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Research Article

PREVALENCE OF BLOOD PARASITES OF DOGS (PET AND STRAY) IN BHAKTAPUR MUNICIPALITY

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ABSTRACT

A cross-sectional study was conducted from September 2022 to February 2023 to investigate the prevalence of blood parasites in dogs (pet and stray) in Bhaktapur municipality. Altogether, 150 blood samples, 50 from pets and 100 from stray dogs, were collected with the help of a trained veterinary assistant from the saphenous or cephalic veins of dogs. Subsequently, the blood was examined microscopically by using a thin blood smear method staining with Giemsa's stain. The overall prevalence of blood parasites was recorded at 27.33% in pet and stray dogs. On individual analysis, 21% of pet samples and 31% of stray dogs were found to be infected with various types of blood parasites. Altogether, there are 4 species of parasites observed in the study, *Anaplasma* spp. (18.67%), *Babesia* sp. (6.67%), *Ehrlichia* sp. (2.67%), and *Hepatozoon* sp. (1.33%). Younger dogs recorded higher infections (31.25%), followed by older dogs (27.2%) and puppies (22.22%). Females showed a higher prevalence (27.65%) than males (27.18%). Higher infection rates of parasites were recorded in the local breed (21.33%), followed by the Japanese breed (1.33%). In the study, the burden of parasites was slightly higher in dogs infested by ticks (52.30%) than in tick-free dogs. A single infection of blood parasites was higher (25.33%) than a double (2%). There was no significant association between factors such as age, sex, breed, and tick infestation and the prevalence of blood parasites ($p > 0.05$). However, significant relationships were found between species of parasites and the concurrency of parasites ($p < 0.001$).

Keywords: *Analpasm*, *Babesia*, blood parasites, dogs

INTRODUCTION

The dog was the first animal that people kept as a pet (Traub *et al*., 2005; Gracenea *et al*., 2009; Ahmed *et al*., 2014). They are affected by various types of endo and ectoparasites such as many protozoa, bacteria, and helminths, which are transmitted by a diverse range of arthropod vectors, including ticks, fleas, lice, triatomines, mosquitoes, tabanids, and phlebotomine sand flies (Dantas-Torres, 2008). The disease caused by these arthropod vectors is called canine vector-borne disease (CVBP), which causes poor performance in dogs and economic losses to the owner (Soulsby, 1982; Pam *et al*., 2013). Dogs serve as reservoirs, carriers, and transmitters of many pathogens, including parasites (Robertson *et al*., 2000). Dogs exposed to ectoparasites transmit hemoparasites, which are zoonotic, important common dermatological diseases of dogs and cats (Moriello, 2003). In tropical and subtropical regions across the world, tick-borne hameoprotozoan diseases such as Babesiosis, Hepatozoonosis, and Trypanosomiasis are majorly affecting canine species because of the high prevalence of tick vectors (Adhikari *et al*., 2016).

Canine babesiosis is a worldwide significant tick-borne disease (Solano-Gallego *et al*., 2016) and an emerging infectious disease (Solano-Gallego and Baneth, 2011), where members of the genus *Babesia* readily parasitize red blood cells, causing progressive anemia and/or a severe inflammatory reaction (Koster *et al*., 2015). Babesiosis is intraerythrocytic, commonly called piroplasms due to their pear-shaped forms (Telford *et al*., 1993; Smith *et al*., 2013). The larger species, *Babesia canis* (approximately 3-5 μm), transmitted by the tick *Dermacentor reticularis*, is considered endemic in Europe (Solano-Gallego and Baneth, 2011), whereas the smaller *B. gibsoni* (approximately 1-3 μm) transmitted by *Haemaphysalis bispinosa* and *H. Longicornis* is considered endemic in Asia. Similarly, *B. rossi* is transmitted by tick *H. elliptica* in South Africa, *B. vogeli* is transmitted by *Rhipicephalus sanguineus* in tropical and sub-tropical regions (Irwan, 2009).

Anaplasma and *Ehrlichia* species belong to the family Anaplasmataceae. *Anaplasma* is an intracellular rickettsial organism that causes canine Anaplasmosis. Two species of Anaplasma have been identified as pathogenic in dogs. It infects granulocytes, predominantly neutrophils but also eosinophils, where they exist and reproduce in membrane-bound vesicles, forming microcolonies called morulae (Carrade *et al*., 2009; Atif, 2016; Said *et al*., 2018). The disease caused by *Ehrlichia* species is called Ehrlichiosis, which is found around the world and is mainly found in tropical and subtropical regions that afflict dogs, humans, and other domestic and wild animal species alike. (Lanza-Perea *et al*., 2009).

Most dogs in Nepal are free to roam and suffer from malnutrition, disease, and a lack of basic health care (Massei *et al*., 2016). Only a very few studies were conducted on blood parasites of dogs in Nepal.

