Wioletta Rożej-Bielicka[1], Hanna Stypułkowska-Misiurewicz[2], Elżbieta Gołąb[1]

HUMAN BABESIOSIS

[1]Department of Medical Parasitology
National Institute of Public Health – National Institute of Hygiene in Warsaw
[2]Department of Virology
National Institute of Public Health – National Institute of Hygiene in Warsaw

ABSTRACT

Babesiosis is an emerging parasitic, anthropo-zoonotic tick-borne disease, seldom diagnosed in humans. Caused by *Protozoa, Babesia* (also called *Piroplasma*) intraerytrocytic piriform microorganism. Infection of vertebrates is transmitted by ticks. Out of more than 100 *Babesia* species/genotypes described so far, only some were diagnosed in infected humans, mostly *B. microti*, *B. divergens* and *B. venatorum* (*Babesia* sp. EU1). Infection in humans is often asymptomatic or mild but is of a particular risk for asplenic individuals, those with congenital or acquired immunodeficiencies, and elderly. Infections transmitted with blood and blood products raise concerns in hemotherapy. Epidemiological situation of babesiosis varies around the world. In Europe, no increase in the number of cases was reported, but in the USA its prevalence is increasing and extension of endemic areas is observed.

The aim of this publication is to describe the problems connected with the current epidemiological situation, diagnosis and treatment of human babesiosis with regard to clinical status of patients.

Key words*: babesiosis,* Babesia *sp., human babesiosis, diagnosis, treatment*

Babesiosis is a new emerging, anthropo-zoonosis, a parasitic disease caused by protozoa of the genus *Babesia*, also called *Piroplasma* due to the pear-shaped appearance of trophozoites at one of the life cycle stages. They are small organisms (ø = 1–5 μm) that invade erythrocytes. There are two hosts in the life cycle of these parasites: ticks, mainly *Ixodidae*, are the definitive host and various vertebrates, including humans, which are intermediate host.

Until now, more than 100 *Babesia* species and genotypes were documented (1, 2, 3), but only few were reported as pathogenic for humans. In humans, the most prevalent are infections caused by *Babesia microti* and less frequently – *B. divergens, B. duncani* or *B. venatorum* (formerly known as *Babesia* sp. EU1) (4). Infections in humans are associated with an increased activity of ticks, but sometimes, rather rarely, through transfusion of infected blood, blood products or transplantation of infected organ (2). Cases of congenital babesiosis were also documented (5).

PREVALENCE OF BABESIOSIS

Babesiosis is noted worldwide. In the North America, infections with *B. microti* predominate, where the tick *Ixodes scapularis* is the vector of the pathogen. In other continents, *B. microti* is also common as a parasite of rodents, however, infections in humans caused by this species are rare. Infections introduced by persons returning from endemic areas elsewhere, were noted so far mainly the USA (6). CDC (*Centers for Disease Control and Prevention*) epidemiological data suggest a geographical expansion and stable increase in the number of *Babesia* infections. In 2011–2013, a total of 3797 new cases were reported in the USA (7).

In the tropical and subtropical countries, where malaria is common, babesiosis rarely is diagnosed. Probably, it occurs much more frequently as it was demonstrated by the results of the studies conducted in 2012–2013 in China in patients presenting with fever and living in the Yunan County. From a detailed analysis of 449 cases, 8 infections with *B. microti* and 2 co-infections were identified, i.e. *B. microti/Plasmodium*

falciparum and *B. microti*/*Plasmodium vivax*. Infection only by *Plasmodium* spp. (mainly *P. vivax* and *P. falciparum*) alone was detected in 63 persons (8).

B. divergens is the most common etiological agent of babesiosis in Europe (1) and *Ixodes ricinus* is the vector. This tick is prevalent in the whole northern hemisphere. It may also transmit *B. microti* as well as *B. venatorum* which are pathogenic for humans (9). Literature data provide descriptions of about 50 cases of confirmed babesiosis which were reported in the European countries, mainly in patients with a history of splenectomy or with impaired immunity (1, 2, 10, 11). Infections with *B. venatorum* (formerly known as *Babesia* sp. EU1) were more commonly reported (12). So far, one case of symptomatic infection with *B. microti* imported from Brasil (13) and some asymptomatic cases were noted in Poland (14).

