchange is indicated for cases with high parasitemia (defined as $\geq$10% of RBCs being infected), severe anemia, or renal, hepatic, or pulmonary compromise, while the most frequent indication for RBC exchange is ongoing severe hemolysis (43).

DIAGNOSIS

The diagnosis of *B. microti* infection and its differentiation from other blood-borne parasites and tick-borne pathogens are essential for effective therapy. The current "gold standard" for the diagnosis of infection is conventional light microscopy, in which permanent blood films (smears) stained with Giemsa stain or Wright's stain are examined for the presence of characteristic organisms (44, 45). However, nucleic acid-based and immunological methods are available in a few hospitals and reference laboratories. Diagnosis is assisted by a comprehensive history, including travel to areas in which the pathogen is endemic, activities associated with tick exposure, immunosuppressive conditions, and blood transfusion.

The diagnostic sensitivity and specificity of light microscopy are highly dependent on the proficiency of the examiner; however, light microscopy is generally considered a sensitive, specific, and inexpensive method for diagnosis of infection. It is not possible to identify *Babesia* to the species level using light microscopy, but differentiation between small species (0.5 to 2.5 μm), such as *B. microti*, and large species ($\sim$3.0 to 5.0 μm), including many animal-associated species, may be feasible (46). Thick and thin blood films are typically prepared using anticoagulated venous blood. Ideally, the specimens should be collected before the initiation of therapy and smears should be prepared within 1 hour after collection. Larger amounts of blood are used during the preparation of thick films than in the preparation of thin films, thus increasing the possibility of detecting light infections, and RBCs are lysed, enabling white blood cells and parasites to be observed clearly. It has been suggested that thick films have limited utility for the diagnosis of *B. microti* infections, due to the small size of the organism (4). This contrasts with our experience, in which thick films have proved essential for diagnosing infections with low levels of parasitemia, particularly in immunosuppressed individuals. The morphological characteristics of parasites are best observed in thin films because they are fixed and RBC morphology is retained, permitting extracellular (extraerythrocytic) organisms to be visualized.

To optimize detection and identification, both thick and thin films should be prepared and the percentage of infected RBCs calculated to guide treatment and determine response to therapy (extracellular forms are not included in the calculation). At least 300 oil immersion fields on both films must be examined before results are reported as negative. Since the degree of infection may vary, a single negative set of

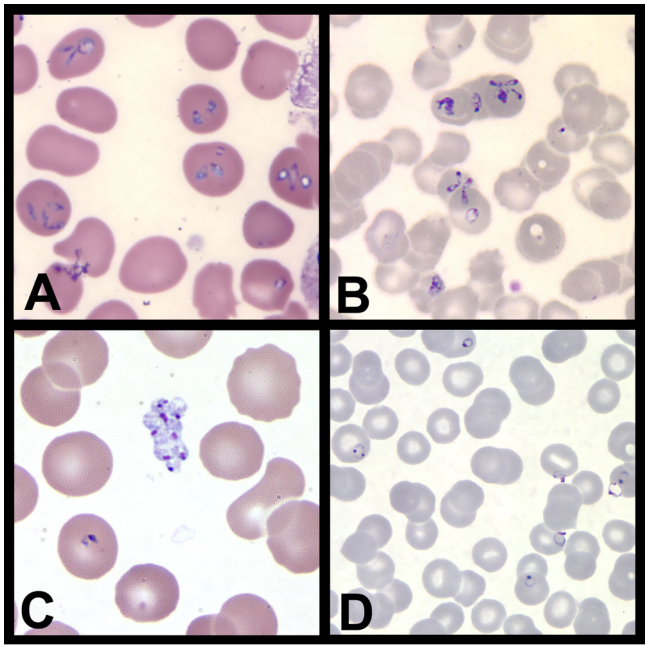

FIG 2 Photomicrographic images of *Babesia* and *P. falciparum* in thin blood films. (A) Images of *Babesia* showing small, pleomorphic, vacuolated rings. (B) *Babesia* organisms exhibiting pleomorphic rings and characteristic tetrad ("Maltese cross") forms. (C) Extracellular forms of *Babesia* parasites. (D) Ring-form trophozoites of *P. falciparum*, for comparison, showing multiply infected RBCs, appliqué forms, and thin cytoplasm with double-chromatin dots. Original magnification for all images, ×1,000, with oil immersion.

blood films does not rule out infection, and specimens should be collected every 6 to 8 h for up to 3 days to increase the likelihood of detecting organisms.

