CASE REPORT

Annals of Agricultural and Environmental Medicine 2015, Vol 22, No 1, 51–54
www.aaem.pl

First report of two asymptomatic cases of human infection with *Babesia microti* (Franca, 1910) in Poland

Renata Welc-Falęciak[1], Agnieszka Pawełczyk[2], Marek Radkowski[2], Sławomir A Pancewicz[3], Joanna Zajkowska[3], Edward Siński[1]

[1] *Department of Parasitology, Faculty of Biology, University of Warsaw, Poland*
[2] *Department of Immunopathology of Infectious and Parasitic Diseases, Medical University of Warsaw, Poland*
[3] *Department of Infectious Diseases and Neuroinfections, Medical University in Białystok, Poland*

Welc-Falęciak R, Pawełczyk A, Radkowski M, Pancewicz SA, Zajkowska J, Siński E. First report of two asymptomatic cases of human infection with Babesia microti (Franca, 1910) in Poland. Ann Agric Environ Med. 2015; 22(1): 51–54. doi: 10.5604/12321966.1141394

Abstract

Human infection by *Babesia microti* has been recognized as an emerging zoonosis with important public health implications worldwide. In Europe the reported cases of human babesiosis have been attributed mostly to *B. divergens* infection, with only sporadic cases of the disease caused by *B. microti* or *B. venatorum*. This study, based on molecular methods (PCR, R-T PCR, DNA sequencing and phylogenetic analysis), reveals for the first time in Poland, asymptomatic infection with *B. microti* in immunocompetent healthy individuals working in forest ecosystems. Of the 58 professional foresters examined, two (3.4%) were identified as *B. microti*-positive by specific PCR. The results of this study also provide strong evidence that in eastern Poland, where tick-borne diseases (TBDs) are endemic, there is a potential risk of acquiring human babesiosis due to zoonotic *B. microti* parasites commonly found in rodents and *I. ricinus* ticks. The potential public health importance of this finding is discussed.

Key words

Babesia microti, asymptomatic infection, immunocompetent forester workers, forest ecosystem, eastern Poland

INTRODUCTION

Human babesiosis is an emerging zoonotic tick-borne disease (TBD), caused by intraerthrocytic protozoa of the genus *Babesia* within the Piroplasmoridae [1]. It is found on all inhabited continents, with an increasing number of cases recognized every year. In some regions of the northern hemisphere, the incidence of human infections with *Babesia* parasites has increased dramatically in the last decade, from 4–20-fold [2]. Most reported cases in Europe have been attributed to *B. divergens*, and the first recorded case of infection with this species occurred in a splenectomized farmer in Croatia [3]. However, a few were caused by *B. venatorum* and *B. microti* [4].

The primary vectors of the majority of zoonotic species of *Babesia* are hard exophilic ticks of the *Ixodes ricinus-persulcatus* 'complex' [5, 6]. A few blood transmission acquired cases of babesiosis, often caused by *B. microti*, have been reported in the USA [7]. Another route of *Babesia* transmission, which occurs sporadically, is vertical infection from mother to infant [8]. In Europe, however, *B. microti* is primarily transmitted by nymphs and adults of the tick *I. ricinus*.

Babesiosis is frequently asymptomatic in healthy immunocompetent people, but severe and often fatal cases may occur in immunodeficient individuals, especially in asplenic subjects, those of advanced age (> 50 years old), and other patients with compromised immunity [4, 9]. The clinical manifestation of babesiosis can range from subclinical infection to fulminate disease, often resulting in death [2]. Most symptomatic patients become ill 1–4 weeks after the bite of a *Babesia*-infected tick, or 1–9 weeks after transfusion of contaminated blood products [10]. Asymptomatic infection with low parasitemia may persist for several months or for more than a year if the infected person does not receive therapy [4]. There is a direct correlation between the immunological status of the infected person and the severity of illness they experience following babesial infection. However, clinical cases are rare, and the risk of severe babesiosis is rather low compared with other TBDs. The main symptom of babesiosis is fever, with a peak temperature of over 40 °C, which may be accompanied by chills, sweats, fatigue, headache and myalgia.

