

Study of Canine Babesiosis in Pet and Stray Dogs of Kathmandu Valley

A. Gautam.^{1*}, C. Regmi¹, P. Neupane¹, S. Shah², T.P. Prasai³

¹Paklihawa Campus, Institute of Agriculture and Animal Sciences (IAAS), Rupandehi

²Veterinary Teaching Hospital, Paklihawa Campus, IAAS, Rupandehi

³Central Referral Veterinary Hospital, Tripureshwor, Kathmandu

*Corresponding author: A. Gautam, anushagautam25@gmail.com

ABSTRACT

Canine babesiosis is caused by protozoan parasites which is usually transmitted via various species of ticks. The parasites ultimately parasitize the erythrocytes of host body, resulting in fever, anemia, and hemoglobinuria. From June 2021 to September 2021, a cross-sectional study using the purposive sampling method was conducted. A total of 140 samples were collected from dogs and were examined for the presence of Babesia spp. using thin blood smear test stained in Giemsa. Regression analysis was done to compare categorical and outcome variables. The overall prevalence of babesiosis was found to be 14.3%. The result showed significantly higher prevalence of babesiosis in tick infected dogs ($p < 0.05$). Sex, age, type and breed wise prevalence were found to be statistically insignificant ($p > 0.05$). In hematological parameters, RBC, Hb, PCV, and platelets declined significantly in positive cases ($p < 0.05$). In TLC, the changes in the Babesia positive cases were statistically insignificant ($p > 0.05$), whereas in DLC of the infected cases, changes only in neutrophils and lymphocytes were statistically significant ($p < 0.05$). The mean temperature in positive cases was found to be 103.44 ± 0.69 . This research intends to add some information on the status of babesiosis inside Kathmandu valley with majorly concluding ticks as major cause of diseases. Tick control is therefore critical in order to reduce the prevalence.

Keywords: Prevalence, Disease, Ticks, Hematology

INTRODUCTION

Canine Babesiosis is one of the major tick-borne illnesses in dogs caused by protozoan parasite. (Irwin, 2010). This disease represents a considerable diagnostic challenge for veterinarians, as the clinical signs are often diffused and overlapped (Andersson et al., 2017). Due to the possible transmission of protozoa, human babesiosis has also been lately recognized as an emerging zoonosis with considerable attention (Schnittger et al., 2012). Ticks play an important role in transmitting parasites to the host body during

their blood meal (Shaw et al., 2001). Members of the genus *Babesia*, which are typically transmitted by vector ticks of the *Rhipicephalus* species, *Haemaphysalis* species, and *Dermacentor* species, are one of the major protozoan disease causing agents in canine species (Schnittger et al., 2012). *Babesia* multiplies in erythrocytes by asynchronous binary fission, resulting in considerable pleomorphism (Schetters et al., 2009). The three large species of *Babesia*, namely *B. canis*, *B. vogeli*, and *B. rossi* and the three small species, namely *B. gibsoni*, *B. microti*, and *B. annae* are documented (Irwin, 2010). However, the most frequently found species infecting dogs are *B. canis* and *B. gibsoni* in Europe and Asian countries, respectively (Bhaskaran Ravi et al., 2016). The common clinical features that are observed during infection are fever, lethargy, lymphadenopathy, muscle pain, and hemoglobinuria (Welc-Faleciak et al., 2009). The hematological parameters in disease cases generally show marked thrombocytopenia and a decrease in packed cell volume (PCV) (Schetters et al., 2009). In total leukocytic count (TLC) there is an insignificant change, whereas the differential leukocytic count (DLC) shows marked neutropenia, eosinophilia, and lymphocytosis with an insignificant change in monocytes and basophils (Hornabrook & Field, 1969). Since, the diagnosis of such diseases by blood smear examination is not a common procedure in the Nepalese context, the disease cases are mostly identified symptomatically and get supportive treatment (Phyual et al., 2006).

Table 1: List of drugs used in treatment of canine babesiosis (Schoeman, 2009)

Drug	Dose	Route of administration	Duration of therapy
Diminazene aceturate (Berenil®)	3.5 mg/kg	IM/SC	One dose
Trypan blue (Trypan blue®)	10 mg/kg	Strictly IV	One dose followed by imidocarb or diminazene 1 week later
Imidocarb dipropionate (Imizol®)	7 mg/kg	IM	Two doses, 14 days apart

Hence, Giemsa-stained blood-smear method was used to study the prevalence of canine babesiosis among the pet and stray dogs in Kathmandu valley. Additionally, hematological parameters between the *Babesia* positive and negative individuals compared in the study.

