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Research Article
Prevalence of Blood Parasites in Hyperthermic Dogs of Kathmandu Valley, Nepal

[1]Thaneshwor Bhatta, [2]Narayan Acharya, [3]Krishna Prasad Acharya and [4]Bala Ram Thapa

[1]Institute of Agriculture and Animal Science (IAAS), Rampur, Chitwan, Nepal
[2]Texas Tech University, Lubbock, Texas, USA
[3]Regional Veterinary Disease Diagnostic Laboratory (RVDDL), Pokhara, Nepal
[4]Ministry of Livestock Development, Singhadurbar, Kathmandu, Nepal

Abstract

Background and Objective: Blood parasites are one of the important contributing factors for hyperthermia in dogs. However, differential diagnosis of hyperthermia with hemoprotozoal infections is not commonly considered by Nepalese veterinarians. This study was designed to monitor the prevalence of blood parasites in hyper-thermic dogs of Kathmandu valley, Nepal. **Materials and Methods:** A total of 150 blood samples were taken from hyper-thermic (>103°F) dogs admitted to Central Veterinary Hospital, Animal Nepal and Veterinary Clinics of Kathmandu valley. Screening for the presence of blood parasites was done by Giemsa stained thin smear technique for *Babesia* and *Anaplasma* and both wet mount and buffy coat techniques were employed for *Trypanosoma* sp. Also, hematological examinations i.e., total leukocyte count (TLC), total erythrocyte count (TEC), packed cell volume (PCV), haemoglobin (Hb) and differential leukocyte count (DLC) were done to study the hematological profile of hyper-thermic dogs. Data were analyzed by SPSS 16 ((SPSS, Inc., Chicago, IL, USA) to test the significance. **Results:** Four different blood parasites were observed i.e. *Babesia* sp. (2%), *Ehrlichia* sp. (10.66%) *Anaplasma* sp. (1.34%) and *Trypanosoma* sp. (0.66%) with overall prevalence of 14.66%. The age and breed of the animal was found to have no statistically significant effect on prevalence of hemoparasites whereas a significant ($p<0.05$) difference in prevalence was noted between the sex groups. Hematology revealed a significant decrease in TLC, DLC, TEC, PCV, Hb and a significant increase in eosinophil count($p<0.05$). Regarding the species of parasites, samples with *Ehrlichia* sp and *Babesia* sp infection were found to have significantly low level of TLC and significantly high eosinophil count. **Conclusion:** The high prevalence of blood parasites in hyperthermic dogs suggests parasitic infection could be a potential cause of the hyperthermia or fever. Thus, differential diagnosis of hyperthermic cases with hemoprotozoan infections is of optimal importance which is made easier by finding the changes in blood parameters and presence of parasites in the blood. If the diagnosis of the case is done with due consideration of these causation, it will increase the success rate of treatment of febrile dogs.

Key words: Hemoparasites, hyperthermia, prevalence, pet enthusiasts

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Corresponding Author: Krishna Prasad Acharya, Regional Veterinary Disease Diagnostic Laboratory (RVDDL), Pokhara, Nepal
Tel: 00977-61-520419/00977-9861601519 Fax: 00977-61-525863

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Dog is a multipurpose highly demanding companion animal worldwide[1]. Almost all of the cultures around the world keep the pet and the dog is the first pet animal reported to have been kept since 15,000 years back[2]. In Nepalese context, sufficiently close bonding exits between the dog and its owner and are reared not only as a pet, but also for a reliable guard and some other distinct purposes like chasing away the animal interlopers such as monkeys and wild animals. Dogs play an important role in physical development as well as behavioral well being of children and elderly[3] in addition to emotional advantages[4]. However, from the public health point of view, dogs have been sources of zoonotic pathogens[5] including hemoparasites[6]. Many protozoal (e.g. *Babesia*, *Leishmania* and *Trypanosome* sp), bacterial (e.g. *Anaplasma* and *Ehrlichia* sp)[6], vector borne diseases affect dogs and those of zoonotic diseases trigger public health concerns[7]. Recent studies have shown that Nepal is endemic country for total of 17 zoonotic diseases including seven potentially endemic diseases[8]. The neurocysticercosis and toxoplasmosis have shown highest presence while the others have imposed relatively less burden to Nepalese community[8]. Although the pet adoption practice has been a common tradition these days, still there is huge population of dog is roaming around as a community or stray dog in Kathmandu valley. Several organizations which advocate animal welfare are being actively involved in preventive health care and management practices of ownerless animals besides, sufficient number of private or government pet veterinarian availability in Kathmandu. There are increasing number of cases of hyperthermic dogs in both pet and stray are being reported from different public and private clinics. Every day a number of dogs are taken to the clinics but the diagnosis of a disease as per the blood examination report is not a common practice in Nepalese context and hemoparasites are usually disregarded as a potential cause of illnesses. However, their consideration is of optimum importance for the successful treatment of hyperthermic cases. Fortunately, some cases are diagnosed properly in time and treated whereas most of them are treated symptomatically and supportive with the help of antibiotics. Some cases are luckily recovered while some go through the protracted period of illness and ultimately pass away. Febrile cases that are non-responsive to antibiotics treatment may have been caused by hemoparasites that if diagnosed in time can be cured easily with low cost of treatment. Sadly, there is meager information on prevalence of blood parasites in canines of Nepal, which is the main reason why veterinarians become confused and often misdiagnose the case resulting into treatment failure. Thus, present study was undertaken to determine the status of hemoparasites infections and their association with hyperthermia and change in hematological profile which will be helpful on timely diagnosis and proper treatment of febrile cases of canines.