Despite the implementation of molecular analysis to study the phylogeny of *Babesia* parasites, the classification of *B. microti* parasites, which form a species 'complex' within the Piroplasmoridae with *B. microti* [11] as a type species, is still controversial [12, 13].

The purpose of the presented study is to investigate the prevalence and risk of *B. microti* infections in healthy professional foresters working in the forest ecosystem in eastern Poland, where TBDs are endemic.

MATERIALS AND METHOD

Human sample collection and DNA isolation. A total of 58 immunocompetent individuals (41 male, 17 female, mean age 46.4, age range 20–59) were examined in early summer (June) 2011. The study participants were randomly selected forestry workers, employed in the Podlaskie province of eastern Poland. Demographic and epidemiological data were

Address for correspondence: Renata Welc-Falęciak, Department of Parasitology, Faculty of Biology, University of Warsaw, Miecznikowa 1, 02-096 Warsaw, Poland
E-mail: rwelc@biol.uw.edu.pl

Received: 25 June 2013; Accepted: 05 November 2013

collected using a questionnaire, and blood samples taken into 1 mM EDTA for molecular analyses. Genomic DNA was extracted from whole blood using a DNAeasy Blood and Tissue Kit (Qiagen, Crawley, UK) and stored at –20°C prior to analysis.

Polymerase chain reaction (PCR) analysis. Detection and genotyping of *Babesia* isolates were performed by PCR amplification and sequencing of a fragment of the *18S rRNA* gene. The primers and cycling conditions used in this study have been described previously [14]. Reactions were performed in a final volume of 20 µl containing 1 µM of each primer, 1× PCR buffer, 2 mM $MgCl_2$, 0.33 mM dNTPs (Eurobio, Lille, France), 1 U *Taq* DNA polymerase, and 5 µl of the DNA extracted from blood samples. DNA of the *Theileria sergenti, B. canis, B. ovis* and *B. microti* King's College strain isolated from BALB/c mouse blood were used as a positive control. Template DNA was omitted from the negative control. PCR amplicons were analyzed by electrophoresis in 2% agarose gels and visualized using Midori Green stain (Nippon Genetics Europe GmbH). These DNA fragments were purified using an Axygen Clean-up purification kit (Axygen, USA) and sequenced in both directions (Genomed S.A., Poland).

Real-Time PCR (RT PCR) analysis. Quantitative real-time PCR was used to detect the genetic material of *B. microti* in the DNA extracted from whole-blood specimens, employing the same primers as previously. Reactions were performed in a final volume of 20 µl and contained 0.5 µM of each primer, 2 µl of Fast Start DNA Master SYBR Green I (Roche Diagnostic, Mannheim, Germany), 2 mM $MgCl_2$ and 2 µl of template DNA. The amplification was performed using a LightCycler (Roche Molecular Biochemicals, Mannheim, Germany). Thirty cycles: 30 s denaturation at 95 °C, 5 s annealing at 60 °C, and 30 s extension at 72 °C were employed. The specificity of the signal was confirmed by melting curve analysis in relation to positive control samples.

Phylogenetic analysis. DNA sequence alignments and phylogenetic analysis were conducted using MEGA version 5.0 [15]. Phylogenetic trees were created using alignments performed with the Kimura-2 parameter algorithm as a distance method and NJ as the tree construction method. For comparison, sequences of *Babesia*, *Theileria* and *Cytauxzoon* obtained from GenBank (www.ncbi.nlm.nih.gov) were included in the sequence alignment. The stability of the inferred phylogenies was assessed by bootstrap analysis of 1,000 randomly generated sample trees.

Nucleotide sequence accession number. The partial sequence of the 18S ribosomal RNA gene of a new *Babesia microti* isolate (H295) was been deposited in GenBank (Accession No. KC470047).

RESULTS

Among the 58 foresters examined, in two cases (3.4%) an asymptomatic infection with *B. microti* was detected. These two healthy immunocompetent individuals, both over 45 years old, had been bitten several times by *I. ricinus* ticks while working in the forest ecosystem during the last two years. Parasites in their blood were positively identified as *B. microti* by PCR, RT-PCR and sequencing of the amplified *18S rRNA* gene fragment.