MATERIALS AND METHODS

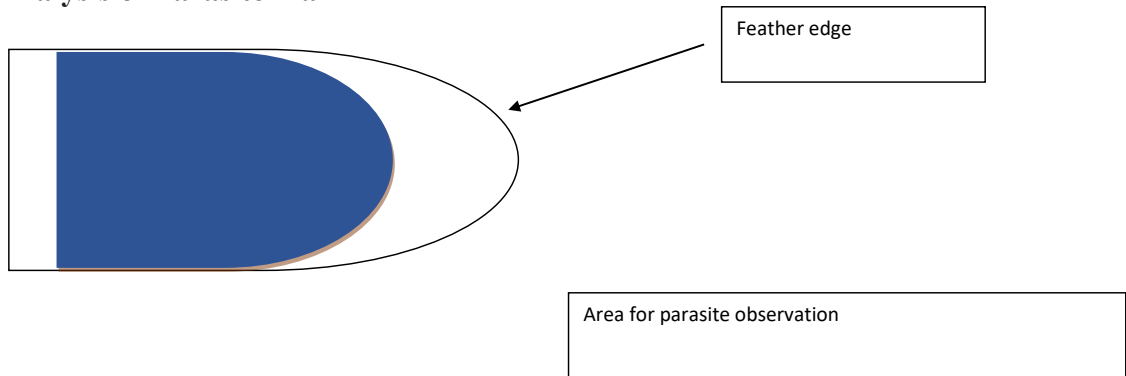
Sampling technique

Random sampling was done to take sample from dogs and the basic parameters such as age, sex and tick infestation status were considered during sampling. Altogether 140 blood samples were collected visiting different places of Kathmandu valley from 70 domesticated and 70 stray dogs.

Giemsa Technique

Giemsa stained blood films examination under light microscopy is considered to be the gold standard for the diagnosis of *Canine Babesiosis* (Njunda *et al.*, 2013). Around 3 ml of blood was collected in a clean ethylenediamine tetraacetic acid (EDTA) tube either from the saphenous, cephalic, or juglar veins with a 22G * 1” needle. Whenever the lab work had to be postponed, it was kept at -4C. The blood was taken to the lab for a complete blood count (CBC) with the help of a hematology analyzer followed by the preparation of blood smear. A drop of blood was taken on a clean glass slide, and by using another slide tilted at an angle of 45 degrees, a thin smear was prepared and air dried immediately. The smear was fixed in methyl alcohol for around 5 minutes. The fixed slide was stained in working solution of Giemsa stain for 35-40 minutes. The slide was washed and dried for examination using a compound microscope under oil immersion.

Analysis of Parasitemia



Positive cases

Babesia spp. are pear-shaped species, which are seemed to be blue due to Giemsa-staining pointed by arrow inside the RBC.

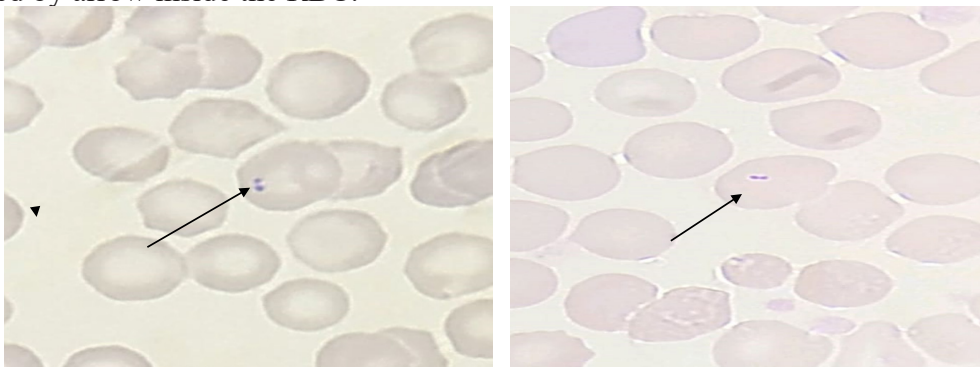


Figure 1: Presence of *Babesia spp* inside RBC

RESULTS

A total of 140 blood samples were collected from pet and stray dogs of CRVH and different animal welfare centers respectively in three districts of Kathmandu valley. Out of 140 blood samples examined, 20 (14.3%) were found to be positive and 120 (85.7%) were negative. In the case of in 60 tick infected sample, 15(33.3%)were found to be positive and 80 samples from the dogs having no ticks present at their body during sampling, 5(6.7%) were found to be positive.