MATERIALS AND METHODS

Study area: The study was conducted in the Kathmandu valley from December, 2015-March, 2016. Kathmandu is a capital city of Nepal located at the lap of hills of Bagmati zone, in Central Development Region. It is a valley made up of 3 districts (viz. Kathmandu, Bhaktapur and Lalitpur) whose geographical location is, from 27' 27"E to 27' 49" E and 85' 10" N 85' 32" N. The altitude of the district ranges between 1262-2732 m.a.s.l.

Study designs and sample collection: Required number of sample size was calculated as per Daniels formulae 1999 and a total of 150 samples were collected from Central Veterinary Hospital, Tripureshwor, Animal Nepal, Chobhar and Veterinary Clinics of Kathmandu valley as per accessibility from December, 2015-April, 2016.

Sampling technique: A total of 150 blood samples were taken purposively from hyperthermic (body temperature $\geq$103°F) dogs from Central Veterinary Hospital, Animal Nepal and Veterinary Clinics of Kathmandu valley. All other information like owner's name, address, age, sex, breed, temperature, clinical signs, de-worming information and all other important information were also taken.

Analysis: All the samples were examined for presence of parasites by making thin blood smear and Giemsa following standard protocols. Both wet mount and buffy coat techniques were employed for the examination of *Trypanosoma* sp. Also, hematological examination i.e., Hb, PCV, TEC, DLC was also done to access the hematological profile of hyper-thermic dogs.

Data analysis: Data were entered and analyzed using MS-Excel, SPSS Ver. 16 (SPSS Inc, Chicago, IL, USA) and Open Epi Ver. 2.3 (CDC, USA). Data regarding the hematology were analyzed by student's t test. Effects of sex, age and breed on prevalence were evaluated by chi- square (x2) test. Values of $p<0.05$ were considered significant at 95% level of confidence.

RESULTS AND DISCUSSION

Blood parasites were found in total of 14.66% of the examined samples. The parasites that were most frequently identified were *Ehrlichia* sp. (10.66%) followed by *Babesia* sp. 2.00%, *Anaplasma* sp. 1.34% and *Trypanosoma* sp. 0.66%. The age wise distribution of prevalence of blood parasites found were 4.34% for dogs 3 months to 2 years of age, 13.63% for those 2-5 years old and 23.07% for those 5-13 years old. However, there was no significance difference between the age groups (Table 1). By breed, parasites were found in 37.5% of the German Shepherds, 8.1% of mongrels, 10.52% of Japanese Spitzes, 25% of Bhotes, 20% of Labrador breeds and 14.20% of Cocker Spaniels (Fig. 1). Male dogs had a higher prevalence of blood parasites (21.87%) than did female dogs (9.3%) (Table 1). The body temperature of the dogs and their hematological profiles were also analysed. The mean temperature for dogs with parasites was 104.3°F and for those without was 103.38°F, suggesting hyperthermia in dogs with parasites. The presence of blood parasites was significantly correlated with the hematological profile of the infected dogs, with decreased TLC, PCV, Hb and TEC and increased eosinophil counts (Table 2). The decrease in TLC was statistically significant ($p<0.05$) in dogs infected with *Ehrlichia* sp. and *Anaplasma* sp. and an increase in eosinophils was statistically significant ($p<0.05$) in dogs with *Ehrlichia* sp. and *Babesia* sp.

The present study demonstrates that blood parasites are one of the factors responsible for hyperthermia in dogs. It supports the idea of investigation of patients' blood for presence of blood parasites as a successful treatment strategy.

