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Relationship between the number of *Hepatozoon canis* gamonts and hematobiochemical values in dogs

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Abstract. The occurrence of canine hepatozoonosis in Thailand is primarily caused by *Hepatozoon canis*. Recently, the relationship of hematology and biochemistry with this disease has been studied, but knowledge regarding the relationship between the quantity of *H. canis* intracellular gamonts and the hematological profile has not yet been reported. The objective of this study was to investigate the clinical, hematological and biochemical profile of *H. canis*-positive dogs and the relationship of the number of *H. canis* gamonts, animal signalment, and hematological and biochemical values. A total of 185 *H. canis*-positive blood samples were examined, including buffy coat smears and comprehensive data. The number of gamonts was randomly counted from buffy coat smears samples (75/185). The dogs infected with *H. canis* presented to the animal hospital mostly for health status checks, anorexia, or accidents. Observations from the physical examination on the first day of registration included systemic abnormalities such as digestive, integument, respiratory, urogenital, etc. Most of the dogs showed clinical signs of systemic abnormality in more than one system. Our study shows that plasma proteins are correlated with the number of *H. canis* gamonts, using Spearman's rho correlation coefficient with significant difference (p <0.05). This finding could be applied to improve the diagnosis and treatment of canine hepatozoonosis.

INTRODUCTION

Canine hepatozoonosis caused by *Hepatozoon* spp., an intracellular protozoan in the phylum Apicomplexa, is mainly discriminated into two distinct species, *H. canis* and *H. americanum* (Baneth *et al.*, 2000). In Thailand, *H. canis* Thailand 1 isolate has been identified using molecular technique (Sungpradit *et al.*, 2016). The primary tick vector of *H. canis* is *Rhipicephalus sanguineus* or brown dog tick (Baneth *et al.*, 2001; Jittapalapong *et al.*, 2006; Juasook *et al.*, 2016). The basic route of transmission of *H. canis* to dogs occurs by ingestion of infected ticks or parts of the tick's body that have mature oocysts (Baneth *et al.*, 2001; Baneth *et al.*, 2007). The gamonts mainly invade circulating neutrophils and these infected leukocytes can be used as a standard diagnostic for canine hepatozoonosis (Eljadar *et al.*, 2012).

Canine hepatozoonosis has a variety of presentations, from asymptomatic to symptomatic and life-threatening. The main nonspecific clinical manifestations of *H.*

canis infection include anorexia, pyrexia, anemia, pain, gait abnormalities, emaciation, lethargy, and popliteal and submandibular lymph node enlargement (Hervás *et al.*, 1995; Gondim *et al.*, 1998; Chhabra *et al.*, 2013; Roopali *et al.*, 2017).

The blood profile in acute onset of *H. canis* infection reported from an adult stray dog showed anemia, thrombocytopenia, hyperproteinemia, polyclonal gammopathy, hypoalbuminemia, and elevated creatinine kinase and alkaline phosphatase activity, and 50% of circulating neutrophils were infected with *H. canis* gamonts (Sakuma *et al.*, 2009).

Hematobiochemical parameters of mild and subclinical signs in canine hepatozoonosis have been studied worldwide. In Thailand, owned dogs infected with *H. canis* presented specific abnormal hematological values, including hypocytic hypochromic anemia, but leukocyte and platelet counts and biochemical values showed nonspecific parameters regarding canine hepatozoonosis (Assarasakorn *et al.*, 2006). Recently, Wongsawang and Jiemthaweeboon (2018) reported that anemia, hypohemoglobinemia, and thrombocytopenia are the major parameters in case of *H. canis* infection (odds ratio = 2.111, 1.645, and 1.554, respectively). In Brazil, O'Dwyer (2006) reported that only hyperglobulinemia was found from serum biochemistry in street dogs infected with *H. canis*, and the highest parasitemia (0.5%) from this study showed slightly leukocytosis. However, of eight owned dogs infected with *H. canis* ($\leq$0.5%; one case showed 2% parasitemia) seven presented with anemia, three showed leukocytosis with neutrophilia, four showed monocytosis, and three had lymphopenia, but all cases had concomitant diseases (Gondim *et al.*, 1998).

