NOTE *Internal Medicine*

Efficacy of Malarone® in Dogs Naturally Infected with *Babesia gibsoni*

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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.
KEY WORDS: *Babesia gibsoni*, doxycycline, Malarone®

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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* infection 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 piroplasms 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

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

No	Breed	age	sex	PCV (%)	PLT ($\times 10^4/\mu l$)	Parasitemia (%)	Clinical signs	Biochemical analysis							Treatment protocol
								ALT (IU/*l*)	ALP (IU/*l*)	BUN (mg/d*l*)	Cre (mg/d*l*)	TP (g/d*l*)	A/G	T-Bil (mg/d*l*)	
1	Welsh Corgi Pembroke	6y	male	23	3	2	Anorexia, Depression	40	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	16	317	15.4	0.6	5.2	0.6	0.4	
3	Miniature Dachshund	unknown	male	35	3	1.5	Anorexia	33	88	22.5	0.7	6	0.8	0.3	
4	Shiba	14y	male	35	3.4	3	Anorexia, Depression	36	996	24.3	0.9	5.8	0.8	0.3	
5	Mixed breed	8y	male	20	10.4	5.5	Anorexia, Hemoglobinuria	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	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	20	209	14	0.4	6.8	0.7	0.2	
8	Chihuahua	7y	male	21	6	3	Pale mucus membrane	30	210	10.5	0.4	8.2	0.5	0.4	

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 at 1- to 30-day intervals for at least 90 days after the first administration. Blood smears were prepared to examine the parasitemia microscopically. Complete blood count analysis was performed using an automated blood cell counter. Serum samples were isolated for biochemical analysis of alanine aminotransferase (ALT), alkaline phosphatase (ALP), blood urea nitrogen (BUN), creatinine (Cre), total protein (TP), albumin/globulin ratio (A/G) and total bilirubin (T-bil). Genomic DNA was extracted from blood samples for a polymerase chain reaction (PCR) assay to detect the parasite p18 gene, and an allele-specific SYBR green real-time PCR assay was performed to quantify the M121I variant population. For evaluating the M121I variant population, copy numbers were calculated for the wild type alleles (363G) and mutated allele (363T) in whole blood samples and were presented as the population ratio of 363G and 363T alleles in the parasites [7].

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 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 population in host blood was measured before and after Malarone® administration. The M121I variant measured less than 0.1% in all dogs at the start of Malarone® administration, but relapsed dogs showed a population of 15–96% of M121I at initiation of the second treatment. Although Dog 1 showed high parasitemia (4.5%) at relapse, the 363T allele was amplified at the Ct value over 22, which was detection limit [7]. When the third treatment was initiated in Dog 3, the M121I population was 91.3% (Table 2). This dog showed a decrease in PCV during the third treatment.