Age-wise prevalence

Out of 140 blood samples, 12 were from dogs of younger than 1 year, 101 were from dogs aged between 1 and 5 years, 23 were from dogs aged between 6 and 10 years, and 4 were from dogs of older than 10 years. Among the 12 samples of dogs younger than 1 year, 1(8.33%) was found positive, and among 101 samples of dogs aged between 1 and 5 years, 13 (14.77%) were found positive. Similarly, among the 23 samples of dogs having an age between 6 and 10 years, 6 (26.08%) were found positive, and among the 4 samples from the dogs with age above 10 years, 0 were found positive. The present study indicated a higher prevalence percentage in the age groups of 6-10 years and 1-5 years, respectively. However, the result found was statistically insignificant ($p=0.351$) meaning that the differences in age group do not play an important role in the occurrence of babesiosis. The effect of the age was only 0.6% on disease occurrence.

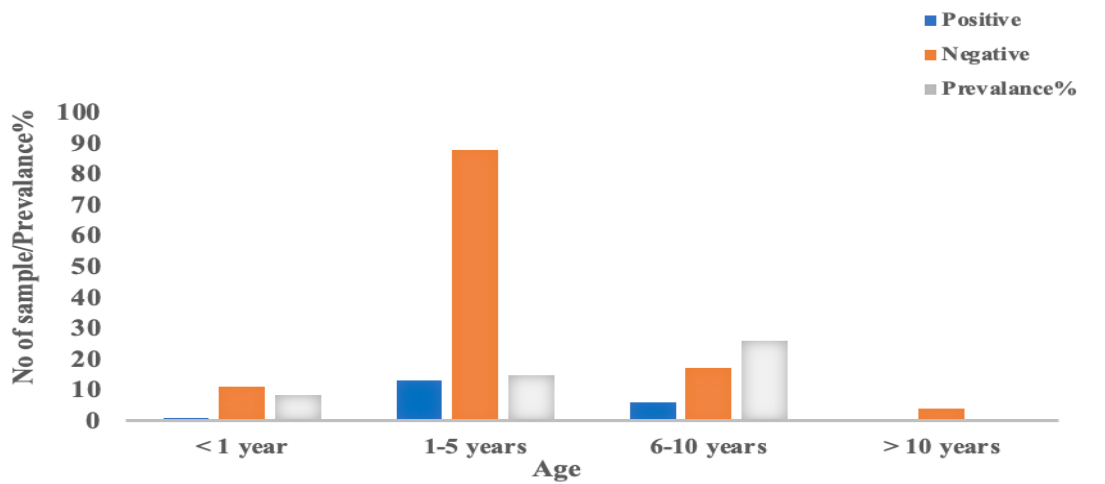


Figure 2: Prevalence of babesiosis on the basis of age

Sex-wise prevalence

Out of 140 blood samples, 81 samples were from male dogs and 59 samples were from female dogs. Among 81 samples from male dogs, 10 (14.08%) were found positive, and

among 59 samples from female dogs, 10 (20.41%) were found positive. The present study showed a higher prevalence percentage in females than in males. However, the result was found to be statistically insignificant ($p=0.446$) meaning that the sexes do not play an important role in the occurrence of babesiosis. The effect of sex was only 0.4% on disease occurrence, given by R square.