Trypanosoma was reported from Makwanpur district by Maharjan and Mishra (2006). Subedi and Shrestha (2012) reported *Babesia canis*, *Babesia gibsoni,* and *Ehrlichia* spp. Similarly, Phuyal *et al.* (2017) surveyed the prevalence of blood parasites in dogs of Kathmandu Valley and reported 12% infection with *Babesia canis* and *Ehrlichia* spp. In the same manner, the research done by Bhatta *et al.* (2018) in hyperthermic dogs of Kathmandu Valley reported *Babesia* sp., *Ehrlichia* sp., *Anaplasma* sp., and *Trypanosoma* sp. The study conducted by Daiz-Reganon *et al.* (2020) on stray dogs in Nepal reported *Hepatozoon canis* (31.43%), *Anaplasma platys* (31.43%), *Ehrlichia canis* (27.14%), *Leishmania donovani* (18.57%), *Theileria* spp. (12.86%), *Babesia vogeli* (12.86%), and *Babesia gibsoni* (2.86%).

Bhaktapur municipality is a rapidly urbanizing municipality of Bhaktapur districts. With rapid urbanization, the number of dogs is also increasing daily as they live closely associated with humans. Urbanization has created several issues for stray animals. People are much more concerned about their pet's health as well as some of the stray dogs of their community and take them to the clinic or hospitals for their treatment. However, dogs carried to clinics/hospitals were treated symptomatically without using any diagnostic methods. But most dogs that don't show any symptoms can also act as reservoirs and carriers of blood parasites. There is no organized research on canine blood parasites in this municipality. So, in this study, investigation effort has been focused on the prevalence of blood parasites of dogs (pet and stray) in Bhaktapur municipality, to increase pet owners' awareness about dog blood parasites, and to recommend preventive measures for canine blood parasites.

MATERIALS AND METHODS

Study Area

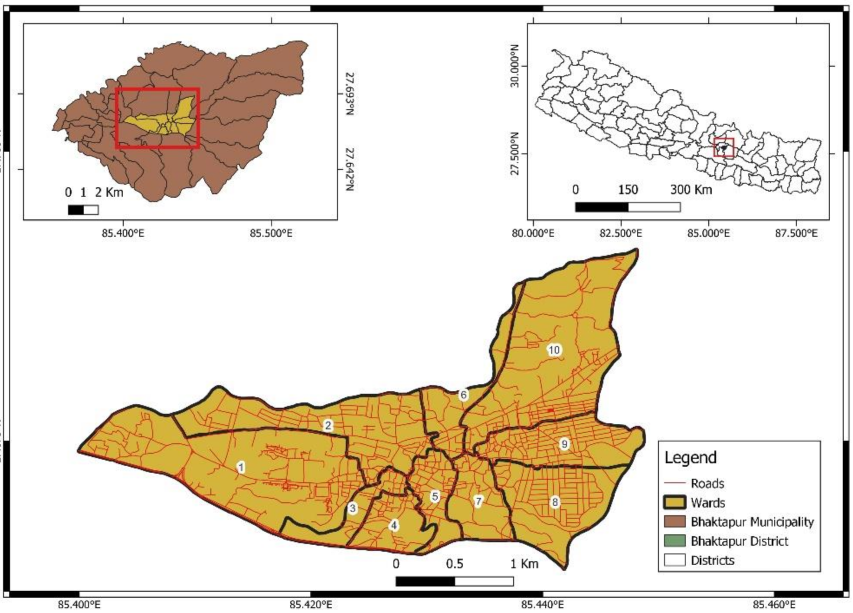

Figure 1. The upper right map shows the location of Bhaktapur district in Nepal, the upper left shows the location of Bhaktapur Municipality in the Bhaktapur District, and the lower map shows different wards within the Bhaktapur Municipality.

One of the seventy-seven districts of the landlocked nation of Nepal is Bhaktapur district, which is a part of Bagmati province. It covers an area of about 123.1 km^2. Its headquarters is in Bhaktapur. It lies at an elevation of 27.672222°N and 85.427778°E. Bhaktapur municipality is one of the municipalities of Bhaktapur district, which is administratively divided into 10 wards. It covers an area of 16.89 km^2. It is in a warm temperate area of the country where the climate is fairly temperate as it lies at an altitude of 1401 meters above sea level. According to census 2021, the total population of Bhaktapur municipality is 77,136. The population of stray dogs is about 2000.

Blood sample collection

A cross-sectional study was conducted from different areas of ten wards of Bhaktapur municipality. Blood samples were collected irrespective of age, sex, and breed, from September 2022 to February 2023. Altogether, 150 samples were collected; 100 samples were from stray dogs, and 50 samples were from pet dogs. With the help of a veterinary assistant, from each dog, 3 ml blood was collected aseptically with the help of a disposable syringe from the saphenous or cephalic vein of the dog by direct vein puncture. The collected samples were placed in a clean ethylene diamine tetraacetic acid (EDTA) tube, which was used as an anticoagulant, and labeled with the sample number. All the information, like owner name, address, sex, age, and breed, was recorded from pet dogs, whereas address, sex, age, and breed were recorded from stray dogs. Dogs were classified into three age groups: puppies (zero to six months), younger dogs (>six months to 12 months), and older dogs (>12 months), as described by Bone, (1988). The age of the stray dogs was identified with the help of local people, as the local people feed food to them and through a veterinary assistant as well. Similarly, health conditions and the presence or absence of ticks were also recorded.