There is a lack of information on the relationship between number of *H. canis* gamonts, clinical signs, and hematological values in the literature, and some studies have stated that the hematological and biochemical parameters in *H. canis* infection were nonspecific and similar to other hematozoa cases (Mundim *et al.*, 2008; Vojta *et al.*, 2012) Therefore, the objective of this study was to investigate the relationship of the number of *H. canis* intraleukocyte gamonts, the hematological profile, and biochemistry values. The results could be applied to improve a novel diagnostic and treatment method and to further confirm its success or failure after treatment of *H. canis*-infected cases.

MATERIALS AND METHODS

Ethics

The study protocol (No. MUVS-2011-19) received approval by the Faculty of Veterinary Science Animal Care and Use Committee (FVS-ACUC), Mahidol University, Thailand.

Buffy coat smear examination

A total of 8700 routine buffy coat smears (approximately 55.4 µl per blood sample) stained using modified Wright–Giemsa stain, were requested from Prasu-arthorn Animal Hospital, Mahidol University, Nakhon Pathom Province, Thailand, for microscopic examination of blood parasites by the parasitology unit between May 2010 and April 2011. In this study, single infection of 185 *H. canis*-positive results were obtained, 75/185 (40.5%) stained buffy coat smears were randomly selected, and the gamonts of each buffy coat smear were counted under light microscope (Nikon Eclipse E200, Tokyo, Japan) at a magnification of 1000X and calculated for the number of gamonts in one milliliter of *H. canis*-infected blood.

Comprehensive data collection

Comprehensive data, including signalment (age, sex, breed, and weight), chief complaint, physical examination, and hematological and biochemical profiles from 185 dogs were gathered retrospectively from medical records of Prasu-arthorn Animal Hospital, Mahidol University, on the first day of registration.

Statistical analysis

This study used SPSS version 19.0 statistics program (SPSS 19.0 for Windows, Chicago, IL, USA) for data analysis. The data as numeric variables for clinic and laboratory

were tested for normal distribution using the Kolmogorov–Smirnov test. The influence of age, sex, and clinical parameters (e.g., temperature, heart rate, and body condition score) on the number of gamonts in each buffy coat smear was studied, and the distribution by normal probability plot (Q-Q plot) was checked. Comparisons between datasets that were not normally distributed were undertaken using Spearman's rho to find the relationship of data as a nonparametric correlation. Probabilities <0.05 were considered significant. Results were reported as minimum, maximum, and 25, 50, and 75% quartile.

RESULTS

The prevalence of *H. canis* infection in this study was 2.13% (185/8700). A total of 185 *H. canis*-positive canine blood samples recruited for the study was divided into 94 males (50.81%), 85 females (45.95%), and 6 unknown sex (3.24%) (no record in the animal hospital's document) (Table 1). There were 21 breed: mixed (24.86%), poodle (23.24%), Thai (19.46%), golden retriever (8.65%), terrier group (3.78%), cocker spaniel (2.16%), miniature pinscher (2.16%), Pomeranian (2.16%), Shih Tzu (2.16%), Dalmatian (2.13%), Siberian husky (1.62%), Chihuahua (1.08%), dachshund (1.08%), Rottweiler (1.08%), Spitz (1.08%), basset hound (0.54%), bichon frise (0.54%), Doberman (0.54%), German shepherd (0.54%), Jack Russell (0.54 %), and pug (0.54%).

The major reasons why owners brought dogs to the animal hospital were health status check (17.3%), anorexia (9.3%), and accident (8.4%). Physical examinations recorded by the veterinary practitioners showed digestive (19.3%), integument (18.8%), respiratory (12.6%), urogenital (12.6%), eye (12.6%), musculoskeletal (9.4%), nervous (6.7%), cardiovascular (6.3%), lymphatic (1.3%), and ear (0.5%) problems.

The recorded parameters of the dog patients on the day of registration for recruited cases and identified gamont cases are shown in Table 1. In brief, they were normal appetite (36.2% and 45.3%, respectively) and water intake (40.5% and 52%), pink mucous membrane (49.7% and 53.3%), normal hydration status (45.4% and 48.0%), general appearance as alert (48.6% and 56.0%), normal urination (46.5% and 56.0%), normal feces (43.2% and 52.0%), and adequate platelet count (59.4% and 54.7%).

