BABESIOSIS IN HUMANS: A BRIEF LITERATURE REVIEW

Zornitsa Traykova

National Centre of Infectious and Parasitic Diseases

ABSTRACT

Babesiosis is a tick-borne parasitic disease caused by the intraerythrocytic protozoan *Babesia* spp. and transmitted primarily by *Ixodes* ticks. The geographical distribution of the parasites coincides with the regions where their tick vectors are prevalent. More than 50 cases of human babesiosis have been reported in Europe, mainly associated with *Babesia divergens*, which causes acute disease in cattle and is transmitted by *Ixodes ricinus*. In contrast, the incidence of the disease in the USA is approximately 2000 cases per year, with the main causative agent being *Babesia microti* and the tick vector being *Ixodes scapularis*. Although babesiosis is primarily an animal disease, humans can also become acutely ill, particularly splenectomized and immunocompromised individuals. Clinical manifestations range from asymptomatic to severe disease with symptoms including fever, chills, hemoglobinuria and anemia. There is a risk of potentially fatal complications such as acute respiratory, renal or multi-organ failure, particularly in vulnerable populations. Diagnosis is primarily based on light microscopy and PCR testing, while serological methods are more appropriate for epidemiological studies. Treatment regimens typically include a 7-10 day course of either atovaquone plus azithromycin or clindamycin plus quinine. Human cases are associated with outdoor activities or living in rural areas during the warm months when tick activity is at its peak. Because of the increasing incidence in endemic regions and the potentially serious clinical consequences, babesiosis should be considered in the differential diagnosis of febrile illnesses of unknown origin.

Keywords: babesiosis; etiology; distribution; treatment

ADDRESS FOR CORRESPONDENCE:
Rumen Harizanov
National Center of Infectious and Parasitic Diseases,
Department of Parasitology and Tropical Medicine
26 Yanko Sakazov bul., 1504, Sofia, Bulgaria
email: traikova@ncipd.org

INTRODUCTION

Babesiosis is an infectious disease caused by the intraerythrocytic protozoa of the *Babesia* species. The principal mode of transmission is via a transmissible vector - the *Ixodes* tick. *Babesia* infects primarily animals, with humans acting as opportunistic hosts. The parasite is also called 'pyroplasma' due to its distinctive pear-shaped morphology observed in infected erythrocytes. The prevalence of *Babesia* infestation is low and confined to specific geographical regions. The majority of individuals do not present with any discernible symptoms; however, in some cases, patients may exhibit severe clinical manifestations and a high mortality rate. In general, the disease affects predominantly patients who have undergone splenectomy, those with a compromised immune system, and the elderly. The diagnosis of babesiosis is not straightforward and requires caution, particularly in regions where the disease is endemic. It is recommended that patients are treated regardless of the presence or absence of symptoms, in order to prevent disease progression and transmission. (1)

Morphology and life cycle

More than 100 different *Babesia* species have been identified (2), of which several have been confirmed to cause human infection: *B. crassa*-like agent, *B. divergens*, *B. duncani*, *B. microti*, *B. motasi*, *B. venatorum*, *Babesia divergens*-like and *Babesia microti*-like pathogens (3).

Babesia spp. can be classified in different ways. Taxonomically, they are part of the phylum *Apicomplexa*, subclass *Piroplasmea*, order *Piroplasmida* (4). Using morphological characteristics, they can be grouped into small *Babesia* (trophozoites are 1.0 to 2.5 μm) and large *Babesia* (2.5 to 5.0 μm) (5), although size has limited taxonomic value (2). Another classification is

based on the capacity for transovarial transmission. *Babesia sensu strictu (s.s.)* demonstrates such transmission, and *Babesia sensu latu (s.l.)* doen not. New molecular taxonomy has classified *Piroplasmidia spp.* into different clades using analysis of 18S rRNA gene sequences obtained from public databases (2). Currently, there are at least ten main lineages, of which four clades include *Babesia spp. – B. microti* is part of Clade I, and *B. divergens* is part of Clade X (6). Morphologically, *Apicomplexan* spp. (like *Babesia, Plasmodium, Toxoplasma, Cryptosporidium* and others) are characterized by the presence of an apical complex (7), located anteriorly in all invasive stages, such as sporozoites and merozoites (6). It consists of multiple structures, including rhoptries and micronemes - membranous organelles containing substances responsible for the attachment to and invasion of host cells. During this process, apicomplexan parasites create a parasitophorous vesicule, in which they reside, using the host cell's plasma membrane (7). Unlike others, the parasitophorous vesicule of *Babesia spp*. disintegrates soon after the parasite invades the host cell, therefore it resides directly in its cytoplasm (6).