Preservation of samples

The collected blood samples were immediately preserved in a cool box after sample collection and carried to the lab of the Central Department of Zoology, Kirtipur, for immediate examination. If the lab work was delayed, then samples were preserved in the refrigerator at 4°C.

Preparation of slides and staining

Each collected blood sample was examined microscopically by using the thin blood smear method and staining with Giemsa's stain (Chatterjee, 2019). At first, a drop of blood was taken on a glass slide with the help of a micropipette at a distance of about half an inch from the right end. A smear was prepared by using another slide tilted at an angle of 45˚ in contact with the drop of blood and pushed gently to the left till the blood was exhausted and was air-dried immediately. Then, the smear was fixed in methanol for three to five minutes by adding two to three drops. This fixed slide was then stained with a working solution (5%) of Giemsa stain in a coplin jar for 40–45 minutes. After that, the slide was washed slowly by running tap water on the slide after being removed from the Giemsa stain, let dry, and examined under a microscope using oil immersion.

Microscopic examination of blood parasite

The stained slide was examined under oil immersion by using a light microscope under high power magnification (10x by 100x), which is considered the best (Njunda *et al.*, 2013). The slides were examined from the tail end to the whole field parasites and focused, and photographs of parasites that were seen were taken. The parasites were identified with the help of experts and from different literature following their morphological characteristics (Hildebrendt *et al.*, 1973; Baneth & Shkap, 2003; Laha *et al.*, 2015; Atif *et al.*, 2021).

Statistical data analysis

The data were coded, and statistical analysis was done through SPSS V25. The association between outcomes and individual explanatory variables was assessed using Pearson's Chi-Square test. The level of confidence was set at 95%, and the value of $p < 0.05$ was considered significant.

RESULTS

Prevalence of blood parasites in dogs

Among collected blood samples, 41 (27.33%) samples were found positive for various types of blood parasites (Fig. 2).

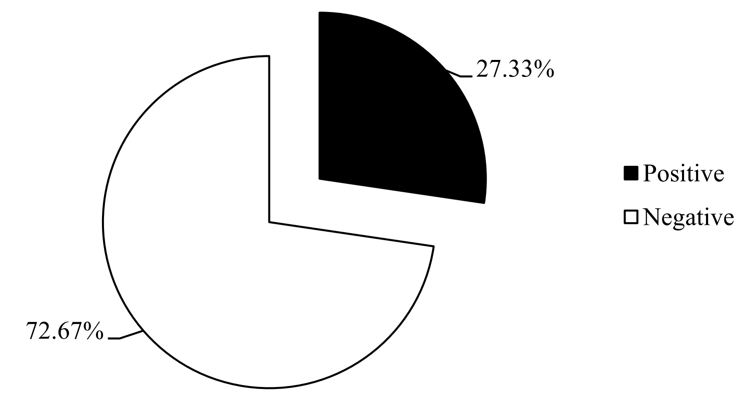

Figure 2. Prevalence of blood parasites in dogs

Prevalence of specific blood parasites

In both pet and stray dogs, altogether 4 species of blood parasites: *Anaplasma* spp., *Babesia* sp., *Ehrlichia* sp., and *Hepatozoon* sp. were found (Figs. 2-6). Among them, *Anaplasma* spp. was found to have the highest prevalence rate (18.67%), followed by *Babesia* sp. (6.67%), *Ehrlichia* sp. (2.67%), and *Hepatozoon* sp. (1.33%), as shown in Table 1.

Prevalence of blood parasites in pet and stray dogs

Among a total of 50 blood samples of pet dogs, 10 (20%) of the samples were found positive. And out of 100 blood samples of stray dogs, 31 (31%) were found to be infected with various types of blood parasites (Fig. 7), which was found to be statistically insignificant ($\chi^2 = 2.03$, d.f. = 1, p = 0.154).

Figure 2. ***Anaplasma*** sp.

Figure 3. ***Anaplasma*** sp

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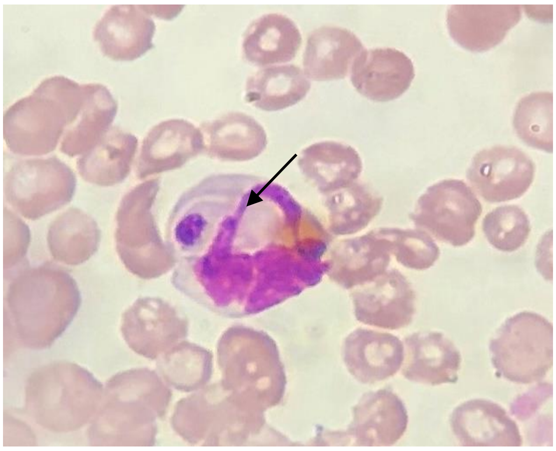

Figure 4. ***Hepatozoon*** sp.

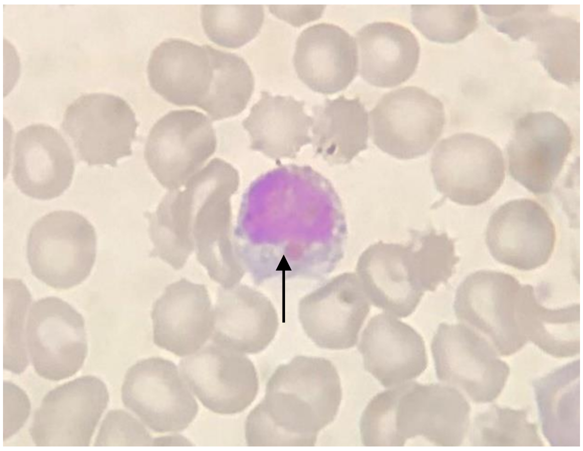

Figure 5. ***Ehrlichia*** sp.

