Human Babesiosis in Romania cause of anemia less known

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Abstract

We present two cases of autochthonous human babesiosis in Romania. The first, documented in 1993, was a case of a splenectomized, 30 year-old woman patient, resident in a country side area of Northern Romania. The infection source in this case was Ixodes, an ectoparasite berried by a buffalo from her personal farm. As a consequence of some aggravating circumstances (included in the highest risk group for Babesia divergens infection as a prior splenectomized, the absence of early diagnosis and treatment), the most severe symptoms and complications are registered leading to death. The second case, detected in 2009, a 36 year-old woman, non-splenectomized, apparently immunocompetent is a German resident, but of Romanian origin. The infection was accidental and the source was Ixodes from a Romanian field where this is present in the environment. The parasites were inoculated into the host by tick bite. The patient presented clinical symptoms of infection after two weeks, although the tick was immediately removed and its hypostome extracted. In both cases, the patients developed: fever, fatigue, headache, arthralgia, hemolytic anemia, thrombocytopenia, splenomegaly, hepatomegaly, but there were differences in the severity of symptoms, and for the splenectomized patient, babesiosis was fatal. The diagnosis was established by microscopic examination of Giemsa-stained thin and thick blood smears, when we noticed typical intraerythrocytic parasites, especially ring and oval forms, and even paired piriform bodies, without parasitic pigment. Blood exam revealed poikilocytosis (crenate erythrocyte or burr cells, codocytes or target cells, spherocytes, ovalocytes, teardrop or pear-shaped cells), anisocytosis, and hypochromic erythrocytes.

Keyword: human babesiosis, Babesia species, Ixodes vectors, intraerythrocytic parasites, tick-borne disease

Introduction

Babesiosis is a parasitic disease caused by protozoan parasites of the genus *Babesia* (Apicomplexa, Babesiidae), which infect red blood cells. First *Babesia* species was described in cattle in 1888 by the Romanian biologist Victor Babeş (1) (after whom the genus and family were named). Due to the pear shaped forms, the parasites are commonly called *Piroplama*, and family, Piroplasmidae. The firsts human babesiosis was identified in 1957, by Skrabalo & Deanovic (2) in Europe, in the former Yugoslavia (near Zagreb), in 1966 in California (3), and in 1969 in Nantucket Island off the coast of Massachusetts.

Worldwide have been reported more than 100 species of *Babesia*, which infect many mammalian and avian species, but only a few infect humans. Most cases of human babesiosis have been reported from the United States (over 500) and Europe (approximately 40 reported mostly in Ireland, the United Kingdom, and France). Sporadic case reports of babesiosis in Japan, Korea, China, India, Mexico, South Africa, and Egypt have also been documented (4).

In many European countries, most human infections with babesiae are believed to be caused by *Babesia divergens* (5), that occur especially in splenectomized individuals (6), with high mortality, and some cases are due to *Babesia venatorum* Herwaldt et al., 2003 (EU-1 strain), identified in asplenic patients from Italy and Austria, reported also from Germany, Netherlands, Switzerland, Slovenia (7), Sweden (8), Poland (9). Some autochthonous cases of human infection with *Babesia microti* (10; 11), or this species presence in Ixodidae, in Slovenia, Switzerland, Germany, Hungary, Poland, Lithuania, Russia, Czech Republic (12) have recently been confirmed.

In USA, babesiosis is due mainly to *Babesia microti*, but other strains have been reported from Washington (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 (13) was described. WA-1 strain *Babesia* is closely related to canine species *Babesia gibsoni*

and MO-1 strain showed affinity to the cattle parasite *B. divergens* (14).

The cases from Africa, Mexico, Japan (15,16,17), Taiwan and India (18) were attributed to *B. microti* and to unidentified *Babesia* species. In Taiwan, the *Babesia* isolate (TW1) is morphologically indistinguishable from and serologically related to the rodent parasite of *B. microti* (19). In Korea was detected a new type of Babesia (KO1), similar to ovine *Babesia* (20).

Babesiosis is a tick-borne disease, the vectors for *Babesia* being the haematophagous Ixodidae species. In Europe, *Ixodes ricinus*, the most common tick species, with the highest prevalence, registered a latitudinal and altitudinal extension, fostered by the global climate warming (21). In Eastern Europe also *I. persulcatus* serve as vectors for *B. divergens*. In the northeastern part of the United States the vectors for *B. microti is Ixodes scapularis (= Ixodes dammini)* and on the West Coast, *I. pacificus*.

