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REVIEW ARTICLE

# Relapsing Babesiosis With Molecular Evidence of Resistance to Certain Antimicrobials Commonly Used to Treat *Babesia microti* Infections

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Human babesiosis cases are emerging with an increased incidence and a wider geographic range worldwide. Relapsing babesiosis cases are becoming more frequently encountered in clinical practice associated with the use of immunosuppressive medications. The 2020 Infectious Diseases Society of America babesiosis guideline recommends at least 6 weeks of antimicrobial treatment for highly immunocompromised patients with *Babesia microti* infection. Nevertheless, cases have relapsed even after 6 weeks of treatment. Genetic mutations regarded as the potential cause of antimicrobial resistance in *B microti* have been identified in certain relapsing cases. A few alternative antimicrobial regimens have been used successfully to achieve cure for some of these cases, but other cases have had fatal outcomes. In this review, we discuss the molecular evidence of genetic resistance to certain antimicrobials commonly used to treat *B microti* infections based on an evaluation of 9 patients with relapsing infection.

**Keywords.** *Babesia microti*; babesiosis; persistent babesiosis; relapsing babesiosis; resistance.

Babesiosis is an emerging zoonotic infectious disease caused mainly by the protozoan *Babesia microti* and transmitted by the *Ixodes scapularis* tick in the United States [1–3]. The number of hospitalized cases with babesiosis has increased in the United States from 676 in 2010 to 1415 in 2013, with an estimated mortality rate of 1.6% [4]. Relapsing babesiosis is defined as the recurrence of *Babesia* infection after a recommended standard course of antimicrobials. Treatment duration is usually 7–10 days for the immunocompetent host, and at least 6 weeks for highly immunocompromised patients [5, 6]. Relapsing babesiosis is due to persistent infection, that is, failure to completely eradicate the infection, which usually only occurs in highly immunocompromised patients, such as patients with AIDS. The most common predisposing factor is being treated with immunosuppressive drugs, particularly anti-CD20 monoclonal antibodies such as rituximab [7–9]. Despite treatment with combination therapy using atovaquone and azithromycin, the most commonly used treatment regimen currently, and which has a lower side effect profile than quinine plus clindamycin [10], there are cases in which the parasite is not completely eliminated from the blood even after several rounds of treatment [9] (based on either microscopy or nucleic acid amplification testing using polymerase chain reaction [PCR]). Of course, PCR positivity alone does not establish parasite viability. In cases with residual viable parasites, there is the potential for a rise in the level of parasitemia in association with the development of recurrent clinical symptoms, which may result in fatality [11]. Thus, other more novel drug regimens, such as the fixed combination antimalarial drug proguanil plus atovaquone (Malarone), or the antimalarial drug tafenoquine, have been included in salvage therapies anecdotally but with limited experience to date [12–18] and with mixed results for tafenoquine in particular [12–14]. The resistance mechanisms of *B microti* against the commonly used antimicrobials have still not been established unequivocally, but multiple potentially relevant genetic mutations have been found, some based on inferences from studies conducted on drug resistance found in malaria parasites [19]. In this review, we summarize the reported cases of persistent or relapsing babesiosis in which there was evidence of genetic mutations in the parasite believed to be causing drug resistance to certain antimicrobials commonly used to treat babesiosis.

## METHODS

We searched PubMed through February 2023 to identify relevant studies that provided data on genetic changes in *B microti* that might explain the cause of treatment failures leading to persistence, often with a relapsing clinical course

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in human infections. The electronic search strategy was as follows: parasite (*B microti*, *Babesia* spp, babesiosis) AND associated conditions (relapsing, resistance, persistent infection, immunosuppression, immunocompromised, anti-CD20 therapy, chemotherapy, rituximab). To identify additional candidate studies, we reviewed the reference lists of the publications identified, including those of case reports, case series, and reviews. The screening process was performed by the authors who evaluated the potential eligibility based on the published abstracts and titles of the articles that were found by the search strategy. Eligible publications were related to studies of patients with persistent or relapsing babesiosis for whom genetic mutations for resistance to antimicrobials were investigated. The full text of all studies regarded as eligible based on the published abstracts were then reviewed. Relevant studies were those that discussed cases of relapsing or refractory human babesiosis, in which testing was conducted to determine certain molecular genetics of the parasite in regard to the parasite being resistant or partially resistant to the relevant antimicrobials. Publications that did not conduct genetic resistance testing on the parasite were not included in this review.

## RESULTS AND DISCUSSION

Of 114 articles that matched the search criteria, 95 were excluded based on evaluation of the title and abstract. A total of 19 articles were screened by reviewing the full text of the papers. Of these 19 papers, 5 met the inclusion criteria outlined above.

As of the end of February 2023, a genetic evaluation of the *B microti* parasites causing persistent or relapsing infection had been reported for 9 patients (Table 1) [12, 15, 16, 20]. Parasite variants found in association with relapsing disease include those with amino acid substitutions in the atovaquone-binding regions of cytochrome b (CYTb) and/or the azithromycin-binding region of the ribosomal protein L4 (RPL4) [20], as well as in domain V of the *B microti* 23S ribosomal RNA gene, which is a probable cause of resistance to both azithromycin and clindamycin [12].

