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Clinical and laboratory aspects of human babesiosis, a poorly investigated life-threatening parasitosis in Romania

Irina Teodorescu[1], Marian Ghervan[2], Diana Toma [3], Didona Vasilache [2]

[1]Faculty of Biology, University of Bucharest, Romania
[2]Fundeni Clinical Institute, Bucharest, Romania
[3]MedLife, Bucharest. Romania

*corresponding author e-mail address: ***teodorescubiologie@yahoo.com***

ABSTRACT

We present a number of 16 cases (two symptomatic and 14 asymptomatic) of *Babesia* infections, detected in Romania. One symptomatic case was of a previously splenectomized woman, resident in Northern Romania. The infection source was *Ixodes*, by a buffalo from her personal farm. As a consequence of previous splenectomy and the absence of early diagnosis and treatment, the patient died. Other symptomatic case was a non-splenectomized, apparently immunocompetent woman, German resident, with Romanian origin. The infection originated from an *Ixodes* bite in a Romanian touristic zone. After two weeks she presented clinical symptoms of infection, although the tick was removed and its hypostome extracted. To eliminate possible other infection causes, hematologic, bacteriologic, biochemical, coproparasitologic examinations and ELISA tests were made. Were detected: anemia, hypereosinophilia and thrombocytopenia. ELISA tests for other parasites or pathogens were negative and HIV infection was absent. In the two symptomatic cases, the patients developed: fever, fatigue, headache, arthromyalgia, hemolytic anemia, thrombocytopenia, hepatomegaly, but with differences in the symptoms severity. The diagnosis was based especially on the typical intraerythrocytic *Babesia* trophozoites in the thin peripheral blood smears stained with May-Grünwald-Giemsa. To differential diagnosis with malaria, pyriform bodies on the blood film, and lack of pigment in erythrocytes, were very important. Blood examination revealed poikilocytosis, anisocytosis, and hypochromic erythrocytes. There were detected another 14 cases with *Babesia* asymptomatic infections from 335 patients investigated in 2010-2017 to detect other infections with bacteria and parasite worms. The severity of infection (fulminate disease, moderate illness or asymptomatic infections), must be correlated with health status of patients, because *Babesia* with its opportunistic behavior can cause severe infection only if the host immune system is lowered, and if the tardiness diagnosis and treatment.

Keywords: *human babesiosis, parasitosis, babesia infections.*

1. INTRODUCTION

Babesiosis is a hemolytic parasitic disease caused by an obligate intraerythrocytic protozoan of the genus *Babesia*, also called *Nuttallia* (Apicomplexa, Sporozoea, Piroplasmida, Babesiidae). *Babesia* species have a complex life cycle that involves asexual reproduction in the mammalian host erythrocytes and sexual reproduction in their Ixodidae arthropod vectors. *Babesia* was first defined by Romanian biologist Victor Babeş, in 1888, discovered in erythrocytes of cattle. Babeş named this agent as *Haematococcus bovis* and in their honor, later the genus name has changed in *Babesia*, and family, *Babesiidae*. Because of the pear-shaped forms, the parasites genus was also called *Piroplasma*, and the family, Piroplasmidae. Worldwide, over 100 *Babesia* species have been reported, with a wide host range, including hundreds of mammal species, some bird species and men. Babesiosis is an anthropozoonosis, distributed especially in areas with a warm climate, having an important negative impact on domestic animals. Humans are infecting by few *Babesia* species and human babesiosis is not a frequent disease. The first cases of human babesiosis were identified in Europe by Skrabalo & Deanovic, in 1957, in a splenectomized farmer in the former Yugoslavia (near Zagreb), later in America, in 1966 in California [1], and in 1969 in Nantucket Island off the coast of Massachusetts [2]. From the United States have been reported approximately 1,000 cases, and in Europe only over 40 cases, number probably underestimated, as a result of a lack of investigations in many countries and a large proportion of asymptomatic infected persons. In Europe, most human infections are believed to be caused by the cattle parasite *Babesia divergens* (M'Fadyean & Stockman, 1911), documented in Austria, Belgium, Denmark, Finland, France, Germany, Ireland, Montenegro, Norway, Netherlands, Portugal, Russia, Slovenia, Spain, Sweden, Switzerland, United Kingdom and former Yugoslavia [3, 4, 5, 6, 7, 8]. Species occur especially in splenectomized or immunocompromised individuals, with high mortality rate. *Babesia divergens* was detected also in *Ixodes ricinus* vector.

