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Prevalence of Blood Protozoa in Street Dogs of The Bharatpur Metropolitan Area of Nepal.

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ABSTRACT

Different hemoparasites are linked to companion animals like dogs and cats, which can pose substantial health risks and have a significant financial impact on owners. Nepal's major cities have huge number of stray dog populations. Due to its extreme pathogenicity, a haemoprotozoan infection is one of the most significant tick-borne infections. Hemoprotozoan diseases caused by many Trypanosoma species include trypanosomosis, theileriosis, babesiosis, and anaplasmosis. Ticks thrive in the hot, humid climate, which also serves as a dependable source of infection for vulnerable animals like dogs. A research was carried out in the Bharatpur Metropolitan City of Chitwan, Nepal during June and July 2022 to assess the incidence of tick-borne blood protozoa in stray dogs. Blood samples were randomly collected and the smears were examined under microscope by the Giemsa staining method. Out of 105 street dogs only 14 samples tested positive for blood protozoa. Among these three types of bloodprotozoa were detected, Theileria spp. (10.47%) was found to have the highest prevalence rate followed by Babesia spp. (1.9%) and Ehrlichiacanis (0.95%) with an overall prevalence of 13.33%. The prevalence rate was higher in males (8.5%) than in females (4.76%). The relation was not statistically significant.Infected dogs had considerably lower mean hemoglobin levels than non-infected dogs.

Keywords: BharatpurMetropolitian city, Hemoprotozoan, Prevalence and Stray dogs.

INTRODUCTION

The domestic dog (Canis lupus familiaris), a member of the Canidae family of carnivore mammals, is descended from the wolf *(Wang et al., 2008)*. Before the development of agriculture, some 15,000 years ago, hunter-gatherers tamed dogs as the first animal *(Laurent et .al, 2020)*. It appears that a number of canine vector-borne diseases, including filariasis, babesiosis, and ehrlichiosis, are common throughout India and other South Asian countries, as identified mostly by morphological techniques, despite the paucity of study on canine ectoparasites(Rani et al., 2010). Dog ectoparasites are a major group of parasites because they commonly ingest blood, produce pruritis, and can serve as transmission routes for different illnesses *(Jajere et al., 2022)*. Different hemoparasites are linked to companion animals like dogs and cats, which can pose substantial health risks and have a

significant financial impact on owners *(Manandhar & Rajawar, 2008)*. Dogs are becoming more domesticated in Nepal as their importance for defense and companionship grows.Stray dog populations are considerable in Nepal's major cities *(Kato M et al., 2003)*. The majority of dogs who wander freely are emaciated, infected, and do not receive basic medical treatment (such as deworming, antiparasitic medications, or vaccinations) *(Massei G et al., 2017)*.

Ticks are the main vector for the spread of haemoprotozoan parasites, which has historically been a major barrier to the survival of foreign species and is of great economic significance in Asia *(Ananda et al., 2009)*.Haemoprotozoan infections are of high importance among tick-borne diseases due to their extreme pathogenicity *(Adhikari et al., 2016)*. Important hemoprotozoan diseases in animal produced by various Trypanosoma species include trypanosomosis, theileriosis, babesiosis, and anaplasmosis *(Tewari et al., 2001)*. In tropical and subtropical regions, Babesia species, Ehrlichia species, and Hepatozoan species are prevalent and are the main pathogens that cause fever, anemia, thrombocytopenia, and jaundice in dogs *(Irwin et al., 2004)*. Ticks thrive in the hot, humid climate, which also serves as a constant source of infection for vulnerable animals *(Maharana et al., 2016)*.

The clinical signs of haemoprotozoan illnesses range from moderate to severe, and if not recognized and treated promptly, can result in death *(Guan et al., 2010)*.Tick-borne infections are a growing concern in dogs.They are now well acknowledged as a cause of serious disease in tropical and semi-tropical nations, as well as a source of illness in dogs in temperate climates and urban settings *(Stella C. W. Self et al., 2019)*.Dogs and other companion animals are linked to a number of hemoparasites, which pose a serious health danger to people worldwide *(Manandhar & Rajawar, 2008)*.The majority of infections are identified by the use of Giemsa stained smears made from likely cases based on common clinical symptoms, which provide microscopic evidence of infective phases in blood or tissue fluids *(Gupta et al., 2005)*.