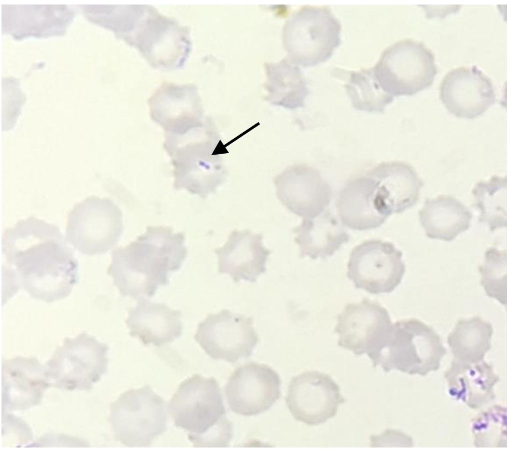

Figure 6. ***Babesia*** sp.

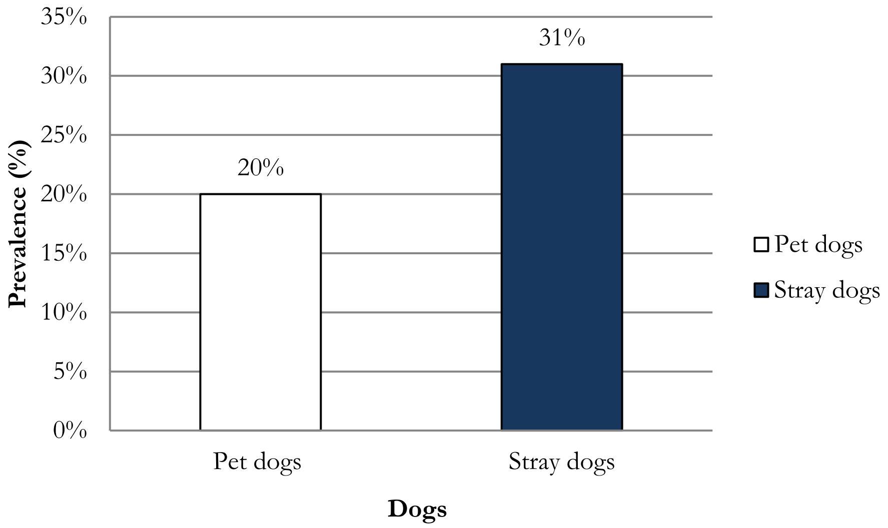

Figure 7. Prevalence of blood parasites in pet and stray dogs

Table 1. Prevalence of specific blood parasites

S.N.	Genera of blood parasites	Total samples	Total positive samples	Prevalence rate
1.	*Anaplasma* spp.	150	28	18.67%
2.	*Ehrlichia* sp.		4	2.67%
3.	*Hepatozoon* sp.		2	1.33%
4.	*Babesia* sp.		10	6.67%

Prevalence of specific blood parasites in pet and stray dogs

The analysis done between pet and stray dogs showed that stray dogs have a higher prevalence rate of blood parasites (*Anaplasma* spp. = 20%) as compared to pet dogs and a lower prevalence of blood parasites (*Ehrlichia* sp.) in pet dogs (2%) and *Hepatozoon* sp. (2%) in stray dogs (Table 2). There was a significant difference between specific blood parasites and the prevalence of blood parasites in stray and pet dogs.

Table 2. Prevalence of specific blood parasites in pet and stray dogs

Genera of blood parasites	Parasitic prevalence in pet dogs (n = 50)	Parasitic prevalence in stray dogs (n = 100)	Chi-square value	p-valve
Anaplasma spp.	8 (16%)	20 (20%)	$\chi^2 = 150$	0.001
Ehrlichia sp.	1 (2%)	3 (3%)		
Hepatozoon sp.	-	2 (2%)		
Babesia sp.	3 (6%)	7 (7%)		

Age-wise prevalence of blood parasites

A total of nine samples from puppies (zero to six months), 16 samples from younger dogs (>six months to 12 months), and 125 samples from older dogs (>12 months) were collected during the study period. The overall prevalence of different age groups of dogs showed that samples collected from younger dogs recorded a higher infection rate (31.25%), followed by older dogs (27.2%), and a lower in puppies (22.22%), which is shown in Fig. 8.

A total of four, seven, and 39 samples were collected from puppies, younger dogs, and older dogs. Among them, one (25%), two (28.57%), and seven (17.94%) of the samples were found to be infected with different species of blood parasites. Similarly, in stray dogs, there were five, nine, and 86 samples collected from each age group, which found one (20%), three (33.33%), and 27 (31.39%) samples infected. Age-wise prevalence analysis showed that a higher prevalence was found in stray dogs of younger age groups and a lower prevalence in pet dogs of older age groups (Fig. 9). There was no significant relationship between age of both pet and stray dogs and prevalence (Pet: χ^2 = 0.486, d.f. = 2, p = 0.784) (Stray: χ^2 = 0.311, d.f. = 2, p = 0.856).

