Journal of Travel Medicine

International Society of Travel Medicine
Established 1991
Promoting healthy travel worldwide

*Journal of Travel Medicine*, 2017, 1–8
doi: 10.1093/jtm/tax073
Original Article

Original Article

# Case report of the patient source of the *Babesia microti* R1 reference strain and implications for travelers

**Philipp Stahl, MD, PhD[1,2], Yves Poinsignon, MD[3], Pascal Pouedras, PharmD[4], Vasilica Ciubotaru, MD[3], Laurence Berry, PhD[5], Brinda Emu, MD[6], Peter J. Krause, MD[7], Choukri Ben Mamoun, PhD[6], and Emmanuel Cornillot, PhD[8,9]***

[1]Institute of Virology, Parasitology Unit, University of Marburg, Marburg, Germany, [2]Department of Internal Medicine, Section of Gastroenterology and Infectious Diseases, University Hospital Gießen and Marburg, Marburg, Germany, [3]Internal Medicine Department, Centre Hospitalier Bretagne Atlantique, Vannes, France, [4]Microbiology Department, Centre Hospitalier Bretagne Atlantique, Vannes, France, [5]Dynamique des Interactions Membranaires Normales et Pathologiques, UMR5235 CNRS, Université Montpellier, Montpellier, France, [6]Department of Internal Medicine, Section of Infectious Diseases, Yale School of Medicine, New Haven, CT, USA, [7]Yale School of Public Health and Yale School of Medicine, 60 College St., New Haven, CT 06520, USA, [8]Institut de Biologie Computationnelle (IBC), Montpellier, France and [9]Institut de Recherche en Cancérologie de Montpellier (IRCM – INSERM U1194), Institut régional du Cancer Montpellier (ICM) & Université de Montpellier, France

*To whom correspondence should be addressed. Email: emmanuel.cornillot@umontpellier.fr

Submitted 8 May 2017; Revised 19 September 2017; Editorial decision 3 October 2017; Accepted 14 October 2017

## Abstract

**Background:** In 2002, a previously healthy 69-year-old man travelled to France from the United States and presented to our hospital with a febrile illness that subsequently was determined to be babesiosis. The blood isolated from this patient served as a source for propagation of the *Babesia microti* R1 strain with subsequent sequencing and annotation of the parasite genome.

**Methods:** Upon admission, we obtained a medical history, performed a physical examination, and examined his blood for the presence of a blood borne pathogen by microscopy, PCR and indirect immunofluorescence antibody testing. Once the diagnosis of babesiosis was made, we reviewed the literature to assess the distribution of *B. microti-associated* babesiosis cases in immunocompetent patients from outside the USA.

**Results:** The patient recalled a tick bite during the previous month on Cape Cod, Massachusetts. The diagnosis was confirmed by identification of *Babesia*-infected red blood cells on blood smears, amplification of *B. microti* DNA in blood by PCR and the presence of *B. microti* antibody in the serum. This strain was the first isolate of *B. microti* to be fully sequenced and its annotated genome serves as a reference for molecular and cell biology studies aimed at understanding *B. microti* pathophysiology and developing diagnostic tests and therapies. A review of babesiosis cases demonstrates a worldwide distribution of *B. microti* and identifies potential emerging endemic areas where travelers may be at risk of contracting *B. microti* infection.

**Conclusion:** This case provides clinical information about the patient infected with the R1 isolate and a review of travel risk, diagnosis and treatment of babesiosis in endemic and non-endemic areas.

**Key words:** Babesiosis, *Babesia microti*, R1 strain, tick-borne disease

© International Society of Travel Medicine, 2017. Published by Oxford University Press. All rights reserved. For Permissions, please e-mail: journals.permissions@oup.com

## Introduction

*Babesia* species are intra-erythrocytic protozoan parasites that cause babesiosis, a tick-borne infectious disease that occurs worldwide.[1] These parasites belong to the phylum Apicomplexa, which encompasses other protozoan parasites such as those that cause malaria, toxoplasmosis and cryptosporidiosis. *Babesia microti*, the causative agent of most human babesiosis cases, is transmitted to humans by hard bodied (Ixodes) tick vectors, the same ticks that transmit the agents of Lyme disease (*Borrelia burgdorferi*) and human granulocytic anaplasmosis (*Anaplasma phagocytophilum*).[2] *Babesia microti* also can be acquired through blood transfusion and is the most common transfusion-transmitted pathogen in the United States.[3] Transfusion-transmitted babesiosis (TTB) is a risk to the blood supply in endemic and non-endemic areas because infected blood from asymptomatic donors from endemic regions may be transported to non-endemic areas and donors that are asymptomatically infected in an endemic area may return to a non-endemic area and donate blood.[4,5] Various screening strategies of blood supply have been implemented and have helped to reduce TTB incidence.[3–6] Human babesiosis due to *B. microti* is endemic in the northeastern and northern midwestern United States and sporadically reported in Asia, Australia, Europe and South America.[2,7–18] In 2011, the Institute of Medicine in the United States designated babesiosis an emerging health threat[19] and the United States Centers for Disease Control and Prevention (CDC) designated babesiosis as a nationally notifiable disease.[20] In 2012, the sequencing and annotation of the full *B. microti* genome was completed, an important advance in our understanding of *B. microti* biology and evolution.[21–23] The R1 strain that was sequenced is currently used as a reference genome to characterize the genetic diversity and pathogenicity of *B. microti* strains and to develop new diagnostic tests and therapies.[24] This manuscript describes the clinical case that led to the isolation of the *B. microti* R1 isolate and provides important information about babesiosis management in endemic and non-endemic areas.

