

Prevalence of *Dirofilaria* and Hemato-biochemical Effect in Street Dogs of Kathmandu Valley and Siddharthanagar Municipality, Bhairahawa, Nepal

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ABSTRACT

A cross sectional study was conducted to determine the prevalence of dirofilariosis in street dogs of Kathmandu valley and Siddharthanagar, Rupandehi from January 2019 to March 2019. Blood sample from 155 dogs of Kathmandu Valley and 150 from Siddharthanagar municipality were collected and examined by Wet Smear, Modified Knott Technique and Buffy Coat methods. PCV value and biochemical parameters were analyzed. Data were analyzed to determine the Prevalence of *Dirofilaria* and correlation of infections with age, sex and biochemical parameters. The prevalence in Kathmandu valley was 0% (0/156) while the prevalence in Siddharthanagar was 19.33%, while the prevalence in direct smear, buffy coat and modified Knott technique were found to be 16%, 16% and 19.33% respectively. The prevalence were significantly ($p < 0.05$) higher in old age and higher in 3-5 years of age i.e. 51.70%. Prevalence was higher in female (21.70%) than male (17.3%). AST and ALT were significantly ($p < 0.05$) increased in infected dogs. Total protein, ALP, BUN, Creatinine level were elevated in the infected dogs.

Keywords: *Dirofilaria*, Dog, Biochemical

INTRODUCTION

Dirofilaria is an intravascular nematode parasite of the superfamily Filarioidea that consists of mostly two species *Dirofilaria immitis* and *Dirofilaria repens* affecting canine soulsby, 2012. Dirofilariosis is prevalent in tropical, subtropical and temperate region worldwide.

Transmission occurs through blood sucking mosquitoes of *Aedes*, *Culex* and *Anopheles* species (Soulsby, 2012). Over 60 mosquito species have been shown to be able to *Dirofilaria* under experimental or natural conditions (Norma *et.al.*, 1998). The mosquito vectors that can transmit *Dirofilaria* commonly present in Kathmandu valley and Rupandehi are *Aedes albopictus*, *Culex quinquefasciatus*, *Aedes aegypti* (Darsie *et.al.*, 1990). Development of *D. immitis* to L3 larval stage in mosquitoes occurs at threshold temperature 14 °C (Genchi *et.al.*, 2010). Pathological changes in *Dirofilaria immitis* has been reported in reductions of RBC, haemoglobin and PCV along with alterations of BUN, Creatinine, ALP, AST and GGT (Bahadori *et.al.*, 2010).

The prevalence rate of heart worm in Assam and Mizoram area of Northeastern state of India is 11.38% out of 413 sample of stray dog (Borthakur *et.al.*, 2015). The prevalence of *Dirofilaria immitis* in Busan, Korea in 77 stray dogs was 6.5% (Byein *et.al.*, 2007). Wang *et.al.*, reported prevalence of *Dirofilaria* in Henan province of central China from March 2015 to Feb 2016 with

the overall recorded seroprevalence of *D. immitis* in dogs in 13.18%. In case of Nepal, Singh *et. al.*, (2018) has reported a case study of heartworm with chylothorax at Veterinary Teaching hospital, Bhairahawa. Pradhan *et.al.*, (2018) reported the case of *Dirofilaria* in anterior chamber of eye in human in Biratnagar Eye Hospital.

Dirofilaria immitis is routinely treated in canines under the guidelines prescribed by the American Heartworm Evaluation of Growth Society using the adulticidal therapy, Melarsomine. The three-dose protocol is the treatment of choice due to its increased safety and efficacy (Humpreys, 2015). *Dirofilaria* is also a zoonotic parasite and humans are dead end hosts. *D. repens* infection in human is associated with the subcutaneous lump in the affected area i.e. face and conjunctiva of the eye while human *D. immitis* infection has been associated with pulmonary dirofilariosis and is usually asymptomatic (Reddy, 2013).