CYTb, a highly conserved mitochondrial protein of *B microti*, is the target of atovaquone. Amino acid changes that occur in the atovaquone-binding regions of CYTb correspond to potential atovaquone resistance mutations, such as M134T and M134I [16, 20]. RPL4 is a 50S ribosomal protein that is the target for azithromycin. Several mutations have been found in the CYTb and/or the RPL4 of the *B microti* parasites associated with relapses and persistence of this infection (Table 1). These mutations were found in immunocompromised patients with babesiosis after receiving antimicrobials. Being immunocompromised likely negatively impacted clearance of the parasitemia, allowing the opportunity for the development of mutations in the genes associated with the atovaquone- and azithromycin-targeted proteins in the CYTb and the 50S ribosomal subunit of the parasite, respectively. In addition to these 9 patients, an additional immunocompromised patient has been reported (but not included in Table 1 because the patient was not reported to have relapsing babesiosis) [21], who developed *B microti* infection while receiving atovaquone as prophylaxis to prevent *Pneumocystis jirovecii* infection. This patient was found to have 4 different amino acid changes in CYTb, thought to reduce susceptibility to atovaquone (Y272S, V141A, M134I, and M134 T).

The 2020 Infectious Diseases Society of America babesiosis treatment guideline recommends at least 6 weeks of treatment for highly immunocompromised patients with babesiosis [6]. Antiparasitic drugs should potentially be continued even longer, since this therapy should not be discontinued until blood smears have become negative for 2 weeks [9]. However, there have been cases of relapsing disease in some patients who cleared the blood parasitemia based on blood smear examinations and even after negative whole blood PCR tests [13, 14, 16, 17]. Currently, there is no commercial test to determine before initiating antiparasitic drug therapy whether the strain of *B microti* causing the infection may already carry 1 of the

**Table 1. Summary of Cases With Relapsing Babesiosis and Resistance Mutations for Antimicrobials Frequently Used to Treat *Babesia microti* Infections**

| Age/Sex | Immunosuppression | Resistance Mutations | Antimicrobial Resistance | Reference |
|---|---|---|---|---|
| NA | NA[a] | RPL4 (C103Y) | Azithromycin | Lemieux et al, 2016 [20] |
| NA | NA[a] | RPL4 (R86C) | Azithromycin | Lemieux et al, 2016 [20] |
| NA | NA[a] | CYTb (L277P) and RPL4 (S73L) | Atovaquone and azithromycin | Lemieux et al, 2016 [20] |
| NA | NA[a] | CYTb (L277P) and RPL4 (R86H) | Atovaquone and azithromycin | Lemieux et al, 2016 [20] |
| NA | NA[a] | CYTb (M134I) | Atovaquone | Lemieux et al, 2016 [20] |
| 81/Male | Rituximab | CYTb (Y272C) and RPL4 (R86C) | Atovaquone and azithromycin | Simon et al, 2017 [15] |
| 64/Female | Rituximab/HCT | CYTb (M134 T) | Atovaquone | Rosenblatt et al, 2021 [16] |
| 36/Male | Rituximab | CYTb (Y272C) and RPL4 (R86C) | Atovaquone and azithromycin | Marcos et al, 2022 [13] |
| 80/Male | Rituximab | 23S rRNA (A1915G)<br>CYTb (V141A) | Azithromycin and clindamycin<br>Atovaquone | Rogers et al, 2023 [12] |

Abbreviations: CYTb, cytochrome b; HCT, hematopoietic cell transplantation; NA, not available.

[a]Details not available but presumed to be immunocompromised.

recognized antimicrobial resistance gene mutations. If this information were known, it might impact the choice of drugs used, the number of drugs used, and/or the drug dosages.

To date, rituximab is the most common anti-CD20 biologic associated with relapsing babesiosis [8, 9, 13, 17, 22, 23]. The anti-CD20 biologic ocrelizumab (used for multiple sclerosis) has also been associated with the development of relapsing disease [24–26]. Some patients with relapsing disease had had removal of the spleen due to lymphoma along with receiving rituximab as a chemotherapy agent. The role of the asplenia, per se, in these cases is unclear [27], as relapsing disease has occurred in a patient who had received rituximab for granulomatosis with polyangiitis and also in a patient being treated for rheumatoid arthritis, both of whom had intact spleens [13, 17]. Overall, anti-CD20 therapy, especially rituximab, seems to be a major risk factor for developing relapsing babesiosis. Rituximab therapy can reduce the number of B lymphocytes, thereby negatively impacting antibody production to *B microti*; lack of an antibody response to *B microti* can last for more than a year after discontinuing rituximab [13, 17, 28].

New therapeutics are urgently needed for patients with babesiosis. Exchange transfusion may potentially reduce mortality from babesiosis in immunocompromised patients with high levels of parasitemia [6, 29–31]. Clofazimine, in conjunction with atovaquone, has been shown to successfully clear *Babesia* parasites in mice [32]. Other animal studies have evaluated other potential therapeutic regimens [33, 34]. Although tafenoquine as a single agent seems promising, more data are needed given the ambiguous results in the currently available case reports [12–14].

Limitations of this study are that very few *B microti* strains were tested and that there was no actual determination of resistance per se, only the potential mechanism of resistance. In addition, the level of resistance was not determined, which is relevant to the question of whether a higher dose of an antimicrobial might have overcome the resistance. A major gap in babesiosis research is the lack of an in vitro culture technique for *B microti* that could be used to determine antimicrobial drug susceptibility.

In conclusion, resistance mutations to 3 antiparasitic antimicrobials (azithromycin, atovaquone, and clindamycin) have been demonstrated in *B microti* strains associated with persistent or relapsing babesiosis thus far, based on studying 9 patients. Further studies are needed to optimize therapy for immunocompromised patients with babesiosis and thereby prevent the emergence of resistance, especially for those patients who have received rituximab.

## Notes

***Author contributions.*** Both authors provided conceptualization and writing—review and editing.

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