Efficacy of Malarone® in Dogs Naturally Infected with Babesia gibsoni

Aiko IGUCHI1), Nobuyuki SHIRANAGA2), Aya MATSUU3)* and Yoshiaki HIKASA1)

1) Laboratory of Veterinary Internal Medicine, Joint Department of Veterinary Medicine, Faculty of Agriculture, Tottori University, Tottori 680–8553, Japan
2) Shiranaga Animal Hospital, Yamaguchi 745–0806, Japan
3) Transboundary Animal Disease Research Center, Joint Faculty of Veterinary Medicine, Kagoshima University, Kagoshima 890–0065, Japan

(Received 16 March 2014/Accepted 15 May 2014/Published online in J-STAGE 9 June 2014)

ABSTRACT. The efficacy of Malarone® alone and in combination with doxycycline (DOXY) against Babesia gibsoni infections was examined in 8 dogs. In all dogs except one treated with Malarone®, parasitemia decreased, and anemia improved soon after initiation of treatment. However, 3 of 4 dogs treated with Malarone® relapsed, and relapse was inhibited in 2 of 4 dogs treated with Malarone® and DOXY. All relapsed dogs responded well to the second treatment, but 1 dog relapsed again and did not respond to the third treatment. Malarone® may be useful for acute stage of B. gibsoni infections, and at least second repeating treatment might be effective.

KEYWORDS: Babesia gibsoni, doxycycline, Malarone®

doi: 10.1292/jvms.14-0139; J. Vet. Med. Sci. 76(9): 1291–1295, 2014

Babesia gibsoni infects dogs’ red blood cells and typically causes hemolytic anemia. This condition is often accompanied by fever, jaundice, hemoglobinuria and an enlarged spleen [3, 5]. The parasite is distributed throughout the world, including Asia, Africa, Europe, America and Australia [11, 17]. In Japan, B. gibsoni mostly occurs in the western part of the country [6, 9, 10] and recently has spread to the eastern region [16]. Although various treatment modalities have been described [2, 12, 21], a definitive strategy to eliminate B. gibsoni has not been established.

In Japan, diminazene aceturate has been mainly used to treat acute onset of babesiosis caused by B. gibsoni infections; however, it often fails to eliminate the parasite and causes severe adverse effects, such as pain at the injection site and nervous symptoms due to cerebral hemorrhage [2, 22]. Some studies have reported the effectiveness of clindamycin-metronidazole-doxycycline (DOXY), but this regimen takes a long time and often requires supportive therapy [13, 21]. It is reported that atovaquone (ATV) monotherapy was effective for acute canine B. gibsoni infection; however, it resulted in relapse and proliferation of ATV-resistant parasites with a single nucleotide polymorphism at nt363 in cytochrome b, which in turn caused the replacement of methionine with isoleucine (M121I) [14]. Therefore, combination therapy with other drugs has been recommended. Azithromycin has been examined as a combination drug with ATV in some studies [8, 14, 15, 19], but it could not completely eliminate the parasites.

Malarone® (GlaxoSmithKline, London, U.K.), which is commercially available for treating malaria in humans, contains ATV and proguanil (PG). PG is a highly protein-bound molecule exhibiting weak antimalarial activity [18]. However, when used with ATV, PG enhanced the action of ATV that collapses the mitochondrial membrane of protozoa [20]. In our previous study, the interaction between ATV and PG proved synergistic against both ATV-sensitive and ATV-resistant B. gibsoni in vitro [8]. In addition, Malarone® showed clinical efficacy in the treatment of B. gibsoni experimentally infected dogs, although this experimental therapy could not eliminate the parasite or inhibit its recurrence with the M121I variant. To gather more clinical information on using Malarone® for B. gibsoni infections, an expanded study using a greater number of dogs is needed.

In the present study, we evaluated the efficacy of Malarone® against B. gibsoni in the acute stage of naturally infected dogs. We also assessed whether the addition of DOXY to Malarone® can inhibit recurrence and emergence of resistance against ATV.