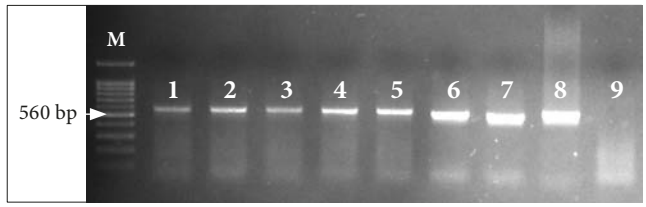

Figure 1. *Babesia microti 18S rRNA* gene fragment amplified from DNA extracted from infected human blood and *I. ricinus* ticks, analyzed by 2% agarose gel electrophoresis. The following sources of template DNA were used in the PCRs: M- 100 bp Ladder DNA marker (100; 200; 300; 400; 500; 600; 700; 800; 1000; 1500; 2000; 3000 bp), 1 and 2 – *B. microti*-infected human blood, two individuals; 3 and 4 – *B. microti*-infected *I. ricinus* ticks, nymph and female; 5 – *B. microti* positive control; 6 – *Babesia canis* positive control; 7 – *Babesia ovis* positive control; 8 – *Theileria sergenti* positive control; 9 – negative control

A 560-bp fragment of *18S rRNA* gene fragment was amplified by PCR using DNA isolated from the blood of the infected individuals (Fig. 1). Phylogenetic analysis showed 100% homology of the obtained sequence with the *18S rRNA* gene of *B. microti* Jena/Germany (EF413181), a zoonotic strain infectious to humans (Fig. 2). The specificity and sensitivity of *Babesia* detection in the two identified cases were additionally confirmed by RT PCR and melting curve analysis of the amplified products (Fig. 3).

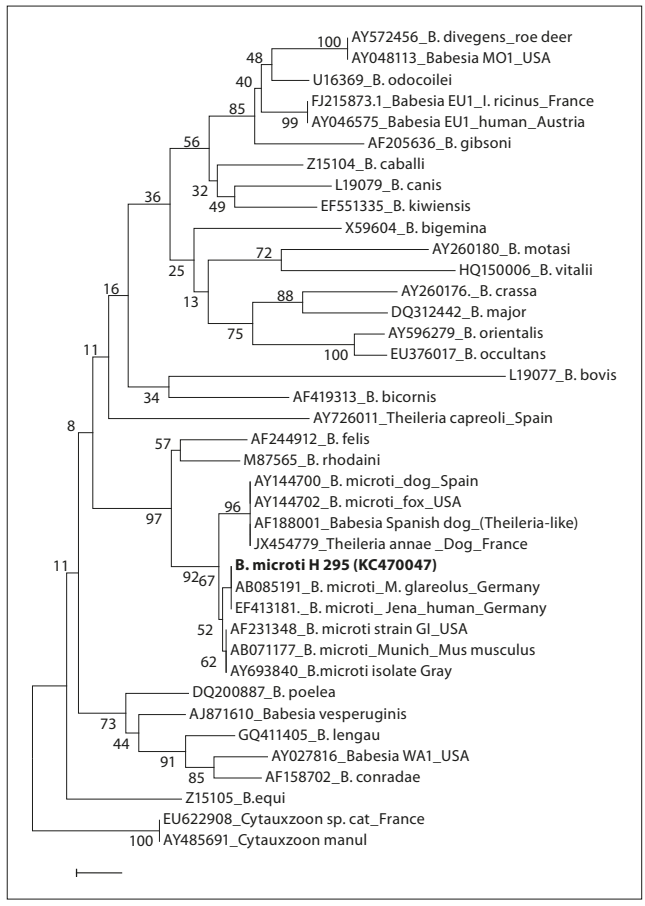

Figure 2. Phylogenetic tree of *Babesia* isolates identified and selected isolates from GenBank, based on the sequence of the *18S rRNA* gene. Numbers at the nodes of the tree indicate bootstrap values (1,000 replicates). The nucleotide sequence of *Cytauxzoon* spp. were used as an outgroup. Isolates marked in bold