It was found that the dogs ranged in age from 1 month to 18 years (median 5.95 years). Body weight ranged from 0.4 to 44 kg (median 8.8 kg). The data showed that 31/174 (17.82%) *H. canis*-infected samples were in dogs younger than 1 year, and the rest were in several age groups up to 18 years old (no age was recorded for 11 samples). The medians of variables from the *H. canis*-positive or recruited cases and the selected *H. canis* cases for gamont counting or identified gamont cases, such as physical examination data (e.g., age, body weight, temperature, heart rate, and respiratory rate), hematological profile, and blood chemical profile, were compared and there were no statistical differences between the two groups. The variables, including physical data and hematobiochemical profiles, of both groups are shown in Table 2. All parameter medians were in the reference range except plasma protein, 9.2 g/dL and 9.4 g/dL for the recruited and identified gamont cases, respectively, with a reference range of 6–7.5 g/dL. In addition, this study obtained *H. canis* gamont counting data, and the median number from the identified cases was 2527.07 gamonts per one milliliter of whole blood (range: 36.1–86,642.60). To calculate the percentage of parasitemia, the median of segmented neutrophils (9336 cell $\times 10^6$ mL), which was the main target of *H. canis* gamonts in the identified gamont group, was used for calculation. The median percentage of parasitemia was 0.027% (range: 3.87×10^{-4}% to 0.93%).

The correlation between the number of gamonts and various parameters revealed that plasma protein showed a statistically significant difference ($p = 0.016$), whereas the other parameters such as age, body weight, total white blood cells, RBC count, Hb, Hct, MCV, MCH, MCHC, RDW, platelet count, ALT, and creatinine, were not demonstrated a statistically significant

Table 1. Comparison of gender, appetite, water intake, pulse quality, mucous membrane color, hydration status, attitude, general appearance, coughing, urination, feces and platelet count. Statistical differences between groups were tested by Chi-square test

Variables	Recruited cases (N = 185)		Identified gamont cases (N = 75)	
	Frequency	%	Frequency	%
Gender				
- Male	94	50.8	35	46.7
- Female	85	45.9	38	50.7
- No record	6	3.2	2	2.7
Appetite				
- Normal	67	36.2	34	45.3
- Decreased	37	20	12	16
- Anorexia	20	10.8	10	13.3
- Increased	2	1	0	0
- Not observed	59	31.9	19	25.3
Water intake				
- Normal	75	40.5	39	52
- Decreased	33	17.8	11	14.7
- Increased	10	5.4	3	4
- Not observed	67	36.2	22	29.3
Pulse quality				
- Normal	50	27	15	20
- Cannot palpate	2	1.1	0	0
- No data	133	71.9	60	80
Mucous membrane				
- Blue	1	0.5	0	0
- Pale	15	8.1	4	5.3
- Pale pink	33	17.8	17	22.7
- Pink	92	49.7	40	53.3
- Yellowish	1	0.5	0	0
- Not observed	43	23.2	14	18.7
Hydration status				
- Normal	84	45.4	36	48
- Dehydration 3–5%	11	5.9	6	8
- Dehydration 5%	13	7	4	5.3
- Dehydration 5–7%	1	0.5	1	1.3
- Dehydration 7%	4	2.1	2	2.6
- Dehydration 10%	4	2.1	1	1.3
- Not observed	68	36.8	25	33.3
Attitude				
- Normal	68	36.8	31	41.3
- Abnormal	12	6.5	4	5.3
- Not observed	105	56.8	40	53.3
General appearance				
- Depressed	53	28.6	19	25.3
- Alert	90	48.6	42	56
- Hyper-excitable	2	1	1	1.3
- Seizure	1	0.5	1	1.3
- Stupor	1	0.5	0	0
- Aggressive	1	0.5	1	1.3
- Not observed	37	20	11	14.7
Coughing				
- Yes	11	5.9	4	5.3
- No	80	43.2	31	41.3
- Not observed	94	50.8	40	53.3

Table 1 continued...

Urination				
- Normal	86	46.5	42	56
- Increased	6	3.2	2	2.7
- Decreased	8	4.3	3	4
- Anuria	1	0.5	0	0
- Not observed	84	45.4	28	37.3
Feces				
- Normal	80	43.2	39	52
- Increased	1	0.5	1	1.3
- Decreased	2	1.1	1	1.3
- Abnormal	13	7	5	6.7
- Not observed	89	48.1	29	38.7
Platelet count				
- Adequate	110	59.4	41	54.7
- Decreased	63	34	29	38.7
- Increase	4	2.1	4	5.3
- Not observed	8	4.3	1	1.3

difference ($p > 0.05$) as presented in Table 3.