Objectives:

1. ***General Objectives:***
   - To know the prevalence of blood parasites in street dogs of Bharatpur Metropolitan City.
2. ***Specific Objectives:***
   - To determine the different blood protozoa in street dogs.
   - To know the effect of blood Protozoa in street dogs.
   - To know the relationship between ticks and blood parasites.

MATERIAL AND METHOD

1. Study Area :

The blood smples were tookfromBharatpur Metropolitan area from June, 2022 to July, 2022, While additional laboratory testing was done at theNepal Polytechnic Institute-Veterinary Teaching Hospital (NPI-VTH).Bharatpuris located at an elevation of about 251 meters above sea level with temperature ranges from 10°C to 40°C*(Bharatpur, Nepal, 2023)*, which is suitable for the survival of ectoparasites that transmit the blood protozoan.

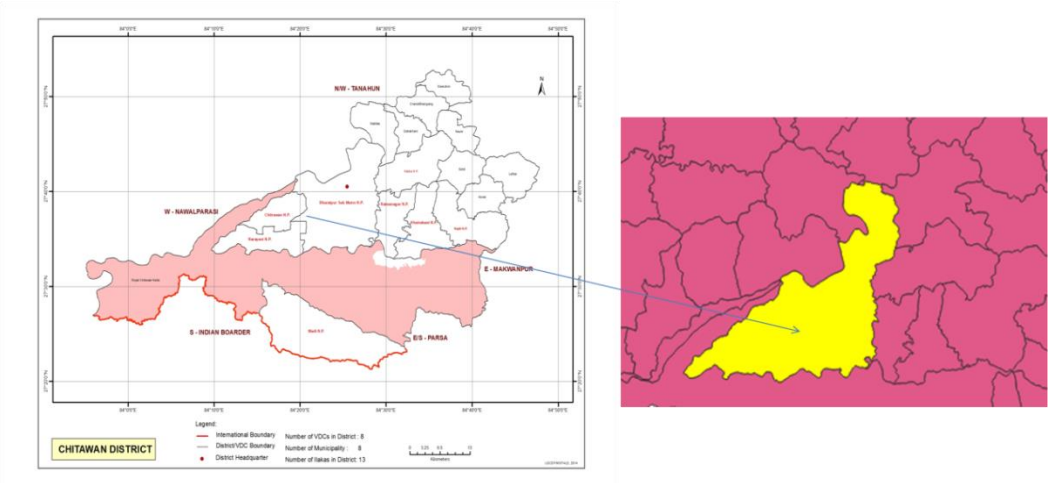

Figure no. (1): Chitwan district and Bharatpur metropolitan city in zoom**.** Source: lgcdp.gov.np

2. Study Designs and Selection of dogs:

The Yamane formula was used to calculate the necessary sample size.105 blood samples of street dogs infected with the ectoparasiteswere collected in vacutainercontainingEDTA. Simple random sampling was done from various Bharatpur locations.

3. Laboratory Tools:

Sterile syringe EDTA vial, Glass slides, Cotton, Colorimeter, and chemicals like Spirit, Giemsa'sstain (Himedia), Methanol, Haemoglobin diluting fluid, and Immersion oil were used for this study.

4. Collection of Blood Samples:

Blood (1.5 t o2 ml) was collected from cephalic vein of animals using a disposable syringe and stored in EDTA vials. Other clinical signs like presence or absenceof tick on body surface and the anemic condition were also checked.Each dog's skin and coat were checked for presence of ticks for one minute, and the dental pad and eye mucous membrane were examined to determine whether the dog was anemic.