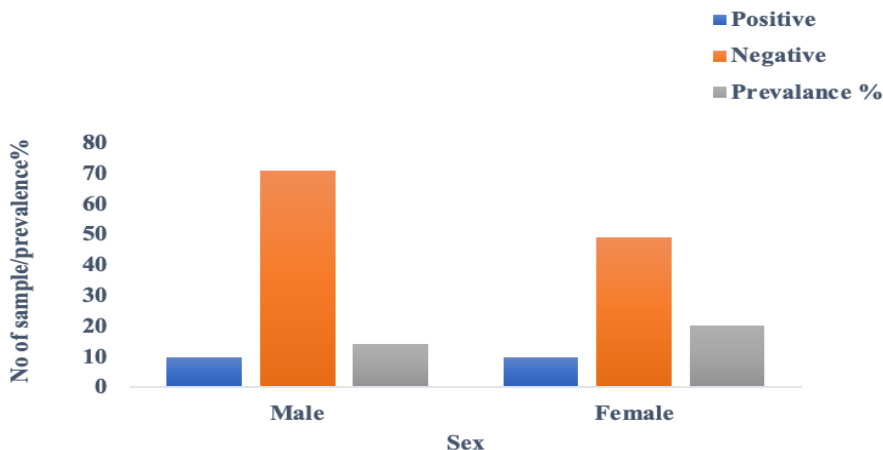


Figure 3: Prevalence of babesiosis on the basis of sex

Prevalence on the basis of presence of ticks

Out of 140 blood samples, 60 were from tick infected dogs, and 80 didn't have tick infestations. Among 60 tick-infected dogs, 15 (33.3%) were found positive, and among 80, 5 (6.7%) were found positive. Out of 5 dogs, that were babesia positive having no ticks present at the time of sample collection, 3 were from street dogs and 2 were from pet dogs. The present study indicated a higher prevalence percentage of *babesiosis* in tick-infected dogs, and the result was found to be statistically significant ($p= 0.002$) meaning that the presence of ticks in the body plays an important role in the occurrence of babesiosis.

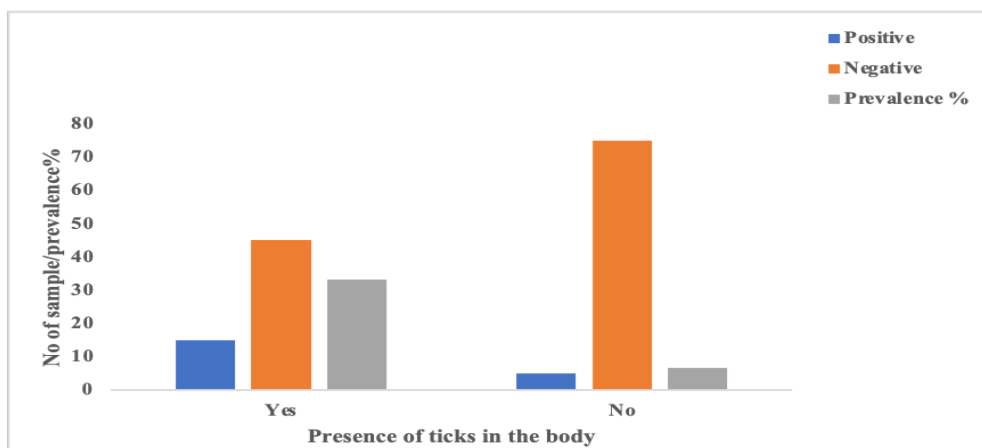


Figure 4: Prevalence of Babesiosis on the basis of presence of ticks in the body

Prevalence on the basis of types of dogs

Out of 140 blood samples, 70 were from pets and 70 were from stray dogs. Among 70 samples of pet dogs, 9 (14.75%) were found positive, and among 70 samples of stray dogs, 11 (18.6%) were found positive. The present study showed that stray dogs had a higher prevalence percentage of *Babesiosis* than pet dogs. However, the result was found to be statistically insignificant ($p=0.632$) meaning that the types of dogs do not play an important role in the occurrence of babesiosis.

