Short Communication

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Clinical observation of *Babesia gibsoni* infection with low parasitemia confirmed by PCR

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Babesia gibsoni is a hemoprotozoan parasite reported to cause clinically important hemolytic anemia in dogs. This *Babesia* parasite distribute in many regions including Asia, Africa, Europe and America (Farwell and others 1982, Lobetti 1998). Generally, the *Babesia* species is identified under a microscope by demonstrating the organisms in blood smears after Romanowsky staining (Lobetti 1998). However, the detection of *B. gibsoni* in blood smears is sometimes very difficult, because of its low parasitemia. Thus, *B. gibsoni* infection with low parasitemia is easily misdiagnosed as immune mediated hemolytic anemia (IMHA), and sometimes inappropriately treated with immune suppressive drugs. Although clinical observation of canine *B. gibsoni* infection has been well documented in several reports (Farwell and others 1982, Lobetti 1998, Macintire and others 2002), most cases reported in previous papers were diagnosed by the demonstration of parasites on blood smear films. Recently, polymerase chain reaction (PCR) has been developed to diagnose the *B. gibsoni* infection with higher sensitivity and specificity (Ano and others 2001, Fukumoto and others 2001, Macintire and others 2002, Stegeman and others 2003, Inokuma and others 2004). After the introduction of PCR, clinically diagnosed animals with *B. gibsoni* infection showing low parasitemia have been easily diagnosed. However, physical and laboratory findings of animals demonstrating low parasitemia have never been precisely documented. Thus, clinical data from an animal with acute *B. gibsoni* infection demonstrating low parasitemia confirmed by PCR are evaluated and compared with those of animals with apparent *B. gibsoni* infection or IMHA using a retrospective case series.

A total of 35 animals with acute *B. gibsoni* infection and 8 with IMHA treated between 1998 and 2003 in the Veterinary Teaching Hospital, Yamaguchi University, were used in this study. These animals showed symptoms suspected for acute *B. gibsoni* infection including anorexia, anemia, icterus, hematuria or fever at presentation. In each animal, more than 100 fields of blood smear stained with Giemsa were observed under high magnification (x1000) to detect *Babesia* parasites. Twenty-five dogs of 35 were easily diagnosed as having *B. gibsoni* infection by demonstration of parasites in the blood smears (*B. gibsoni* infection with moderate to severe parasitemia). The remaining 10 dogs were not confirmed at presentation because apparent parasites could not be detected in the blood smear. Positive reactions to diminazen aceturate, or the appearance of *Babesia* parasites in the blood films after treatment with immune suppressive drugs later caused these animals to be diagnosed with *B. gibsoni* infection (*B. gibsoni* infection with low parasitemia). Total DNA was extracted from each canine sample and *B. gibsoni*-specific PCR amplification was performed using a primer set of Gib599F and Gib1270R (Inokuma and others 2004). In our preliminary experiments, this PCR for *B. gibsoni* was able to detect approximately 10 infected red blood cells in 1 μl of peripheral blood. To confirm the PCR findings, nucleotide sequences of randomly selected products of the *B. gibsoni*-specific PCR were determined by direct sequence analysis using the method previously described (Inokuma and others 2003). All the analyzed sequences showed 100% homology with *B. gibsoni* Asia-1 (GenBank accession number AF175300). Animals with IMHA showed acute onset of symptoms similar to those of *B. gibsoni* infection such as hemolytic and regenerative anemia, icterus, anorexia, or hematuria at presentation. All 8 animals with IMHA were judged negative for *B. gibsoni* infection by PCR. Information obtained from all 35 babesiosis and 8 IMHA cases included histories, findings of physical examination, CBC, serum biochemical analysis, the radiography of the

abdomen. To compare data from dogs with low parasitemia with those of *B. gibsoni* infection with moderate to severe parasitemia or IMHA cases, chi-square tests and one-factor ANOVA were performed by using StatView Ver.5.0 (Hulinks).