Other few cases in Europe (in Austria, Italy and Germany) were due also to *Babesia venatorum* Herwaldt et al., 2003, found naturally in deer, that given initial the designation EU-1 strain (because the first three cases occurred in patients from European Union). Infections with *B. venatorum* have been documented in splenectomized, immunosuppressed and co-infected patients. In Belgium, France, Germany, Italy, Netherlands, Poland, Slovenia, Switzerland, *B. venatorum* parasite was identified also in *Ixodes ricinus* vector [9]. Recently, in Europe, some autochthonous cases of human infection with *Babesia microti* Franca, 1910 or their presence in Ixodidae have been confirmed in Belarus, Belgium, Czech Republic, Denmark, France, Germany, Hungary, Lithuania,

Netherland, Norway, Poland, Portugal, Russia, Slovenia, Sweden, Switzerland [10- 17].

In USA, babesiosis is due mainly to *Babesia microti* Franca, 1910, with rodents and insectivores primarily hosts. Other strains of *Babesia* have been reported from Washington (designated WA-1), California (CA-5) and Missouri (MO-1). Based on the characterization of isolates WA-1 and CA-5, obtained from human patients, a new species, *Babesia duncani* Conrad et al., 2006 was described [18]. WA-1 strain *Babesia* is closely related to the canine *Babesia gibsoni* Patton, 1910 and MO-1 strain showed affinity to the cattle parasite *B. divergens* (Schuster, 2002). In the USA also related human cases with *Babesia divergens*-like organisms [19].

Cases from Taiwan, Korea, have been caused by *B. microti*-like species [20], in Taiwan, the *Babesia* isolate (TW-1) being morphologically indistinguishable and serologically related to *B. microti* and in Korea this new type of *Babesia* (KO-1) is similar to *Babesia ovis* [21]. In

Japan, India, China, babesiosis have been caused by *B. microti*-like species [22- 25] but in China also related cases with *Babesia divergens* and *B. venatorum* [26, 27].

In Australia, the first case was diagnosed in 2010 [28, 29], and has recently been proven babesiosis with *B. duncani* and *B. microti* along the eastern coastline of the continent.

The cases from South Africa, Egypt, and Mexico were attributed to *B. microti* and to unidentified *Babesia* species [30, 31, 32 and 33].

Vectors for *Babesia* are the haematophagous Ixodidae species, babesiosis being a tick-borne disease. In Europe, *Ixodes ricinus* (Linnaeus, 1758) is the most common tick species vector for *B. divergens*, *B. venatorum* and *B. microti* [34, 35 and 36]. In the last years, the distribution of *I. ricinus* was registered a northern latitudinal and higher altitudinal extension, attributed to the global climate warming [37, 38, 39 and 40]. In Europe, species *Ixodes persulcatus* (Schulze, 1930) serve also as a vector for *B. divergens*. In the northeastern part of the United States, the vector for *B. microti* is *Ixodes scapularis* Say, 1821 (previously named *I. dammini* Spielman et al., 1979) and on the West Coast, *Ixodes pacificus* Cooley & Kohls, 1943. In East Asia and Japonia vector is *Ixodes ovatus* Neumann, 1899.

2. EXPERIMENTAL SECTION

From the 16 cases with symptomatic and asymptomatic infections with *Babesia*, two women patients, 30 and 36 years old develop symptomatic babesiosis, one in 1995 and the other in 2009, both infected by tick bites. In the case from 1995, the authors established the diagnosis (by microscopic examination of thin peripheral blood smears stained with May-Grünwald-Giemsa and discover typical intraerythrocytic *Babesia* parasites) and were suggested to be treated with chloroquine, clindamycin and quinine. In the case from 2009, hematologic, bacteriologic, biochemical, coproparasitologic examinations and ELISA tests for the differential diagnostic of *Toxoplasma gondii*, *Toxocara canis*, *Cysticercus* sp., *Entamoeba histolytica*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Mycoplasma pneumoniae*, *Lysteria monocytogenes* and *Borrelia burgdorferi*, were made in the Clinical Laboratory, Department of Parasitology and Immunology, of the Fundeni Clinical Institute, Bucharest. Patient survey and the recommended treatment were made in Romania (at the Center of Hematology and Bone Marrow, of the Fundeni Clinical Institute, in Bucharest) and in Germany. From the other 335 patients investigated in 2010-2016 period, 1,233 ELISA tests were made and a number of 14 asymptomatic infections with *Babesia* were detected.