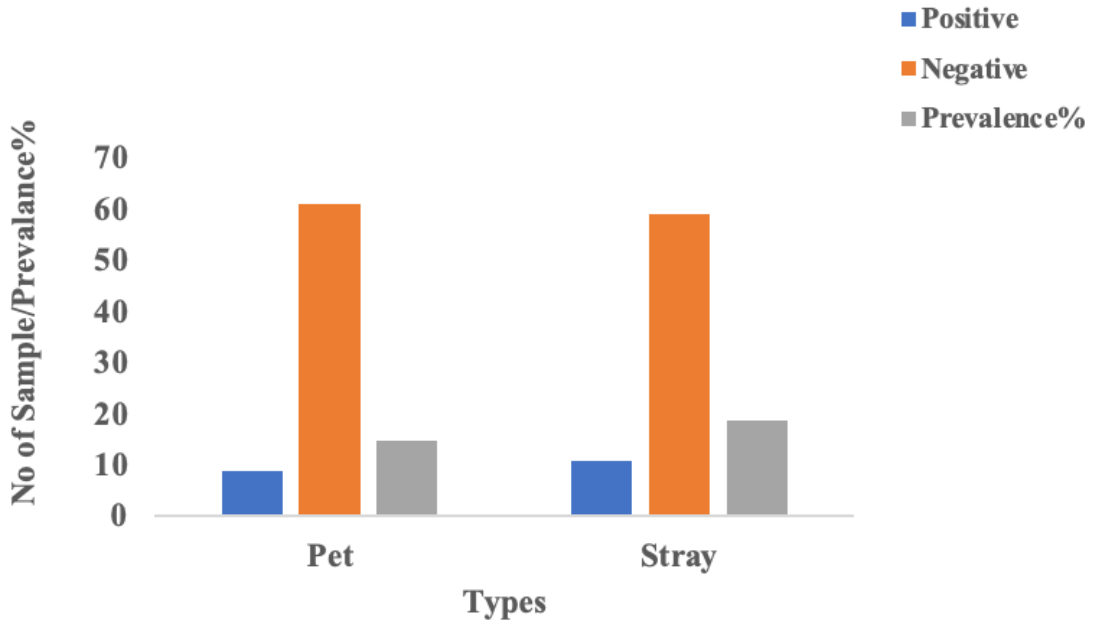


Figure 5: Prevalence of babesiosis on the basis of types of dog

Prevalence on the basis of breed

Out of 140 blood samples, 60 samples were of pure breed, 83 were mongrel and 16 were of cross breed. The breed that showed true characters of certain breed were classified as pure breed, the breed that showed mixed character of two pure breeds were classified as mongrel and the breed which were not characterized as specific breed or mixed one were classified as local breed. Among 60 samples from pure breed, 9(17.65%) were found positive, among 83 samples from mongrel, 10(13.7%) were found positive and among 16 samples from cross breed 1(6.67%) were found positive. The present study indicated the higher prevalence percentage in pure breed followed by mongrel and cross breed dogs. However, the result was found to be statistically insignificant ($p=0.631$). meaning that the nature of breed of dog do not have important role in the occurrence of babesiosis.