5. Preparation of Slide:

A drop of blood was placed on a clean glass slide, and a thin smear was made using a slide that had been tilted at an angle of 450 degrees. The smear was then quickly air dried. The smear was then let to dry in methyl alcohol for two to three minutes. After being fixed, the slide was stained for 30 to 35 minutes in a Coplin jar with a working solution of Giemsa stain (Himedia) (10%). For further analysis, the slide was cleaned and allowed to air dry.

6. Examination of Slide:

A binocular compound microscope set to 100X was used to view the slide after addition of immersion oil. To determine whether a blood parasite was present, the smear was inspected. Particular attention was paid to the RBCs and WBCs. *Babesia spp.*were detected inside RBCs, while*Trypanosoma spp*. were foundbetween RBCs. On the other hand, *Ehrlichia spp*.were identified in WBCs (particularly monocytes). The edge of the smear was mostly studied.

7. Estimation of Hemoglobin:

Hemoglobin was estimated by hemoglobin reagent (HEMOCOR–D) by the cyanmethemoglobin method in aSquare Benchtop Photo Colorimeter. In a large test tube, 5 ml of hemoglobin was combined with 20 µl of blood sample and thoroughly mixed. At 540nm, the diluted solution was observed.Calculation = reading in colorimeter/ 0.4

8. Data Analysis:

EViews Student ver. 12 and MS-Excel 2010 were used to enter and evaluate the data. T-test analysis was used to examine the hematological data. the influence of

sex on prevalence was assessed by using the chi-square (χ_2) test. Values of $p<0.05$ were considered statistically significant at a 95% level of confidence.

RESULT AND ANALYSIS

1. Overall Prevalence of Study Sample

Out of 105 samples examined, 14 samples were found positive for blood protozoa. So, prevalence percentages of the study were 13.33%.

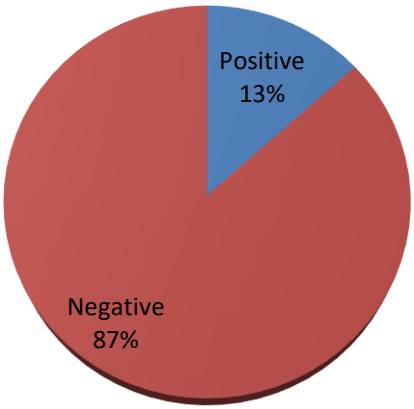

Figure 2: Overall Prevalence of study samples

Source: Field survey 2022

2. Blood parasitic Species Isolated in Positive Blood Samples of Dogs:

In the total fourteen positive samples, only three species of blood protozoa were identified in the Nepal Polytechnic Institute Veterinary Hospital. Protozoa that were found in the study were *Theileria* spp. *11(10.47%), Babesia* spp. *2(1.9%)* and *Ehrlichiacanis 1(0.95%),* as shown in the figure no. 3

