

Symptomatic co-infection with *Babesia microti* and *Borrelia burgdorferi* in patient after international exposure; a challenging case in Poland

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Abstract

The report presents a well-documented case of symptomatic co-infection of *Babesia microti* and *Borrelia burgdorferi* in a Polish immunocompetent patient after travelling to Canada and the USA.

Key words

immunocompetent host, Babesiosis, Lyme borreliosis, symptomatic coinfection

INTRODUCTION

Babesiosis is a tick-borne zoonosis caused by haemoparasites of the protozoan genus *Babesia*, transmitted by bites from the infected ticks, especially *Ixodes*. The clinical presentation of babesiosis in humans varies from asymptomatic to mild flu-like symptoms to a severe malaria-like disease [1, 2]. In Europe, symptomatic disease occurs in most cases in immunocompromised patients, especially splenectomized, and is caused mainly by *Babesia divergens* [3]. Symptomatic infection by *Babesia microti* occurs in the USA in either immunocompetent or immunocompromised individuals [1, 2, 4].

Lyme disease (LD) or Lyme borreliosis (LB), an emerging infectious zoonosis transmitted by ticks of the genus *Ixodes*, has a worldwide distribution. With approximately 20,000–30,000 confirmed cases reported in 2003–2012 (incidence 7.0/100,000 in 2012) (www.cds.gov/lyme/stats), it has become the most common vector-borne disease in the USA. In Poland, 12,779 (33.2 per 100,000 population) human cases of LB were noted in 2013 (www.pzh.gov.pl) with the highest incidence (100.2/100 000) reported in the north-eastern region (Podlaskie Province). The causative agent of the disease is *Borrelia burgdorferi* sensu lato (s.l.), a bacteria of the family *Spirochaetaceae*. While in Europe, at least five species of the *B. burgdorferi* s.l. complex are pathogenic for humans: *B. afzelii*, *B. garinii*, *B. bavariensis*, *B. spielmanii* and *B. burgdorferi* sensu stricto (s.s.) [5], the latter genospecies is the only human-pathogenic species known in North America [6].

The case is presented of a symptomatic co-infection of *B. microti* and *Borrelia burgdorferi* in a Polish immunocompetent patient after travelling to Canada and the USA.

CASE REPORT

A 48-year-old woman was admitted to the Hospital of Infectious Diseases in Warsaw with a fever of 39–40 °C, chills, and a headache lasting 10 days. Her previous medical history was non-contributory. Symptoms started during a 5-week-long trip to Canada and the northeastern seashore of the USA, including the White Mountains of New Hampshire, where the patient hiked and camped. Due to the fever and headache, she was started on a 7-day course of norfloxacin by a physician in the USA, but experienced no improvement. During admission, aside from high fever, no abnormalities were found in the physical examination. Her blood at the time revealed the following values: haemoglobin level of 14 g/l, haematocrite value of 42% and white blood cells count of 4.2 G/l. C reactive protein level was high (251 mg/dl), procalcitonin slightly elevated (1 mg%), and elevated liver enzymes activity: ALT – 135 U/l, AST-65 U/l, GGT 222 U/l and ALP 270 U/l. Blood culture was negative.

The patient was treated with a wide spectrum antibiotic regimen: ceftriaxone, amikacin and metronidazole. Body temperature decreased to 37 °C, but after 4 days the fever returned. The antibiotic regimen was changed to meropenem and vancomycin. One week after admission, the patient developed facial nerve palsy and stiffness of the neck. In CSF, mild inflammatory changes were found (31 white blood cells/μl, 83% lymphocytes). In blood and in CSF, IgM antibodies against *B. burgdorferi* by ELISA (Borrelia IgM Recombinant Antigen, Biomedica, Austria) were detected. IgM antibody levels were greater in CSF. IgG antibodies were negative. Results were confirmed by Western blot test (recomLine Borrelia Mikrogen, Germany). The recomLine immunoassay was based on recombinant antigens for the determination of IgM and IgG antibodies against *Borrelia burgdorferi* sensu lato in human serum or CSF. In these tests, the OspC homologues of 4 genospecies (*B. burgdorferi* s.s., *B. garinii*, *B. afzelii*, *B. spielmanii*) were detected. Reaction IgM with p100, p41 and OspC of four *Borrelia*

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genospecies – the strongest with *B. garinii* and the weakest with *B. burgdorferi* s.s, was observed.

