pISSN 2508-1357, eISSN 2508-139X
J Biomed Transl Res 2019;20(3):071-074
https://doi.org/10.12729/jbtr.2019.20.3.071
Received 10 Sep. 2019, Accepted 19 Sep. 2019

Case Report

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

Hyung-Mo An[1], Joong-Hyun Song[1], Su-Jin An[1], DoHyeon Yu[1], Donghyun Han[2], Young Joo Kim[3], Dong-In Jung[1*]

[1]*Institute of Animal Medicine, College of Veterinary Medicine, Gyeongsang National University, Jinju 52828, Korea*
[2]*Section of Veterinary Research and Academic Consulting, Veteran, Inc., Seoul 06254, Korea*
[3]*College of Veterinary Medicine, Western University of Health Sciences, California 91766-1854, USA*

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 babesiacidal agents (such as diminazene aceturate and imidocarb diproprionate), 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].

***Corresponding author:** Dong-In Jung
Institute of Animal Medicine, College of Veterinary Medicine, Gyeongsang National University, Jinju 52828, Korea
Tel: +82-55-772-2361, Fax: +82-55-772-2330, Email: jungdi@gnu.ac.kr

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.

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 (Fullgram, 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 babesiacidal agents (such as diminazene aceturate and imidocarb diproprionate), 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, babesiacidal 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 diproprionate) 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. gibsoni* 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. gibsoni* infections [12]. In a previous study, clindamycin, doxycycline, and metronidazole combination therapy successfully eliminated *B. gibsoni* 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. gibsoni* 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-imidocarb, and doxycycline-enrofloxacin-metronidazole [16–18]. As a result of *in vitro* research on the growth inhibitory effect of *B. gibsoni*, 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. gibsoni* 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.

ORCID

Hyung-Mo An, https://orcid.org/0000-0003-3792-9326
Joong-Hyun Song, https://orcid.org/0000-0001-9961-6451
Su-Jin An, https://orcid.org/0000-0003-4232-2799
DoHyeon Yu, https://orcid.org/0000-0001-7645-6926
Donghyun Han, https://orcid.org/0000-0003-3258-244X
Young Joo Kim, https://orcid.org/0000-0002-0507-707X
Dong-In Jung, https://orcid.org/0000-0002-5116-6006

References

1. Conrad P, Thomford J, Yamane I, Whiting J, Bosma L, Uno T, Holshuh HJ, Shelly S. Hemolytic anemia caused by *Babesia gibsoni* infection in dogs. J Am Vet Med Assoc 1991;199:601-605.
2. Kumara WRB. Antibabesial treatment protocols against canine babesiosis. Sri Lanka Vet J 2016;63:15-21.
3. Hwang MJ, Lee HS, Lee KW. Haematological and serum chemical findings following repeated medication of diminazene aceturate in canine babesiosis. Korean J Vet Clin Med 2000;17:349-358.
4. Miller DM, Swan GE, Lobetti RG, Jacobson LS. The pharmacokinetics of diminazene aceturate after intramuscular administration in healthy dogs. J S Afr Vet Assoc 2005;76:146-150.
5. Jefferies R, Ryan UM, Jardine J, Robertson ID, Irwin PJ. *Babesia gibsoni*: detection during experimental infections and after combined atovaquone and azithromycin therapy. Exp Parasitol 2007;117:115-123.
6. Sakuma M, Setoguchi A, Endo Y. Possible emergence of drug-resistant variants of *Babesia gibsoni* in clinical cases treated with atovaquone and azithromycin. J Vet Intern Med 2009;23:493-498.
7. Suzuki K, Wakabayashi H, Takahashi M, Fukushima K, Yabuki A, Endo Y. A possible treatment strategy and clinical factors to estimate the treatment response in *Bebesia gibsoni* infection. J Vet Med Sci 2007;69:563-568.
8. Abdullah AS, Sheikh-Omar AR, Baggot JD, Zamri M. Adverse effects of imidocarb dipropionate (Imizol) in a dog. Vet Res Commun 1984;8:55-59.
9. Birkenheuer AJ, Levy MG, Breitschwerdt EB. Efficacy of combined atovaquone and azithromycin for therapy of chronic *Babesia gibsoni* (asian genotype) infections in dogs. J Vet Intern Med 2004;18:494-498.
10. Wulansari R, Wijaya A, Ano H, Horii Y, Nasu T, Yamane S, Makimura S. Clindamycin in the treatment of *Babesia gibsoni* infections in dogs. J Am Anim Hosp Assoc 2003;39:558-562.
11. Vercammen F, De Deken R, Maes L. Prophylactic treat-

ment of experimental canine babesiosis (*Babesia canis*) with doxycycline. Vet Parasitol 1996;66:251-255.

12. Fowler JL, Ruff MD, Fernau RC, Furusho Y. *Babesia gibsoni*: chemotherapy in dogs. Am J Vet Res 1972;33:1109-1114.
13. Chalker VJ, Waller A, Webb K, Spearing E, Crosse P, Brownlie J, Erles K. Genetic diversity of *Streptococcus equi* subsp. *zooepidemicus* and doxycycline resistance in kennelled dogs. J Clin Microbiol 2012;50:2134-2136.
14. Goodwin A, Kersulyte D, Sisson G, Veldhuyzen van Zanten SJ, Berg DE, Hoffman PS. Metronidazole resistance in *Helicobacter pylori* is due to null mutations in a gene (rdxA) that encodes an oxygen-insensitive NADPH nitroreductase. Mol Microbiol 1998;28:383-393.
15. Schreckenberger PC, Ilendo E, Ristow KL. Incidence of constitutive and inducible clindamycin resistance in *Staphylococcus aureus* and coagulase-negative staphylococci in a community and a tertiary care hospital. J Clin Microbiol 2004;42:2777-2779.
16. Lee DK, Kim YG, Yun YM, Lee KK. Therapeutic effects of atovaquone/proguanil in combination with azithromycin in dogs naturally infected with *Babesia gibsoni*. J Vet Clin 2016;33:16-20.
17. Lin EC, Chueh LL, Lin CN, Hsieh LE, Su BL. The therapeutic efficacy of two antibabesial strategies against *Babesia gibsoni*. Vet Parasitol 2012;186:159-164.
18. Lin MY, Huang HP. Use of a doxycycline-enrofloxacin-metronidazole combination with/without diminazene diaceturate to treat naturally occurring canine babesiosis caused by *Babesia gibsoni*. Acta Vet Scand 2010;52:27.
19. Matsuu A, Yamasaki M, Xuan X, Ikadai H, Hikasa Y. *In vitro* evaluation of the growth inhibitory activities of 15 drugs against *Babesia gibsoni* (Aomori strain). Vet Parasitol 2008;157:1-8.
20. Sands M, Kron MA, Brown RB. Pentamidine: a review. Rev Infect Dis 1985;7:625-634.