Babesia spp. need two hosts to complete their development - vertebrates (including man) and tick vectors. The parasite's life cycle is complex, with two asexual and one sexual reproduction cycles. Stages of development include merogony, gamogony and sporogony, which are typical for the phylum *Apicomplexa* (6).

Babesia-infected ticks introduce sporozoites into vertebrae hosts by blood meal. Sporozoites enter the host's erythrocytes and develop into trophozoites (ring forms), which undergo merogony and turn into merozoites. They egress, infect other erythrocytes and either undergo a further merogony cycle or a sexual commitment to transition into intraerythrocytic gametocytes. In further blood meal, the host's erythrocytes containing gametocytes enter the tick and develop into gametes, referred to as ray-bodies, inside the vector's gut lumen (6). *Babesia spp.* exhibit two gamete types (different from other piroplasmidian micro- and macrogametes) (8), the fusion of which creates an ookinete. Ookinets invade the tick gut cells, undergo a meiotic division and develop into kinetes. Kinetes reach other organs in the tick, including ovarian cells, which mediates transovarial transmission in *Babesia sensu stricto* species (6). Primary kinetes multiply asexually to create secondary kinetes, which invade the salivary gland cells, where they turn into a multinucleated sporoblast. This syncytium is dormant during the tick's ecdysis and mediates babesial transstadial transmission. Infective sporozoites are procured by sporogony once the adult tick initiates blood-feeding on a naive host, during which multiple sporozoites get inoculated in the vertebrate bloodstream (8).

Hosts

B. divergens was thought to have a narrow host range and to be typical for cattle (9). Now it has been proven to have one of the widest host ranges for a *Babesia* species through experimental infections in different splenectomized and non-splenectomized animals, including primates, deer, sheep, gerbils and rodents. Furthermore, naturally infected reindeer, red deer and roe deer have been reported, although it has not been proven with absolute certainty that the causative agent is *B. divergens* (10).

Bovine babesiosis is the most economically significant arthropod-transmitted pathogen affecting cattle due to the mortalities, abortions, and reduced production of meat and milk caused by this disease. The most economically relevant pathogens are *B. bovis* and *B. bigemina* in tropical and subtropical areas, as well as *B. divergens*, mainly found in Europe from Scandinavia to the Mediterranean, but also in North Africa (2).

B. divergens is transmitted by *Ixodes ricinus*. The species' habitat is restricted to areas with an average annual rainfall of 100cm or more due to its requirement for high humidity. A suitable microhabitat can be found in woodland, rough hill scrub and damp low-lying land, unlike well-maintained pastures, where the conditions needed are rarely provided (9). According to different studies, this tick is found primarily in urban and peri-urban areas such as city parks, gardens and forest patches. It should be noted that climate change leads to a wider distribution of *I. ricinus* in regions with higher latitudes and altitudes (11).

There is a geographical overlap between countries reporting human babesiosis cases, regions with

infected cattle, and *I. ricinus*-infested areas. Human cases occur between May and September, which corresponds with the peak activity of *I. ricinus* ticks (11). People at the highest risk of acquiring babesiosis are those who visit rural areas where cattle are kept, such as farmers, foresters and hikers (12). Furthermore, the risk of infection through a tick bite increases because all stages (larvae, nymph and adult) can transmit *B. divergens* and *B. venatorum* (11).

Ixodes scapularis is the main tick vector in North America and is found in the north- and southeast, upper Midwest and mid-Atlantic states of the United States and in Canada, but *I. ricinus* is also proven to be a competent vector for *B. microti* and *B. venatorum* (11). The main reservoir of *B. microti* is the white-footed mouse (*Peromyscus leucopus*) (13).

Epidemiology

The first case of human babesiosis was described in 1957. In former Yugoslavia (now Croatia), a 33-year-old splenectomized farmer died after an acute illness, characterized by fever, hemoglobinuria and anemia. Parasites found in blood smear were identified as piroplasms, resembling *B. bovis* (14). The second case of human babesiosis was reported in California in 1968 when a splenectomized individual exhibited malaria-like symptoms without any evidence of malaria exposure. A diagnosis of babesiosis was later confirmed through serological evidence (17). In the U.S., the primary causative agent of human babesiosis is *Babesia microti*, which is endemic mainly in the Northeast and upper Midwest (13). The incidence is 2000 cases a year, although the number is believed to be higher (18).