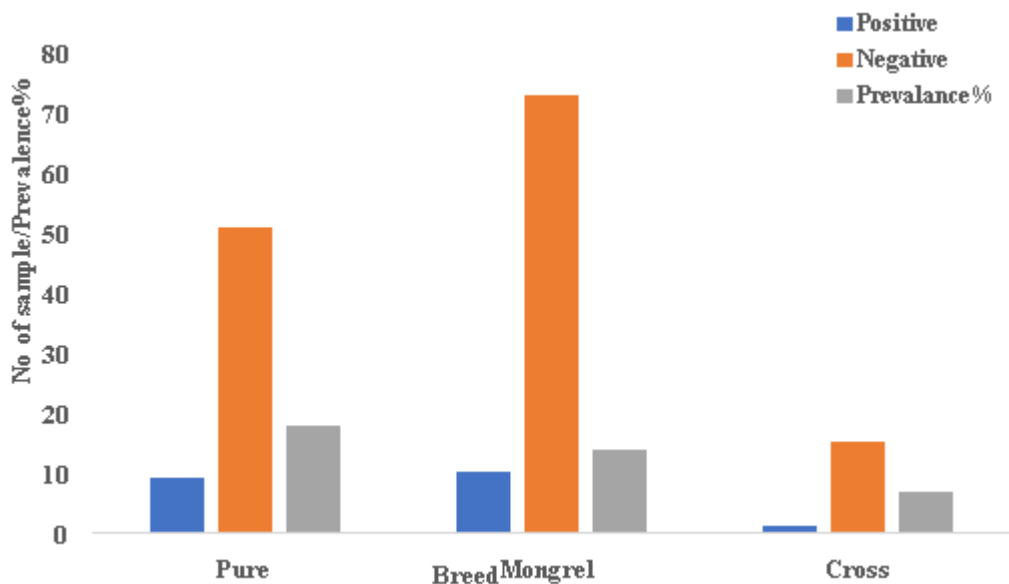


Figure 6: Prevalence of babesiosis on the basis of breed of dog

Comparison of hematological parameters

The hematological parameters of *Babesia* infected and non-infected dogs were analyzed. The result found that the mean value for RBC was $5.06 \pm 1.10 \times 10^{12}/l$, Hb was 11.29 ± 2.21 gm/dl, PCV was $32.29 \pm 2.61\%$, TLC was $13.75 \pm 1.64 \times 10^{12}/l$, neutrophil was $59.52 \pm 9.08\%$, lymphocyte was $31.97 \pm 8.73\%$, monocyte was $4.34 \pm 1.10\%$, eosinophil was $3.66 \pm 0.38\%$, basophil was $0.55 \pm 0.05\%$ and platelet was 235.35 ± 42.23 in non-infected dogs. Similarly, the result found that the mean value for RBC was $5.50 \pm 1.14 \times 10^{12}/l$, Hb was 9.57 ± 2.31 gm/dl, PCV was $27.63 \pm 1.72\%$, TLC was $11.99 \pm 1.38 \times 10^9/l$, neutrophil was $44.7 \pm 7.17\%$, lymphocyte was $45.55 \pm 7.65\%$, monocyte was $5.1 \pm 1.55\%$, eosinophil was $4.15 \pm 0.13\%$, basophil was $0.5 \pm 0.06\%$ and platelet was 150.4 ± 18.68 in infected dogs. The result showed that mean value of RBC, PCV, Hb, and platelet decrease significantly ($p < 0.05$) with the presence of disease in the body. Similarly, R square value of 5.8%, 6.9%, 6.0%, and 20.2% specified the percentage responsible for decrease in RBC, PCV, Hb, and platelet, respectively. In the case of TLC, the mean value decreased insignificantly ($p > 0.05$), whereas in DLC, the mean value of neutrophil decreased significantly ($p < 0.05$) with R square 7.1% and lymphocyte increased significantly ($p < 0.05$) with R square 6.2%. The fluctuation in monocytes, basophils, and eosinophils was insignificant ($p > 0.05$).

Table 2: Comparison of complete blood count in *Babesia spp.* infected cases and in non-infected cases (Reference Source: MSD Veterinary Manual, 11th edition)

Parameters and reference values	Non-infected cases	Infected cases	P-value	R square
RBC (5.6-8.7*10 ¹² /l)	5.06±1.10	5.50±1.14	0.04	5.8
Hb (11.9-18.9gm/dl)	11.29±2.21	9.57±2.31	0.02	6.9
PCV (35-57%)	32.29±2.61	27.63±1.72	0.04	6.0
TLC (5-14.1*10 ⁹ /l)	13.75±1.64	11.99±1.38	0.198	1.2
Neutrophil % (58-85%)	59.52±9.08	44.7±7.17	0.01	7.1
Lymphocytes% (8-24%)	31.97±8.73	45.55±7.65	0.03	6.2
Monocytes% (2-10%)	4.34±1.10	5.1±1.55	0.126	1.7
Eosinophil% (0-9%)	3.66±0.38	4.15±0.13	0.393	0.5
Basophil% (0-1%)	0.55±0.05	0.5±0.06	0.696	0.5
Platelets (211-621*10 ⁹ /l)	235.35±42.23	150.4±18.68	0.000	20.2

The mean temperature was 101.33±1.28 °F in non-infected dogs and 103.44±0.69°F in infected dogs. The present study indicated a greater increase in temperature in infected dogs than in non-infected ones, and the result was found to be statistically significant (p=0.01) which clearly explains that there is a rise in the body temperature with the presence of the disease. The effect of babesiosis in the increment of body temperature of body temperature was 4.7% given by R square.

DISCUSSION

Out of 140 samples, the overall prevalence of babesiosis was found to be 14.3%. This prevalence is higher as compared with the findings of Maharjan *et al.* (2014) who found a prevalence of babesiosis of 9.09% in feral dogs in Lalitpur district. This may be due to seasonal variation differences in the selection of dogs. But the result resembles the findings of Díaz-Regañón *et al.* (2020) who found the prevalence of babesiosis at 15.71% in stray dogs in Nepal.