DISCUSSION

To our knowledge, this study is the first report of *H. canis* prevalence in owned dogs in the central part of Thailand using the microscopic technique. The prevalence of canine hepatozoonosis in owned dogs residing in Nakhon Pathom Province and the neighborhood was 2.13%. In Thailand, a previous surveillance of stray dogs in Bangkok showed that 2.6% of the samples were positive for *H. canis* by the same technique (Jittapalapong *et al.*, 2006). Recently, the prevalence of *H. canis* from the Nakhonpranom animal quarantine station was 32.56% by light microscope (Juasook *et al.*, 2016). Using high sensitivity and specificity molecular techniques such as polymerase chain reaction (PCR), multiplex PCR, and loop-mediated isothermal amplification (LAMP) assay not only shows an increased prevalence of the disease, (Singh *et al.*, 2019), but also discriminates between single *H. canis* and concurrent infection with other blood parasites (Kledmanee *et al.*, 2009), which should confirm the hematobiochemical findings from particular *H. canis* infection (Singh *et al.*, 2017).

Most recruited and identified gamont *H. canis* cases gathered from this study showed nonrelated and nonspecific clinical examination of canine hepatozoonosis, as in a previous review (O'Dwyer 2011). The main problems of dogs infected with *H. canis* presenting to the animal hospital were health status investigation, anorexia, and traffic accidents. In our study, the gamonts were primarily obtained by accidental findings in health checkup samples, and gamonts were also observed in patients with abnormalities in the digestive, integument, respiratory, and urogenital systems. Our information is similar to previous studies in that the occurrence of gamonts in blood circulation varied from accidents in normal dogs to disability (Gondim *et al.*, 1998). However, the hypocytic hypochromic anemia was demonstrated as the main clinical pathology and around thirty dog patients showed PCV less than 20% (Assarasakorn *et al.*, 2006). To study the clinical and hematobiochemical parameters, the experimental infection should be performed (Baneth *et al.*, 1998) in order to exclude the other canine hematozoa and the diseases which affect the blood profiles.

The median percentage of parasitemia from asymptomatic or subclinical conditions of *H. canis*-infected dog samples calculated by this study was 0.027% (range: 3.87×10^{-4}% to 0.93%) or 2.53 gamonts/µL (range 0.036–86.642 gamonts/µL), which represents low

Table 2. Comparison of age, physical examination data, hematological profile, and blood chemical profile of recruited *H. canis* cases and the selected cases for gamont counting. Statistical differences between groups were tested by the Mann–Whitney U test

Variables	N	Min	Max	Quartile			Reference range
				25%	Median	75%	
Age (years)							N/A
Recruited cases	172	0.08	18	1.43	5.95	9	
Identified gamont cases	72	0.08	14.92	1.41	5	8.39	
Body weight (kg)							N/A
Recruited cases	169	0.4	44	4.6	8.8	19.1	
Identified gamont cases	67	0.4	44	4.2	8.6	18.8	
Temperature (°F)							99.5-102.5
Recruited cases	118	98	106	100.8	101.2	102	
Identified gamont cases	48	98	105	100.6	101	101.95	
Heart rate (bpm)							60-140
Recruited cases	48	60	200	103	120	140	
Identified gamont cases	18	60	180	113.5	120	140	
Respiratory rate (bpm)							10-35
Recruited cases	26	20	52	25.5	30	38.5	
Identified gamont cases	9	20	48	24	28	39	
Total white blood cells (cells/µL)							6 000-17 000
Recruited cases	184	200	57 000	8 450	11 600	17 275	
Identified gamont cases	75	200	47 000	8 600	13 200	17 300	
Segmented neutrophils (cells/µL)							2 060-10 600
Recruited cases	177	715	39 292	5 472	9 000	14 290	
Identified gamont cases	72	1 764	35 720	5 638.5	9 336	13 312.50	
Band neutrophils (cells/µL)							0-300
Recruited cases	177	0	5 915	0	0	0	
Identified gamont cases	72	0	2 600	0	0	0	
Monocytes (cells/µL)							0-840
Recruited cases	177	0	26 220	0	202	570	
Identified gamont cases	72	0	9 850	0	248.50	569.75	

Table 2 continued...

