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PREVALENCE OF BLOOD PARASITES IN DOGS OF KATHMANDU VALLEY

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ABSTRACT

Study in blood parasitic diseases in dog was carried out from August 2014 to November 2014 to find out the prevalence of haemoparasites in clinical cases of hyperthermia in dogs. Blood sample from 50 cases of hyperthermia were collected and examined for any blood parasites by the smear method. The haematological parameter (RBC, WBC, PCV, Hb, & DLC) of each sample was also assessed and analyzed. Data was analyzed to determine prevalence of various species of blood parasites to establish the correlation of the infections with age, sex and breed. Out of 50 samples examined, blood parasites were determined in 12 percent samples of which 2(4%) were positive for Babesia canis and 4 (8%) were positive for Ehrlichia spp. The percentage of infection was greater in female 4(16.67%) than male 2(7.14%). The prevalence of blood parasite was higher in German shepherd. The prevalence of blood parasite was higher in 2-4 years of dogs. To determine significant difference between the hematological parameter of infected and non-infected cases, t-test was need (R, version 3.0.3). The confidence level for the analysis was set at 95percent with significance level assessed at p< 0.05. The study between the infected and non-infected host revealed statistically significant difference in PCV, Neutrophil and Eosinophil. Whereas other parameters did not have any significant difference. The mean PCV was significantly low (P <0.05) in infected dog than in non-infected dog. Mean Neutrophil was significantly decreased whereas Eosinophil was increased in infected dogs than in non-infected dogs.

Keywords: breed, dog, haemoparasites, haematological parameter, hyperthermic, sex

INTRODUCTION

Endoparasitic infestations have always been a major health problem in animal species. These blood parasites affect the blood vascular system, which may be the intraerythrocytic parasite, the intraleukocytic parasite or those living freely (Urquhart, 1987). The common diseases caused by blood parasite in dog are babesiosis, trypanosomiasis and ehrlichiosis (Urquhart, 1987). The principal vector for *E. canis* is *Rhipicephalus sanguineus* (brown dog tick*). E. canis* is passed only transtadially in the tick, so unexposed ticks must feed on a rickettsemic dog to become infected and perpetuate the disease. *E. canis* is maintained in the environment by passage from ticks to dogs. These blood parasites are mostly diagnosed and identified by blood smear examination. Companion animals such as dogs are associated with different haemoparasites and can pose serious health concern worldwide (Manandhar, 2008) and has significant economic impact from veterinary standpoint.

Study conducted by (Maharjan *et al*., 2014), the prevalence of haemoparasites in feral dogs in Lalitpur district was found to be of 10%. In the study, 10(9.3%) samples were detected positive for the *Babesia spp*. and 2(1.8%) samples were positive for *Ehrlichia spp.* Dogs negative for haemoparasites had Packed Cell Volume (PCV) within normal range (35-51%) whereas PCV was decreased in infected dogs.

The organism parasitizes the leucocytes mainly monocytes and lymphocytes. Some strains, which parasitize neutrophils have been reported, which are like*E. equi* (Kirk & Bistner, 1985). (Bhattarcharjeeand sarmah*,* 2013), showed the prevalence of 57.3 percent in the hospital population comprising pet (58.03%) and working (54.54%) dogs and 63.64% in stray dog population.(Tuna and Ulutas, 2009) Investigated the prevalence of *Ehrlichia canis* infection in thrombocytopenic dogs. Blood samples were collected from 224 dogs of different ages and of both sexes. Eighty-one (36.2%) of 224 dogs were positive to *E. canis* infection.*E. canis* infection occurs in the average of 5.22 years in the dogs and the range has been found within 2 months to 14 years (Tilley *et al*., 1997).

Diagnosis of a disease as per blood examination is not a common procedure in Nepalese context. Fortunately, some cases are diagnosed in time and treated whereas most of the cases are treated symptomatically and supportively with the help of antibiotics and some cases are luckily recovered whereas some remain ill because of disease which may be caused by haemoprotozoan. Though the occurrence is low it is sometimes confused with other similar disease and the veterinarian overlooked the hemoprotozoan disease. So this research was mainly conducted to know the prevalence of blood parasites namely *Babesia spp., Trypanosomes spp.*, and *Ehrlichia spp* in dog based on breed wise, age wise & to compare the hematological parameters in blood parasites positive and negative cases of dogs.