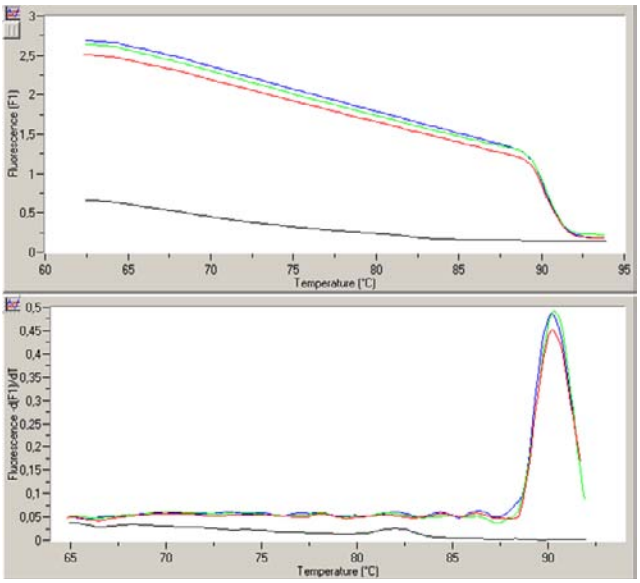

Figure 3. Melting curve analysis of *18S rRNA* gene fragments amplified by Real-Time PCR from DNA isolated from *B. microti*-infected human blood samples using specific primers. The y-axis indicates fluorescence intensity, the x-axis indicates temperature. Melting temperature of the DNA fragment is 90 °C.
Blue – sample 1; Green – sample 2; Red – positive control; Black – negative control

DISCUSSION

In the last decade of the 20th century, new tick-borne diseases (TBDs) emerged as threats to public health in Europe. These TBDs are mostly due to infections with *Borrelia burgdorferi* s.l., *Anaplasma phagocytophilum*, tick-borne encephalitis virus (TBEV) and *Babesia* spp: pathogens primarily transmitted by *I. ricinus-persulcatus* 'complex' ticks. Human babesiosis in Europe is mostly caused by *B. divergens*, but some have been caused by *B. venatorum* and *B. microti*. To date, approximately 40 cases have been reported, mainly in France, Ireland and the UK, with a few cases described in Sweden, Switzerland, Spain, Portugal and Croatia [6, 16, 17, 18]. To-date, only three cases of *B. microti* infection in humans have been reported in Europe [19, 20, 21], one of which remains controversial [22]. Infection with *B. venatorum*, formally designated *Babesia* sp. (EU1), in Europe, was first described in two asplenic patients from Italy and Austria [23], and two further cases have since been documented, one in a splenectomized and immunosuppressed (Rituximab) patient from Germany [24], and the other in a person from Poland who was co-infected with *Borrelia burgdorferi* [25].

The presented study, based on molecular analysis, is the first to describe asymptomatic *B. microti* infection without any subclinical symptoms in immunologically competent persons in Poland. Moreover, no subclinical or clinically confirmed cases of *Babesia* infection reported in Poland have previously been verified by molecular assays. This study demonstrates that the quantitative RT PCR assay is highly effective as a diagnostic tool for the detection of *B. microti* infection in whole blood.

Phylogenetic examination of the two isolates of *Babesia* identified in the present study, based on a *Babesia 18S rRNA* gene sequence, revealed their close relationship with *B. microti* Jena/Germany, a zoonotic strain pathogenic for humans. Both are grouped as sister clades within the piroplasms represented by *Theileria annae* and the other *Babesia* spp. of carnivores, referred to by Criado-Fornelio et al. [26] as Archaeopiroplasmids. Recently, the first genome-wide phylogenetic analysis of *B. microti* indicated that this species is significantly distinct from species of either the *Babesia* or *Theileria* genus, and suggested that it should be classified as a new clade in the phylum Apicomplexa [12].