Neuroborreliosis was diagnosed. The patient was febrile with malaise and developed anaemia (haemoglobin 8.6 g/dl) and decrease of platelets count (30 G/l). The malaria antigen test was negative. Examination of blood smears stained with Giemsa revealed *Babesia* spp. infection (ring forms and tetrad forms) with parasitemia of 3% (Fig. 1).

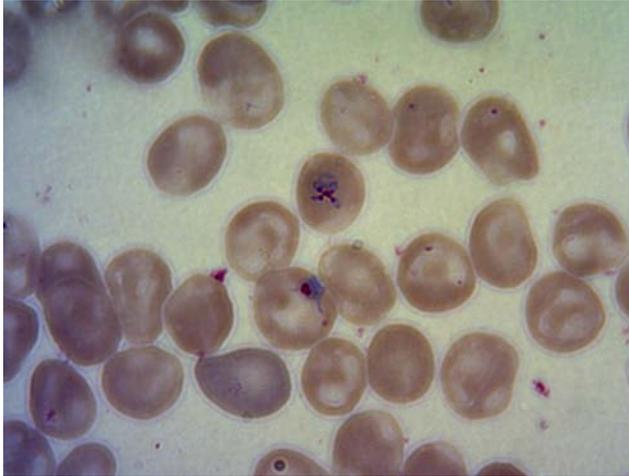


Figure 1. *Babesia microti* in a thin blood smear stained with Giemsa. Typical tetrad form (Maltese Cross) and trophozoite. X 1000

To confirm microscopic findings and to differentiate the species of *Babesia*, molecular studies were performed. Two primer sets: Div up / Div down, EU1 up / EU1 down, designed to amplify 353-bp and 362-bp fragments 18S rRNA gene, were used for the detection of *B. divergens* and *Babesia* spp. EU1 (*B. venatorum*), respectively [7]. For detection of *B. microti*, species-specific outer primers Bab1 and Bab4, and inner primers Bab2 and Bab3 amplifying 238-bp and 154-bp fragments, respectively, of a gene encoding the nuclear small sub-unit ribosomal RNA (ss-rDNA) (18S rRNA) were used [8]. PCR results revealed the presence of the DNA of *B. microti*. Positive PCR products generated by Bab1/Bab4 primers were then sequenced analyzed with an ABI PRISM 3100 genetic Analyser (Applied Biosystem 850) according to the manufacturer's protocol.

The obtained DNA sequence of 236-bp (KJ783438) was compared with sequences of *Babesia* spp. deposited in the GenBank database using NCBI BLAST network service. This showed the 100% identity with the corresponding fragment of 18S rRNA gene of the North American zoonotic *B. microti*: reference strain GI (AF231348) and the isolate Gray (AY693840), isolated from human blood and 98.7% similarity (3 bp difference) to *B. microti* isolate Jena (EF413181) from a German patient. The deposited sequences of Gray and Jena isolates, when compared, were almost identical (99.8%) as they differ by only 4 nucleotides. However, 3 differences occurred in the fragment amplified and analyzed, which helped identify the detected *B. microti* as the most similar to isolate Gray, rather than to isolate Jena.