Material and methods

Two women patients were investigated, 30 and 36 years old, one in 1993 and the other in 2009, both infected by ticks bite. In the 1993 case, the authors' contribution consisted only in establishing the diagnosis (by microscopic examination of Giemsa-stained thin and thick patient blood smears, and discovering typical intraerythrocytic *Babesia* parasites), studying a lot of specialized literature and suggest to administer chlorochin, clindamycin and quinine treatment. In the 2009 case, hematologic, bacteriologic, biochemical, coproparasitologic exams and ELISA tests for *Toxoplasma gondii, Toxocara canis, Cysticercus, Entamoeba histolytica, Chlamydia pneumoniae, Chlamydia trachomatis, Mycoplasma pneumoniae, Lysteria monocytogenes, Borellia burgdorferi* were made in the Clinical Laboratory, Department of Parasitology and Immunology, of the Fundeni Clinic Institute, in Bucharest. Patient survey and the recommended treatment were made in Romania (at the Center of Hematology and Bone Marrow, of the Fundeni Clinic Institute, in Bucharest) and in Germany.

Results and discussions

By microscopic examination of Giemsa-stained thin and thick blood smears, two human babesiosis cases were detected. In both, typical intraerythrocytic parasites ring, round, oval forms, single pear shaped or binary trophozoites (pyriform bodies), and even very rare tetrad form (Maltese cross) and without parasitic pigment from hemoglobin were discovered (Figs. 1-2).

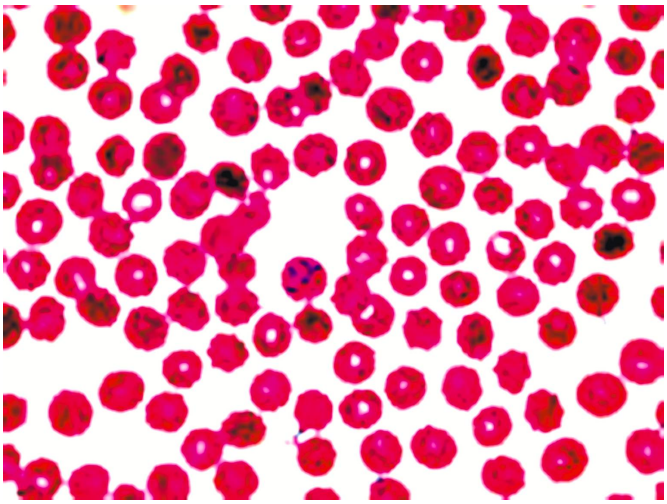

Fig. 1

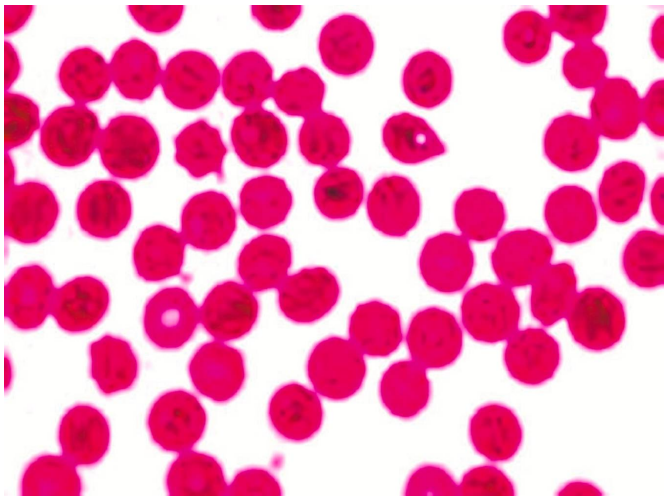

Fig. 2

Figs. 1, 2: Giemsa-stained thin blood smear with Babesia parasites and erythrocytes modifications

The absence of the hemozoin pigment within parasites cytoplasm, serve to not confuse the *Babesia* ring form, with *Plasmodium falciparum* ring form. In most of cases, the parasites positions in human erythrocytes are subcentral.

It was not possible to determine the species of *Babesia*, and to verify the species of *Ixodes*.

Blood exam revealed the same erythrocytes modifications: abnormal variation in shape (poikilocytosis, for example crenate erythrocyte or burr cells, codocytes or target cells, spherocytes, ovalocytes, teardrop or pear-shaped cells), inequality in size (anisocytosis), and erythrocytes paler than normal, with less concentration of hemoglobin (hypochromic erythrocytes) (Figs 3-5).