Eight dogs with acute onset of B. gibsoni infection, exhibiting anemia and with microscopic evidence of small proplasms in their red blood cells, were enrolled in this study. Observation of blood smears did not reveal the presence of other pathogens. The 8 dogs (dogs 1–8) were diagnosed with canine babesiosis during 2012–2013 at Shiranaga Animal Hospital in Yamaguchi Prefecture, Japan. The breed, age, sex, symptoms and blood parameters of the dogs are shown in Table 1. Dogs 1, 5, 6 and 7 had no relevant case history. Dog 2 had a history of kennel cough; Dog 3, slipped disk and accidental ingestion of foreign body in gullet and stomach; Dog 4, prostatic hypertrophy and spondylosis deformans. These histories had resolved, and the dogs were not administered any treatment for these when they were diagnosed with canine babesiosis. Dog 8 showed idiopathic hypoglycemia
and administrated corticosteroid before admission to the animal hospital. During the treatment for babesiosis, this dog was not administered any other drugs. All 8 dogs had not received any antiprotozoal drugs before their admission to the animal hospital.

After diagnosis, 4 dogs (dogs 1–4) received Malarone® therapy (ATV 17–25 mg/kg, PG 7–10 mg/kg, twice daily for 10 days); the other 4 dogs (dogs 5–8) received combination therapy of Malarone® for 10 days and DOXY for 30 days (5 mg/kg, twice daily). After initiation of therapy, blood samples were collected with EDTA as the anticoagulant (5 mg/kg, twice daily). After initiation of therapy, blood in the parasites [7]. Presented as the population ratio of 363G and 363T alleles and mutated allele (363T) in whole blood samples and were numbers were calculated for the wild type alleles (363G). Assay was performed to quantify the M121I variant population. In 3 of 4 cases treated with Malarone® therapy (dogs 1, 2 and 3); and in all cases treated with the combination therapy (dogs 5–8), clinical signs of babesiosis, anemia and the number of PLTs improved soon after initiation of treatment (Fig. 1). Because dog 4 showed progressive anemia although parasitemia decreased during Malarone® therapy, observation was terminated. This dog was treated with diminazene aceturate 3 times (2 mg/kg/day, every other day). After this therapy, PCV increased to 34% on day 37. Two dogs treated with combination therapy (dogs 5 and 6) did not relapse, whereas the other 3 dogs treated with Malarone® therapy (dogs 1, 2 and 3) and 2 dogs with combination therapy (dogs 7 and 8) relapsed during the observation period. These 5 dogs showing relapse received a second round of Malarone® therapy, particularly Dog 1 received combination therapy at relapse as per the owner’s choice; the dog responded well to this therapy. However, dog 3 relapsed again after the second treatment. This dog was administered Malarone® for the third time, but had a progression of anemia. Then, the observation was stopped. In all dogs, supportive therapies, such as blood transfusion, were not required, and any evident adverse effects resulting from these treatment protocols were not detected. Dog 4 showed high ALP activity (996 U/l) before starting the Malarone® therapy. The laboratory data did not disclose any other significant biochemical changes.

PCR assays showed that the B. gibsoni p18 gene was detected intermittently until the last day of observation, when it was discovered in all dogs. The M121I variant population in host blood was measured before and after Malarone® administration. The M121I variant measured less than 0.1% in all dogs. The M121I population in host blood decreased during Malarone® therapy, observation was terminated. This dog was administered Malarone® for the third time.