Sex-wise prevalence of blood parasites

Out of 103 blood samples collected from male dogs, 28 (27.18%) samples were found to be infected, and out of 47 samples from female dogs, 13 (27.65%) samples of blood were found to be infected with different blood parasites (Fig. 10).

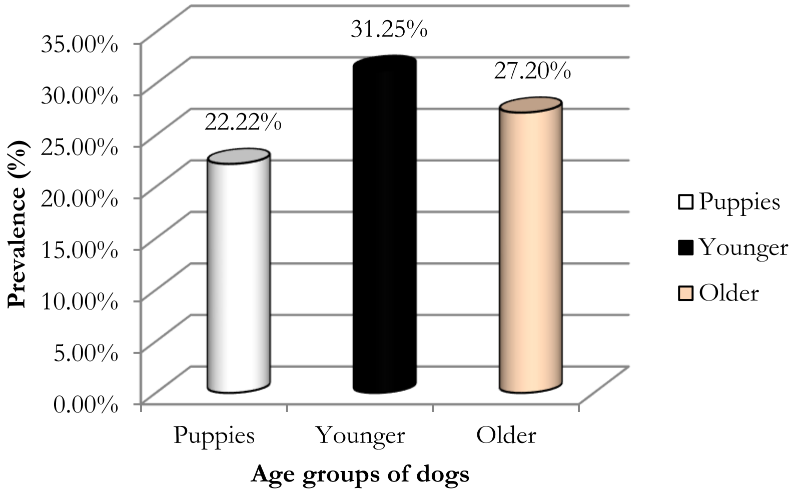

Figure 8. Age-wise prevalence of blood parasites

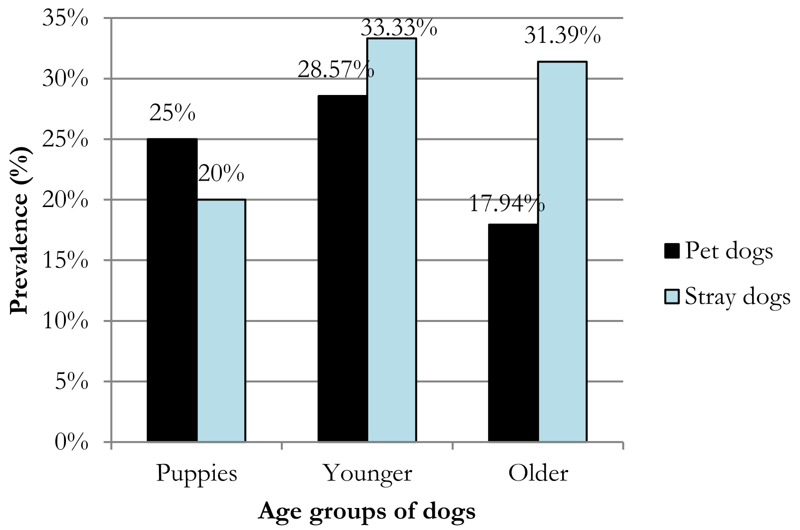

Figure 9. Age-wise prevalence of blood parasites in pets and stray dogs

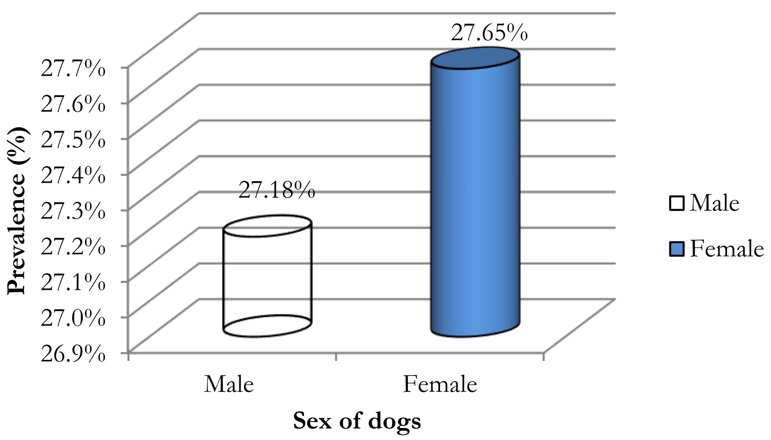

Figure 10. Sex-wise prevalence of blood parasites

During the study, 72 male and 28 female blood samples were collected from stray dogs, and 31 and 19 samples were collected from pet dogs. During analysis, female stray dogs showed a higher prevalence (39.28%) among both pet and stray dogs, whereas in pet dogs, males showed a higher infection (25.80%) as compared to females (10.58%), as shown in Fig. 11. However, there was no significant relationship between the prevalence of blood parasites and the sex of pet and stray dogs (pet: p = 0.190, stray: p = 0.264).