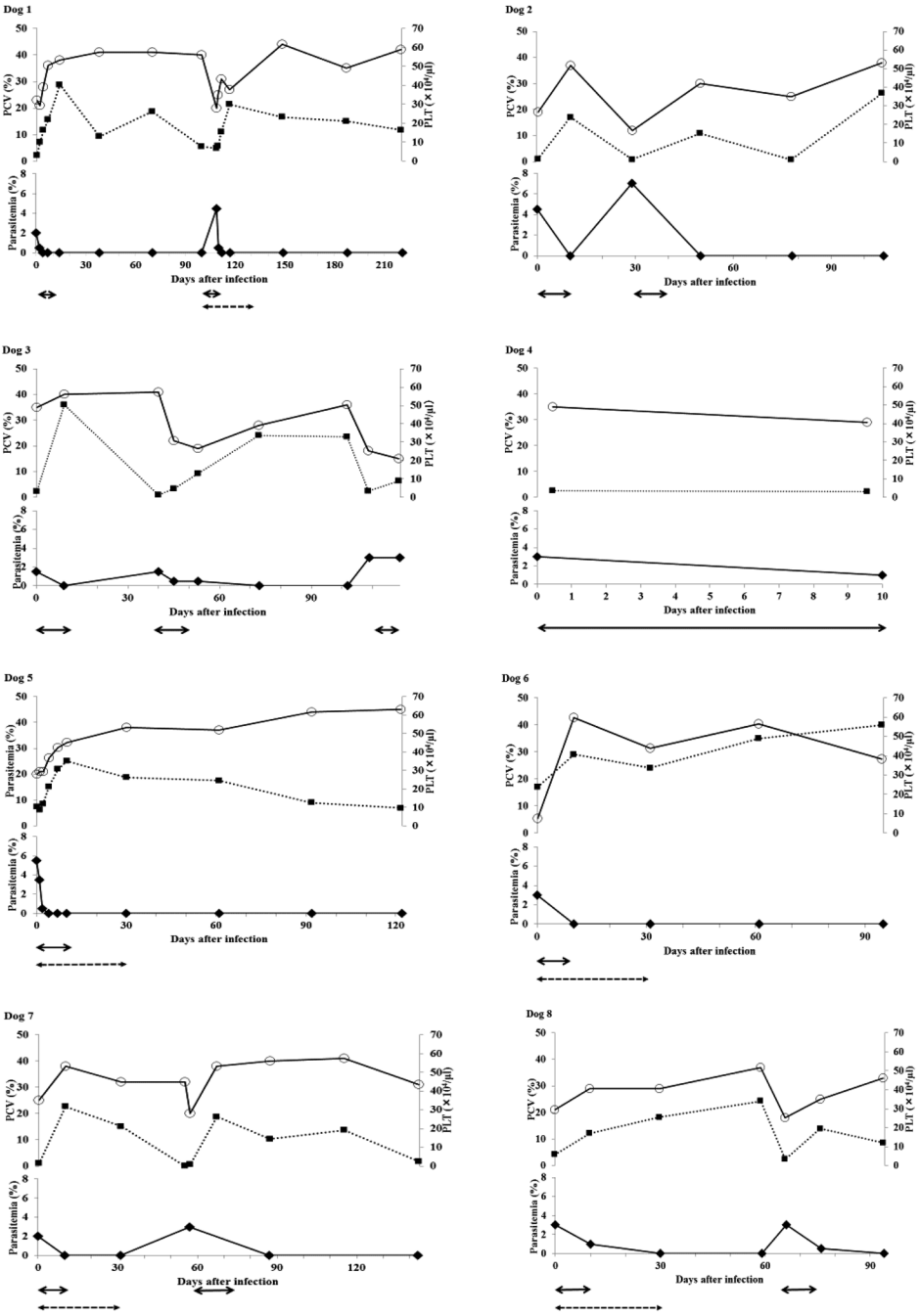

Fig. 1. Changes in PCV (○), number of PLT (■) and parasitemia (♦) in the 8 dogs. The period of Malarone® (solid line) and/or DOXY (dot line) treatment is shown as bows under the figure.

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

Table 2. The M121I variant population at before and after each treatment (%)

Dogs	First treatment		Second treatment		Third treatment	
	Pre	Post	Pre	Post	Pre	Post
1	0.02	ND[a)]	ND	ND	-	-
2	0.06	ND	14.5	13.9	-	-
3	0.06	ND	58.7	ND	91.3	84.4
4	0.33	0.34	-[b)]	-	-	-
5	0.61	ND	-	-	-	-
6	0.09	ND	-	-	-	-
7	0.52	ND	80.1	80.1	-	-
8	0.7	ND	96.1	95.9	-	-

a) ND: Not detected, b)-: No relapse.

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. gibosni* 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. The efficacy of repeating the administration of Malarone® in dogs infected with *B. gibsoni* has not been reported. These results indicate that a second administration of Malarone® at the same dose as the first time in relapse cases could inhibit the parasite growth and improve the anemia in dogs, but the efficacy of this therapy when administered more than 3 times needs further examination.

In our previous study, a culture strain of ATV-resistant *B. gibsoni* with over 99% of the M121I variant population showed a six-fold low sensitivity to ATV [7]. This indicated that the second ATV dosage to the relapsed dogs predicted to carry M121I *B. gibsoni* should have been increased. The optimal dosage for the second round of ATV therapy has not evaluated until today. It is reported that ATV and azithromycin in combination were needed to prolong the treatment period in 3 of 5 relapsed dogs [19]. Furthermore, there is no report about repeated dosing therapy of Malarone® in human or dogs. A further study using a larger number of dogs is required to establish optimal treatment based on dosage and period.

In this study, one dog (dog 4) developed anemia and did not respond to the first Malarone® treatment. 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.

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