Since then, more than 50 cases of human babesiosis have been reported in Europe, of which nearly 1/4th in France. The other cases were registered in the following countries: Great Britain, Ireland, Spain, Portugal, Italy, Germany, Austria, Switzerland, Russia, Poland, the Czech Republic, Sweden, Denmark, Norway, Finland, Turkey (15) and Hungary (16). The majority were caused by *B. divergens*; 5 were caused by *B. venatorum* and 24 by *B. microti* , 11 autochthonous and 13 imported cases acquired in the Americas (15).

An imported case of human babesiosis was described in Bulgaria in 1995. The patient was 34 years old and had a history of residing in Sudan for six months, as well as a tick bite two months before the onset of the illness. Symptoms included fever, chills, fatigue, loss of appetite, jaundice and hepatomegaly for more than a week before hospitalization. Laboratory tests showed hemoglobinuria and elevated CRP, AST, ALT, total and direct bilirubin levels. *Babesia* infection was diagnosed using light microscopy. The patient was treated with Chloroquine orally for five days, with doses of 1g on the first day and 500mg on the subsequent days. The treatment was successful, and the patient was discharged in improved condition. Since then, no further cases of human babesiosis have been registered in Bulgaria (19).

Clinical manifestations and complications

B. divergens infection

Splenectomized and immunocompromised patients

B. divergens affects mainly splenectomized (15,20,21) or hyposlenic individuals (22,23). The incubation period varies from 1 to 3 weeks after vector bite (20). The onset is sudden with fever (22, 24-26), chills (24,26,27), sweats, headache (28), myalgia (24), abdominal (25-27) and back pain (22). (20) Hemoglobinuria and jaundice (23, 25-27) are commonly seen due to massive intravascular hemolysis (20). Other symptoms include nausea (23-25), anorexia (25), malaise (23), vomiting (26,29), diarrhea (29), cough (24,26), hematuria (25,27), oliguria (22), proteinuria (25), fatigue (22,24,28), arthralgia (24), skin rash (24), petechiae (27).

Life-threatening complications can occur, such as acute respiratory (25-27), renal (23, 25-27) and multi-organ failure (22), coma (27), DIC (30), shock (26), hemophagocytic syndrome (31), hospital-acquired pneumonia (23), atrial fibrillation (27), QT-prolongation (26). Rapid renal failure due to babesiosis has been associated with pulmonary edema (20).

The most common laboratory findings are anemia, lymphopenia, thrombocytopenia, elevated levels of ASAT and evidence of inflammation (24). Higher levels of LDH (26,28) and signs of hemolysis (22,26,28) can also be established.

Normosplenic patients

Acute illness caused by *B. divergens* has also been reported in normosplenic individuals, some of whom with no prior remarkable medical history. In those cases, the clinical manifestation was moderate (32) to severe (33-35). One case had a lethal outcome (36).

Symptoms included fever with chills, headache, arthromyalgia (32) in milder cases, fatigue (35), nausea, abdominal pain, dark urine (33), jaundice, (35) mild hepatomegaly, acute renal failure (34), cough and dyspnea (35) in more severe cases. One of the patients experienced a relapse of the disease after 18 days of treatment (34). Laboratory findings included leukopenia, elevated liver enzymes and CRP in milder cases (32) and anemia (34), thrombocytopenia (33,34), leukocyte left shift with immature neutrophils (35), low haptoglobin, hematuria (34), elevated creatinine, total bilirubin, direct bilirubin and LDH (34) in more severe ones.

Furthermore, seropositivity for *B. divergens* in individuals without a diagnosis of human babesiosis has been shown in different serological surveys: in Belgium (33% in patients with tick-borne disease) (37), in Italy (5,1 % in individuals with professional risk and 1,4% in less exposed individuals) (38), in Midwestern Germany (3,6% in individuals with clinical or serological evidence of Lyme borreliosis) (39), in Sweden (6,9% in individuals positive for *Borrelia burgdorferi* antibodies and 1% in healthy volunteers) (40).

B. microti infection

Studies in white-footed mice and hamsters suggest that the time for transmission after tick attachment may be 36 to 54 hours, but this has not been studied in humans. The incubation period for tick-borne disease varies from 1 to 4 weeks (41).