LIFE CYCLE OF *BABESIA*

Following the invasion of the intermediate host bloodstream, *Babesia* sporozoites enter the red blood cells. They achieve trophozoite stage there, proliferate in schizogonic divisions and mature to the stage of merozoites (12). Red blood cells collapse and released merozoites enter new red blood cells. This cycle is repeated. Having entered the red blood cells, a part of merozoites differentiate into gametocytes which are infective for the tick. In the tick gut, gametocytes released from the digested red blood cells develop into gamonts (gametes) which then form zygotes in a reproductive cycle. Zygotes mature to the stage of ookinete which is able to move, and consequently, allows the pathogen to enter the hemolymph from the tick gut. The hemolymph transports the ookinetes to different parts of tick organism, including salivary glands, where the next stage of life cycle may be observed, i.e. sporogony, in which sporozoites infective for intermediary hosts are formed (15). It was demonstrated that transmission of *B. microti* from the tick occurs 24 hours following its feeding. This process has not been studied with other *Babesia* species (16).

TRANSFUSSION-TRANSMITTED BABESIOSIS

Babesiosis, transmitted through infected blood and blood products, constitutes a serious problem. The blood cell, being an environment for the development of *Babesia*, is a niche which increases the probability of pathogen transmission during transfusion. It was demonstrated experimentally that the piroplasmas survive in the erythrocytes outside the host organism. Studies were conducted under conditions providing additional stress for the red blood cells, i.e. in tubes containing anticoagulant (EDTA), not in the specific bags that increase the red blood cells survival by optimizing gas exchange. Under experimental conditions, *B. microti* remained alive for at least 21 days in tubes stored at 4°C (17). It was also determined that *Babesia* survive process of cryo-conservation of blood preparations (18).

An infective dose, which may cause the disease, is relatively low. Administration of 30 infected red blood cells caused babesiosis in 40% of healthy hamsters while 10-fold higher dose resulted in infection of 100% of studied animals (4).For individuals with an impaired immunity system, even single erythrocyte containing pathogens, may be a potential source of babesiosis (19). In such persons, an exceptionally severe manifestation of infection was observed, i.e. resistant to a standard chemotherapy, usually resulting in death (20). In 2005–2008, 12 fatal cases of transfusion-transmitted babesiosis were registered in the USA (4, 19). It is suggested to include tests for babesiosis in the differential diagnosis of transfusion-associated anemia or fever of unknown origin (5).

CLINICAL MANIFESTATIONS OF BABESIOSIS

Usually, clinical symptoms appear between weeks 1 and 4 following the bite of tick infected with *Babesia* (2). For transfusion-transmitted babesiosis, this period may be extended up to 9 weeks and even up to 6 months in extreme cases (5).

Initially, disease is manifested by general malaise and fatigue. Then, influenza-like symptoms appear, including: fever, chills, sweating, joint and muscle pain (4, 21). These symptoms are similar to malaria. and many other infectious diseases. Along with the exacerbation of symptoms, hemolytic anemia, intravascular coagulopathy, hepatomegaly and splenomegaly may occur. Babesiosis complications may include respiratory distress syndrome, heart failure, inflammation of the central nervous system and even death in extreme cases (2, 16).

Complete blood count reveals the disorders of parameters resulting from excessive erythrocyte lysis. Low hematocrit, low hemoglobin concentration, thrombocytopenia and reticulocytosis are observed. Biochemical examination reveals an increased activities of transaminases, alkaline phosphatase, indirect bilirubin and lactate dehydrogenase (2, 10, 22).

In immuno-competent individuals, symptoms usually resolve within a few weeks without any treatment, however, malaise and fatigue may persist for even several months. Severe manifestations of disease, which

require hospitalization, and babesiosis fatal cases are reported in patients with considerably impaired immunity. Immunosuppressed patients, individuals with a history of splenectomy, those with hemoglobinopathy, suffering from malignant tumors, infected with HIV and persons at advanced age are at the risk of severe babesiosis (23).

Babesiosis course and its prognosis are dependent on the immunological status of the patient as well as *Babesia* species which caused the disease. Infection with *B. venatorum* is usually of mild to moderately severe course, with a good prognosis also for asplenic patients and those with autoaggressive diseases (12, 24), while infection with *B. divergens* is frequently of fulminant. Life-threatening symptoms appear immediately (2, 10, 11). The majority of fatal infections with *B. divergens* resulted in death within 4–7 days following the onset of symptoms of hemoglobinuria or multiple organ dysfunction syndrome (2, 21, 25, 26).

Undiagnosed *Babesia* infections may co-exist with other tick-transmitted diseases, resulting in exacerbation and disturbances in the course of disease. Co-infection with *Babesia* was identified in 10% of patients with Lyme disease from the New England (USA) (27). Thus, in the USA, on babesiosis endemic territories, patients with borreliosis accompanied by complications are recommended to undergo laboratory testing for *Babesia* infection and antiprotozoa therapy is also indicated (22).