## Case Presentation

A 69-year-old previously healthy Caucasian male was in good health until he developed chills during a flight from the United States to France in the summer of 2002. Despite self-administration of non-steroidal anti-inflammatory drugs (NSAIDs), he subsequently developed a frontal headache and purpuric rash, which continued until presentation to the Centre Hospitalier Bretagne Atlantique in Vannes, Brittany, France. He was a resident of New York City with a second residence in Cape Cod, Massachusetts. Over the course of 30 years the patient had traveled to Europe, South America, Asia and Australia. One month prior to hospitalization, he spent 2 weeks at his Cape Cod residence where he reported multiple tick bites. The patient was on no medications. His past medical history included appendectomy.

On physical examination, the patient was awake, alert and oriented to person, place and time and in no acute distress. He was febrile to 105 °F (rectal) with normal blood pressure and heart rate. There was no evidence of meningismus. Eye exam revealed no icterus. Skin examination revealed diffuse purpura of the lower extremities. The remainder of the patient's clinical examination was unremarkable.

Initial laboratory findings showed anemia with hemoglobin of 12.2 g/dl; a normal mean corpuscular volume (MCV); thrombocytopenia with platelet count of 77 000/mm$^3$; normal leukocyte and neutrophil counts of 4800/mm$^3$ and 2450/mm$^3$, respectively, mild lymphopenia of 1250/mm$^3$ with 16% activated lymphocytes, reticulocytosis of 3% (119 100/mm$^3$) and an elevated erythrocyte sedimentation rate (ESR) of 100 mm/h. Prothrombin time (PT) was slightly prolonged with an INR of 1.3. Serum electrolytes revealed a sodium of 134 mmol/l and potassium of 3.4 mmol/l. Serum creatinine was 1.37 mg/dl. Liver enzymes alanine aminotransferase (ALT), gamma-glutamyl transpeptidase (GGT), alkaline phosphatase and total bilirubin were in normal range, and haptoglobin was undetectable. Aspartate transaminase (AST: 75 U/l) and lactate dehydrogenase (LDH: 700 U/l) levels were elevated. Marked elevations were noted in the acute phase protein fibrinogen (6600 mg/dl) and C-reactive protein (CRP: 286 mg/l).

Three blood cultures were performed and were negative. Lumbar puncture analysis revealed clear CSF with no cells and normal total protein and glucose. CSF culture revealed no growth of organisms. Urine analysis showed no evidence of leukocytes, nitrites or erythrocytes. Electrocardiography was normal. Abdominal echography showed a normal spleen and no other abnormalities except a hemangioma of the liver in segment VI. Tranthoracic echocardiography revealed normal ejection fraction, morphology and valvular function without evidence of pericardial effusion. Chest radiography demonstrated a mild interstitial infiltrate at the right lung base.

An initial peripheral blood smear suggested *Plasmodium falciparum* with a 3% parasitemia. Re-evaluation of the blood smear and morphology of parasites within the patient's red blood cells *Babesia* infection (Figure 1). *Babesia microti* DNA was amplified from blood using a *Babesia* PCR using the primer pair PIRO-A (5′AATACCCAATCCTGACACAGGG 3′) and antisense oligonucleotide primer PIRO-B (5′TTAAATACGAA TGCCCCCAAC 3′) amplifying a variable section of the 18S encoding gene.[25] Sequencing of the PCR product showed 95% homology to the 18S rDNA sequence of *B. microti*.

Several *Babesia* serologic tests were performed using indirect immunofluorescence antibody tests (IFT) with polyvalent anti-human secondary antibody (Sigma, F6506) to discriminate between antibodies to different *Babesia* species. Markedly elevated antibody titer was detected for *B. microti* (1:16 400) with the presumption that reactivity to *Babesia divergens* (titer: 1:2048) and *Babesia canis* (titer: 1:512) were a result of cross-reactivity of *B. microti* antibody to antigens of these other *Babesia* species. The initial serological analysis for malaria showed negative IgM but positive IgG. The results of antibody testing for ehrlichiosis, syphilis (*Treponema pallidum*) and for HIV were negative. Lyme disease serology was performed to assess possible coinfection with *B. burgdorferi*. *Borrelia burgdorferi* IgG antibody was detected but no IgM. The patient had no previous history of Lyme disease. A diagnosis of acute Lyme disease was ruled out based on the absence of an erythema migrans rash and the absence of IgM antibody confirmed by western blot analysis.

The patient was diagnosed with severe babesiosis caused by *B. microti* and was given intravenous quinine (20 mg/kg body

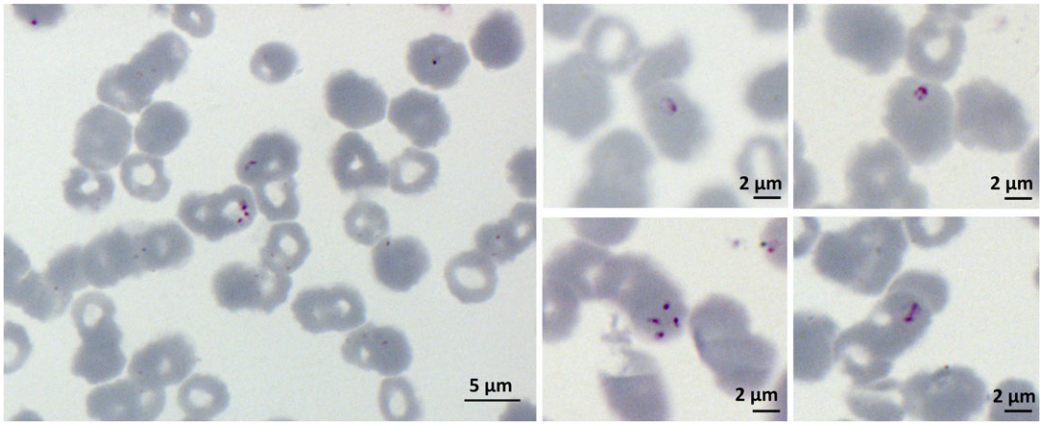


**Figure 1.** Peripheral blood smear of R1 isolate infected patient. The patient was treated at the Centre Hospitalier Bretagne Atlantique in Vannes, Brittany, France. He had a residence in Cape Cod, Massachusetts