Lymphocytes (cells/µL)							690-4 500
Recruited cases	177	126	8 428	1 335	2 223	3,610	
Identified gamont cases	72	261	8 428	1 350	2 335.50	3 635.50	
Eosinophils (cells/µL)							0-1 200
Recruited cases	177	0	5 850	0	180	397	
Identified gamont cases	72	0	5 850	0	222	516	
Basophils (cells/µL)							0-150
Recruited cases	177	0	13 790	0	0	0	
Identified gamont cases	72	0	13 790	0	0	0	
Red blood cell count (RBC) $\times 10^6$ (cells/µL)							5-9
Recruited cases	184	1	59	4.4	5.23	6.4	
Identified gamont cases	75	1.4	59	4.4	5.5	6.3	
Hemoglobin (Hb) (g/dL)							10-18
Recruited cases	184	2.6	20	9.2	11.3	13.47	
Identified gamont cases	75	3.6	19.8	9.5	11.5	13.4	
Hematocrit (Hct) (%)							35-55
Recruited cases	184	8.4	65	29	35.25	42	
Identified gamont cases	75	10.8	61	31	37	42	
Mean corpuscular volume (MCV) (fL)							60-77
Recruited cases	184	46	99	65	69	71	
Identified gamont cases	75	46	78	66	69	72	
Mean corpuscular hemoglobin (MCH) (pg)							20-25
Recruited cases	184	13.6	221.4	20.5	21.8	22.77	
Identified gamont cases	75	13.6	25.8	20.5	22	23	
Mean corpuscular hemoglobin concentration (MCHC) (g/dL)							32-36
Recruited cases	184	22.8	37.2	31.1	32	32.57	
Identified gamont cases	75	22.8	37.2	31	32	32.4	
Platelet $\times 10^3$ (cells/µL)							200-500
Recruited cases	184	6	753	114	195.5	277.5	
Identified gamont cases	75	6	753	117	200	317	
Red cell distribution width (RDW) (%)							12-15
Recruited cases	184	6.8	115.9	14	15	16.37	
Identified gamont cases	75	6.8	115.9	14.2	15.1	16.3	

Table 2 continued...

Plasma protein (g/dL)							6-7.5
Recruited cases	184	0	12	8.6	9.2	10.4	
Identified gamont cases	75	0	12	8.6	9.4	10.4	
Albumin (g/dL)							2.7-3.8
Recruited cases	5	2.5	3.2	2.55	2.9	3.05	
Identified gamont cases	2	2.5	2.9	2.5	2.7	–	
Alkaline phosphatase (U/L)							23-212
Recruited cases	55	0.8	4 646	51	139	311	
Identified gamont cases	26	11	4 646	47.35	136	278.5	
Alanine transaminase (ALT) (U/L)							10-100
Recruited cases	164	5	1 427	23	43	82	
Identified gamont cases	68	5	1 185	28	45.2	82	
Blood urea nitrogen (mg/dL)							7-27
Recruited cases	43	2.2	7 140	10.4	16.2	37.7	
Identified gamont cases	22	8	223	12.5	15.45	38.55	
Creatinine (mg/dL)							0.5-1.8
Recruited cases	160	0.2	710	0.7	0.9	1.28	
Identified gamont cases	66	0.2	10.3	0.7	0.9	1.38	
Potassium (mmol/L)							3.5-5.8
Recruited cases	9	2.89	5.68	3.82	4.7	5.27	
Identified gamont cases	6	3.74	5.68	3.86	4.75	5.49	
Total protein (g/dL)							5.2-8.2
Recruited cases	6	4.8	9.2	5.02	7.75	8.52	
Identified gamont cases	2	4.8	8.3	4.8	6.55	–	
Number of gamonts (counted gamonts)							N/A
Recruited cases	–	–	–	–	–	–	
Identified gamont cases	75	2	4 800	33	140	893	
Total gamonts (gamonts/mL)							N/A
Recruited cases	–	–	–	–	–	–	
Identified gamont cases	75	36.1	86 642.60	595.66	2 527.07	16 119.13	

Table 3. Spearman's rho correlation coefficient between variables and number of *H. canis* gamonts