The results of the present study are in close agreement with many other studies[9-10], where they found a prevalence of blood parasites 17.14,14, 15.33 and12%, respectively, in dogs of Kathmandu valley. The similarity in results is probably due to similarity in the animal selection method i.e., hyper-thermic dogs brought to clinic and sample examination method i.e., Geimsa staining. The results of the present study indicated a greater prevalence of parasites in dogs than did the results of Bashir *et al.*[11], who reported a prevalence of 2.62% in dogs in Lahore. This higher value could be due to sampling hyper-thermic dogs in this study compared to sampling from both apparently healthy and hyper-thermic dogs it his study. The prevalence obtained in this study was lower than that found by Nwoha *et al.*[12], who recorded 71.4% of dogs with blood parasites. This variation may be due to their low sample size and demographic variations. The pattern of parasite species observed in this study was similar to the findings of Manandhar and Rajawar[9], (11.43% *Ehrlichia* sp., 5.71% *Babesia* sp.), Bogicevic *et al.*[13] (*Ehrlichia canis* 11.06%) and Phuyal[10] (4% *Babesia canis,* 8% *Ehrlichia* sp.), but vary from the results of Subedi[14] (10% *Babesia* sp., 4% *Ehrlichia* sp.), (10% *Babesia* sp., 3.4% *Ehrlichia* sp., 2.0% *Anaplasma* sp.) and Gadahi *et al.*[15] (5% *Babesia canis*). The present findings also vary from those of Nwoha *et al.*[12], who found *Babesia* sp. (94.4%), *Trypanosoma* sp. (5.0%) and *Anaplasma* sp. (45.0%) in Ikwuano local government area of Abia State and those of Bhattacharjee and Sarmah[16], who found *B. gibsoni* (47.72%), *E. platys* (4.54%), *E. canis* (2.27%), *E. ewingii* (2.27%) and *H. canis* (2.27%) in the blood of stray dogs of Khanapara, Guwahati, India. The high prevalence in that study was due to sampling from the stray dog population.

Table 1: Prevalence of blood parasites by age and sex

Parameters	Age group	Sample (N)	Positive N (%)	Negative N (%)	Chi square value	p-value
Age	3 months-2 years	23	1 (4.34)	22 (95.66)	4.23	0.12
	2-5 years	88	12 (13.63)	76 (86.37)		
	3-5 years	39	9 (23.07)	30 (76.93)		
Sex	Male	64	14 (21.87)	50 (78.13)	2.73	0.0313
	Female	86	8 (9.30)	78 (90.7)		

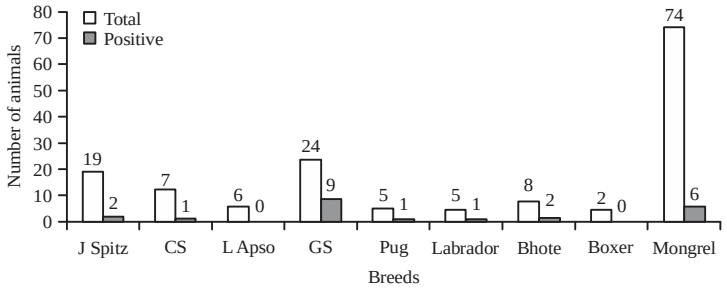

Fig. 1: Breed-wise prevalence of blood parasites

Table 2: Influence of different blood parasites on TLC, DLC, TEC, Hb and PCV values

	Total leucocyte count		Neutrophils		Lymphocytes		Monocytes		Eosinophils		RBC		PCV		Hb	
	+Ve	-Ve	+Ve	-Ve	+Ve	-Ve	+Ve	-Ve	+Ve	-Ve	+Ve	-Ve	+Ve	-Ve	+Ve	-Ve
E. canis	7.01±1.16	11.7±0.56	55.43±2.62	62.33±1.81	29.62±2.31	30.61±1.71	5.5±0.54	4.63±0.28	9.43±0.88	2.41±0.20	3.5±0.15	6.9±0.15	21±0.92	41.66±0.88	7±0.30	13.85±0.30
p-value	p<0.05		p>0.05		p>0.05		p>0.05		p<0.05		p<0.05		p<0.05		p<0.05	
Babesia sp.	7.88±1.43	11.28±0.53	59±8.08	61.65±1.68	25.66±4.37	30.60±1.58	6±4.16	4.7±0.25	9.33±1.33	3.04±0.26	2.22±0.47	6.64±0.15	13.66±2.84	39.98±0.92	4.55±0.94	13.29±0.31
p-value	p>0.05		p>0.05		p>0.05		p>0.05		p<0.05		p<0.05		p<0.05		p<0.05	
Anaplasma	1.81±1.61	11.34±0.52	39±19	61.9±1.65	50±20	30.24±1.55	3.5±1.15	4.74±0.26	7.5±0.5	3.1±0.27	2.5±0.83	6.61±0.15	15±5	39.79±0.93	5±1.66	13.22±0.31
p-value	p<0.05		p>0.05		p>0.05		p>0.05		p>0.05		p<0.05		p<0.05		p<0.05	
Trypanosoma sp.	5	11.25±0.53	56	61.63±1.66	30.55	24±1.56	4.7	8±0.25	3.1	12±0.26	6.58	2.5±0.15	15	39.62±0.94	5	13.17±0.31
p-value	p>0.05		p>0.05		p>0.05		p>0.05		p>0.05		p<0.05		p<0.05		p<0.05	