LABORATORY DIAGNOSIS OF BABESIOSIS

Babesiosis case is considered to be confirmed if pathogen or its genetic material is detected in peripheral blood. Microscopic examinations of peripheral blood smears are most frequently performed in the laboratory diagnosis. Percentage of infected red blood cells in patients is usually low. It rarely exceeds 5% in immunocompetent persons, but, in case of asplenic patients it may amount to 85% (28).

In the diagnosis of babesiosis in the USA, commercial serological tests are used, e.g. immunofluorescence assay (IFA) for the presence of IgM and/or IgG antibodies against *B. microti.* Due to a high species specificity of *Babesia* antigens, these tests are hardly applicable in Europe, where infections in humans are mostly caused by the *B. divergens* and *B. venatorum*. Serological tests are not recommended to be used in the case of persons with impaired immunity system as false negative test results may appear. In case of patients with bacterial, viral infections, autoimmune disorders of connective tissue, infected with *Plasmodium* or *Toxoplasma gondii*, false positive test results may occur (2, 29).

To confirm babesiosis, a biotest was also performed, consisting in the inoculation of patient's blood to the peritoneum of laboratory rodent. Nowadays, such method is hardly employed due to its low efficiency and long period of waiting for the test result, amounting to about 2–4 weeks (2, 30).

Currently, polymerase chain reaction (PCR) is a reference method for diagnosis of babesiosis (31). PCR is recommended if the species of pathogen cannot be identified based on the blood smear or if the diagnosis is uncertain and medical interview and clinical symptoms are indicative of babesiosis (2). In Europe, certified commercial PCR tests intended for the laboratory diagnosis of human babesiosis are not accessible. Having considered the tests elaborated by reference laboratories for their own purposes, a gene sequence encoding for small ribosomal subunit (18S rRNA) of *Babesia* is the most frequently employed genetic marker of babesiosis. PCR sensitivity for the 18S rRNA gene was assessed at 5–10 pathogens/1μl of blood which corresponds to 0.0001% parasitemia (32).

Based on the initiative of the National Chamber of Laboratory Diagnosticians (NCLD), the Recommendations of the Working Group concerning laboratory diagnosis of tick-borne diseases, including babesiosis were issued in 2014. Document, which is accessible on the NCLD website in the section Recommendations, contains information on the infections with *Babesia* and methods of babesiosis diagnosis together with a list of reference laboratories performing laboratory testing for babesiosis in our country (www.kidl.org.pl).

The recommendations regarding the diagnostic algorithm for cases suspected of babesiosis are on Figure 1.

TREATMENT

The majority of recommendations and indications for the treatment of human babesiosis are with regard to the infections caused by *B. microti* or *B. divergens*. Antimalaria drugs and some antibiotics are used in chemotherapy. A list of drugs of choice includes: atovaquone, azithromycin, clindamycin and quinine. Due to a possibility of antimicrobial resistance of *Babesia,* it is recommended to initiate combination therapy with the use of quinine preparations with clindamycin or atovaquone with azithromycin (9, 33). Drugs are usually administered for 7 to 10 days. However, in about 1/3 of patients, side effects of chemotherapy may be so strong that it is necessary to modify the therapy regimen or considerably reduce the dosage. Drugs which are associated with fewer side effects may not always be used in the treatment of babesiosis with an example being chloroquine. It causes fewer side effects compared to quinine, however, it is ineffective in the treatment of babesiosis caused by *B. microti* (34).

