Case Report

Clindamycin-doxycycline-metronidazole combination therapy in a refractory canine babesiosis case

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An 8-year-old castrated male Maltese dog (patient) was referred to our institute with refractory canine babesiosis. The patient had previously responded to conventional treatment with atovaquone and azithromycin; however, anemia had recurred at six weeks after treatment withdrawal. No effect was observed on the administration of the same medication along with diminazene aceturate. On blood analysis, mild anemia was identified, with the absolute reticulocyte count indicating a markedly regenerative state. On Diff-Quik-stained peripheral blood film examination, the parasitic protozoan Babesia gibsoni was observed, and based on further laboratory examinations, a relapse of babesiosis was confirmed. Based on a previous study of drug-resistant variants of B. gibsoni and therapeutic trials, the treatment was then changed to a combination therapy of clindamycin, doxycycline, and metronidazole. Subsequently, the patient’s condition improved rapidly — B. gibsoni was not detected in the blood film and the PCR analysis for it was negative. This treatment was discontinued at six weeks after treatment initiation; however, at seven weeks after the treatment withdrawal, another relapse of babesiosis was confirmed and treatment was restarted with the same protocol. This treatment was effective again and lasted for 12 weeks. However, anemia recurred again at five weeks after withdrawal of the previous treatment and was corrected by restarting the same treatment protocol. This third treatment continued for 24 weeks and was finally stopped at the request of the client. The patient has reportedly been doing well with no manifestation of clinical signs and symptoms. This case report demonstrates that the clindamycin-doxycycline-metronidazole combination therapy against atovaquone and azithromycin-resistant B. gibsoni may be effective in improving the clinical manifestation of symptoms of canine babesiosis and this therapy may be an alternative treatment strategy.

Key words: Babesia gibsoni, clindamycin, dog, doxycycline, metronidazole

Introduction

Canine babesiosis is a tick-borne hemoprotozoan disease majorly caused by Babesia gibsoni across Asia, including in Korea [1]. B. gibsoni infects erythrocytes and causes hemolytic anemia accompanied by several clinical signs and symptoms (including fever, lethargy, anorexia, splenomegaly, and jaundice) [1]. Many drugs, including babsiacidal agents (such as diminazene aceturate and imidocarb dipropionate), antiprotozoal agents (such as atovaquone), and antibiotics have been used in the management of canine babesiosis, but no single drug can eliminate the B. gibsoni found in the blood of infected patients [2]. Previously, diminazene aceturate was the preferred choice of drug for treatment of canine babesiosis; however, frequent relapse occurred in B. gibsoni infected patients, while severe adverse side effects such as cerebellar hemorrhage, necrosis at the injection site, and hepatotoxicity were also occasionally induced [3, 4]. Additionally, the addictive or synergistic effects of these drugs in these patients have also been under investigation. Lately, atovaquone-azithromycin combination therapy has been the main choice for treatment, and although it has shown good effectiveness, resistant variants of Babesia have yet been reported [5, 6]. There are reports that the clindamycin-doxycycline-metronidazole combination therapy is effective against a B. gibsoni infection and no cases of resistance have been reported to date [6, 7].

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**Case Description**

An 8-year-old castrated male Maltese dog (patient), weighing 4.5 kg, was referred to our institute with refractory canine babesiosis. The patient had initially visited a local animal hospital for treatment of anemia and was diagnosed with an infection of *Babesia B. gibsoni* following PCR analysis (Idexx laboratories). The patient had previously responded to conventional treatment with atovaquone and azithromycin; however, anemia had recurred at six weeks after treatment withdrawal. Although the PCR analysis was positive for *B. gibsoni*, no effect was observed on the administration of the same medication of diminazene aceturate.

A complete blood count revealed mild anemia (Hematocrit [Hct]: 35.2%; reference range: 36%–55%), a markedly regenerative state (Reticulocyte: 495.2 × 10^3 / μL; reference range: 10–110 × 10^3 / μL), leukocytosis (White blood cells: 34.9 × 10^3 / μL; reference range: 5.05–16.76 × 10^3 / μL). On Diff-Quik-stained peripheral blood film examination, the parasitic protozoan *B. gibsoni* was observed, and based on further laboratory examinations, a relapse of babesiosis was confirmed.

Based on a previous study of drug-resistant variants of *B. gibsoni* and therapeutic trials, the treatment was then changed to a combination therapy of clindamycin (Fullram, Samjin Pharm, Seoul, South Korea; 25 mg/kg, PO, q12H), doxycycline (Doxycycline, Young Poong Pharm, Incheon, South Korea; 5 mg/kg, PO, q12H), and metronidazole (Flasinyl, CJ HealthCare, Seoul, South Korea; 15 mg/kg, PO, q12H). Subsequently, the patient’s condition improved rapidly with no manifestation of clinical signs and symptoms observed. Furthermore, *B. gibsoni* was not detected in the blood film and the results of the PCR analysis for it were negative (Idexx laboratories). This treatment was discontinued after six weeks of initial therapy, and three weeks following its discontinuation, the hematocrit value was determined to be within the reference range (Hct: 42.0%). However, at seven weeks after treatment withdrawal, another relapse of babesiosis was confirmed, with moderate anemia (Hct: 23.0%) and *B. gibsoni* being detected in the blood film again. Thereafter, treatment was restarted with the same protocol and was effective again, lasting for 12 weeks.