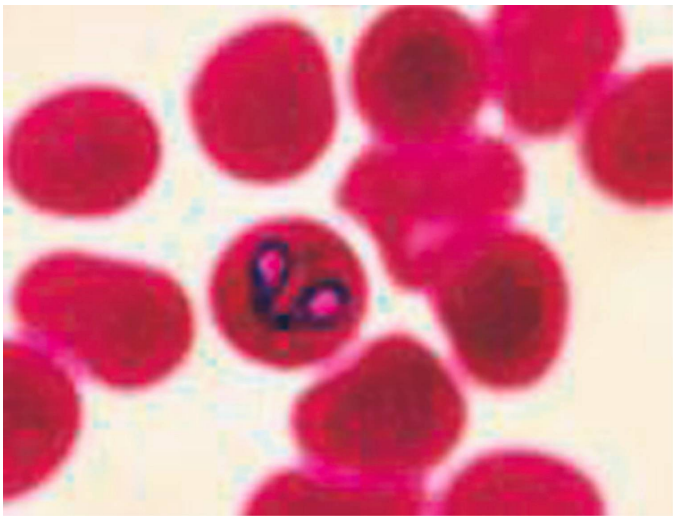

Fig. 3

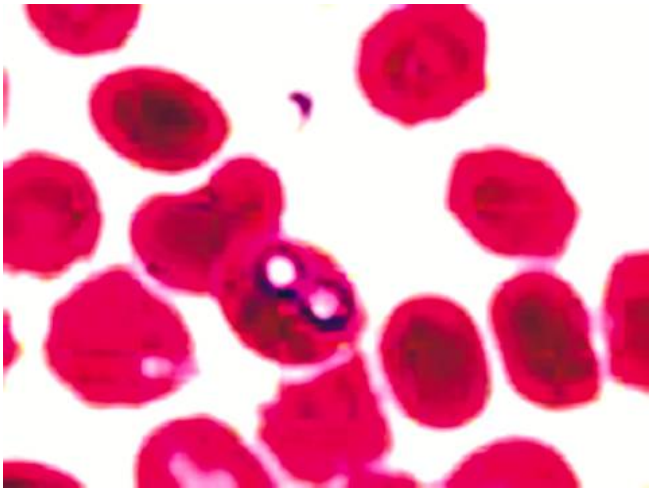

Fig. 4

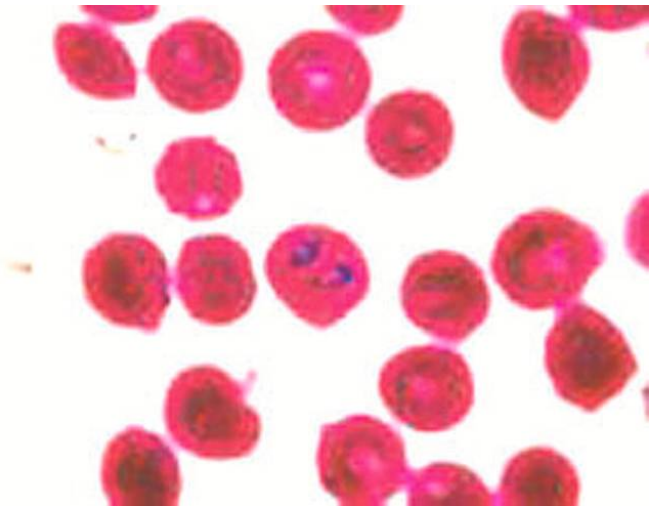

Fig. 5

Figs. 3, 4: Giemsa-stained thin blood smear with binary Babesia trophozoites and erythrocytes modification
Fig. 5: Giemsa-stained thin blood smear with paired pyriform Babesia and erythrocytes modifications

The first case, documented in 1993, was a splenectomized prior to infection, 30 year-old woman patient, resident in Negreşti Oaş, a locality in Maramureş County (side area of Northern Romania), hospitalized successively in Szeged (Hungary), Cluj and Bucharest (Romania). The infection source in this case was *Ixodes* ecto-parasite berried by a buffalo from her personal farm. In this case, *Babesia* species has bovine origin. The authors analyzed Giemsa-stained thin and thick blood smears, and discovered typical intraerythrocytic parasites, especially ring forms but even characteristic paired pyriform bodies, without parasitic pigment As a result of the parasite presence, blood exam also revealed red blood modifications: poikilocytosis, anisocytosis, and hypochromic erythrocytes. In the Bucharest hospital, the attempt to administer chlorochin, clindamycin and quinine, and whole-blood exchange transfusion remained ineffectual.

As a consequence of some aggravating circumstances (the absence of an early diagnosis and treatment and mainly included in the greatest risk group of acquiring clinical infection with *Babesia divergens* as a splenectomized patient), the most severe symptoms and complications registered ended in death.

The correlation between the disease severity

and spleen status was proving by differences in susceptibility of infection from normosplenics members of this woman family that develop only asymptomatic or subclinical forms of babesiosis.

The second case, detected in 2009, was of a 36 year-old woman, normosplenic, previously healthy and apparently immunocompetent, residing in Germany, but of Romanian origin.