weight loading dose, followed by 10 mg/kg body weight every 8 h) for 4 days. The patient experienced a rapid defervescence following treatment with resolution of thrombocytopenia but experienced persistent hemolysis and mild renal insufficiency. After completion of 4 days of intravenous quinine, he was given oral quinine 500 mg thrice daily and clindamycin 300 mg thrice daily for another 10 days. By the end of the fifth hospital day, the patient recovered well with resolution of hemolysis and mild renal dysfunction. He was discharged with no residual symptoms.

## Review and Discussion

The primary objective of this report is to describe the clinical presentation, diagnosis and therapy of human babesiosis case that led to the isolation of the R1 isolate, which provided the first completed genome sequence of *B. microti*.[21] The sequencing, assembly and annotation of the genome of this isolate was performed in collaboration between the Genoscope (Evry, France) and our teams at University of Montpellier in France and Yale University in the USA. The patient traveled to France where he was diagnosed to have babesiosis at the Centre Hospitalier Bretagne Atlantique in Vannes, Brittany. This report further highlights frequently encountered problems in the diagnosis of babesiosis, particularly in non-endemic regions of the world.

### *B. microti* R1 genome analysis: biological and clinical implications

The assembly and annotation of the *B. microti* R1 genome made it possible to understand the metabolic functions of the parasite, identify new antigens for development of diagnostic assays, and identify targets for development of novel therapies.[24,26] The parasite was first isolated from the blood of the patient and propagated in gerbils or hamsters.[21] The genome was then sequenced, annotated and made available on public genome databases.[21] Subsequent analyses included whole genome comparison between R1 and Gray strain isolates using optical mapping, determination of the structure and composition of the mitochondrial and apicoplast genomes.[22,23] The size of the *B. microti* R1 nuclear genome is 6.5 MB, making it the smallest nuclear genome among apicomplexa.[21,22] The genome consists of 4-chromosomes, has a G + C content of 36% and encodes 3567 protein-coding genes, most of

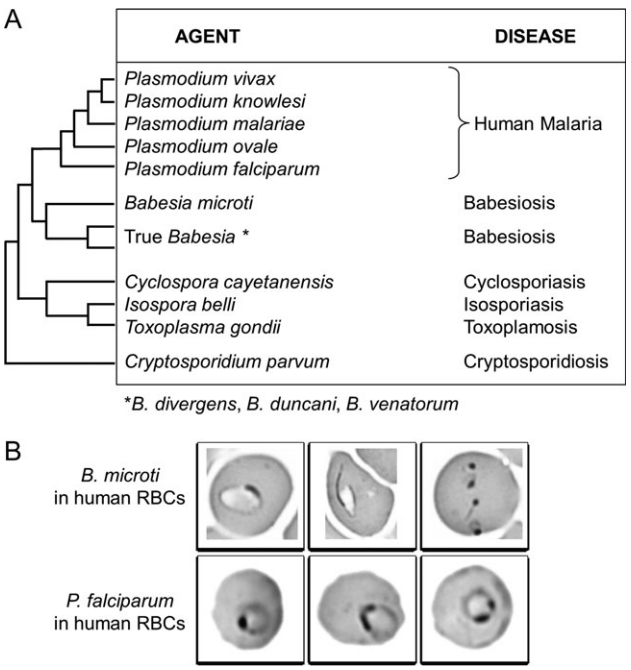


**Figure 2.** Difficulty in distinguishing between *Babesia microti* and *Plasmodium falciparum* on blood smear. (A) *Babesia microti* and *P. falciparum* are the only challenging apicomplexa. *Plasmodium falciparum* is only species for which confusion was described among all *Plasmodium* species responsible for human malaria. Dendogram shows clade organization of human infecting apicomplexa. *Babesia microti* is the type species of parasites that get separated early in piroplasmida evolution. *Babesia divergens, B. duncani* and *B. venatorum* are the major species infecting human among the major branch of piroplasmida encompassing true *Babesia* sp. *Plasmodium* and piroplasmida are hemoparasites. Other apicomplexa are intestinal parasites. (B) Challenging diagnosis of *B. microti* vs *P. falciparum*. Blood smears from patients with babesiosis or malaria are similar. Similarity is true for *B. microti* during human infection only

which are expressed during parasite development in mammalian red blood cells. Phylogenetic analyses reveal that *B. microti* represents a new lineage within the phylum Apicomplexa, distinct from lineages encompassing *Plasmodium* sp. and true *Babesia* sp. (Figure 2A). Despite its distinct evolution and until future renaming, the genus *Babesia* is still used for *B. microti* taxonomy and

closely related species like *B. rodhaini* and *B. felis*. Other *Babesia* species (including those that infect humans, such as *B. divergens*) are phylogenetically related and are designated as *Babesia sensu stricto* or true *Babesia* sp. as the group encompasses type species used to specify the genera. Comparison of 18S rDNA sequences revealed that the *B. microti* clade is diverse with evidence of differences in mammalian host specificity among different groups.[27] Following sequencing of the R1 isolate, further efforts by our group and others have led to the sequencing of several isolates from human, ticks and infected animals.[28–30] The overall size of the genomes of the *B. microti* isolates sequenced so far ranges between 6.3 and 6.9 MB. Available data suggest that sub-population of *B. microti* might have evolved new properties that allow infection of humans.[28,29]