The variation in the prevalence of different types of parasites may depend on geographical region, different sample sizes and seasons.

In the current study, the prevalence of blood parasites was significantly higher in male dogs than in females ($p<0.05$). This finding is in agreement with previous research[9-14,17], while in contrast with findings of Maia *et al.*[18], Gadahi *et al.*[15], Phuyal[10], Nwoha *et al.*[12] and Batmaz *et al.*[19]. The variation in prevalence of hemo-protozoans in dogs by sex may be due to differences in immune status of animals, different geographical regions sampled and different sample sizes. Some other studies, such as a study done in Ludhiana district of Punjab state of India[20] and a study from Serbia[13] has clearly stated that there was no association found between the seropositivity of hemoprotozoan *Ehrlichia canis* and sex. The differences could also be mediated through hormonal responses. Oestrogen promotes increased cytokine and antibody production, which may provide resistance to infection[21], while androgens (testosterone and 5-alpha-hydroxy-testosterone) inhibit the humoral and cell-mediated immune responses[22-23]. High testosterone levels cause increased locomotor activity and reduced innate and acquired resistance to tick feeding[24] thereby high chance of acquiring tick borne diseases. The present study showed a high prevalence of blood parasites in dogs 5–13 years old, (23.07%), followed by 13.63% in 2–5-year-old dogs and 4.34% in 3-months-old to 2-year-old dogs. These findings are supported by similar findings of previous research by Manandhar and Rajawar[9] , Subedi[14], Gadahi[15], Jalali *et al.*[25] and Akhtardanesh *et al.*[26]. The higher prevalence of parasites in older dogs may be due to poor immune status, frequent exposure to vector ticks and chronic courses of blood parasitemia[27]. In agreement with the findings of Subedi[14], Manandar and Rajawar[9] and Phuyal[10], German Shepherds had high prevalence compared to other breeds. This may be due to an inadequate immunogenic response in the former[28-29].

The presence of blood parasites, particularly *Ehrlichia* sp. and *Anaplasma* sp., was significantly correlated with a decrease in TLC ($p<0.05$), which is consistent with results by Subedi[14], Phuyal[10] and Irwin and Hutchinson[30], but in contrast to results by Manandhar and Rajawar[9]. Lower TLC with infections of Anaplasmosis and Babesiosis could be due to direct damage to hematopoietic precursor cell and suppression of leucocyte proliferation. The presence of blood parasites was also significantly correlated with the level of blood TEC, PCV and Hb ($p<0.05$). However, there was no significant correlation with neutrophil, lymphocyte or monocyte levels. These results are similar to the findings of Manandhar and Rajawar[9], Subedi[14], Kamani *et al.*[31] and

Phuyal[10]. However, there was a significant increase in eosinophil counts in dogs with infections of *Ehrlichia* sp. and *Babesia* sp. Similar reports have been reported from other studies too[9-10]. This significant decrease in PCV and Hb highlights the deleterious effects of hemo-parasites in blood responsible for immune-compromised condition and anemia related diseases.

CONCLUSION

The higher occurrence of blood parasites in samples from the dogs with high temperature suggested that blood parasites are one of the important contributing factors for hyperthermia in dogs. So, blood examination is necessary for their rational treatment. It is crucial to perform study throughout a year that includes large sample size and all seasons helping to proper evaluation of risk factors so that appropriate preventive and control measures can be established. Moreover, detailed study on the detection and molecular characterization of hemo-protozoan parasites is strongly suggested.

SIGNIFICANCE STATEMENT

This study highlights the prevalence of blood parasites in hyperthermic dogs of Kathmandu valley that can be beneficial for the judicial diagnosis and treatment of hyperthermic cases brought to clinics or in the field condition. Further, this study will help clinical veterinarians to analyze the blood parameters during hemoprotozoal infection. Additionally, this study uncovers epidemiology of blood parasites in canine population of Kathmandu valley which will be valuable information to researchers as well as to the pet enthusiasts.

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