In the present study, all 35 animals with *Babesia* showed positive PCR reaction for *B. gibsoni.* When the clinical and laboratory findings of *Babesia* infected animals showing low parasitemia were compared with those of dogs with moderate to severe parasitemia, nothing was different significantly among the 3 groups (Table 1). Although the numbers of dogs that showed icterus and the mean total bilirubin level in dogs with more severe parasitemia were slightly higher than those of dogs with low parasitemia, the differences were not significant. Oxidative damage to erythrocytes induced by *B. gibsoni* infection is thought to result in severe anemia even in the presence of low parasitemia (Otsuka and others 2002). The degree of parasitemia in *B. gibsoni* infection did not relate to clinical and laboratory findings. The clinical and laboratory findings of *B. gibsoni* infection with low parasitemia were also compared with those of IMHA (Table 1). Some dogs with IMHA showed more severe clinical findings than those with *B. gibsoni* infection, and the mean RBC, PCV and hemoglobin value in IMHA were slightly lower than those in *B. gibsoni* infection without significant differences. Although hematuria is not a specific finding for IMHA (Honeckman and others 1996, Miller 2000), the number of dogs with IMHA that showed hematuria was significantly higher than those with *B. gibsoni* infection with low parasitemia (Table1). The mean body temperature of animals with *Babesia* infection was significantly higher than that of those with IMHA, but 2 of 8 dogs with IMHA showed fever (>39.5 C) at presentation. Most animals with IMHA were also positive on direct Coomb's-test showing significant numbers of spherocytes in blood smears; however, these results were also found in some animals with *B. gibsoni* infection. Thus, it is very difficult for clinical veterinarians to confirm *B. gibsoni* infection and differentiate it from IMHA, if there were no evidence of *Babesia* parasites in blood smears. In conclusion, *B. gibsoni*-specific PCR would be highly useful tool for diagnosing acute *B. gibsoni* infection in dogs.

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Table 1. Comparison of physical examination and laboratory findings of *B. gibsoni* infection with low parasitemia comfirmed by PCR with those of middle to high parasitemia cases and IMHA

	Acute type of *B.gibsoni* infection		IMHA
	Low parasitemia	Middle to high parasitemia	
Numbers of dogs	10	25	8
a) Physical examination			
Fever (>39.5 C)*	4	14	2
Anemia *	8	21	8
Icterus *	1	8	4
Splenomegaly *	7	16	3
Hematurea *	0	1	4 #
Body temperature (C)**	39.6 (0.5)	39.7 (0.7)	38.8 (0.8) #
b) Laboratory findings **			
RBC ($x10^4/\mu l$)	334 (159)	303 (145)	204 (177)
Hb (g/dl)	7.5 (3.4)	6.9 (3.0)	5.0 (2.0)
PCV (%)	23.1 (9.2)	21.2 (6.8)	16.1 (5.1)
Platelets ($x10^4/\mu l$)	7.0 (6.4)	4.9 (4.1)	15.5 (12.3)
WBC (/μl)	13178 (10329)	15083 (10178)	31525 (30993)
Total bilirubin (mg/dl)	0.57 (0.55)	1.89 (4.06)	8.1 (14.5)
BUN (mg/dl)	15.5 (6.4)	22.4 (13.1)	37.7 (15.1)
Creatinine (mg/dl)	0.6 (0.3)	0.8 (0.3)	0.7 (0.3)
AST (U/l)	46 (24)	98 (216)	166 (226)
ALT (U/l)	173 (262)	146 (256)	60 (29)
ALP (U/l)	1697 (2119)	536 (622)	726 (421)
CRP (mg/dl)	6.2 (2.2)	7.4 (3.6)	9.2 (6.5)

*: Numbers of dogs that showed the specific findings

**: Mean (standard deviation)

#: significant differences ($p<0.05$) compared with the data of cases with low parasitemia