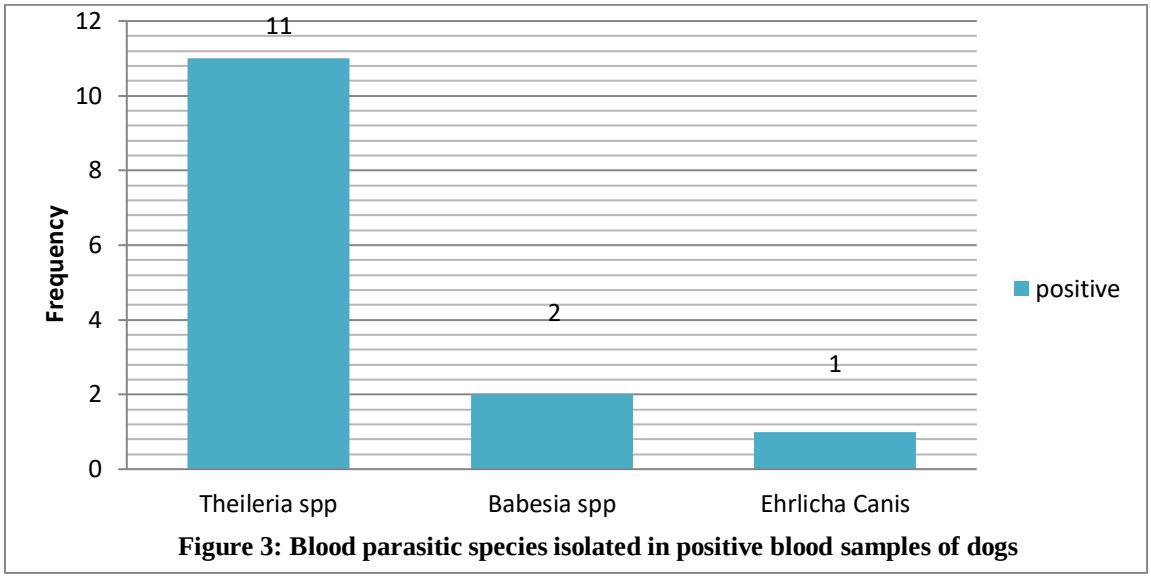

Figure 3: Blood parasitic species isolated in positive blood samples of dogs

Source: Field survey 2022

3. Sex-wise prevalence:

Out of 105 samples, the prevalence of blood protozoan was found to be 8.57% in males and 4.7% in female dogs.

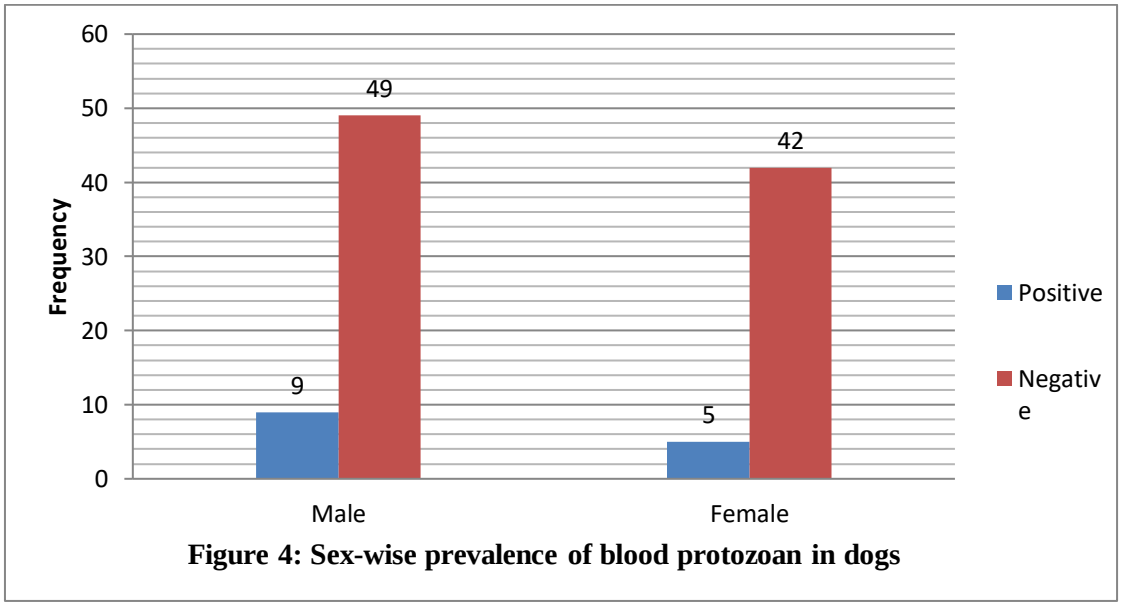

Figure 4: Sex-wise prevalence of blood protozoan in dogs

Source: Field survey 2022

4. Mean Hb Estimation:

The mean value of hemoglobin in blood protozoa-positive dogs was 9.5g/dl which was below the normal value.