Generally in Europe, there is a dearth of molecular data to confirm human cases of babesiosis, and the real prevalence in humans is probably underestimated because of subclinical infection or co-infection with other TBD pathogens, such as *Borrelia burgdorferi* s.l. or *Anaplasma phagocytophilum*. Field surveys in the north-east of Poland have shown that between 12–23% of wild rodents in forest ecosystems are infected with *B. microti* [27, 28]. In these ecosystems, the prevalence of infection in the abundant nymphs and adults of *I. ricinus* ticks was over 1%. It is noteworthy that a zoonotic genotype of *B. microti* (Jena strain) was recently identified in *Babesia*-infected ticks from this region [29].

The findings of the presented study confirm the existence of natural foci of human babesiosis. Foresters as a group are occupationally exposed to zoonotic *B. microti* parasites in the Polish forest ecosystem, and thus are at potential risk of infection.

Acknowledgements

The research forms part of Project No. NN 404 795240, supported by the Polish Ministry of Science and Higher Education in Warsaw.

REFERENCES

1. Kjemtrup AM, Conrad PA. Human babesiosis: an emerging tick-borne disease. Int J Parasitol. 2000; 30: 1323–1337.
2. Vannier E, Gewurz BE, Krause PJ. Human babesiosis. Infect Dis Clin North Am. 2008; 22: 469–488.
3. Škrabalo Z, Deanovic Z. Piroplasmosis in man: report on a case. Documenta de Medicina Geographica et Tropica 1957; 9: 11–16.
4. Vannier E, Krause PJ. Current concepts: human babesiosis. New Eng J Med. 2012; 366: 2397–2407.
5. Tsao JL. Reviewing molecular adaptation of Lyme borreliosis spirochetes in the context of reproductive fitness in natural transmission cycles. Vet Res. 2009; 40: 1–42.
6. Gray JS, Zintl A, Hildebrandt A, Hunfeld KP, Weiss L. Zoonotic babesiosis: overview of the disease and novel aspects of pathogen identity. Ticks Tick Borne Dis. 2010; 1: 3–10.
7. Leiby DA. Transfusion-transmitted *Babesia* spp.: bull's-eye on *Babesia microti*. Clin Microbial Rev. 2011; 24: 14–28.
8. Joseph JT, Purtill K, Wong SJ, Munoz J, Teal A, Madison-Antenucci S, Horowitz HW, Aguero-Rosenfeld ME, Moore JM, Abramowsky C, Wormser GP. Vertical transmission of *Babesia microti*, United States. Emerg Infect Dis. 2012; 18: 1318–1321.
9. Krause PJ, Gewurz BE, Hill D, Marty FM, Vannier E, Foppa IM, Furman RR, Neuhaus E, Skowron G, Gupta S, McCalla C, Pesanti EL, Young M, Heiman D, Hsue G, Gelfand JA, Wormser GP, Dickason J, Bia FJ, Hartman B, Telford SR 3rd, Christianson D, Dardick K, Coleman M, Girotto JE, Spielman A. Persistent and relapsing babesiosis in immunocompromised patients. Clin Infect Dis. 2008; 46: 370–376.
10. Herwaldt BL, Linden JV, Bosserman E, Young C, Olkowska D, Wilson M. Transfusion-associated babesiosis in the United States: a description of cases. Ann Int Med. 2011; 155: 509–519.
11. Franca C. Sur la classification des piroplasmes et description de deux de ces parasites. Arq R Inst Bact Camara Pestana. 1910; 3: 11–18.
12. Cornillot E, Hadj-Kaddour K, Dassouli A, Noel B, Ranwez V, Vacherie B, Augagneur Y, Brès V, Duclos A, Randazzo S, Carcy B, Debierre-Grockiego F, Delbecq S, Moubri-Menage K, Shams-Eldin H, Usmani-Brown S, Bringaud F, Wincker P, Vivarès ChP, Schwarz RT, Schetters TP, Krause PJ, Gorenflot A, Berry V, Barbe V, Mamoun ChB. Sequencing of the smallest Apicomplexan genome from the human pathogen *Babesia microti*. Nucleic Acids Res. 2012; 40: 9102–9114.