## Areas of travel risk for babesiosis

Human babesiosis is endemic in the United States and China,[1,31] while sporadic cases occur in Europe (Figure 3). *B. microti* infection is endemic in the northeastern and northern Midwestern United States with the number of cases increasing significantly since the disease has become nationally notifiable in 2011.[2] In 2016, more than 1600 were reported to CDC. Babesiosis is not a notifiable disease outside the USA and incidence of this disease is likely to be underestimated. *Babesia divergens* is the most common etiologic agent of babesiosis in Europe and splenectomized persons are at greater risk.[1] Most *B. microti* cases reported in Europe are found in travelers coming from the USA.[7,8,11–13,16] Serosurveys suggest that local transmission of *B. microti* could happen in Europe, but with little to no clinical signs.[1–3,32–36] Indigenous *B. microti* infection has been observed in an immunocompromised patient in Germany.[37] Surveys in local areas, often linked with poverty and presence of rodents have revealed *B. microti* infection in China, Mongolia and Bolivia.[38–40] Case of *B. microti* in South America was reported for a traveler coming back in the USA.[17] A patient traveling from Uruguay to Spain presented mild symptoms of *B. microti* infection a few days after he return to his home but the origin of the parasite remains uncertain.[18] *Babesia venatorum* infection is endemic in Heilongjiang province, the most northeastern province of China.[31,41] Both *B. microti* infection and malaria are found in Yunnan province in southwestern China along the Myanmar border.[31,38] There is concern that babesiosis may be misidentified as resistant malaria, given that both *Plasmodium* and *Babesia* parasites have a similar appearance on microscopic examination following Giemsa staining (Figure 2B) and that the *Babesia* parasites are less sensitive to chloroquine and mefloquine than *Plasmodium* parasites.[34] Physicians and other health care workers in areas where both babesiosis and malaria are endemic should order Babesia and malaria PCR, in addition to blood smears.[17,42]

## Babesiosis diagnosis

Babesiosis presents as a malaria-like illness without an easily recognizable sign. The diagnosis is therefore often delayed, especially

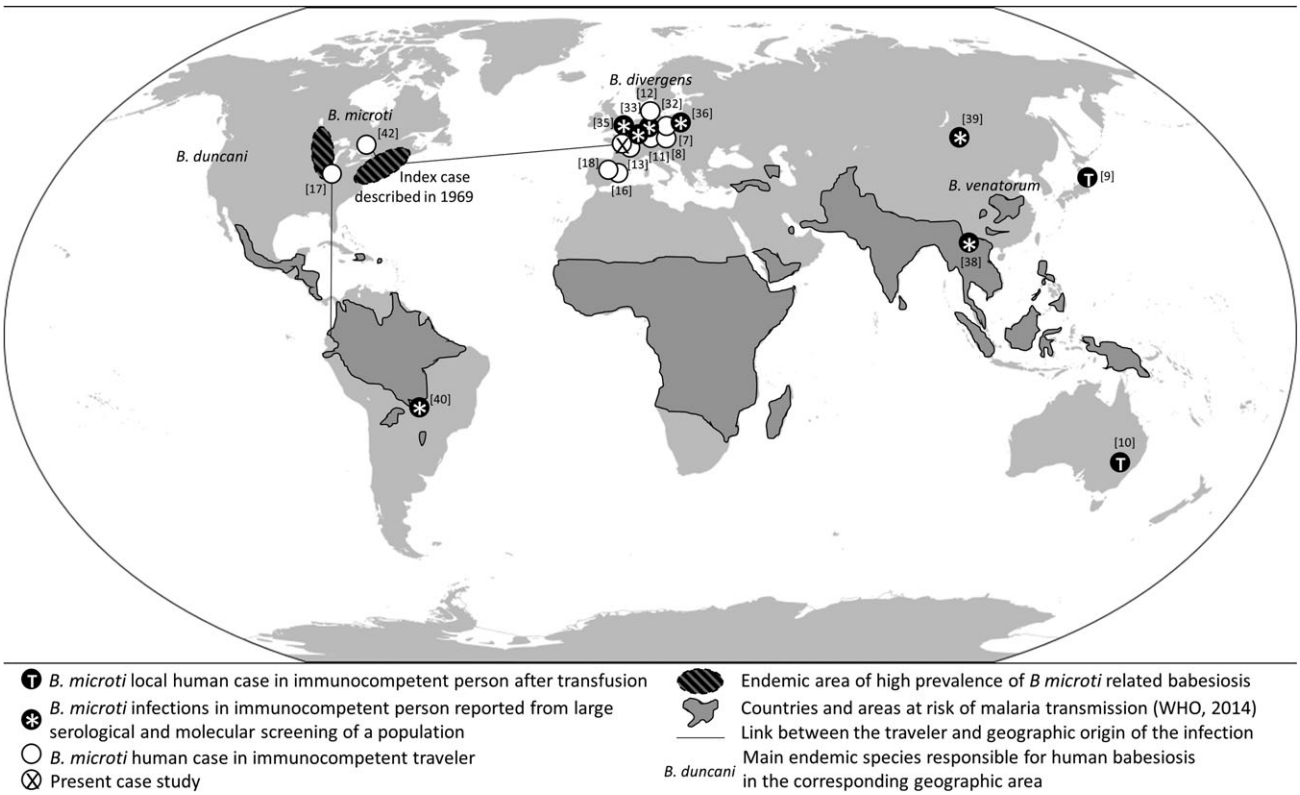


**Figure 3.** Endemic areas and sporadic cases of *B. microti*-human babesiosis and relationship with malaria transmission areas. Most travelers in Europe that experimence babesiosis due to *B. microti* acquired their infections in the USA. Babesiosis is not a notifiable disease outside the USA. We refer only to *B. microti* cases concerning immunocompetent patients. *Babesia divergens, B. duncani, B. microti* and *B. venatorum* are the major species infecting human across the world. Reference number is given in brackets

