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Citation	Japanese Journal of Veterinary Research, 66(3), 221-225
Issue Date	2018-08
DOI	10.14943/jjvr.66.3.221
Doc URL	http://hdl.handle.net/2115/71345
Type	bulletin (article)
File Information	p221-225 Hisashi_Inokuma.pdf



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Effects of low-dose diminazene aceturate injection followed by clindamycin administration for treating canine *Babesia gibsoni* infection

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Received for publication, December 3, 2017; accepted, April 2, 2018

Abstract

A total of 242 dogs diagnosed with acute *Babesia gibsoni* infection were administered three subcutaneous injections of low-dose diminazene aceturate (DA). After the initial DA treatment, 20 dogs in grave condition were excluded, and the remaining 222 were divided into 2 groups for the evaluation of clindamycin treatment from days 8 to 29: the clindamycin group, which received oral clindamycin and the control group, which received no drugs. Between days 8 and 29, relapse was observed in 13 of 80 dogs in the clindamycin-group, as compared to 42 of 142 dogs in the control. The relapse rate in the clindamycin-group was significantly lower compared to the control. Low-dose DA injection followed by oral clindamycin administration could effectively prevent relapse.

Key Words: *Babesia gibsoni*, clindamycine, relapse

Canine babesiosis is a hematologic disease caused by protozoal parasites, *Babesia gibsoni* and *Babesia canis*. These parasites infect the red blood cells of dogs, typically leading to the onset of hemolytic anemia. Infection with *B. gibsoni* in dogs can result in more severe clinical symptoms, including disseminated intravascular coagulation (DIC) and acute renal failure, as compared to infection with *B. canis*. Canine babesiosis in mainland Japan is commonly caused by *B. gibsoni*^{5,11,13)} and represents one of the important infectious diseases of dogs in Japan.

Diminazene aceturate is a main drug used

for the treatment of canine *B. gibsoni* infection. Reportedly, two intramuscular injections of 3 to 5 mg/kg diminazene aceturate (q7d) are usually administered to dogs with *B. gibsoni* infection^{2,12,14,16,17)}. However, this drug has been associated with serious adverse events, including cerebellar hemorrhage^{2,4,13,14,15)}. Another disadvantage of diminazene aceturate is the high rate of relapse^{1,4,18,19)}. Due to these disadvantages, several treatment protocols using other antiprotozoal drugs such as atovaquone, proguanil, doxycycline, clindamycin, metronidazole and enrofloxacin, have recently been reported.

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doi: 10.14943/jjvr.66.3.221

Meanwhile, the use of low-dose diminazene aceturate (2 mg/kg) with three subcutaneous injections, which was originally proposed by Namikawa *et al.*,¹³⁾ has become popular among veterinarians in endemic areas in Japan to avoid serious adverse events. Concomitant therapy with clindamycin for preventing relapse has also been performed by local veterinarians in those areas¹⁶⁾. However, the effect of low-dose diminazene aceturate followed by clindamycin administration has never been evaluated in a systematic manner. Moreover, there are scarce data regarding the associated adverse events.

Against this backdrop, this study was conducted to evaluate the effects of low-dose diminazene aceturate injection followed by clindamycin administration for treating acute canine *B. gibsoni* infection in an endemic area in Japan.

A total of 242 dogs diagnosed with acute *B. gibsoni* infection that were brought to the Shiranaga Animal Hospital in Yamaguchi Prefecture between 2000 and 2009 were included in this study. These dogs had no history of *B. gibsoni* infection and had not received any treatment. Diagnosis of acute *B. gibsoni* infection was determined based on both clinical symptoms (e.g., anorexia, depression, and darkening of urine) and the presence of *B. gibsoni* in a blood smear, as detected by microscope observation. All animals were examined for CBC and C-reactive protein (CRP).

The initial low-dose administration of diminazene aceturate consisted of three subcutaneous injections of Ganaseg[®] (Novartis Animal Health, Tokyo) at a dose of 2 mg/kg (q48h). All dogs received an intravenous drip infusion of glucose-lactated Ringer's solution (SOLURACT D[®]; Terumo, Tokyo) at an infusion speed of 5–10 ml/kg/h for 2 to 3 hours before and after diminazene treatment as a supportive therapy. Among 242 dogs, 13 dogs died without any response to the treatment; 7 dogs received a blood transfusion and adrenocortical hormone, given the high risk of severe anemia. These 20