Although statistically insignificant, the prevalence of disease was found higher in the age group of 6-10 years, followed by 1-5 years. The findings are similar to those of the study reported by Mahalingaiah *et al.* (2017), who found higher prevalence in the age group of 1-8 years and less in dogs of age groups less than 1 year and more than 8 years. According to the study done by Schoeman (2005), babesiosis occurs mostly in young dogs, although dogs of all ages can be affected. The differences in the result might be due to the unequal selection of dogs of various age groups.

This study revealed that there is no significant difference in the sex-wise prevalence of babesiosis, but female dogs have a higher prevalence than male ones. The findings resemble those of studies conducted by Shitta *et al.* (2012) and Phuyal *et al.* (2017), who found higher prevalence of *Babesia spp.* in females than in males. Likewise, prevalence of babesiosis in tick infected dogs was higher than in non-infected dogs and the result was also found to be statistically significant which is slightly higher than the findings of (Obeta *et al.*, 2020) who found 17.3% prevalence in tick-infested dogs and 3.5% prevalence in non-tick infested dogs. The higher prevalence could be attributed to the location differences in which the study was carried out.

There was no significant relationship between disease prevalence and dog type; however, street dogs had a higher risk of infection than pet dogs due to exposure to an unsanitary environment. The findings agree with the study conducted by Díaz-Regañón *et al.* (2020) and Bhattacharjee & Sarmah (2013), who states that stray animals are prone to vector borne diseases as they lack basic animal health care.

In case of breed, prevalence was higher in pure breed followed by mongrel and cross breed, however the result was statistically insignificant and the findings resembles with the results obtained by Okubanjo *et al.* (2013). But the study contradicted to the findings made by Opara *et al.* (2017) who found higher prevalence on mongrel dogs. This discrepancy could be attributed to the fact that the study was focused specifically in local dogs unlike this study which focused on all types of dogs.

The above results showed that the mean temperature in positive cases was $103.44 \pm 0.69^\circ\text{F}$ which was higher than in normal cases and statistically significant. The result agreed with the study conducted by Richardson *et al.* (1964), who state that the rise in body temperature is due to a biological response to the blood parasitic diseases. The hematological parameters of the study revealed statistically significant lower RBC, Hb, and PCV in infected cases than in non-infected cases. This finding agrees with the findings of Zamokas *et al.* (2014), who also observed a lower mean RBC, Hb, and PCV in the infected dogs than in non-infected dogs. The result could be attributed to the higher production of superoxide in the infected cells and, thus clarifying the RBC damage due to oxidative damage (Otsuka *et al.*, 2001).

It has been reported statistically no significant difference in the TLC between infected and non-infected dogs. This study resembled the findings of Phuyal et al. (2017), which also observed minor differences in TLC. In differential leucocyte count (DLC), there was a statistically significant difference in neutrophil counts and lymphocyte counts between infected and non-infected cases, whereas monocyte, eosinophil, and basophil counts did not vary significantly. Neutropenia was observed in infected cases, which satisfies the findings of Weiser et al. (1991), which stated neutropenia is usually present in a hemoprotozoan infection. Similarly, lymphocytosis was observed in positive cases which resembled with the findings of Hornabrook & Field (1969) which observed lymphocyte increase in atypical shape.

The hematological parameter of the study revealed statistically significant lower platelets in infected cases than in non-infected ones. This finding resembles studies conducted by Phuyal et al. (2017), Schetters et al. (2009), Kumar & Kumar (2020), and Irwin (2010), which describe thrombocytopenia as a hallmark of disease prevalence.

CONCLUSION

Overall prevalence of canine babesiosis was 14.3% examined by blood smear examination. Although rapid test kits are developed for the diagnosis, they are expensive to carry out in case of research based study. So, Geimsa stained blood smear analysis by direct microscopy is one of the useful tool, that is relatively cheap and effective for recognizing infectious diseases like *babesiosis* (Nayal et.al., 2012). A higher prevalence of the disease could be attributed to the increased exposure to vector ticks. Hence, proper control measures for the control of vectors should be followed in dogs to decrease the prevalence of hemoparasites in Nepal. For this, the active involvement and cooperation of concerned authorities from the veterinary and ailing professions is required.

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