in non-endemic regions. Because the malaria-like symptoms are non-specific (see below), health care workers in non-endemic areas often will not think of babesiosis and the diagnosis may first be made by laboratory personnel who recognize *Babesia* on a thin blood smear that was ordered as part of a complete blood count (CBC) or a peripheral smear requested for workup of malaria. Consequently, health care workers and laboratory personnel must include babesiosis in their differential diagnosis, especially because of the worldwide emergence of the disease and the increase in international travel.

Babesiosis should be considered in any patient experiencing a malaria-like illness, including fever, fatigue, chills, sweats, headache and/or muscle aches. Other symptoms include anorexia, nausea, arthralgia and emotional lability.[1,2] There are few findings on physical examination other than fever and occasional splenomegaly and/or hepatomegaly. A thorough travel history is important to obtain in such patients, including travel within any *Babesia*-endemic areas. It is also important to ask about blood transfusion within the previous 6 months because of the possibility of having received a transfusion of *Babesia* contaminated blood. *Babesia microti* is the most common pathogen transmitted by blood transfusion in the USA.[1–3] Due to the absence of routine screening of blood for *Babesia* infection and the transport of blood products from endemic to non-endemic areas, *Babesia* infection from blood transfusion can occur in both endemic and non-endemic areas.[4–6]

Useful laboratory screening tests for patients include a CBC that usually shows hemolytic anemia and thrombocytopenia.[43] Liver enzymes are often elevated. Of note, thrombocytopenia and elevated transaminases also may be observed during *A. phagocytophilum* infection. Specific diagnosis is made by identification of *Babesia* on a Giemsa or Wright-stained thin blood smear, or PCR amplification of *Babesia* DNA with specific primers. There is a risk of mis-interpretation of intra-erythrocytic microorganisms on blood smear as *B. microti* or *P. falciparum* (Figure 2B). Distinguishing features of *Babesia* include tetrad merozoite forms ('Maltese Cross') that are pathognomonic for *Babesia* and the absence of pigment inclusions and visible gametocytes. Again, a travel history is often useful in distinguishing between babesiosis and malaria. PCR is more sensitive and specific than blood smear so that infections with as few as three parasites per 100μl of blood can yield a positive result.[44–47] PCR usually is performed with two specific primers design in the 18S gene such as piroA/piroB,[25] as used for our patient or BAB1/BAB4.[44] Use of specific PCR primers or DNA sequencing of PCR products is necessary to identify various *Babesia* species such as *B. microti*, *B. divergens*, *B. duncani* and *B. venatorum*. Serology with IFA or ELISA is another useful laboratory test that may be positive at presentation because diagnosis is often delayed, giving time for production of antibody. Alternatively, the presence of IgG antibody may be due to past infection. In the case of the R1 patient, the serological analysis for *B. microti* was positive for IgM and IgG while that for malaria was negative for IgM but positive for IgG, most likely due to cross-reactivity of IgG against *P. falciparum* antigens. Cross-reactivity between antigens of different hemoparasites has previously been described for *Babesia bovis* and *P. falciparum*.[48]

*Babesiosis therapy*: Current therapy for the treatment of *B. microti*-human babesiosis consists primarily of the combination of atovaquone and azithromycin.[49,50] Clindamycin and quinine is used for severe disease. This combination is recommended for babesiosis resulting from *B. divergens* infections which are almost always severe.[50] The recommended duration of therapy is 7–10 days in most cases. Clinical improvement is usually observed within 48–72 h. In highly immunocompromised patients with severe disease who do not respond well to a standard duration of therapy, a more extended course of at least 6 weeks (including 2 weeks with repeatedly negative blood smears) is recommended.[1,2,51] Such patients include those with malignancy and rituximab treatment (especially B cell lymphoma and asplenia), HIV/AIDS, autoimmune disease treated with rituximab and organ transplantation treated with immunosuppressive agents.[51] The risk–benefit ratio of more prolonged therapy must be considered, especially with quinine that can lead to gastrointestinal distress, hearing impairment including tinnitis and/or cardiac impairment.[2,49] Intravenous quinine or quinidine treatment may be given in the early phase of severe infection but should only be given in an ICU with cardiac monitoring. Our patient initially had insufficient improvement on quinine monotherapy so that addition of clindamycin to the quinine regimen was used to clear the infection.

The current antibiotics used for treatment of babesiosis generally are effective but newer antimicrobial regimens are needed, especially for severe disease. Although quinine is often used for treatment of severe babesiosis, the side effects associated with this drug, the lack of evidence that *B. microti* degrades hemoglobin, and the inability of the compound to inhibit growth of the parasite in mice all suggest that its use for babesiosis treatment should be reevaluated. Atovaquone is a useful anti-*Babesia* drug with fewer side effects. The drug irreversibly binds to the mitochondrial cytochrome bc1 complex and blocks the electron flux in the mitochondrial inner membrane, which plays a central role in parasite physiology. However, resistance alleles have been found in the cytochrome b (*cytb*) gene on the mitochondrial genome.[48] The combination of atovaquone with endochin-like quinolone (ELQ) has shownpromise in preclinical studies[52] and awaits clinical evaluation. Azithromycin and clindamycin are suspected to target the ribosome of the apicoplast or the mitochondria, and are used in separate combination therapy regimens. Sensitivity of *B. microti* to artemisinin has been demonstrated in rodent models but efficacy in humans is still unknown.[53]