dogs were excluded from the evaluation of clindamycin treatment from day 8. The owners of the remaining 222 dogs received an explanation regarding clindamycin administration following the three diminazene aceturate injections. Of these, 80 owners provided informed consent to have their dogs undergo clindamycin treatment. The 80 dogs were given clindamycin (Dalacin; Pfizer, Tokyo; DC group) orally at a dose of 25 mg/kg twice daily from days 8 to 29. The remaining dogs were not administered any drugs from days 8 to 29 (control group). Red blood cells (RBC), packed cell volume (PCV), hemoglobin (Hb), platelet counts, and CRP were monitored on days 1, 8 and 29. Relapse was diagnosed when clinical symptoms recurred post-treatment and the presence of *B. gibsoni* was confirmed by observation in a blood smear by veterinarians. When relapse was diagnosed, three additional subcutaneous injections of 2 mg/kg diminazene aceturate were administered at intervals of 2 days as a rescue therapy.

Differences in RBC, PCV, Hb, platelet counts, and CRP between the DC and control groups on days 1, 8 and 29 were analyzed with one-way ANOVA. The date of relapse in each group was also compared. In addition, differences in RBC, PCV, Hb, platelets and CRP between relapse and non-relapse groups were analyzed in each group. All statistical analyses were performed using Microsoft Excel software (XLSTAT; Addinsoft, New York, USA). In addition, the chi-square test was used to examine the recurrence rate after clindamycin treatment. Differences with p-values less than 0.05 were considered statistically significant.

Frequent clinical symptoms and chief complaints at the initial presentation were anorexia (87.8%), depression (83.3%), color change of urine to dark brown (49.9%), nausea (15.4%), diarrhea (14.1%) and body pain (12.0%), which were consistent with previous reports¹⁶⁾. Fever was noted in 41.5% of the affected animals. The laboratory analysis revealed that anemia (86.3%), thrombocytopenia (98.3%), and bilirubinemia

Table 1. Changes in red blood cells, packed cell volume, hemoglobin, platelets, and C-reactive protein in the clondamycin administration (DC) and control groups on days 1, 8, and 29

	N		DC group			Control group		
			Total	Non-relapse	Relapse	Total	Non-relapse	Relapse
			80	67	13	142	100	42
Day 1	RBC	($\times 10^6/\mu\text{l}$)	3.5 \pm 1.5	3.4 \pm 1.5	3.9 \pm 1.5#	3.8 \pm 1.6	3.8 \pm 1.5	4.2 \pm 1.6
	PCV	(%)	24.8 \pm 9.0	24.5 \pm 9.1	26.5 \pm 8.4	25.1 \pm 8.9	24.3 \pm 8.9	27.1 \pm 8.8
	Hb	(g/dl)	8.4 \pm 2.7	8.4 \pm 3.6	8.6 \pm 2.9#	8.4 \pm 3.0	8.1 \pm 2.9	9.2 \pm 3.0
	Platelets	($\times 10^3/\mu\text{l}$)	45.8 \pm 39.6	49.8 \pm 40.7	24.5 \pm 2.4	43.5 \pm 28.0	43.6 \pm 33.1	43.1 \pm 25.6
	CRP	(mg/dl)	13.3 \pm 5.2	13.0 \pm 5.1	14.7 \pm 5.5	13.4 \pm 5.5	13.4 \pm 4.8	13.2 \pm 5.7
Day 8	RBC	($\times 10^6/\mu\text{l}$)	4.4 \pm 1.2*	4.4 \pm 1.2	4.2 \pm 1.2#	4.7 \pm 1.3	4.6 \pm 1.2	4.9 \pm 1.3
	PCV	(%)	33.2 \pm 7.1	33.6 \pm 6.9	31.4 \pm 7.6#	32.6 \pm 7.5	31.9 \pm 7.5	34.1 \pm 7.4
	Hb	(g/dl)	10.4 \pm 2.7	10.5 \pm 2.6	10.0 \pm 2.9	10.1 \pm 2.7	9.9 \pm 2.5	10.6 \pm 2.7
	Platelets	($\times 10^3/\mu\text{l}$)	204.1 \pm 150.1	214.0 \pm 157.1	153.1 \pm 98.0	170.9 \pm 112.5	162.9 \pm 140.4	190.3 \pm 98.0
	CRP	(mg/dl)	NE	NE	NE	NE	NE	NE
Day 29	RBC	($\times 10^6/\mu\text{l}$)	5.7 \pm 1.6*	5.9 \pm 1.5**	4.4 \pm 1.7#	5.2 \pm 1.8	5.9 \pm 1.4**	3.4 \pm 1.4
	PCV	(%)	39.4 \pm 9.6*	41.2 \pm 8.4**	30.5 \pm 10.4#	35.6 \pm 11.2	39.9 \pm 10.0**	25.3 \pm 8.5
	Hb	(g/dl)	12.6 \pm 3.6	13.0 \pm 3.4**	10.0 \pm 3.6#	12.2 \pm 8.8	13.6 \pm 3.5**	8.5 \pm 9.9
	Platelets	($\times 10^3/\mu\text{l}$)	234.3 \pm 17.8	269.2 \pm 168.5**	56.8 \pm 10.8	219.7 \pm 186.5	290.0 \pm 39.5**	42.1 \pm 174.7
	CRP	(mg/dl)	3.6 \pm 5.0	2.0 \pm 2.5**	11.8 \pm 6.5	5.6 \pm 7.0	1.7 \pm 5.8**	15.5 \pm 1.5