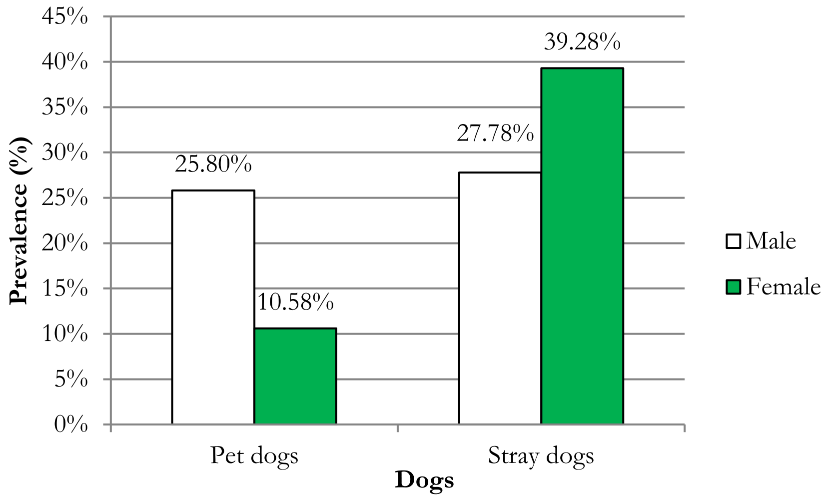

Figure 11. Sex-wise prevalence of pet and stray dogs

Breed-wise prevalence of blood parasites in dogs

In pet dogs, altogether seven breeds of dogs were found, whereas in stray dogs, only local breeds of dogs were recorded. A higher prevalence of parasites was recorded in local dogs (21.33%) among both pet and stray dogs, whereas in pet dogs only, Japanese breeds have a higher (6%) prevalence of parasites. But there was no significant association between the breed of dogs and the prevalence of parasites (χ^2 = 3.101, d.f. = 6, p = 0.796).

Table 3. Breed-wise prevalence of blood parasites in dogs

S.N.	Breed	Pet dogs (n =50)	Stray dogs (n = 100)	Total (150)
1.	Cross breed (n = 10)	2 (4%)	-	2 (1.33%)
2.	Golden retriever (n = 8)	2 (4%)	-	2 (1.33%)
3.	Japanese breed (n = 17)	3 (6%)	-	3 (2.0%)
4.	Cocker spaniel (n = 7)	1 (2%)	-	1 (0.67%)
5.	Bilati (n = 1)	0 (0%)	-	0 (0%)
6.	Local (n = 105)	1 (2%)	31 (31%)	32 (21.33%)
7.	Pop (n = 2)	1 (2%)	-	1 (0.67%)

Prevalence of blood parasites in tick infestation and non-tick infestation dogs

Out of 150 dogs that were chosen for sample collection, 21 dogs were infected by ticks and 129 dogs were non-infected. Both types of dogs that are infected by ticks and those that are not infected by ticks are found to be infected with blood parasites. The samples collected from 21 dogs infected by ticks resulted in 11 (52.3%) samples being positive, whereas 129 samples taken from non-infected dogs resulted in 30 (23.26%) samples being infected with blood parasites (Fig. 12).

In both pet dogs and stray dogs, samples collected from the dogs that were infested by ticks showed a higher prevalence of blood parasites (66.67% and 50%) as compared to non-tick infestation dogs (Table 4), which was found to be statistically insignificant.

Occurrence of species of blood parasites in tick infestation dogs

Out of 21 blood samples collected from tick infestation dogs, 11 samples were found positive for three different species of blood parasites: *Anaplasma* spp., *Babesia* sp., and *Hepatozoon* sp. Among these blood parasites, *Anaplasma* spp. showed a higher infection (33.33%), followed by *Babesia* sp. (19.05%) and *Hepatozoon* sp. (4.76%) in tick-infested dogs, which are shown in Fig. 13.

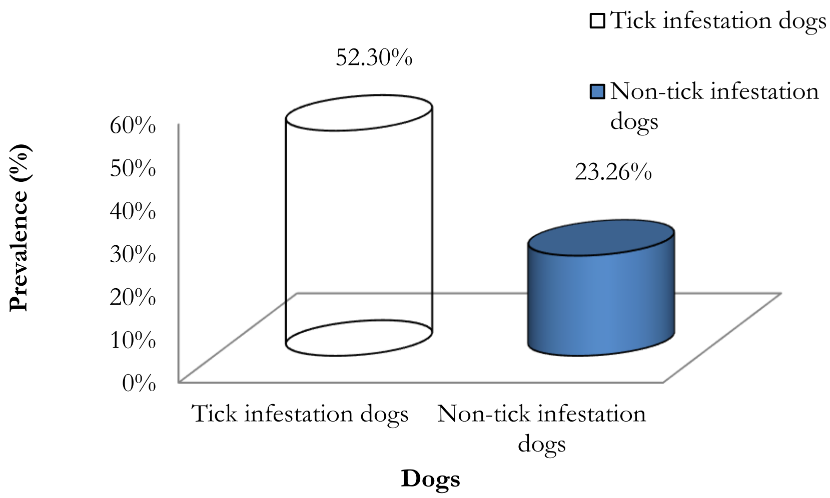

Figure 12. Prevalence of blood parasites in tick infestation and non-tick infestation dogs

Table 4. Prevalence of blood parasites in tick infestation and non-tick infestation in pet and stray dogs