Partial or complete exchange blood transfusion is recommended in patients with high grade parasitemia (≥10%), significant hemolysis, or renal, hepatic or pulmonary compromise. Patients who are susceptible to severe babesiosis include those with asplenia, cancer, organ transplantation, HIV, hemoglobinopathies, those who acquire babesiosis through blood transfusion, are on immunosuppressive drugs, have chronic heart, lung or liver disease, or are neonates or elderly. Mortality rates of many such patients are about 20% even with antibiotic therapy.[1,51]

## Coinfection in tick-borne disease complicates both diagnosis and therapy

Ixodid ticks transmit an array of pathogens including *A. phagocytophilum*, *B. microti*, *B. burgdorferi*, *Borrelia miyamotoi*, *Borrelia mayonii*, *Ehrlichia muris*-like agent, and Powassan virus.[54–57] A

single tick can carry more than one agent. Accordingly, several reports have shown evidence of coinfection in reservoir hosts and humans. The distribution of these agents varies geographically. Patient travel history and knowledge about tick-borne diseases prevalent in the patient's place of residence are thus very important and may help better understand the clinical presentation and determine best therapeutic strategies. Coinfection may have a significant impact in natural hosts and in humans. Recent evidence suggests that *B. burgdorferi* coinfection in the natural reservoir host (*Peromyscus leucopus* mice) increases *B. microti* parasitemia and the transmission of *B. microti* to other mice and to humans.[58,59] Lyme disease coinfection may therefore enhance the emergence of babesiosis. Lyme disease patients who are coinfected with either *B. microti* or *A. phagocytophilum* experience a greater number of symptoms for longer duration than those with Lyme disease alone.[60] Coinfection should be considered in patients with more severe Lyme disease or patients who do not respond well to standard Lyme disease therapy. Finally, gene transfer between coinfecting microbes may occur. Genome sequencing of the *B. microti* R1 isolate provided evidence for lateral transfer of a *Bartonella* gene encoding a putative thiamin pyrophosphokinase into the *B. microti* genome.[21] The possible impacts of these genetic events on clinical severity or antibiotic resistance remains unknown.

The diagnosis of Lyme disease and coinfection with babesiosis was considered in the R1 patient because of the detection of *B. burgdorferi* IgG antibody. The absence of an erythema migrans rash and IgM antibody indicated that the patient did not have acute Lyme disease but had experienced Lyme disease in the past. Simultaneous infection with *Babesia* and non tick-borne pathogens may occur.

## Conclusions

The *B. microti* R1 parasite isolated from the blood of the patient described in this report sat the stage for the genomic analysis of this parasite, which in turns has helped advance our understanding of the parasite's biology and pathogenesis and identify new targets for development of more sensitive diagnostic assays and more effective therapies. The diagnosis of babesiosis in non-endemic areas requires knowledge of the disease among health care workers and microbiology laboratory personnel. A travel history or history of blood transfusion within the 6 months preceding clinical evaluation is often important in making a correct diagnosis. Health care workers should consider coinfection with other *Ixodes*-borne pathogens in any patient with babesiosis. Severe disease may occur in immunocompromised hosts and those who acquire the infection through blood transfusion. Current treatment of babesiosis consists of atovaquone and azithromycin or clindamycin and quinine as an alternative treatment for severe disease. Current therapy for babesiosis is generally effective but new therapies, especially for severe disease are needed.

## Abbreviations

| | |
|---|---|
| ALT | alanine transaminase |
| AST | aspartate transaminase |
| CDC | Center for Disease Control |
| CSF | cerebrospinal fluid |
| HIV | human immunodefiency virus |
| ICU | intensive care unit |
| LDH | lactate dehydrogenase |
| NSAID | non-steroidal anti-inflammatory drug |
| PCR | polymerase chain reaction |
| TTB | transfusion-transmitted babesiosis |
| WB | western blot |

## Funding

Emmanuel CORNILLOT was supported by the Agence Nationale de la Recherche "Investissements d'avenir/Bioinformatique": ANR-11-BINF-0002 "Institut de Biologie Computationnelle". In memoriam of Fabrice Legros, former director of the Centre National de Référence des Maladies d'Importation.

**Conflict of interest:** None declared.

## Contributors

Y.P. and V.C. were the primary physicians and managed the patient. P.P. made the diagnosis of babesiosis. P.S. wrote the initial report. L.B. set up Figure 1. B.E. reviewed initial manuscript and provided data for Figure 2. E.C., C.B.M. and P.J.K. collected all information and wrote the manuscript. All co-authors participated in the writing and editing of the report.