13. Lack JB, Reichard MV, Van Den Bussche RA. Phylogeny and evolution of the Piroplasmida as inferred from 18S rRNA sequences. Int J Parasitol. 2012; 42: 353–363.
14. Bonnet S, Jouglin M, L'Hostis M, Chauvin A. *Babesia* sp. EU1 from roe deer and transmission within *Ixodes ricinus*. Emerg Infect Dis. 2007;13: 1208–1210.
15. Tamura K, Dudley J, Nei M, Kumar S. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol Biol Evol. 2007; 24: 1596–1599.
16. Uhnoo I, Cars O, Christensson D, Nystroem-Rosander C. First documented case of human babesiosis in Sweden. Scand J Infect Dis. 1992; 24: 541–547.
17. Centeno-Lima S, do Rosario V, Parreira R, Maia AJ, Freudenthal AM, Nijhof AM, Jongejan F. A fatal case of human babesiosis in Portugal: molecular and phylogenetic analysis. Trop Med Int Health. 2003; 8: 760–764.
18. Martinot M, Zadeh MM, Hansmann Y, Grawey I, Christmann D, Aguillon S, Jouglin, M, Chauvin A, De Briel D. Babesiosis in immunocompetent patients, Europe. Emerg Infect Dis. 2011; 17: 114–116.
19. Meer-Scherrer L, Adelson M, Mordechai E, Lottaz B, Tilton R. *Babesia microti* infection in Europe. Curr Microbiol. 2004; 48: 435–437.
20. Hildebrandt A, Hunfeld KP, Baier M, Krumbholz A, Sachse S, Lorenzen T, Kiehntopf M, Fricke HJ, Straube E. First confirmed autochthonous case of human *Babesia microti* infection in Europe. Eur J Clin Microbiol Infect Dis. 2007; 26: 595–601.
21. Ramharter M, Walochnik J, Lagler H, Winkler S, Wernsdorfer WH, Stoiser B, Graninger W. Clinical and molecular characterization of a near fatal case of human babesiosis in Austria. J Travel Med. 2010; 17: 416–418.
22. Gray JS. Identity of the causal agents of human babesiosis in Europe. Int J Med Microbiol. 2006; 40: 131–136.
23. Herwaldt BL, Cacciò S, Gherlinzoni F, Aspöck H, Slemenda SB, Piccaluga PP, Martinelli G, Edelhofer R, Hollenstein U, Poletti G, Pampiglione S, Karin Lõschenberger K, Tura S, Pieniazek NJ. Molecular characterization of a non-*Babesia divergens* organism causing zoonotic babesiosis in Europe. Emerg Infect Dis. 2003; 9: 942–948.
24. Häselbarth K, Tenter AM, Brade V, Krieger G, Hunfeld KP. First case of human babesiosis in Germany-clinical presentation and molecular characterisation of the pathogen. Int J Med Microbiol. 2007; 297: 197–204.
25. Welc-Falęciak R, Hildebrandt A, Siński E. Co-infection with *Borrelia* species and other tick-borne pathogens in humans: two cases from Poland. Ann Agric Environ Med. 2010; 17: 309–313.
26. Criado-Fornelio A, Martinez-Marcos A, Buling-Sarana A, Barba-Carretero JC. Molecular studies on *Babesia*, *Theileria* and *Hepatozoon* in southern Europe Part II. Phylogenetic analysis and evolutionary history. Vet Parasitol. 2003; 114: 173–194.
27. Siński E, Bajer A, Welc R, Pawełczyk A, Ogrzewalska M, Behnke JM. *Babesia microti*: prevalence in wild rodents and *Ixodes ricinus* ticks from the Mazury Lakes District of north-eastern Poland. Int J Med Microbiol. 2006; 296 (S1):137–143.
28. Welc-Falęciak R, Bajer A, Behnke JM, Siński E. Effect of host diversity and the community composition of hard ticks (Ixodidae) on *Babesia microti* infection. Int J Med Microbiol. 2008; 298 (S1): 235–242.
29. Welc-Falęciak R, Bajer A, Paziewska-Harris A, Baumann-Popczyk A, Siński E. Diversity of *Babesia* in *Ixodes ricinus* ticks in Poland. Adv Med Sci. 2012; 57: 364–369.