### Table 1. The clinical information of dogs before starting the treatment and their treatment protocol

| No  | Breed                        | age | sex | PCV (%) | PLT (×10⁴/µl) | Parasitemia (%) | Clinical signs | Biochemical analysis | Treatment protocol |
|-----|------------------------------|-----|-----|---------|---------------|-----------------|-----------------|---------------------|-------------------|
| 1   | Welsh Corgi Pembroke         | 6y  | male | 23      | 3             | 2               | Anorexia, Depression | ALT (IU/l) 134 12.4 0.3 7.7 0.5 0.4 | Malarone® therapy (ATV 17–25 mg/kg, PG 7–10 mg/kg, twice per day, for 10 days) |
| 2   | Pug                         | 2y  | female | 19     | 1.4           | 4.5             | Anorexia         | ALP (IU/l) 1637 15.4 0.6 5.2 0.6 0.4 |
| 3   | Miniature Dachshund          | unknown | male | 35     | 3             | 1.5             | Anorexia         | BUN (mg/dl) 33 88 22.5 0.7 6 0.8 0.3 |
| 4   | Shiba                       | 14y | male | 35     | 3.4           | 3               | Anorexia, Depression | Cre (mg/dl) 36 996 24.3 0.9 5.8 0.8 0.3 |
| 5   | Mixed breed                  | 8y  | male | 20     | 10.4          | 5.5             | Anorexia, Hemoglobulinuria | TP (g/dl) 19 176 14.7 0.6 5.9 0.5 0.2 | Combination therapy (Malarone® for 10 days and DOXY for 30 days (5 mg/kg, twice pre day)) |
| 6   | American Cocker Spaniel      | 3y  | female | 17    | 7.5           | 3               | Anorexia, Depression | A/G 19 152 17.1 0.6 4.9 0.6 0.3 |
| 7   | West Highland White Terrier  | 6y  | male | 25     | 1.5           | 2               | Anorexia, Depression | T-Bil (mg/dl) 20 209 14 0.4 6.8 0.7 0.2 |
| 8   | Chihuahua                    | 7y  | male | 21     | 6             | 3               | Pale mucous membrane | ALT (IU/l) 30 210 10.5 0.4 8.2 0.5 0.4 | Malarone® therapy (ATV 17–25 mg/kg, PG 7–10 mg/kg, twice per day, for 10 days) |

Relapse of babesiosis was defined in this study as the reappearance of B. gibsoni in blood smear and/or a decrease in PCV of less than 25%. At relapse, Malarone® therapy was reinitiated at the previous dosages for 10 days. If the dogs vomited more than twice or anorexia was diagnosed, treatment and observation were terminated.

As shown in Table 1, the 8 dogs in this study showed mild to severe anemia and thrombocytopenia. In 3 of 4 cases treated with Malarone® therapy (dogs 1, 2 and 3); and in
Clinical signs in the acute stage of *B. gibsoni* infection in 7 of 8 dogs were well controlled by the Malarone® or combination therapy, without the need for supportive therapies. In these 7 dogs, parasitemia decreased, and on day 10, PCV increased when administration of Malarone® was completed. Malarone® appears to be clinically useful for treating *B. gibsoni* infection. Nevertheless, 3 of the 4 dogs treated with Malarone®, on the other hand, 2 of the 4 dogs treated with combination therapy did not relapse during the observation period. In our previous study, DOXY inhibited the growth of both wild-type (WT) and ATV-resistant *B. gibsoni* [7]. It is also reported that daily administration of DOXY may provide satisfactory prophylaxis against *B. canis* [4]. The efficacy of DOXY monotherapy against *B. gibsoni* infections
has not been reported. The addition of DOXY to Malarone® for long period might be efficient for inhibiting some of the recurrence of the canine *B. gibsoni* infections. Because the number of dogs in this study was low, further studies using a greater number of dogs are needed.