	Parasitic prevalence in tick-infected dogs (n = 3, 18)	**Parasitic prevalence in tick non-infected dogs (n = 47, 82)**	**Chi-square value**	**p value**
Pet dogs	2 (66.67%)	8 (17.02%)	4.344	0.37
Stray dogs	9 (50%)	22 (26.82%)	3.705	0.054

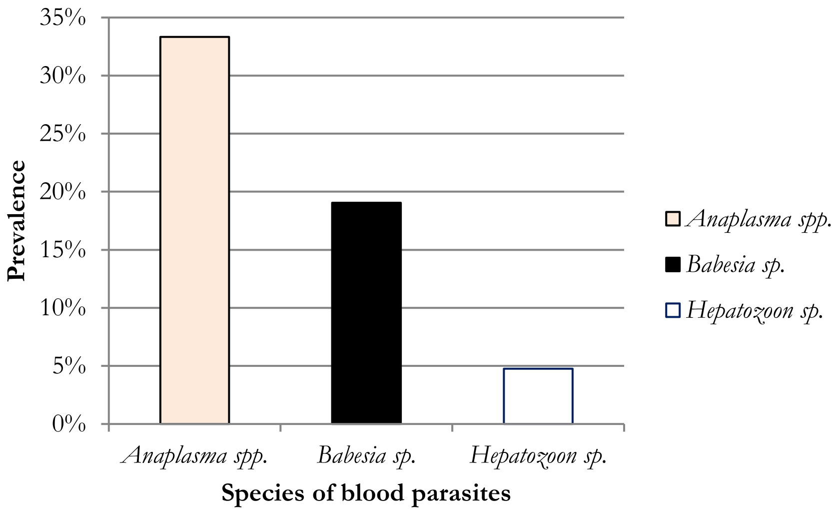

Figure 13. Occurrence of species of blood parasites in tick infestation dogs

Concurrency of blood parasites in pets and stray dogs

Out of 150 samples, single infection was found to be higher (25.33%) and double infection was lower (2%). In individual analyses of pet dogs and stray dogs, similar results were obtained: single infections were higher than double infections (Table 5), which showed a significant difference ($p < 0.001$).

Table 5. Concurrency of blood parasites in pets and stray dogs

Dogs	Total samples	Single infection	Double infections
Pet	50	8 (16%)	2 (4%)
Stray	100	30 (30%)	1 (1%)

DISCUSSION

In the present study, it has been observed that out of 150 samples collected from pets and stray dogs, the overall prevalence of blood parasites was found to be 27.33%. This finding was slightly similar to the study done by Hii *et al.* (2012) in Australia (29.13%), to that of the Belgrade area (31.32%) by Pavlovic *et al.* (2017), and in Sri Lanka (25.3%) by Rajakaruna *et al.* (2021). The current finding was higher than the study conducted by Kumar *et al.* (2009) on the prevalence of hemoprotozoans in canines in Chennai city, which records an 11.6% prevalence of parasites. Similarly, studies done in hyperthermic dogs of Kathmandu Valley, Nepal by Subedi and Shrestha (2012) and Phuyal *et al.* (2017) by taking total samples number of 50, and Bhatta *et al.* (2018) with samples 150, also showed a lower prevalence of parasites (14%, 12%, and 14.66%) as compared to the present study. This may be because the current study includes both stray and pet dogs, whereas the prior study included pet dogs. In the present study, stray dogs have a higher (31%) prevalence of parasites as compared to pet dogs (20%). The study done by Bhattacharjee and Sarmah (2013) in Assam and North-East India supports the present findings, recording the prevalence of blood parasites at 58.03% in pet dogs, 54.54% in working dogs, and 63.64% in stray dogs.

Different species of blood parasites, such as *Anaplasma* spp., *Babesia* sp., *Ehrlichia* sp., and *Hepatozoon* sp. were found in the current study. Among the four species of parasites, *Anaplasma* spp. was recorded as having the highest (18.67%) prevalence of parasites, followed by *Babesia* sp. (6.67%), and the least was *Hepatozoon* sp. (1.33%). Research conducted by Sathish *et al.* (2021) supports the current findings, revealing *Anaplasma* sp. higher, followed by *Babesia* spp. Similar findings were recorded by Dantas-Torres *et al.* (2020) in two Brazilian regions: Sao Joaquim da Bicas showed a similar prevalence (16.5%) as the present study, whereas the Goiana area recorded a higher prevalence (45.1%) than the current study. This may be due to the presence of a favorable environment for the spread of these parasites (Irwin & Jefferies, 2004).

In age-wise overall prevalence, younger dogs showed a higher prevalence (31.25%) of parasites, followed by older dogs (27.2%), and puppies (22.22%). This finding was supported by Oduye and Dipeolu (1976), who have researched the blood parasites of dogs in Ibadan. Similarly, the research conducted by Sahu *et al.* (2014) also records 17.07% prevalence in less than one-year-old dogs and 9.73% above one-year-old dogs. In stray dogs as well, younger dogs (33.33%) have higher infections, followed by older dogs (31.39%), and puppies (20%), whereas pet dogs have a higher prevalence of parasites in younger dogs (28.57%), followed by puppies (25%), and then older dogs (17.94%), but it was found to be statistically insignificant. This may be due to vertical transmission (Fukumoto *et al.*, 2005) or the fact that the maternal colostrum's of dogs were either non-protective or short-lived protection which leads to weak immunity against blood parasites (Oduye & Dipeolu, 1976).