Regenerative mild anemia (Hct: 36.2%, reticulocyte: 335.8 × 10^3 / μL), however, recurred again at five weeks after withdrawal of the previous treatment and was corrected by restarting the same treatment protocol. At 16 weeks after the third treatment initiation, the patient still reported normal hematocrit levels and an asymptomatic course, despite the results of the PCR analysis being positive for *Babesia spp* (Idexx laboratories). This third treatment continued for 24 weeks and was finally stopped at the request of the client. The patient has reportedly been doing well with no manifestation of clinical signs and symptoms.

**Discussion**

Conventional treatments used for canine babesiosis include babesiidal agents (such as diminazene aceturate and imidocarb dipropionate), antiprotozoal agents (such as atovaquone) and antibiotics. There is, however, no treatment protocol that can eliminate a *B. gibsoni* infection, and therefore, dogs generally become chronic carriers, with frequent relapse also occurring after treatment [2]. Additionally, babesiidal agents have a narrow margin of safety and could induce severe adverse side effects — imidocarb, for instance, can cause severe inflammation, while diminazene aceturate can cause side effects such as seizure and neurological symptoms due to cerebellar hemorrhage and hepatotoxicity [3, 4, 8]. Furthermore, it is currently difficult to use these two drugs (diminazene aceturate and imidocarb dipropionate) in Korea. Concurrently, atovaquone (an antiprotozoal agent) and azithromycin (a macrolide antibiotic) combination therapy has shown good effectiveness but could cause the emergence of resistant variants of *B. gibsoni* [6, 9]. Similarly, in our case, despite the patient’s response to early atovaquone-azithromycin combination therapy being effective and the improved clinical symptoms, anemia had recurred six weeks after treatment withdrawal. When the same protocol was reused, the clinical symptoms did not improve. During this time, the client consistently implemented procedures for the extermination of ectoparasites after the first infection, and was particularly cautious while walking outside with the patient, so we considered this recurrence to be a relapse rather than a reinfection. Therefore, this was suspected to be due to the resistance of *B. gibsoni* to the atovaquone-azithromycin combination therapy.

As a novel alternative treatment strategy for this patient, we used clindamycin (25 mg/kg, PO, q12H), doxycycline (5 mg/kg, PO, q12H), and metronidazole (15 mg/kg, PO, q12H) combination therapy [7]. Clindamycin, which is a
type of lincomycin-derived antibiotic, stimulates both cellular and humoral immunity by damaging B. gibboni and has been shown to be effective against human babesiosis [10], while doxycycline, which is one of the tetracycline antibiotics, has been reported to have a prophylactic effect against B. canis infections [11], and metronidazole, one of the antitrichomonal agents, has been shown to have a therapeutic effect against B. gibboni infections [12]. In a previous study, clindamycin, doxycycline, and metronidazole combination therapy successfully eliminated B. gibboni in three out of four of the infected dogs [7]. Our combination therapy had a synergistic effect and was further evaluated to be economical as well as less toxic.

In our case, the relapse was identified when administration of the combination was withdrawn, and yet the results of the PCR analysis were positive for Babesia spp; therefore, it should be noted that B. gibboni cannot be removed completely. However, the clinical manifestation quickly improved again once treatment was restarted, and the recurrence of the clinical symptoms did not occur during this administration. Therefore, we can infer that the resistance for this combination therapy had not occurred yet, and that the parasitemia can be suppressed effectively when this combination protocol is implemented successfully. Since there have been many reports on resistant mutations for each of the drugs that were used in the combination, the combination itself also needs more research on the possibility of the emergence of resistant variants of Babesia spp, and therefore, it we considered that careful monitoring of the patient receiving this combination therapy will be required [13-15].

Many other combination therapy strategies have also been reported, including atovaquone-proguanil-azithromycin, clindamycin-diminazene aceturate-para-amino, and doxycycline-enrofloxacin-metronidazole [16-18]. As a result of in vitro research on the growth inhibitory effect of B. gibboni, pentamidine was observed to show an inhibitory effect in its lowest dosage, after atovaquone and diminazene aceturate [19]. Therefore, we considered that additional research on combination therapy with other drugs using pentamidine could also be beneficial for a new therapeutic option [20].

This case report describes the application and management of clindamycin-doxycycline-metronidazole combination therapy against a B. gibboni infection that is refractory to atovaquone-azithromycin combination therapy. Our combination therapy was effective in improving clinical signs and symptoms without any side effects observed. However, it should be noted that the patient needs careful monitoring due to the possibility of relapse. This combination therapy can be an alternative treatment strategy for babesiosis that is refractory to conventional therapy.

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