MATERIAL AND METHODS

Materials such as sterile syringe, EDTA vial, glass slides, cotton, spirit, Giemsa's stain (Himedia), methanol, RBC and WBC diluting fluids, wintrobe's tube were used for this study.

Selection of dogs

Blood samples was taken from dogs with hyperthermia (temperature >103-degree F). These samples were randomly selected (irrespective of age, sex and breed) from hospital and clinics of different district

Collection of blood

About 2 ml of blood was withdrawn in sterile blood collecting vial containing EDTA. The blood was immediately transferred to a sterile vacutainer. The tube was kept under refrigeration at -4 ˚C until further laboratory works were performed. Collection of blood, preparation of slide and staining, RBC count, DLC, PCV and hemoglobin determination will be performed as per Benjamin (2010).

Preparation of slide and staining

A drop of blood was taken in a clean glass slide and by using another slide tilted at an angle of 45 degree, a thin smear was prepared and air dried immediately. Then the smear was fixed in methyl

alcohol for 2- 3 minutes. This fixed slide was then stained in working solution of Giemsa stain (Himedia) in coplin jar for 20-30 minutes. The slide was washed and dried for further examination.

Examination of slide for blood parasite

The slide was examined under oil immersion by using binocular compound microscope (Olympus). The smear was examined for the presence of blood parasite. Mainly the RBC's and WBCs was carefully looked. In the RBCs protozoans like *Babesia spp*., and WBCs, especially monocytes for the presence of *Ehrlichia spp*., and *Trypanosoma spp*. were looked between the blood cells. Mainly the periphery of the smear was examined.

Babesia species appeared as blue stained piroplasms having V or comma shape. They are singly or multiple present in the RBCs. *Ehrlichia spp*. was morulae in monocytes, lymphocytes and sometimes neutrophils.

RBC count and WBC count

RBC and WBC diluting pipette were taken separately, and blood and dilution fluid were drawn as per required. After mixing it well, a drop of the mixture was charged on a hemocytometer (Hawksley) and it was fixed in 100X microscope (Olympus). RBC and WBC were then counted.

Differential leukocyte count (DLC)

The same Giemsa stained smear was used for differential leukocyte count.

Packed cell volume (PCV) determination

PCV was determined by microhematocrit tube to separate the cells from plasma.

Hemoglobin

Hemoglobin was estimated by acid hematin method using Sahli's hemoglobinometer (Singhla Company).

Estimation of prevalence

The prevalence of infection was assorted in terms of breed, age and sex. The basic formula for calculating the prevalence in percentage is given below:

$$\text{Prevalence} = \frac{\text{Number of individual having a disease at a particular point of time}}{\text{Total number of individual in the population at risk sampled at that point of time}} \times 100$$

Data analysis

The collected data were entered spreadsheets and analysed with statistical software; a Statistical Package for Social Sciences (SPSS, version 16). Data were summarised as descriptive statistics (mean and percentages) and displayed as tables. Data regarding the hematology was analyzed by studentst test using R, version 3.0.3 (R Core Team, 2014). The confidence level for the analysis was set at 95 percent and $p < 0.05$ was considered significant**.**

RESULT

Overall prevalence of blood parasites

In the study done in 50 pyrexic dogs, 6 (12%) were found to be caused by blood parasites.

Blood parasitic species isolated in positive blood samples of dogs
Two different blood parasites were observed in 6 positive blood samples. *E. canis* was found highest 4(8%) followed by *Babesia canis* 2 (4%).

Sex-wise prevalence of blood parasites
The study comprised of 28 males and 22 females. Among 28 males, 2(7.14%) and among 22 females, 4 (16.67%) in infected cases.

Breed wise prevalence of blood parasites
Out of the 6 total positive cases 4 cases (20%) were encountered in German Shepherd breed of dog followed by 2 cases (15.38%) were encountered in Mongrel.

Age wise prevalence of blood parasites
Out of 50 blood sample, 29 samples were from age 1-5 years and 21 sample were from dog greater than 5 years. The age group of 1 –5 years i.e. 12 percent of canine population were infected.

Temperature
The mean temperature of the infected cases was found to be 103-105°F, whereas for the non-infected cases was 103.73 °F.