Even though an allele-specific SYBR green real-time PCR assay showed various levels of M121I (15–96%) in each relapsed dog at initiation of the second treatment, these dogs responded well to the second Malarone® treatment, with or without DOXY. In our previous study, the combination of ATV and PG had a synergistic effect against ATV-resistant as well as WT *B. gibsoni in vitro*. The second Malarone® treatment at the same dosage as the first treatment was effective for inhibiting the parasites and relieving the clinical signs in a relapsed dog with a 75% population of M121I parasites [8]. Results of the present study are similar to our previous study. However, in the present study, dog 3 developed anemia during the third course of Malarone® therapy. It is reported that a disadvantage of ATV is its poor bioavailability. In human patients with decreased intestinal absorption, ATV effectiveness may be reduced, particularly during management of acquired immune deficiency syndrome (AIDS) patients with *Pneumocystis carinii* pneumonia or toxoplasmosis [1]. Dog 4 showed hyperphosphatasemia on day 0. Although underlying disease could not detect based on the owner’s interview, if hepatic disorders coexist with *B. gibsoni* infection, it could affect the bioavailability of ATV. On the other hand, it is possible that the sensitivity of parasites against ATV in Dog 4 was low. In this study, parasites’ ATV sensitivity was evaluated on the basis of M121I population alone. However, the mechanism of action of ATV against *B. gibsoni* is not well understood, and factors other than M121I could affect the sensitivity against ATV. Furthermore, this study was performed in an area where *B. gibsoni* is endemic. Therefore, although prior infection may not have been identified by the owner or veterinarian, it is possible that some of the dogs had subclinical infection, which may have relapsed with age or immunosuppressive treatment. This may be one of the reasons why the efficacy of Malarone® treatment in Dog 4 was low.

Our findings suggest that Malarone® therapy is a clinically effective treatment in the acute stage of naturally *B. gibsoni*-infected dogs. However, Malarone® treatment could not completely eliminate *B. gibsoni* from the patients in our study. Moreover, the addition of DOXY could not completely inhibit the M121I variant. At least twice administration for the relapsed patient Malarone® at the same dose as the first time could inhibit the parasite growth and improve the anemia of dogs.

### REFERENCES

1. Baggish, A. L. and Hill, D. R. 2002. Antiparasitic agent atovaquone. *Antimicrob. Agents Chemother.* 46: 1163–1173. [Medline]  [CrossRef]
2. Boozer, A. L. and Macintire, D. K. 2003. Canine babesiosis. *Vet. Clin. North Am. Small Anim. Pract.* 33: 885–904. [Medline]  [CrossRef]
3. Farwell, G. E., Legrand, E. K. and Cobb, C. C. 1982. Clinical observations on *Babesia gibsoni* and *Babesia canis* infections in dogs. *J. Am. Vet. Med. Assoc.* 180: 507–511. [Medline]
4. Vercammen, F., De Deken, R. and Maes, L. 1996. Prophylactic treatment of experimental canine babesiosis (*Babesia canis*) with doxycycline. *Vet. Parasitol.* 66: 251–255. [Medline]  [CrossRef]
5. Groves, M. G. and Dennis, G. L. 1972. *Babesia gibsoni*: field and laboratory studies of canine infections. *Exp. Parasitol.* 31: 153–159. [Medline]  [CrossRef]
6. Ikada, H., Tanaka, H., Shibahara, N., Matsu, A., Uexchi, M., Itoh, N., Oshiro, S., Kudo, N., Igarashi, I. and Oyamada, T. 2004. Molecular evidence of infections with *Babesia gibsoni* parasites in Japan and evaluation of the diagnostic potential of a loop-mediated isothermal amplification method. *J. Clin. Microbiol.* 42: 2465–2469. [Medline]  [CrossRef]
7. Iguchi, A., Matsu, A., Ikada, H., Talukder, H. and Hikasa, Y. 2012. Development of *in vitro* atovaquone-resistant *Babesia gibsoni* with a single-nucleotide polymorphism in cytb. *Vet. Parasitol.* 185: 145–150. [Medline]  [CrossRef]
8. Iguchi, A., Matsu, A., Fujii, Y., Ikada, H. and Hikasa, Y. 2013. *The in vitro interactions and in vivo efficacy of atovaquone and proguanil against Babesia gibsoni* infection in dogs. *Vet. Parasit-
9. Inokuma, H., Yoshizaki, Y., Shimada, Y., Sakata, Y., Okuda, M. and Onishi, T. 2003. Epidemiological survey of Babesia species in Japan performed with specimens from ticks collected from dogs and detection of new Babesia DNA closely related to Babesia odocoilei and Babesia divergens DNA. J. Clin. Microbiol. 41: 3494–3498. [Medline] [CrossRef]