In case of Nepal, there are no research published on prevalence of *Dirofilaria*. Climatic factors, Vectors and geographical location supports the prevalence of *Dirofilaria* in Nepal. Its presence have been observed in necropsy findings in 4 street dogs in Veterinary Teaching Hospital, Paklihawa, Siddharthanagar. As the disease when prevailed is complex to treat and expensive too, similarly it is a burden for public health as it is zoonotic. No vaccination practice against *Dirofilaria* have been seen in Nepal. So this research was mainly conducted to know the overall prevalence.

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MATERIALS AND METHODS

Study Area: This study was conducted in Siddharthanagar municipality, Rupandehi and Kathmandu valley. 160 samples from Kathmandu valley and 150 samples from Siddharthanagar were collected.

Collection of Blood: About 10 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 degree celsius until further laboratory works were performed.

Techniques Used: Three methods were used as a diagnostic method for the detection of *Dirofilaria* as described by Taylor *et.al.*, (2016):

Wet Smear Method: one drop of venous blood was kept on a clean microscope slide coverslip was placed. The coverslip area was examined under low magnification (10×) of the microscope. Undulating movements of larvae can be observed.

Haematocrit Test: Fresh whole blood was drawn into a Microhematocrit tube and centrifuged for 3 minutes at 1500 rpm. The plasma portion of the separated blood was examined under low magnification (10×). Moving microfilariae was observed in the plasma above the buffy coat.

Modified Knott’s Test: Sample of blood was drawn into a syringe containing EDTA. 1mL of the blood was mixed with 9mL of a 2% formalin solution and the mixture was centrifuged the mixture at 1500 rpm for 5 minutes. Discarding the supernatant, one drop of 0.1% methylene blue was added to the sediment and mixed well. The stained sediment was transferred to a microscope slide using a Pasteur pipette to collect the entire sample and examined using the 10× microscope objective. Microfilariae was observed in an extended position with nuclei stained blue.

Packed Cell Volume Determination: PCV was determined by microhematocrit tube to separate cells from plasma.

Biochemical Test: Serum biochemical values were determined by using Autozyme reagent of Accurex Biomedical PVT. LTD. and Advanced Microprocessor Based Colorimeter.

Data Analysis: Statistical analysis was conducted using Statistical package on social science (SPSS) v.25 with chi square test, one sample T test and compare mean and MS excel was also used for data presentation.

RESULT

Overall Prevalence of *Dirofilaria*:

Overall prevalence in Kathmandu valley was found to be zero while 19.33% was found to be positive among 150 samples in Siddharthanagar.

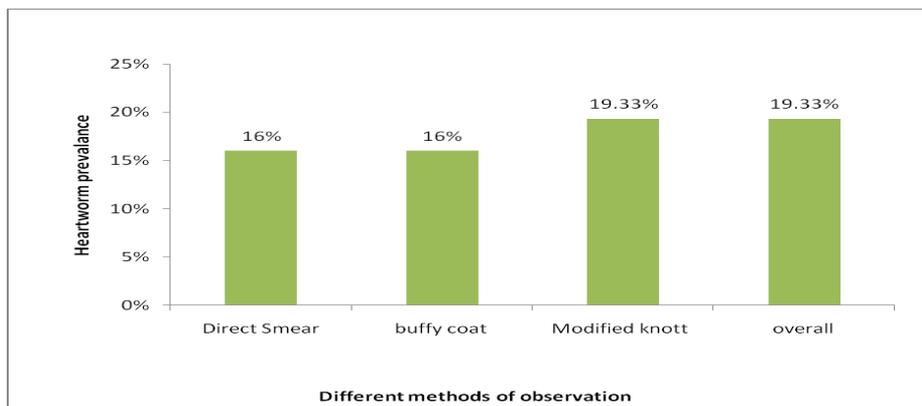


Fig. 1 Prevalence of heartworm infection in street dog of Bhairahawa in 2019

Sex-wise Prevalence of *Dirofilaria*:

This study revealed the prevalence in male 17.3% and in female was 21.70% among the infected dogs out of 150 samples of Siddharthanagar.