A 3-day treatment with atovaquone/proquanil was initiated, and continued with doxycycline, azithromycin and clindamycin for 4 weeks. Therapy with ceftriaxone was continued for 4 weeks as a treatment of neuroborreliosis. The fever, malaise, and headache were resolved in 24

hours. The patient had continuous haemolytic anaemia (minimal haemoglobin 8 g/dl), and developed splenomegaly (178×63 mm by ultrasound) complicated by splenic infarctions. After 3 weeks of treatment, all these symptoms resolved. Facial nerve palsy vanished in 3 weeks. On control three months after discharge patient was symptom-free.

DISCUSSION

The presented case describes a symptomatic case of babesiosis and borreliosis in a Polish immunocompetent patient. Diagnosis of *B. microti* infection was well-documented and finally confirmed by specific PCR. As the patient had travelled to the USA, 2 potential sources of babesiosis and borreliosis had to be taken into consideration. An infection with *B. microti* could be acquired in Poland or in North America. The patient camped and hiked in a known endemic area for babesiosis 10 days before developing fever and 3 weeks before symptoms of neuroborreliosis. Thus it was supposed that the source of both infections was American, although *B. microti* homologous to isolate Gray has also been recently detected in *I. ricinus* from Poland [9]. *B. microti* can cause symptomatic illness in immunocompetent persons and many such cases have been reported in USA [1, 4]. In the Western blot test, reactivity with OspC of 4 *Borrelia* genospecies was observed and the strongest reaction with *B. garinii*, which is the most frequently cause of neuroborreliosis in Europe. It is possible that Lyme disease had a Polish origin and superinfection with *B. microti* caused the onset of the neurological symptoms.

The majority of described *Babesia* infections in Europe are caused by *B. divergens* [3], while in the USA by *B. microti* [2, 4]. In the USA there are 7 endemic regions, whereas in Europe there is almost no data available about the *Babesia* epidemiology. Although both species have been isolated from *Ixodes* ticks in different European countries, cases of *B. microti* human infection are rare [2, 3]. However, one confirmed and 2 probable autochthonous cases have been recently reported from Germany [10] and Switzerland [11], respectively. The studies on seroprevalence of *Babesia* antibodies in humans exposed to ticks in Germany showed that 5.4% and 3.6% of investigated group (476) were seropositive for *B. microti* and *B. divergens*, respectively [12]. Serum samples from 1.5% of 396 Swiss residents reacted to *B. microti* antigen [13]. These results suggest that both species can infect the European population more frequently than previously believed.

In Poland, the first imported case of human babesiosis was described in 1997, and concerned a Polish sailor who acquired an infection with *B. microti* in Brazil [14]. Recently, babesiosis has been diagnosed in a patient with ulcerative colitis [15]. Different *Babesia* sp. stages were observed on a Giemsa-stained smear of peripheral blood. The infection was then confirmed by the inoculation of laboratory mice with the patient's blood. However, detection and differentiation of *B. microti* and *B. divergens* by PCR failed. The source of infection remained unknown, but the patient, who lived in the vicinity of forests, declared tick bites. Moreover, in a retrospective study of 24 tick-exposed individuals from south-eastern Poland, *Babesia* spp. was detected in the blood of one person infected with *B. burgdorferi*. Further molecular investigations showed that these piroplasms were 98.9% homologous with *B. divergens* and *Babesia* EU1 [16].

In the reported case, diagnosis of babesiosis was delayed because of lack of awareness of the possibility of symptomatic babesiosis in an immunocompetent woman in Poland. Given the presence of both *Borrelia* spp. and *Babesia* spp. in *Ixodes* ticks in Europe, co-infection may exist in Europe. This report highlights the difficulty in diagnosing such cases and the necessity for careful anamnesis to consider appropriate differentials. Babesiosis should be taken into consideration in differential diagnosis in patients with acute febrile disease and history of tick bite or tick exposure. Giemsa-stained smear of peripheral blood is good diagnostic tool even in spite of lack of easy accessibility to specific diagnostic tests, but to make diagnosis sure specific PCR and serologic tests should be done.

The patient gave written permission for publication of this case report.

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