In the present study, overall sex-wise prevalence showed that females were more (27.65%) infected than males (27.18%). However, there was no significant association between sex and the prevalence of parasites. While carrying out individual analyses of stray dogs, it was also found that females have a higher (39.28%) prevalence of parasites than males (27.78%). This finding was supported by Gadahi *et al.* (2008), who recorded 18.6% in females and 9.33% in males. Similarly, research conducted by Shitta (2012) in Jos North and Jos South LGAs of Plateau state, Nigeria, El-Dakhly *et al.* (2013) in Japanese islands and Peninsulas, and Phuyal *et al.* (2017) in Kathmandu also showed higher infection in female than male. In the same manner, Pam *et al.* (2013) recorded 23% of females and 20% of males; similarly. It may be due to stress arising during reproductive activities that leads to low immunity and gets easily attacked by blood parasites, or due to sedentary behavior during nursing their offspring that causes more tick infestation. However, in pet dogs, males showed a higher infection than females. Different articles support these findings, such as Subedi and Shrestha (2012), Bhatta *et al.* (2018), Obeta *et al.* (2020), and Iatta *et al.* (2021). This might be explained by the roaming nature of male dogs in search of mates, which increases their exposure to ticks (Mundim *et al.*, 2008).

Out of 150 blood samples, 105 were from local breeds, 17 from Japanese breeds, and so on. Overall prevalence showed that local breeds have a higher prevalence (21.33%) as compared to other breeds. According to tick infestation and non-tick infestation, the prevalence of blood parasites was found to be higher (52.3%) in tick infestation dogs than in non-tick infestation (23.36%) but statistically found to be insignificant. Out of 21 blood samples collected from tick infestation dogs, 11 samples were found positive for three different species of blood parasites: *Anaplasma* spp., *Babesia* sp., and *Hepatozoon* sp. Among these blood parasites, *Anaplasma* spp. showed a higher infection (33.33%). The dogs get infected due to prolonged stress that leads to weak immunity causing parasitic infection (Romeo *et al.*, 2020).

In cases of concurrency of prevalence of blood parasites, a single infection was found to be higher (25.33%) than double infections (2%). This finding was supported by the research done by Piratae *et al.* (2015) on molecular detection of *Ehrlichia canis*, *Hepatozoon canis,* and *Babesia canis vogeli* in stray dogs in Mahasarakham which showed higher in single infections (32.91%) than co-infection of *E. canis* and *B. canis vogeli*. Similarly, other researchers from different countries (Yabsley, 2008; Hii *et al.*, 2012; Hoffman *et al.*, 2019; Alanazi *et al.*, 2021; Do *et al.*, 2021) recorded single infection higher than co-infections with two or more pathogens. The study carried out by Gaunt *et al.* (2010) and Rawangchue and Sungpradit (2020) in

Thailand showed lower hematocrit, platelet counts, red blood cell, hemoglobin, and lymphocytes count on co-infection with *A. platys* and *E. canis* and with *B. vogeli* and *E. canis* than in single infection which results to the weak body and easily catch by several diseases.

Babesia and *Ehrlichia* are zoonotic parasites. It was supported by the findings of Jefferies *et al.* (2007) from the region of western Victoria in southern Australia. Similarly, El-Bahnasawy *et al.* (2011) recorded that a 12-year-old boy acquired a babesiosis infection from his pet dog in Egypt. Andric, 2014 recorded *Ehrlichia canis* in humans.

CONCLUSIONS

In conclusion, the study recorded the prevalence of blood parasites as 27.33% in pet and stray dogs. Higher infection was found in stray dogs with various types of blood parasites. Altogether, 4 species of parasites *Anaplasma* spp., *Babesia* sp., *Ehrlichia* sp., and *Hepatozoon* sp. were observed in the study. The younger dogs recorded higher infestation followed by older dogs and puppies. In sex-wise prevalence, females showed higher infection as compared to males. Higher infestations of parasites were recorded from the local breed followed by the Japanese breed. A higher occurrence of parasites was found in tick-infected dogs than in non-tick-infected dogs. From tick infestation dogs, *Anaplasma* spp., *Babesia* sp., and *Hepatozoon* sp. blood parasites were recorded. Single infection of blood parasites was more common than double infection. However, there was no significant association between factors such as age, sex, breed, tick infestation, and the prevalence of blood parasites. Significant relationships were found between species of parasites and the concurrency of parasites. Some of these parasites are zoonotic to humans and other animals, so to break the transmission chain of blood parasites, there should be treatment for infected dogs.

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AUTHOR CONTRIBUTIONS

Semsal Tamang: Conceptualization, study design, methodology, data analysis and interpretation, writing draft, critical review and revision of manuscript, final approval, final proof to be published. Janak Raj Subedi: Conceptualization, critical review and revision of manuscript, final approval, final proof to be published.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY

The data that is used to create figures and tables are available upon request to the corresponding author.

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