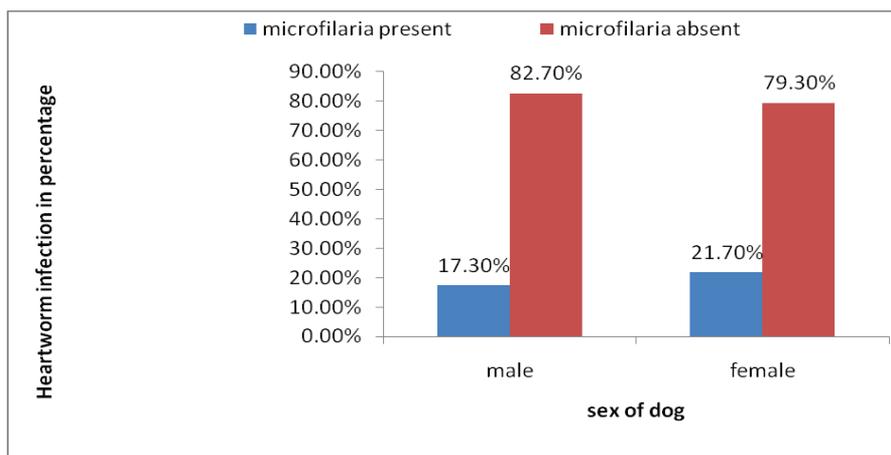


Fig. 2 Heartworm infection in male and female dog on Bhairahawa in 2019

Age-wise Prevalence of *Dirofilaria*:

Out of 150 samples of Siddharthanagar 3.50% were from age below 1 year, 10.30% from 1-5 year, 51.70% between 3-5 years and 34.50% greater than 5 year.

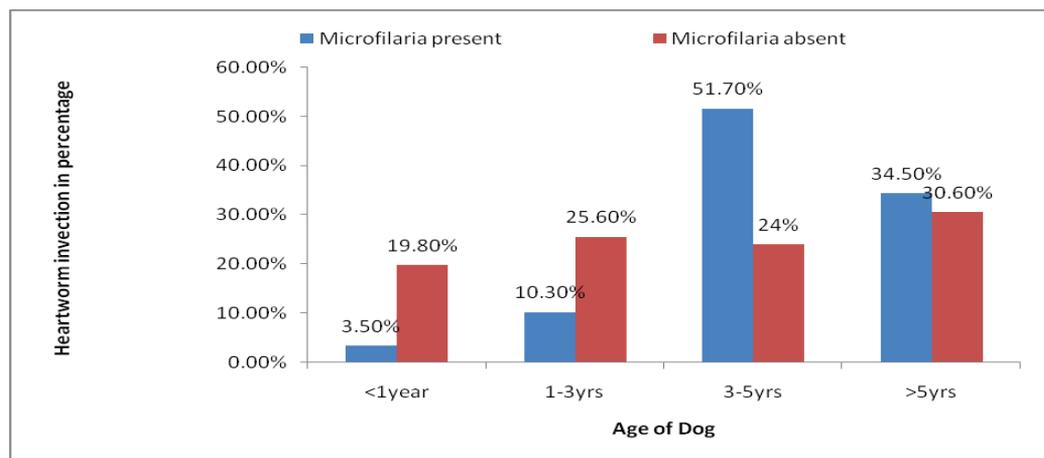


Fig. 3 Heartworm infection against different age of dog on Bhairahawa in 2019

Variation of Prevalence with the Tests:

The prevalence of heartworm microfilaria on direct smear, buffy coat method and modified Knott smear were 16%, 16% and 19.33% respectively on Siddharthanagar municipality, Bhairahawa.

Temperature Variation:

Of the total infected dog, 13.80% have temperature greater than 102.5 degree F while 86.20% have the range between 99.5-102.5 degree F.

Haematological and Biochemical Variations:

Chi square test shows PCV, Total protein, ALP, Urea, BUN and creatinine value insignificant with infection while AST (IU/L) and ALT (IU/L) value were found to be significant with mean value 26.73 and 27.25 in infected dogs and 17.98 and 19.91 in non infected dogs respectively. There was overall increase in AST, ALT, BUN, creatinine and Total protein level in infected dogs.