The course of *B. microti* infections varies from asymptomatic to severe (42). This condition is observed mainly in normosplenic patients (15). Most cases are mild to moderate, with a gradual onset. Clinical manifestations typically include fever, chills and sweats, malaise, fatigue, anorexia, headache, arthromyalgia and cough. Gastrointestinal disturbances (nausea, vomiting, abdominal pain) and other symptoms (e.g. conjunctival injection, sore throat, pallor, weight loss, and depression) are less common. Physical examination shows minimal changes like mild spleno- and hepatomegaly and slight jaundice. Immunosuppressive medication and conditions (HIV-coinfection, malignancies, and splenectomy) are associated with a more severe disease. Furthermore, such patients are more likely to experience a prolonged, relapsing course of illness and have a higher mortality (42). In asplenic individuals, the clinical manifestation is similar to *B. divergens*, often fulminant and lethal (15). Complications include acute respiratory, hepatic, renal and heart failure, DIC and splenic infarction (42).

Asymptomatic parasitemia can be found in individuals who have either no symptoms or a subclinical manifestation, and it may persist for months to years. Such carriers have been identified through different serosurveys, showing a disparity between seroprevalence and the number of reported cases. Asymptomatic parasitemia in blood donors may lead to transfusion-transmitted babesiosis (42), which has a longer incubation period (1 to 6 weeks) (41) and can occur at any time of the year (29).

In Europe, seropositivity for *B. microti* has been found in different countries, for example - in Sweden (10,4% in individuals positive for *Borrelia burgdorferi* antibodies and 1,52% in healthy volunteers) (40), in Poland (23,1% in employees of National Forests) (43), in Belgium (9% in patients with tick-borne disease) (37), in Italy (4,8% in individuals with professional risk; 4,2% in less exposed individuals) (38), in Midwestern Germany (5,4% in of individuals with clinical or serological evidence of Lyme borreliosis) (39).

Diagnosis

Babesiosis should be considered in patients with fever of unknown origin or signs of hemolytic anemia (15), history of residing in *Babesia* endemic areas, tick-bite or exposure to tick-infested areas (5), absence of recent travel to malaria-endemic regions (9), splenectomy (5) and potential immunocompromising factors (15).

Laboratory findings in symptomatic patients may be non-specific, such as normochromic normocytic anemia, thrombocytopenia and occasionally leukopenia, as well as elevated liver enzymes

(aspartate aminotransferase [AST], alanine transaminase [ALT], alkaline phosphatase) (44). Evidence of intravascular hemolysis may be present - elevated LDH levels, total and indirect bilirubin levels, and reduced haptoglobin (18). Furthermore, if alongside with hemolytic anemia, the Coombs test is positive and procalcitonine levels are elevated, babesiosis is to be suspected, and further diagnostic tests should be performed (44).

Light microscopy

Light microscopy of Giemsa-stained thin blood smears is used for detecting *Babesia spp* (44). Various forms can be seen, including rings, pear-shaped parasites and Maltese cross forms (5), which are pathognomonic of babesiosis (18). Tetrads are more commonly seen in *B. microti* but are still rare (44). The size of merozoites varies from 1,5 to 2 µm in *B. microti* and from 1 to 3 µm in *B. divergens*, depending on the host (5), the mean length of pyriforms in human erythrocytes being approximately 2µm (9). Merozoites have a (sub)central position and polyparasititsm is common, with up to 8 parasites in a single erythrocyte (9).

The main differential diagnosis is malaria because *Plasmodium spp.* also shows intraerythrocytic rings. Typical for malaria is the parasitic pigment (hemozoin), although early parasitic stages may lack it (44). Furthermore, schizonts and gametocytes, seen in malaria, are absent in *Babesia* infections (45). Parasitemia in *B. divergens* ranges from 1 to 80% (20), though it should be noted that in the early stages of the disease in immunocompetent patients, it can be lower than 1% (44). Therefore, a review of at least 200–300 fields in thin blood smears is recommended, but the exact number of fields has not yet been standardized. An examination of thick blood smears may be helpful, although parasites could be missed due to their size (18).