10. Inokuma, H., Yoshizaki, Y., Matsumoto, K., Okuda, M., Onishi, T., Nakagome, K., Kosugi, R. and Hirakawa, M. 2004. Molecular survey of Babesia infection in dogs in Okinawa, Japan. Vet. Parasitol. 121: 341–346. [Medline] [CrossRef]

11. Kjemtrup, A. M., Kocan, A. A., Whitworth, L., Meinkoth, J., Birkenheuer, A. J., Cummings, J., Moudreaux, M. K., Stockham, S. L., Iruzarry-Rovira, A. and Conrad, P. A. 2000. There are at least three genetically distinct small piroplasms from dogs. Int. J. Parasitol. 30: 1501–1505. [Medline] [CrossRef]

12. Lin, E. C., Chueh, L. L., Lin, C. N., Hsieh, L. E. and Su, B. L. 2012. The therapeutic efficacy of two antibabesial strategies against Babesia gibsoni. Vet. Parasitol. 186: 159–164. [Medline] [CrossRef]

13. Lin, M. Y. and Huang, H. P. 2010. Use of doxycycline-enrofloxacin-metronidazole combination with/without diminazene diaceturate to treat naturally occurring canine babesiosis caused by Babesia gibsoni. Acta Vet. Scand. 52: 27. [Medline] [CrossRef]

14. Matsuu, A., Koshida, Y., Kawahara, M., Inoue, K., Ikadai, H., Hikasa, Y., Okano, S. and Higuchi, S. 2004. Efficacy of atovaquone against Babesia gibsoni in vivo and in vitro. Vet. Parasitol. 124: 9–18. [Medline] [CrossRef]

15. Matsuu, A., Miyamoto, K., Ikadai, H., Okano, S. and Higuchi, S. 2006. Cloning of the Babesia gibsoni cytochrome b gene and isolation of three single nucleotide polymorphisms from parasites present after atovaquone treatment. Am. J. Trop. Med. Hyg. 74: 593–597. [Medline]

16. Miyama, T., Sakata, Y., Shimada, Y., Ogino, S., Watanabe, M., Itamoto, K., Okuda, M., Verdida, R. A., Xuan, X. N., Nagasawa, H. and Inokuma, H. 2005. Epidemiological survey of Babesia gibsoni infection in dogs in eastern Japan. J. Vet. Med. Sci. 67: 467–471. [Medline] [CrossRef]

17. Muhlnickel, C. J., Jefferies, R., Morgan-Ryan, U. M. and Irwin, P. J. 2002. Babesia gibsoni infection in three dogs in Victoria. Aust. Vet. J. 80: 606–610. [Medline] [CrossRef]

18. Pudney, M., Gutteridge, W., Zeman, A., Dickins, M. and Wolley, J. L. 1999. Atovaquone and proguanil hydrochloride: a review of nonclinical studies. J. Travel Med. 6 Suppl. 1: S8–S12. [Medline]

19. Sakuma, M., Setoguchi, A. and Endo, Y. 2009. Possible emergence of drug-resistant variants of Babesia gibsoni in clinical cases treated with atovaquone and azithromycin. J. Vet. Intern. Med. 23: 493–498. [Medline] [CrossRef]

20. Srivastava, I. K. and Vaidya, A. B. 1999. A mechanism for the synergistic antimalarial action of atovaquone and proguanil. Antimicrob. Agents Chemother. 43: 1334–1339. [Medline]

21. Suzuki, K., Wakabayashi, H., Takahashi, M., Fukushima, K., Yabuki, A. and Endo, Y. 2007. A possible treatment strategy and clinical factors to estimate the treatment response in Babesia gibsoni infection. J. Vet. Med. Sci. 69: 563–568. [Medline] [CrossRef]

22. Wulansari, R., Wijaya, A., Ano, H., Horii, Y. and Makimura, S. 2003. Lymphocyte subsets and specific IgG antibody levels in clindamycin-treated and untreated dogs experimentally infected with Babesia gibsoni. J. Vet. Med. Sci. 65: 579–584. [Medline] [CrossRef]