## References

1. Vannier E, Krause PJ. Human babesiosis. *N Engl J Med* 2012; **366**: 2397–407.
2. Vannier EG, Diuk-Wasser MA, Ben Mamoun C, Krause PJ. Babesiosis. *Infect Dis Clin North Am* 2015; **29**: 357–70.
3. Herwaldt BL, Linden JV, Bosserman E *et al*. Transfusion-associated babesiosis in the United States: a description of cases. *Ann Intern Med* 2011; **155**: 509–19.
4. Leiby DA. Transfusion-associated babesiosis: shouldn't we be ticked off? *Ann Intern Med* 2011; **155**: 556–7.
5. Young C, Chawla A, Berardi V *et al*. Preventing transfusion-transmitted babesiosis: preliminary experience of the first laboratory-based blood donor screening program. *Transfusion* 2012; **52**: 1523–9.
6. Moritz ED, Winton CS, Tonnetti L *et al*. Screening for *Babesia microti* in the U.S. Blood Supply. *N Engl J Med* 2016; **375**: 2236–45.
7. Humiczewska M, Kuźna-Grygiel W. A case of imported human babesiosis in Poland. *Wiad Parazytol* 1997; **43**: 227–9.
8. Nohýnková E, Kubek J, Měst'ánková O *et al*. A case of *Babesia microti* imported into the Czech Republic from the USA. *Cas Lek Cesk* 2003; **142**: 377–81.
9. Saito-Ito A, Tsuji M, Wei Q *et al*. Transfusion-acquired, autochthonous human babesiosis in Japan: isolation of *Babesia microti*-like parasites with hu-RBC-SCID mice. *J Clin Microbiol* 2000; **38**: 4511–6.
10. Senanayake SN, Paparini A, Latimer M *et al*. First report of human babesiosis in Australia. *Med J Aust* 2012; **196**: 350–2.
11. Berens-Riha N, Zechmeister M, Hirzmann J *et al*. Babesiose by einem spenektomierten reisenden aus dem USA. *FTR* 2012; **19**: 113–5.
12. Holler JG, Roser D, Nielsen HV *et al*. A case of human babesiosis in Denmark. *Travel Med Infect Dis* 2013; **11**: 324–8.