3. RESULTS SECTION

(By microscopic examination of thin peripheral blood smears stained with May-Grünwald-Giemsa, two human symptomatic babesiosis cases were detected. In both, typically intraerythrocytic parasites rings, round, oval forms, single or binary pear-shaped trophozoites (pyriform bodies), and erythrocytes without parasitic pigment, were detected (Figures 1-2), all of these confirming *Babesia* presence. The absence of the pigment within erythrocytes, allows to not confusing the *Babesia* ring form with *Plasmodium falciparum* ring. It was not possible to determine the species of *Babesia*, and to verify the species of *Ixodes*.

Blood examination revealed the erythrocyte modifications: abnormal variation in shape (poikilocytosis, for example, crenate erythrocytes or burr cells, codocytes or target cells, spherocytes, ovalocytes, teardrop or pear-shaped cells), inequality in size (anisocytosis), and hypochromic erythrocytes, paler than normal, with less concentration of hemoglobin.

The first symptomatic case, documented in 1995, was a previously spleen removed surgically, 30 year-old woman patient, resident in a locality in Maramureş County (Northern Romania), hospitalized successively in Hungary and Romania (in Cluj Napoca and Bucharest). In this case, *Babesia* species has bovine origin, the infection source being *Ixodes* ticks carried by buffalos from woman personal farm. In the thin peripheral blood smear stained with May-Grünwald-Giemsa were discovered typical intraerythrocytic parasites, especially ring forms but even characteristic oval, paired pyriform bodies, without parasitic pigment. As a result of the long time parasite's presence, blood examination also revealed modifications in the majority of red blood cells: poikilocytosis, anisocytosis, and hypochromic erythrocytes. The administration of chloroquine, clindamycin and quinine, and whole-blood exchange transfusion, was ineffective. As a consequence of aggravating circumstances (the absence of an early diagnosis and treatment and mainly women being included in the greatest risk group for acquiring clinical infection with

Babesia as a splenectomized person), the case ended in death, confirming the fact that babesiosis can be fatal in asplenic patients. The correlation between the disease's severity and spleen status was proven by differences in susceptibility of infection from normosplenic members of this woman's family that developed only asymptomatic or subclinical forms of babesiosis (information obtained from anamnesis). The second symptomatic case, detected in 2009, was a 36 year-old woman, without known medical history and apparently immunocompetent, with intact spleen, residing in Germany, but of Romanian origin. In this case, the infection was acquired on May 2009 from an infected *Ixodes* bite on the left foot, in a touristic zone (Cheia, a mountain resort, surrounded by the Ciucaş and Ziganu mountains, in Prahova County), where the tick was present on the wild plants. The tick was immediately removed by pincers, but its hypostome (covered with curved teeth) remained anchored in the skin and was extracted later at Floreasca Emergency Clinic Hospital in Bucharest. Later, 15 days after receiving the tick bites, the patient was hospitalized in the Clinic of Hematology, of the Fundeni Clinical Institute-Bucharest, with some nonspecific infection symptoms: fever (up to 39^{o}C), fatigue, anorexia, vomiting, sleeplessness, headache, arthromyalgia, diarrhoea, some respiratory symptoms, emotional lability, headaches and photophobia. The physical examination revealed moderate splenomegaly and hepatomegaly. A lot of laboratory examinations have been made to detect the relevant hematologic values to infection with *Babesia*.