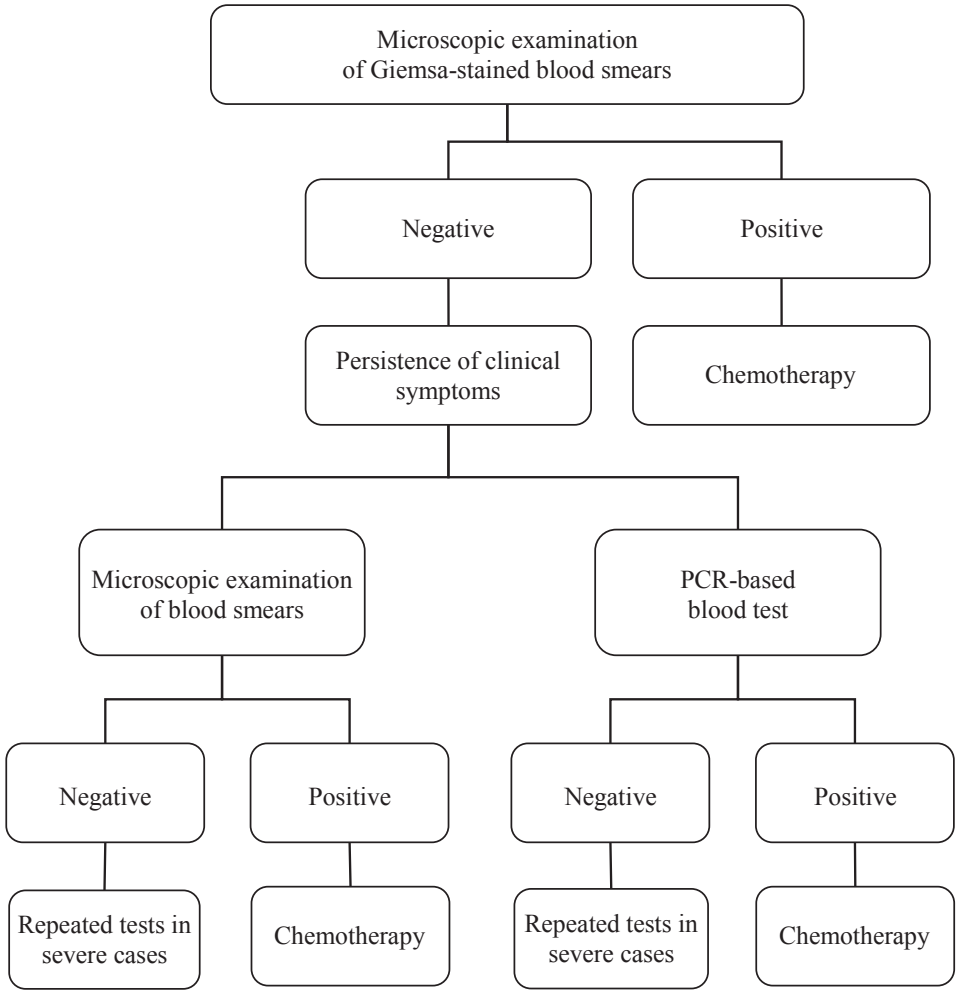

Figure 1. Diagnostic algorithm for suspicion of babesiosis in patients from risk groups, i.e.: (I) individuals living in endemic areas or returning from such areas, (II) patients who had blood transfusion within 6 months since the onset of symptoms, (III) patients with Lyme disease or anaplasmosis, poorly responding to standard treatment or presenting more intense symptoms than usually observed in such cases, based on Vannier and Krause (2012) (2).

Antiprotozoal and antibacterial drugs, including: primaquine, quinacrine, pyrimethamine, sulfadoxine-pyrimethamine, artesunate, sulfadiazine, tetracycline, minocycline, pentamidine or trimethoprim-sulfamethoxazole were ineffective in the treatment of babesiosis caused by *B. microti* and *B. divergens* (25, 33). Indications for combination therapy are the moderate and severe cases as well as the asymptomatic carriage with parasitemia lasting for more than 3 months (18, 25). In severe cases additionally, exchange transfusions may be indicated (26).

Still little is know about the susceptibility of *B. duncani*, *B. venatorum* and *B. divergens*-like in the USA and Europe whose pathogenic potential for humans was confirmed relatively recently (1, 25). However, there is no convincing evidence that considerable differences exist in the susceptibility of these pathogens to the drugs used in the therapy of *B. microti* infection (1, 12, 15, 25). So far, the duration of treatment is not determined. Furthermore, the processes of drug resistance of *B. duncani, B. venatorum,* and *B. divergens*-like were not studied (12).

Babesia may be present in the peripheral blood of patients for some time after the termination of chemotherapy. In case of patients with symptomatic babesiosis, who are treated with clindamycin and quinine, pathogens were still detectable up to 16 days. Untreated asymptomatic, 'silent babesiosis' may last for a number of months (29).

SUMMARY

Epidemiological situation of babesiosis varies worldwide and is a subject to continuous changes. In the UE countries, no increased incidence of human babesiosis is reported, however, there is a high prevalence of borreliosis which is transmitted by ticks. Recently, a nationwide obligation was introduced in the USA to register babesiosis due to a constant increase in the number of cases and extension of territories in which infections are frequently reported.

So far, no effective vaccine against babesiosis was developed. As with other tick-borne disease, prevention

of infections with *Babesia* consists in the usage of personal protective equipment during activities undertaken on forest or grassy areas. Interventions in the natural environment through intensification of farming, land amelioration or river management favour the increase in the number of *Babesia* vectors and may lead to a more frequent occurrence of human babesiosis, also in Europe.

In Poland, the testing for babesiosis is recommended for a person with clinical symptoms of the disease returning from babesiosis endemic areas. Testing for babesiosis should be also considered in case of patients with Lyme disease of acute, atypical course, poorly responding to standard treatment.

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Address for correspondence:
Dr Elżbieta Gołąb, Professor of the NIPH-NIH
Department of Medical Parasitology
National Institute of Public Health – National Institute of Hygiene
Chocimska 24
00-791 Warsaw
email: egolab@pzh.gov.pl