RBC: red blood cells, PCV: packed cell volume, Hb: hemoglobin, CRP: C-reactive protein, NE: not evaluated
Data are presented as mean \pm standard deviation.

*: Significant differences between the DC group and the control group among total.

** : Significant differences between the relapse and non-relapse groups in each group.

#: Significant differences between the DC group and the control group in relapse dogs.

(92.1%) were the common findings of acute *B. gibsoni* infection in dogs. Clinicians should note these higher ratios for differential diagnosis, as the same findings are observed in dogs with Evans syndrome (immune-mediated hemolytic anemia with immune-mediated thrombocytopenia)⁶. Jaundice (28.0%) and intravascular hemolysis (5.1%) were less common.

Except for 20 dogs in grave condition, all dogs showed improved general condition by day 8. Accordingly, the efficacy of three low-dose diminazene aceturate injections for treating acute canine *B. gibsoni* infection was determined to be 91.0 %.

No statistical differences were found in RBC, PCV, Hb, platelet counts, and CRP between the DC and control groups on day 1 (Table 1). Although lower RBC in the DC group compared to the control group was noted on day 8, there were no differences in other parameters and physical condition (Table 1). These data confirmed the randomness of the present study. Relapse

was observed in 13 of 80 dogs (16.3%) in the DC group, as compared to 42 of 142 dogs (29.6%) in the control group. The rate of relapse in the DC group was significantly lower than that in the control group ($P < 0.05$). The mean date of relapse in the DC and control groups were days 27.4 and 27.5, respectively. On day 29, the mean values of RBC and PCV in the DC group were significantly higher compared to the control group; however, there were no significant differences in Hb, platelet counts, and CRP between the two groups (Table 1). The mean values of RBC, PCV, Hb, and platelet counts were significantly lower, and the mean value of CRP was significantly higher, in the relapse group compared to the non-relapse group, in both DC and control groups (Table 1). Furthermore, the mean values of RBC, PCV and Hb of relapse dogs in DC group were significantly higher than those of relapse dogs in control on day 29, although the mean RBC and PCV of relapse dogs in DC group were significantly lower than the control on day 8

(Table 1).

In Japan, diminazene aceturate is the most commonly used drug to treat acute *B. gibsoni* infection in dogs. Although the low-dose administration protocol is a popular and empirically used method in endemic areas in Japan, the effect of the protocol has not been systematically evaluated. Among the 242 dogs, 222 (91.0%) showed improved general condition on day 8 of treatment with low-dose diminazene aceturate. The remaining 20 dogs showed no positive reaction, or died without any improvement in anemia. Renal failure and severe anemia are known to be fatal clinical symptoms of acute *B. gibsoni* infection^{8,20}.

Treatment of *B. gibsoni* infection using a single drug is known to be challenging⁷. The use of a single drug does not completely eliminate the parasite, and the dog usually becomes a carrier of *B. gibsoni*. Relapse is common after diminazene aceturate treatment^{2,3,9}. Clindamycin is an antibiotic that is dose-dependently effective in *B. gibsoni* infection^{9,18,19}. Thus, to examine whether clindamycin could prevent relapse, dogs were followed up from days 8 to 29. Although the date of relapse in DC group was the same as that in control group, a significant lower relapse rate was observed in DC group compared to the control. Furthermore, the anemia found in the relapse dogs in DC group was milder than control group. These findings suggested that the combination therapy of diminazene and clindamycin is a useful choice of treatment to avoid relapse. Some strains of *B. gibsoni* might be resistant to clindamycin treatment¹⁰. Further studies are necessary to clarify clindamycin resistance in those strains.

In conclusion, low-dose diminazene aceturate injection followed by clindamycin administration is a useful and safe method to treat acute *B. gibsoni* infection in dogs in endemic areas.

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