In this case the infection was accidental on May 14th 2009 and source was an infected *Ixodes* from a Romanian field, in a touristic zone (Cheia, a mountain resort, sourrounded by Ciucaş and Zăganu mountains, in Prahova county), where the tick was present in the environment.

The *Babesia* parasites were introduced in the host's left foot by the tick bite. The tick was immediately removed by pincers but its hypostome (covered with recurved teeth) remained anchored into the skin and was extracted later at Floreasca Emergency Clinic Hospital in Bucharest.

After the two weeks of the incubation period, the patient was hospitalized in the Clinic of Hematology, of the Fundeni Clinical Institute-Bucharest, with some nonspecific infection symptoms: fever (38°C-38.5°C), fatigue, inapetency, vomiting, sleeplessness, headache, arthralgy, diarrhea, feeble respiratory insufficiency, emotional lability, and photophobia. The physical examination signaled splenomegaly and hepatomegaly.

Laboratory exams revealed: hemoglobin 10.5g/dl; hematocrit 29 %; eosinophils 28 %; monocytes 12 %; lymphocytes 21 %; platelets (thrombocytes) 110,000/mm3; VSH 62/94; urea 22 mg/dl; glycemia 108 mg/dl; cholesterol 98 mg/dl; AST 24 U/L; ALT 31 U/L; amilase 32 U/L; alkalin fosfatasis 210 U/L; Na 132 mEq/l; K 3.98 mEq/l; PT 7.2 g/l; albumin 2.52 g/l; alfa1 0.78 g/dl; alfa2 1.36 g/dl; beta 0.94 g/dl; gama 2.35 g/dl; seric crioglobulines.

The relevant hematologic values were anemia, hypereosinophilia, thrombocytopennia.

Negative results: AgHBs; HVC; ELISA for *Toxoplasma* IgM 0.339 (cut off 0.500); ELISA for *Toxoplasma* IgG 0.761 (cut off 0.500); ELISA for *Toxocara* IgM 0,092 (cut off 0.360); ELISA for Cysticercosis IgM 0.014 (cut off 0.500); ELISA for *Giardia* IgM 0.117 (cut off 0.400); ELISA for *Chlamydia trachomatis* IgM 0.045 (cut off 0.600); ELISA for *Chlamydia pneumoniae* 0.398 (cut off 0.500); ELISA for *Mycoplasma pneumoniae* 0.271 (cut off 0.500); ELISA for *Borrelia burgdorferi* IgM 0.268 (cut off 0.500), coproparazitologic exam, pharingian exudate, uroculture.

HIV infection was absent.

The diagnosis of *Babesia* infection was established by microscopic examination of thick and thin blood smears stained with Giemsa, and detection of intraerythrocytic parasites: ring form, round, oval, pear-shaped, even paired pyriform and very rarely tetrad form ("Maltese cross").

The same red cells modifications (poikilocytosis, anisocytosis, and hypochromic erythrocytes) were detected.

To verify the diagnosis, the examination of the blood smears was repeated in Germany.

The treatment recommended in Romania (clyndamicin and quinine and whole-blood exchange transfusion) was confirmed and applied in Germany.

After one month, new blood exams were made in Romania and Germany, and they registered no parasite presence. Although all analysis and the symptomatology revealed normal values, to avoid the persistence of low parasitemia after acute phase and the infection reappearance and to consolidate the good result, the patient was recommended to apply a new treatment (atovaquone associated with azithromycin) and blood control in the minimum following two years. Very important is also to verify a co infection with other infectious agent, because this additional immunosuppressive factor can explain *Babesia* opportunistic behavior and the infection severity in this normosplenic patient.

In Romania, Olteanu et al. (22) reports that the first case of human babesiosis was diagnosed by Panaitescu in 1972, and subsequently, another two cases were diagnosed in animal caretakers in Mehedinti County, without other specifications.

Conclusions

Two cases of Babesiosis, a rare parasitic disease, potentially fatal, were detected in Romania.

In one case, as a result of aspleny, Babesiosis conducted to fatality.

The species of Babesia was difficult to determine morphologically.

The infections were acquired through an infected Ixodid tick bite.

Diagnosis was based on clinical manifestation, tick bite, spleen status (splenectomy), and especially on examination of stained blood smear and typical intraerythrocytic Babesia trophozoites detection. To correct diagnosis, very important were the presence of pyriform bodies and Maltese cross and lack of cytoplasm pigment.

Both patients developed fever, fatigue, headache, inapetency, vomiting, arthralgia, hemolytic anemia, thrombocytopenia, splenomegaly and hepatomegaly, but in different degrees of intensity.

The differences in the severity of symptoms, complications and disease evolution were the consequences of the difference in their immunity competence, spleen status (aspleny or intact spleen), and the moment when the diagnosis was established and the treatment was applied (early or undue).

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