On review of blood films, ring-like forms of *Babesia* are pear- or spindle-shaped and often vacuolated (Fig. 2A). The size of infected RBCs is the same as that of uninfected RBCs, and neither stippling nor malarial pigment is evident. Rarely, merozoites are present in a tetrad formation, termed a "Maltese cross," which is pathognomonic for *Babesia* (Fig. 2B), and extracellular ring forms are frequently observed (Fig. 2C). *Babesia* can resemble *Plasmodium*, especially early ring-form trophozoites of *Plasmodium falciparum* (Fig. 2D); however, the ring-like forms of *Babesia* differ in being more pleomorphic and vacuolated, and extracellular forms are rarely observed in *Plasmodium* infections.

Several nucleic acid-based assays that target the *B. microti* 18S rRNA gene and use conventional, real-time, or reverse transcription-PCR approaches for detection of *B. microti* have been developed, with the specimen of choice being EDTA-preserved whole blood (47–51). While identification to the species level does not alter clinical management or treatment (4), these methods are useful if reliable genus-level identification is not possible through microscopic examination due to low levels of parasitemia or poor parasite morphology (as can be observed in drug-treated patients), or if species-level identification is required for epidemiological purposes. Furthermore, nucleic acid-based methods are tolerant of hemolyzed specimens (which are unsuitable for microscopy) and are helpful if there is a delay in collection and laboratory receipt of specimens for microscopic examination. Finally, these methods may allow the recognition of novel *Babesia* species if blood films are positive for characteristic organisms but species-specific molecular methods yield negative results.

In the setting of routine clinical testing, the diagnostic performance of *B. microti*-specific nucleic acid-based methods is excellent, with a sensitivity of 100% and a probable specificity of 100%, compared to microscopy (47, 49). The limit of detection (analytical sensitivity) of microscopy is estimated to be 10 to 50 parasites/μl of blood (0.0002 to 0.001% infected RBCs) under optimal conditions but is routinely about 100

parasites/μl of blood (~0.002% infected RBCs) (52). In contrast, the limit of detection of *B. microti* DNA PCR tests is 1 to 10 parasites/μl (0.00002 to 0.0002% infected RBCs) (47, 49, 51), which is 10- to 100-fold more sensitive than microscopic examination of blood films under routine conditions. This illustrates the utility of molecular assays for detecting *Babesia* infections with low levels of parasitemia, although infections typically present with >0.1% infected RBCs (i.e., significantly greater than the limit of detection of nucleic acid-based assays) (47). Reverse transcription-PCR-based methods that detect 18S rRNA (which is >1,000-fold more abundant than its coding genes) are thought to be able to detect a single organism in ~50 to 500 μl of whole blood (51) and could be very important for screening the U.S. blood supply.

While PCR has been shown to be more sensitive than blood film examination for patients during and following anti-*Babesia* therapy (49), the role of nucleic acid-based technologies in therapeutic management should be approached with caution. Parasite DNA may be detectable for long periods (weeks to months), even after organisms are no longer evident on blood films (4, 27, 47–49), which may reflect detection of DNA from nonviable organisms rather than active infection. Quantitative PCR platforms may allow systematic assessment of the efficacy of anti-*Babesia* regimens (48), but generally the results of nucleic acid-based methods should be interpreted in the setting of clinical findings and, where possible, other diagnostic markers.