Mean RBC count, HB and PCV estimation
The mean RBC, Hb and PCV in infected dogs was 4.7 X 10^6/µl, 9.34 g% and 23.17% and in non-infected dogs was 5.61 X 10^6/µl, 10.8 gm% and 33.31% respectively.

DISCUSSION

The prevalence of blood parasites under study closely resembles the findings of (Maharjan*et al.,*2014; Subedi, 2009; Manandharand Rajawar, 2008) who found 10%, 14% and 17.14%respectively in dogs of Kathmandu valley. This may be due to the selection of hyperthermic dogs for the study or may be due to the seasonal variation (summer) orselection of dogs with tick infestation (Maharjan*et al*., 2014; Subedi, 2012; Manandhar and Rajawar, 2006).

On the other hand, our study reported high prevalence of Ehrlichia canis followed by *Babesia canis*. Occurrence pattern is almost like the findings of Manandhar and Rajawar, (2006) (*Ehrlichia spp*. 11.43% and *Babesia Spp*. 5.71%) but vary from the (Maharjan, 2014) (*Babesia spp.* 9.09% and *Ehrlichia spp.* 1.08%). Under sex wise prevalence of blood parasites in the study higher prevalence in female species than in male species. This finding was similar with findings reported by (Shitta, 2009; Gadahi*et al*., 2008) who observed high prevalence of infection on female dogs than male. But findings of our study were contrast to that of Subedi (2009) who found that male dogs have a significantly higher prevalence than female dogs. This could be attributed to the greater propensity for male dogs as compared to females (Papa, 2016).

In this study, the prevalence of blood parasites was higher in German shepherd followed by Mongrel. This may be due to inadequate immunogenic response and the owner's preference to German shepherd (Subedi, 2009; Manandar *et al*., 2008).

The prevalence of blood parasites was higher in age group 1-5 years of canine population. This may be due to greater attention paid by the pet owners to the dogs before 1 years to control vectors than to the dogs greater than 1 years (Jalali *et al*., 2013; Subedi, 2009)

The above results of the blood tests showed that the temperature in positive cases ranges from 103 - 105°F. This result due to biological response to a blood parasitic disease (Richardson *et al*., 1964).

The hematological parameter under study revealed statistically significant low PCV in infected cases than in non-infected cases whereas there was no significant difference in the RBC count and Hb estimation between infected and non-infected group. This finding agrees with the findings of (Maharjan*et al.,* 2014; Shitta*et al.,* 2012) who also have observed a lower mean PCV in the infected dogs than the non-infected dogs. This may be due to immune mediated damage to the bone marrow stem cells caused by blood parasites. And is attributed due to immunomediated phenomena by autoantibodies directed against component of membrane of infected and uninfected erythrocytes, production of toxic hemolytic factors of the parasite, mechanical damage by trophozoite intra-erythrocytic binary fission, erythrophagocytosis and through release of vasoactive molecules such as kallikrein. (Schetters*et al.,* 2009 & Brockus and Andreasen, 2003)

It has been reported statistically no significant difference in the TLC between infected and non-infected dogs. This study resembles with the findings in which leucopenia is not specific in the positive cases. In differential leucocyte count (DLC) there was statistically significant difference in Neutrophil and Eosinophil counts between infected and non-infected cases, whereas lymphocyte and monocyte count did not vary significantly. Eosinophilia in infected cases may be due to parasitic infection. Neutropenia was observed in infected cases, which satisfies the findings of (Weiser*et al*., 1991) which has stated neutropenia in a hemoprotozoan infection. (Manandhar and Rajawar, 2008).

CONCLUSION

In conclusion, our study identified the prevalence of Babesia spp. and Ehrlichia spp. in clinical cases of dogs with hyperthermia. The study showed higher prevalence of disease in female with respect to male. High occurrence was encountered in German shepherd breed of dog and rest in Mongrel. Eventhough, the prevalence of blood parasite was higher in 2-5 years of dogs. In comparative study of hematological parameter between the infected and non-infected host revealed statistically significant difference ($P<0.05$) in PCV, Neutrophil and Eosinophil whereas other parameter doesnot have any significant difference. The mean PCV was significantly low in infected dogs than in non-infected dogs. Neutropenia and Eosniophiliawas observed in infected dogs. A holistic approach is required for the prevention and control of haemoparasites in Nepal and this will require the active involvement and cooperation of veterinary and allied professionals and government regulatory agencies at all levels.

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