Variable	N	Spearman's rho	*p*-value
Age (years)	72	–0.051	0.670
Body weight (kg)	67	–0.242	0.049
Total white blood cells (cells/µL)	75	0.060	0.607
Segmented neutrophils (cells/µL)	72	–0.009	0.941
Band neutrophils (cells/µL)	72	0.061	0.069
Monocytes (cells/µL)	72	0.088	0.464
Lymphocytes (cells/µL)	72	0.015	0.904
Eosinophils (cells/µL)	72	0.013	0.913
Basophils (cells/µL)	72	0.186	0.117
RBC ($\times 10^6$/µL)	75	–0.093	0.428
Hb (g/dL)	75	–0.146	0.211
Hct (%)	75	–0.105	0.372
MCV (fL)	75	–0.038	0.743
MCH (pg)	75	–0.190	0.103
MCHC (g/dL)	75	–0.160	0.171
Platelet ($\times 10^3$/µL)	75	–0.072	0.542
RDW (%)	75	0.076	0.517
Plasma protein (g/dL)	75	0.277*	0.016
ALT (U/L)	68	–0.077	0.535
Creatinine (mg/dL)	66	–0.163	0.191
Gamonts (number)	75	1.000**	–
Total gamonts (gamonts/mL)	75	1.000**	–

*Correlation is significant at the 0.01 level (two-tailed); ** significant at the 0.05 level (two-tailed).

parasitemia. The proposed criteria of *H. canis* infection with low and high parasitemia, <800 gamonts/µL or <1% of infected neutrophils and >800 gamonts/µL or >1% of infected neutrophils, respectively, was reported (Baneth and Weigler 1997). Our study result was similar to the previous studies which the parasitemia of the infected dogs with lower than 0.1% (O'Dwyer *et al.*, 2006) and 0.5% - 2.0% (Gondim *et al.*, 1998) did not showed the specific abnormality in hemograms. Moreover, various studies have demonstrated the range of parasitemia and severity of the disease as moderate (0–18%) and mild (0–3%) (Karagenc *et al.*, 2006; Chhabra *et al.*, 2013; Kwon *et al.*, 2017). In addition, previous case reports showed that in acute *H. canis* infection, 50% of neutrophils were invaded by gamonts (Sakuma *et al.*, 2009), a male-mongrel dog was also demonstrated 37% of gamont-infected neutrophils but no response after chemotherapy for two weeks (33% of neutrophils still infected) (Kaur *et al.*, 2012), and another report described a dog presenting with 39% parasitemia that died within one day after admission (Karagenc *et al.*, 2006).

Our study found that the plasma protein level in *H. canis* infection cases strongly correlated with the number of gamonts, with a significance of $p = 0.016$, in agreement with previous studies (Shah *et al.*, 2013; Pasa *et al.*, 2009). Pasa *et al.* (2009) reported that *H. canis*-infected dogs had altered plasma protein elevation (>7.1 g/dL) related to hypoalbuminemia (<2.8 g/dL) and hyperglobulinemia (>4.7 g/dL). However, Assarasakorn (2016) reported that total protein and albumin means were variable and not correlated with canine hepatozoonosis, and this study did not calculate the number of gamonts in the blood circulating neutrophils. Hypoalbuminemia in *H. canis* infection might be a consequence of the acute phase protein response or a compensation for hyperglobulinemia (Attipa *et al.*, 2018),

whereas hyperglobulinemia is followed by chronic infectious disease (Baneth and Weigler 1997). Recently, acute phase proteins such as serum amyloid A, haptoglobin, and paraoxonase-1 were investigated in naturally *H. felis*-infected cats. The study reported that serum amyloid A and haptoglobin were significantly increased in symptomatic samples, and paraoxonase-1 was decreased in both symptomatic and asymptomatic *H. felis*-infected samples (Vilhena *et al*., 2017). Biomarkers such as acute phase proteins should be further studied in canine hepatozoonosis for potential next-generation diagnosis and treatment.

CONCLUSION

This study demonstrates a correlation of plasma protein with the number of gamonts in *H. canis* infection. The signalment, hematological profile, and clinical manifestations on the day of first registration were not significantly related to the *H. canis* infection samples. The specific biomarkers and advanced molecular diagnostic methods should be developed for rapid and accurate diagnosis and treatment of canine hepatozoonosis in the future.

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Conflict of Interests
The authors declare that there are no conflicts of interest.

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