Immunological methods for the detection of *B. microti* show promise for screening the U.S. blood supply and have also facilitated investigations of donors in cases of transfusion-acquired *Babesia* (23, 27). Diagnostically, however, their primary role is to confirm infection. Such methods should not replace microscopy or nucleic acid-based platforms for diagnosis, as antibodies may not be present or detectable early in infections or in immunosuppressed individuals and antibodies can persist well beyond the resolution of disease (4, 21). One of the best described diagnostic immunological methods is an indirect immunofluorescent antibody assay that uses *B. microti* parasites as antigens for detecting antibodies to *B. microti* in human sera (53). The assay displays sensitivity of 88 to 96% and specificity of 90 to 100%. Reactivity with other *Babesia* species and *Plasmodium* has been observed, although titers are typically low and occur most often during the acute phase of disease (54, 55). IgG and IgM titers usually exceed 1:1,024 in acute illnesses, but these levels generally decline within 1 year (55).

ANTIMICROBIAL THERAPY AND PREVENTION

In general, treatment options for babesiosis have focused on repurposing antimalarial agents and, while *Babesia* and *Plasmodium* have similar invasion and metabolic pathways, the two differ greatly in their erythrocytic life cycles and mechanisms of host cell modification (10, 11). These differences are important when considering novel parasite interventions and the development of targeted anti-*Babesia* therapies.

Guidelines for the treatment of babesiosis include combination therapy, i.e., azithromycin and atovaquone for mild to moderate disease or clindamycin and quinine for severe disease (4, 21, 56). Treatment duration is 7 to 10 days for immunocompetent hosts. However, numerous case reports have demonstrated the negative side effects of clindamycin and quinine therapy, which severely limit its clinical applications (6, 21, 36). Difficulty in managing babesiosis often occurs after initial treatments fail and individuals present with relapsing disease. Official recommendations for treatment of immunosuppressed patients call for 6 weeks of treatment or 2 weeks beyond the last positive blood film (21). However, there are reported cases of continuing anti-*Babesia* treatment for the presumed duration of medication-induced immunosuppression (5, 6).

With relapsing disease, the choices of therapy, dose, and duration remain undefined, as do the laboratory parameters for monitoring infections. Doxycycline and proguanil have been successfully added to the aforementioned regimens in cases of treatment failure, but such regimens have not been systematically studied (6). Also concerning are recent reports demonstrating the molecular evidence of azithromycin and atovaquone resistance in relapsing infections in immunocompromised patients, further complicating treatment selection (7, 8). In multiple cases of therapeutic failure thought to be

attributable to the development of antimicrobial resistance, resistance appears to have emerged *de novo* while the patient was receiving therapy, suggesting that current regimens may not be universally sufficient to eradicate the parasite from immunocompromised human hosts (5–8).

Because of increased risk of prolonged infection and treatment failure, asplenic individuals and immunosuppressed patients in areas in which the pathogen is endemic should be counseled regarding tick avoidance and tick removal. For babesiosis, this is achieved by avoiding environments such as tall grass (where ticks, deer, and mice reside), applying repellents, wearing clothing that covers the extremities (mainly the lower part of the body), and removing ticks (using tweezers to grasp the mouth parts without squeezing the tick body) on animals and humans as soon as possible after outdoor activities are concluded.

SUMMARY AND FUTURE DIRECTIONS

Babesia microti is a tick-borne pathogen of significant medical importance in the United States, where it is endemic in the Northeast and the upper Midwest, and it is associated with transfusion-transmitted illness and relapsing disease in immunosuppressed populations. Accurate diagnosis is essential for adroit clinical management and is predominantly based on conventional light microscopy. Nucleic acid-based methods have a role if disease is suspected but parasites are not observed or if parasite morphology is poor. Measurement of HDL-C and ferritin levels could be useful in facilitating diagnosis and monitoring treatment efficacy and parasite clearance. Determining optimal treatment regimens and duration is particularly challenging in the setting of immune suppression, where relapsing disease has been observed. Therefore, our hope with this review is to provoke the medical and scientific communities to develop novel diagnostic algorithms and targeted anti-*Babesia* therapies and to incorporate them into diagnostic and treatment recommendations that offer clear guidance for monitoring therapeutic efficacy for all patient populations.

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