13. Poisnel E, Ebbo M, Berda-Haddad Y *et al*. *Babesia microti*: an unusual travel-related disease. *BMC Infect Dis* 2013; **13**: 99.
14. Zhou X, Xia S, Yin SQ, Zhou XN. Emergence of babesiosis in China-Myanmar border areas. *Parasit Vectors* 2015; **8**: 390.
15. Bullar JM, Ahsanuddin AN, Perry AM *et al*. The first case of locally acquired tick-borne *Babesia microti* infection in Canada. *Can J Infect Dis Med Microbiol* 2014; **25**: e87–9.
16. Merino A. Blood film findings in severe babesiosis. *Br J Haematol* 2016; **172**: 839.
17. Al Zoubi M, Kwak T, Patel J, Kulkarni M, Kallal CA. Atypical challenging and first case report of babesiosis in Ecuador. *IDCases* 2016; **4**: 15–7.
18. Arsuaga M, Gonzalez LM, Lobo CA *et al*. First report of *Babesia microti*-caused Babesiosis in Spain. *Vector Borne Zoonotic Dis* 2016; **16**: 677–9.
19. Institute of Medicine of the National Academies. *Committee on Lyme disease and other tick-borne diseases. Critical needs and gaps in understanding. Prevention, amelioration, and resolution of Lyme and other tick-borne diseases. The Short-term and long-term outcomes*. Washington, DC: The National Academies Press, 2011. http://www.nap.edu.
20. CDC. Nationally Notifiable Infectious Conditions, United States 2011, Babesiosis, 2011.
21. Cornillot E, Hadj-Kaddour K, Dassouli A *et al*. Sequencing of the smallest Apicomplexan genome from the human pathogen *Babesia microti*. *Nucleic Acids Res* 2012; **40**: 9102–14.
22. Cornillot E, Dassouli A, Garg A *et al*. Whole genome mapping and re-organization of the nuclear and mitochondrial genomes of *Babesia microti* isolates. *PLoS One* 2013; **8**: e72657.
23. Garg A, Stein A, Zhao W *et al*. Sequence and annotation of the apicoplast genome of the human pathogen *Babesia microti*. *PLoS POne* 2014; **9**: e107939.
24. Cornillot E, Dassouli A, Pachikara N *et al*. A targeted immunomic approach identifies diagnostic antigens in the human pathogen *Babesia microti*. *Transfusion* 2016; **56**: 2085–99.
25. Olmeda AS, Armstrong PM, Rosenthal BM *et al*. A subtropical case of human babesiosis. *Acta Trop* 1997; **67**: 229–34.
26. Lawres LA, Garg A, Kumar V *et al*. Radical cure of experimental babesiosis in immunodeficient mice using a combination of an endochin-like quinolone and atovaquone. *J Exp Med* 2016; **213**: 1307–18.
27. Baneth G, Florin-Christensen M, Cardoso L, Schnittger L. Reclassification of *Theileria annae* as *Babesia vulpes* sp. nov. *Parasit Vectors* 2015; **8**: 207.
28. Lemieux JE, Tran AD, Freimark L *et al*. A global map of genetic diversity in *Babesia microti* reveals strong population structure and identifies variants associated with clinical relapse. *Nat Microbiol* 2016; **1**: 16079.
29. Carpi G, Walter KS, Mamoun CB *et al*. *Babesia microti* from humans and ticks hold a genomic signature of strong population structure in the United States. *BMC Genomics* 2016; **17**: 888.
30. Silva JC, Cornillot E, McCracken C *et al*. Genome-wide diversity and gene expression profiling of *Babesia microti* isolates identify polymorphic genes that mediate host-pathogen interactions. *Sci Rep* 2016; **6**: 35284.
31. Zhou X, Huang J, Tambo E *et al*. Human babesiosis, an emerging tick-borne disease in the People's Republic of China. *Parasit Vectors* 2014; **7**: 509.
32. Hunfeld K-P, Lambert A, Kampen H *et al*. Seroprevalence of *Babesia* infection in human exposed to ticks in midwestern Germany. *J Clin Microbiol* 2002; **40**: 2431–6.
33. Foppa IM, Krause PJ, Spielman A *et al*. Entomologic and serologic evidence of zoonotic transmission of *Babesia microti*, eastern Switzerland. *Emerg Infect Dis* 2002; **8**: 722.
34. Genchi C. Human babesiosis, an emerging zoonosis. *Parassitologia* 2007; **49**: 29.
35. Lempereur L, Shiels B, Heyman P *et al*. A retrospective serological survey on human babesiosis in Belgium. *Clin Microbiol Infect* 2015; **21**: 96.e1.
36. Moniuszko-Malinowska A, Swiecicka I, Dunaj J *et al*. Infection with *Babesia microti* in humans with non-specific symptoms in North East Poland. *Infect Dis (Lond)* 2016; **48**: 537–43.
37. Hildebrandt A, Hunfeld KP, Baier M *et al*. First confirmed autochthonous case of human *Babesia microti* infection in Europe. *Eur J Clin Microbiol Infect Dis* 2007; **26**: 595–601.
38. Zhou X, Li SG, Chen SB *et al*. Co-infections with *Babesia microti* and Plasmodium parasites along the China-Myanmar border. *Infect Dis Poverty* 2013; **2**: 24.
39. Hong SH, Anu D, Jeong YI *et al*. Molecular detection and seroprevalence of *Babesia microti* among stock farmers in Khutul City, Selenge Province, Mongolia. *Korean J Parasitol* 2014; **52**: 443–7.
40. Gabrielli S, Totino V, Macchioni F *et al*. Human Babesiosis, Bolivia, 2013. *Emerg Infect Dis* 2016; **22**: 1445–7.
41. Jiang JF, Zheng YC, Jiang RR *et al*. Epidemiological, clinical, and laboratory characteristics of 48 cases of '*Babesia venatorum*' infection in China: a descriptive study. *Lancet Infect Dis* 2015; **15**: 196–203.
42. Warren T, Lau R, Ralevski F *et al*. Fever in a visitor to Canada: a case of mistaken identity. *J Clin Microbiol* 2015; **53**: 1783–5.
43. Krause PJ. Babesiosis diagnosis and treatment. *Vector Borne Zoonotic Dis* 2003; **3**: 45–51.
44. Persing DH, Mathiesen D, Marshall WF *et al*. Detection of *Babesia microti* by polymerase chain reaction. *J Clin Microbiol* 1992; **30**: 2097–103.
45. Armstrong PM, Katavolos P, Caporale DA *et al*. Diversity of *Babesia* infecting deer ticks (*Ixodes dammini*). *Am J Trop Med Hyg* 1998; **58**: 739–42.
46. Rollend L, Bent SJ, Krause PJ *et al*. Quantitative PCR for detection of *Babesia microti* in *Ixodes scapularis* ticks and in human blood. *Vector Borne Zoonotic Dis* 2013; **13**: 784–90.
47. Wilson M, Glaser KC, Adams-Fish D *et al*. Development of droplet digital PCR for the detection of *Babesia microti* and *Babesia duncani*. *Exp Parasitol* 2015; **149**: 24–31.
48. James MA, Montenegro-James S, Fajfar-Whetstone C *et al*. Antigenic relationship between *Plasmodium falciparum* and *Babesia bovis*: reactivity with antibodies to culture-derived soluble exoantigens. *J Protozool* 1987; **34**: 328–32.
49. Krause PJ, Lepore T, Sikand VK *et al*. Atovaquone and azithromycin for the treatment of babesiosis. *N Engl J Med* 2000; **343**: 1454–8.
50. Kletsova EA, Spitzer ED, Fries BC, Marcos LA. Babesiosis in Long Island: review of 62 cases focusing on treatment with azithromycin and atovaquone. *Ann Clin Microbiol Antimicrob* 2017; **16**: 26.
51. Krause PJ, Gewurz BE, Hill D *et al*. Persistent and relapsing babesiosis in immunocompromised patients. *Clin Infect Dis* 2008; **46**: 370–6.
52. Lawres LA, Garg A, Kumar V *et al*. Radical cure of experimental babesiosis in immunodeficient mice using a combination of an endochin-like quinolone and atovaquone. *J Exp Med* 2016; **213**: 1307–18.
53. Goo YK, Terkawi MA, Jia H *et al*. Artesunate, a potential drug for treatment of *Babesia* infection. *Parasitol Int* 2010; **59**: 481–6.
54. Diuk-Wasser MA, Vannier E, Krause PJ. Coinfection by *Ixodes* tick-borne pathogens: ecological, epidemiological, and clinical consequences. *Trends parasitol* 2016; **32**: 30–42.
55. Nelder MP, Russell CB, Sheehan NJ *et al*. Human pathogens associated with the blacklegged tick *Ixodes scapularis*: a systematic review. *Parasit Vectors* 2016; **9**: 265.
56. Saito-Ito A, Kasahara M, Kasai M *et al*. Survey of *Babesia microti* infection in field rodents in Japan: records of the Kobe-type in new

foci and findings of a new type related to the Otsu-type. *Microbiol Immunol* 2007; **51**: 15–24.
57. Yabsley MJ, Shock BC. Natural history of Zoonotic *Babesia*: role of wildlife reservoirs. *Int J Parasitol Parasites Wildl* 2013; **2**: 18–31.
58. Dunn JM, Krause PJ, Davis S *et al*. *Borrelia burgdorferi* promotes the establishment of Babesia microti in the northeastern United States. *PloS One* 2014; **9**: e115494.
59. Hersh MH, Ostfeld RS, McHenry DJ *et al*. Co-infection of blacklegged ticks with *Babesia microti* and *Borrelia burgdorferi* is higher than expected and acquired from small mammal hosts. *PloS One* 2014; **9**: e99348.
60. Knapp KL, Rice NA. Human coinfection with *Borrelia burgdorferi* and *Babesia microti* in the United States. *J Parasitol Res* 2015; **2015**: 587131.