Serological diagnosis

Serological testing is not suitable for diagnostic purposes (18). On one hand, specific antibodies become detectable 1 week after the onset of acute *B. divergens* infection, which may lead to false-negative results and delay of treatment. On the other hand, false-positive results have been observed in patients with connective tissue disorders such as systemic lupus erythematosus and rheumatoid arthritis. Cross-reactivity between different *Babesia spp.* (*B. divergens* and *B. venatorum*), as well as between *Babesia* and other *Apicomplexa* parasites (*Plasmodium* and *Toxoplasma*) were also described (21). Moreover, the differentiation between active and past infection is challenging because most patients remain seropositive for a year or more after acute illness. Therefore, serological methods are suitable only for epidemiological studies (18).

Molecular diagnosis

PCR assays targeting the 18S rRNA gene can be used to detect *Babesia* parasites. Both clotted and EDTA-treated blood samples can be used for this test (15). These methods have higher sensitivity and equal specificity compared to microscopic examination of blood smears and hamster inoculation (5). The detection limit of PCR assays is approximately 1-3 parasites per µL of blood, which is lower than the microscopic detection limit (15). The detection of *Babesia* DNA indicates the presence of parasitemia (21).

PCR assays for both *B. microti* and *B. divergens* have been developed. These assays typically amplify highly conserved sequences, which contain species-specific regions. By analyzing the sequences of the amplified fragments and comparing them to a database of known sequences, the infective agent can be conclusively identified (5).

Treatment

Currently, atovaquone, azithromycin, clindamycin and quinine are used as antibabesial drugs. The mechanism of action of atovaquone in *Apicomplexa* parasites is through targeting the cytochrome bc1 complex of the mitochondrial electron transport chain. Azithromycin is associated with protein synthesis inhibition, including the translation machinery in the apicoplast. Clindamycin is thought to have the same target of action as azithromycin. The exact mechanism of action of quinine against *Babesia* is different from its action against malaria parasites, where it interferes with hemozoin formation. *Babesia* species do not produce hemozoin, which suggests that quinine's action in babesiosis is mediated through other pathways. According to different studies, quinine may potentially inactivate critical biological

functions in various parasite organelles such as the plasma membrane, endoplasmic reticulum, and mitochondria, or it may act as a DNA intercalator, though the latter hypothesis is less supported (46).

Two reatment regimens are used: atovaquone plus azithromycin, which is preferred, and clindamycin plus quinine as an alternative. Courses should last at least 7–10 days and are longer for immunocompromised patients. Asymptomatic patients typically do not require treatment (47).

For mildly to moderately ill adult patients, treated in outpatient settings, the preferred regimen is atovaquone (750 mg p.o. twice a day) combined with azithromycin (500 mg p.o. on the first day and 250 mg p.o. daily on subsequent days). Alternative regimens are clindamycin (600 mg p.o. three times a day) or quinine (650 mg p.o. three times a day) (47).

For hospitalized adult patients with severe disease, the preferred regimen includes atovaquone (750mg p.o. twice a day) and azithromycin (500mg i.v. daily). Clindamycin (600mg i.v. four times a day) or quinine (650 mg p.o. three times daily) can be used as alternatives. The administration of either regimen should continue until symptoms subside, after which the patient should receive oral medications at outpatient treatment doses to complete the 7 to 10-day course. In severely ill patients and immunocompromised ones, higher doses of azithromycin have been administered - 1000 mg, followed by 500mg daily (47).

Supportive care including antipyretics, vasopressors, blood transfusions, exchange transfusions for high-grade parasitemia (>10%), mechanical ventilation, or dialysis may be necessary for some patients (47).

In immunocompetent patients, symptoms usually resolve during the 7–10 days of treatment, and blood smears become negative. In contrast, highly immunocompromised patients require longer courses and close monitoring, including daily blood smears until parasitemia is below 4%, followed by weekly checks. Treatment should continue until parasites are undetectable on smears for two consecutive weeks (47).

CONCLUSION

Babesiosis is a rare infection globally and in Bulgaria. Diagnosis can be challenging, as the infection can be confused with malaria or other tick-borne diseases. Therefore, it is advisable to adopt a multidisciplinary approach when diagnosing suspected infections. This entails the involvement of specialists in both infectious diseases and medical parasitology, to prevent any potential omissions. In the light of rising incidence of human cases observed in recent years and the potential of babesiosis to manifest as a severe disease with a fatal outcome, it is imperative to consider it in the differential diagnosis of febrile conditions of uncertain origin.

ACKNOWLEDGEMENTS

This research is supported by the Bulgarian Ministry of Education and Science under the National Program „Young Scientists and Postdoctoral Students - 2“.

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