Table (1): Mean Hemoglobin Estimation:

Parameter	Normal Reference ranges	Infected dogs
Hemoglobin	12 ±19	9±11

DISCUSSION

This study, as far as we are aware, was the first to examine hemoprotozoan in street dogs from the metropolitan area of Bharatpur. The prevalence of blood parasites in street dogs around Bhartpurmetropolitan was 13.33%, which is closely related to 10 % *(Manandhar et al., 2008)*, 14%*(Maharjan et al., 2014)*, 17.4% *(Subedi et al., 2009)*, 12% *(Phuyal et al., 2017)*) that were conducted in dogs around the Kathmandu valley. This might be as a result of the study's choice of dogs with tick infestations or as a result of the seasonal change (summer) *(Maharjan et al., 2014)(Subedi et al., 2009)(Manandhar & Rajawar, 2008)*.

This study found that *Theileriaspp* (10%) had the highest prevalence, followed by *Babesiaspp* (1.9%) and *E. canis* (0.95) which is different from the other studies like the one conductedby*(Díaz-Regañón et al., 2020)*.There was a lower prevalence of *Theleiria spp*., which was 12.86%, whereas there was a higher prevalence of *E. canis*, which was 31.43%.This could be due to the fact that their study covers a large area of Nepal (representing the entire country), whereas our study is concentrated on Bharatpur metropolitan area of Chitwan district, Nepal. The finding result of different types of hemoprotozoa is different from previous studies *(Phuyal et al., 2017)*, *(Maharjan et al., 2014)*, and*(Manandhar & Rajawar, 2008)*which might be due to different sample size, study area, and selection of dogs.

The findings of this study were in line with a previous study*(Subedi et al., 2009)*, that discovered male dogs were more infestated with blood protozans than female dogs. Pawer and Gatnestudy findings for hepatozoonosis support the sex-wise susceptibility of dogs to haemoprotozoan parasites found in this investigation. There may be hormonal factors or males' frequent romping behavior that contribute to the increased prevalence of hemoprotozoans *(Adhikari et al., 2014)*.Senthilkumar et al. found no difference in haemoprotozoa susceptibility in dogs based on gender. However, the conclusion contrasted with other studies *(J. A. Gadahi et.al, 2008)*and*(Phuyal et al., 2017)*who discovered that female dogs had an infection rate that was greater than that of male dogs.

The HB calculation found a very low difference between the infected and non-infected groups; this conclusion is consistent with that of an earlier investigation of *(Maharjan et al., 2014)*. This may be due to an immune-mediated condition with severe thrombocytopenia caused by the blood parasites.

CONCLUSION

In BharatpurMetropolitan area of Nepal,street dogs with tick infestations had a significant frequency of hemoprotozoans. *Theileria* spp. was found to have the greatest prevalence rate, followed by *Babesia* spp. and*Ehrlichiacanis*. This study discovered that while males had higher prevalence of the condition than females, the difference was statistically insignificant. Mean hemoglobin levels in infected dogs were also considerably lower than in uninfected dogs.

DECLARATIONS& ETHICAL APPROVAL

I can confirm that the research has not been published elsewhere and is not under consideration for publication in any other journal. We have followed the ethical guidelines and standards in conducting our research, and all necessary permissions have been obtained for the use of any copyrighted material. Additionally, we have also got approval from Animal service department of Bharatpur Metropolitan city to collect blood samples from street dogs.

AVAILABILITY OF INFORMATION AND RESOURCES

Upon reasonable request, the corresponding author will provide access to the data used to support the study's conclusions.

COMPETING INTERESTS

I certify that none of the authors' interests, as defined by Springer, or any other interests might be seen as having the potential to affect the findings and/or conclusions described in this research.

AUTHORS' CONTRIBUTIONS

Dr. Singh Thakuri wrote the entire main manuscript text file while, Dr. Pokhrel supports him during the technical writing aspect as well as data calculation. Dr. Pathak accumulated the samples from the street dog of Bharatpur Metropolitan area with the support of Dr. Kafle. At the last Dr. Shrestha provides lab settings to the team as well under his guidance the results were outcomes.

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