S.N.	Parameter	Infected dog	Non infected dog	P value	T value
1	Total protein (mg/dl)	7.37 ± 0.36	6.56 ± 0.29	p> 0.05	1.166
2	AST (IU/L)	26.73 ± 3.54	17.98± 2.52	P<0.05	2.013
3	ALP (IU/L)	376.11 ± 39.60	337.35± 53.71	p> 0.05	0.581
4	ALT (IU/L)	27.25 ± 2.65	19.91± 2.47	P<0.05	0.049
5	Urea (mg/dl)	22.19.12 ± 1.91	25.24± 2.90	P>0.05	-0.876
6	BUN (mg/dl)	11.39 ± 01.01	12.21 ± 1.49	P>0.05	-0.509
7	Creatinine (mg/dl)	1.63 ± 0.132	1.46± 0.12	p>0.05	0.934

DISCUSSION

The prevalence of *Dirofilaria* in our study in Siddharthanagar is lower than the findings of Retnasbapathy, 1976 i.e. 25.8% in Malaysia and Vieira *et.al.*, 2014 in Portugal with prevalence 27.3%. However the prevalence is higher than the findings of Borthukar *et.al.*, 2014 i.e. 11.38% in Assam, India and Wang *et.al.*, 2015 with prevalence of 13.18% in Henan, Central China. This may be due to the fact that transmission of dirofilariosis is dependent upon the presence of sufficient numbers of infected, microfilaraemic dogs, susceptible mosquitoes, and a suitable climate to permit extrinsic incubation of *Dirofilaria* in the mosquito intermediate host (Medlock *et.al.*, 2007, Genchi *et.al.*, 2009).

Biochemical variation in infected dogs is similar to Zalvaris *et.al.*, 2008 i.e Increase in AST (54.84%), ALT (51.61%), Creatinine (54.84%), Total protein (25.81%), Urea (32.26%) out of 31 infected dogs in Greece. Our findings also coincides with findings of Hashem *et.al.*, 2007 in Egypt who found among 14% infected dogs there was increase in AST, ALT, BUN, Creatinine and Total protein level. Increase in AST and ALT level may be due to loss of renal function while increase in BUN and creatinine level may be due to renal damage result of glomerulonephritis (Zalvaris *et.al.*, 2008 , Hashem *et.al.*, 2007).

In this study among the positive sample of Siddharthanagar, prevalence of male was 17.3% and female was 21.70%. This finding is similar to the findings of Adanir et al. where prevalence rate was 22.8% in male and 21.2% in female. It was higher in the study of Yaman *et.al.*, 2009 in Turkey where prevalence rate in male was 24.5% and female 33.3%. The difference in male and female infection is not statistically significant, difference may be due to the sample size taken from both population (Zarei *et.al.*, 2016).

In this study, prevalence was higher in age group 3-5 years (51.70%). Prevalence was also higher in old age dogs. Adanir et al findings shows higher prevalence of age greater than 7 years i.e 53.3%. The findings of Boonyapakan where the prevalence was higher in 3-6 years of age 33.3% while in old age greater than 10 years was 41.5%. The greater infection rate in older dogs (more than two years old) than younger dogs was possibly due to the longer exposure of older dogs to the mosquito bite (Zarei *et.al.*, 2016).

Prevalence of *Dirofilaria* was found to be negative in Kathmandu Valley despite being favourable temperature for the parasite, presence of vector and large population of dog. However it cannot be said it is 100 % negative. Further research is to be carried out taking large population size and using antigen test kit with high sensitivity and specificity.

CONCLUSION

Our study revealed the prevalence of *Dirofilaria* for the first time in Nepal. Prevalence of *Dirofilaria* in Siddharthanagar i.e. 19.33% is an alarming situation as in Nepal there is no vaccination practice against *Dirofilaria*. Although there was no prevalence in Kathmandu valley, a more detail study is to be carried out. Transmission of disease can take place to the major cities of Nepal as they have favourable environment, favourable temperature and presence of vectors. A holistic approach is required for the prevention in early stage and this will require the active involvement and cooperation of veterinary, allied professionals and government at all levels.

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