The relevant hematological values which can be also present in the infection with *Babesia* were anemia (hemoglobin 10.5g/dl, versus normal value 12.1 - 15.1 g/dL in women; hematocrit 29 % versus about 40 % normal value in women), hypereosinophilia (28 % eosinophils in the peripheral blood, versus normal 1-6 % of white blood cells), thrombocytopenia (100,000 thrombocytes per µL, versus normal count between 150,000 and 450,000 per µL). Negative results were obtained for: AgHBs; HVC; ELISA for *Toxoplasma* IgM; ELISA for *Toxoplasma* IgG; ELISA for *Toxocara* IgM; ELISA for cysticercosis IgM; ELISA for *Giardia* IgM; ELISA for *Chlamydia trachomatis* IgM; ELISA for *Chlamydia pneumoniae* IgM; ELISA for *Mycoplasma pneumoniae* IgM; ELISA for *Borrelia burgdorferi* IgM; coproparasitologic examination; pharingeal exudate; uroculture. HIV infection was absent. The confirmation of *Babesia* infection was established by microscopic examination of thin peripheral blood smears stained with May-Grünwald-Giemsa, revealed intraerythrocytic rings, round, oval, pear-shaped, even paired parasites resulting from trophozoites schizogony. The same red cell modifications (poikilocytosis, anisocytosis, and hypochromic erythrocytes) were detected, but in a small number of red cells. The examination of the blood smears was repeated in Germany, confirming the initial diagnosis. The treatment started in Romania (clindamycin and quinine and whole-blood exchange transfusion) was continued in Germany. After one month, new blood examinations were made in Romania and Germany, and no parasites were detected. Although all analyses and the symptomatology revealed normal values and status, to avoid the persistence of low parasitemia after the acute phase and the reappearance of the infection and to consolidate the cure, a new treatment (atovaquone associated with azithromycin) and blood examination during at least two years were recommended. It is also very important to check on co-infections with other infectious agents, because such possible additional immunosuppressive factors can explain the opportunistic behavior of *Babesia* and the severity of the infection in this normosplenic patient.

During 2010-2016 period, the infection rate with bacteria (Spirochaetes, Chlamydiaceae, Mycoplasmataceae) and worms Nematoda (Toxocaridae, Trichinellidae) and Cestoda (Taeniidae) has been investigated by ELISA in 335 normosplenic patients, with nonspecific symptoms.

Only a few infections with *Babesia* (very low infection rate of 4.179 %) have been detected. In some thin peripheral blood smears stained with May-Grünwald-Giemsa of patients tested for *Toxocara*, *Borrelia*, *Leptospira* and *Mycoplasma* a low level of parasitemia with *Babesia* trophozoites was registered.

Table 1. Results of 1233 ELISA tests performed on 335 normosplenic, most probably immunocompetent patients.

ELISA tests	Number of tests	Positive tests	Unclear	Negative tests	Babesia positive cases
Toxocara IgG	176	23	-	153	2
Chlamydia IgG	130	40	16	74	
Chlamydia IgM	143	63	24	56	
Borrelia IgG	162	107	7	48	9
Borrelia IgM	193	102	4	87	
Leptospira IgG	49	3	-	46	2
Leptospira IgM	54	7	-	47	
Trichinella IgG	30	8	-	22	
Taenia solium IgG	13	1	-	12	
Hydatid cyst IgG	185	47	1	137	
Mycoplasma pneumoniae	98	31	13	56	1
Total	1233	432	63	738	14

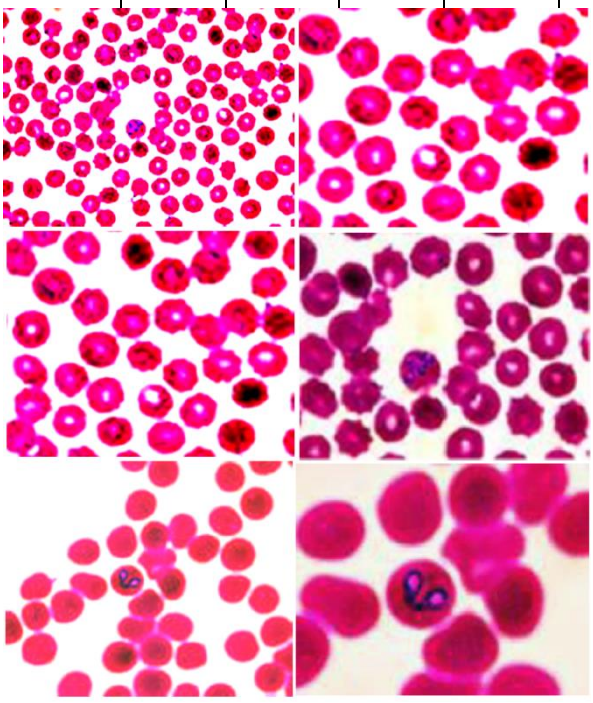

Figure 1. May-Grünwald-Giemsa stained thin peripheral blood smears with *Babesia* parasites and erythrocyte modifications.

Only a few intraerythrocytic rings, round parasites, but typical pear form bodies it is not present. No red cell modifications and no characteristic clinical manifestations were detected (asymptomatic babesiosis). The majority of positive cases with *Babesia* infection

(64.28 %) were detected in patients tested to the spirochete *Borrelia*, other tick-borne obligate parasites, whose normal reservoir is a variety of small mammals.

Canine babesiosis in Romania was reported by many authors [42, 43, 44, 45, 46, 47], and bovine babesiosis with *Babesia bovis*, *Babesia bigemina* and *Babesia divergens* is also common in Romania. Human babesiosis is poorly known in Romania. Olteanu [41] make a commentary that the first case of human babesiosis was detected by Dan Panaitescu in 1972, and subsequently, another two cases detected in animal caretakers in Mehedinţi County, but in both cases without other specifications.

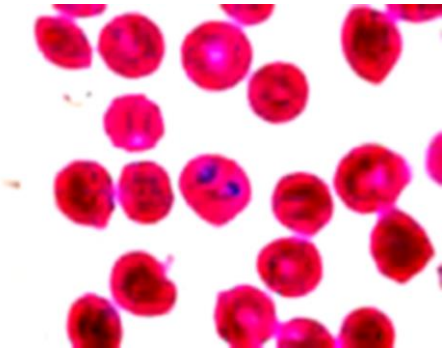

Figure 2. May-Grünwald-Giemsa stained thin peripheral blood smears with binary *Babesia* trophozoites and erythrocyte modifications.

4. CONCLUSIONS

A number of 16 cases (two symptomatic and 14 asymptomatic) of babesiosis, a rare potentially fatal parasitic disease were detected in Romania. In the two symptomatic cases, the infection was acquired through infected ticks bites. The diagnosis was based on the clinical symptoms, spleen status (splenectomy in one case), detection of the typical intraerythrocytic *Babesia* trophozoites, and especially of the presence of pear form bodies on the blood film, and lack pigment in erythrocytes (important to differential diagnosis with malaria).

In the first symptomatic case, as a result of aspleny and delayed treatment, babesiosis evolved to death. In the second symptomatic case, to eliminate other possible infection causes, hematologic, bacteriologic, biochemical, coproparasitologic examinations and ELISA tests were made. The laboratory examinations detected some hematologic values relevant for babesiosis disease: anemia, hypereosinophilia and thrombocytopenia. ELISA tests for other parasites or pathogens were negative and HIV infection was absent.

In both symptomatic patients, the same red cell modifications (poikilocytosis, anisocytosis, and hypochromic erythrocytes) were detected, accompanying the clinical symptoms of fever, fatigue, headaches, lack of appetency, vomiting, arthromyalgia, hemolytic anemia, thrombocytopenia, hepatomegaly, but in different intensity degrees. The species of *Babesia* could not be determined morphologically. In a cohort of 335 patients tested in 2010-2016 to detect other infections with bacteria and parasites worm, there were detected 14 cases with *Babesia* asymptomatic infections, 9 of these in patients tested from *Borrelia* infections. These results suggest that in Romania, *Babesia* infections probably occur more frequently and may affect apparently healthy patients, which appear as asymptomatic.

In all 16 cases, the severity of infection was varying from fulminate disease resulting in death (the case diagnosed in 1995), to-moderate illness (the case diagnosed in 2009), to asymptomatic infections (in some apparently healthy members of a female patient), must be correlated with the health status of patients. *Babesia* with its opportunistic behavior can cause severe infection in the host organism only if the host resistance or immune system is lowered (for example by lack of spleen or by co-infection with other parasites or pathogens).

It is very important the fact that the differences in the severity of symptoms, complications and disease evolution were the consequences not only of the different immune status, including spleen status (aspleny or intact spleen), but also of the moment (early or late) when the diagnosis was established and the treatment was applied.

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