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PREVALENCE OF *Dirofilaria immitis* IN THE CARNIVORES AND ITS VECTORS IN MYMENSINGH MUNICIPAL AREA OF BANGLADESH

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ABSTRACT

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Heartworm is a global problem caused by *Dirofilaria immitis* that occurs in some carnivores in tropics and subtropics including some temperate countries. There is also a public health implication, since infection in human is occasionally reported. We investigated the prevalence of *D. immitis* among carnivores and to identify its vectors and to correlate between them in the study area. We examined the prevalence of *D. immitis* in stray dogs, wild foxes, and community cats, and in their vectors in Mymensingh Municipal Area. In this study, following the guidelines of the animal welfare and experimentation ethical committee, euthanized animals and mosquitoes were investigated for adult parasites and microfilariae, respectively. Among animals investigated 56.0% of dogs and 71.4% of foxes and none of cats were infected with *D. immitis*. Infection in animal below 2 years of age were 46.1% and 66.6% in dogs and foxes respectively compared to 66.6% and 75.0% infection respectively in dogs and foxes above 2 years of age. In male the rate of infections were 61.5% in dogs and 75.0% in foxes compared to 50.0% in female dogs and 66.66% in female foxes. Microfilariae were detected in 44% dogs and 57.14% foxes examined. The *Culex sp.* had 11.3% and the *Anopheles sp.* had 6.1% infection, whereas none of the *Aedes sp.* had any microfilariae. Since these dogs and foxes live near the human habitations in study area, it was considered a serious public health threat to humans. Because of both veterinary and public health significance, further detailed studies on the prevalence of *D. immitis* in Bangladesh are highly emphasized.

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INTRODUCTION

Canine heartworm is now a serious problem throughout the world. *Dirofilaria immitis* (Nematoda: Filariidae) occurs in the dog, cat, fox and wolf in tropics and subtropics and in some temperate countries (Soulsby, 1982) and wild canids harbor important reservoir. *D. immitis* establishes itself in human and causes severe consequences (Saito *et al.*, 1995, Smith *et al.*, 1998). The worms live mainly in the right ventricle and the pulmonary artery, but have also been found in other parts of the body such as lungs and its adjacent vessels (Smith *et al.*, 1998). The intermediate hosts are usually the mosquitoes of the genera *Culex*, *Anopheles*, *Aedes* those maintain the developmental stages of the *D. immitis* (Soulsby, 1982). Many dogs may be infected with *D. immitis* without showing any clinical signs of infection other than microfilariae in the blood. The prevalence of all types of heart disease in the domestic canine population is very common. However, in areas where heartworms are endemic the prevalence of heart disease may be greater. In heavy infections, however, the worms cause circulatory distress, due to mechanical interference and progressive endocarditis (Soulsby 1982). In large numbers they interfere with the function of the heart valves with resultant compensatory hypertrophy of the right ventricle and eventually result in congestive heart failure and chronic passive congestion manifested by liver enlargement, ascites and occasionally peripheral oedema (Soulsby, 1982). There is also public health implications of heartworm of dogs, since human infection with *Dirofilaria* sp. is occasionally reported, most have been recovered from sub-cutaneous tissues of the upper extremities in man, e.g. trunk, face, orbit or conjunctiva and heart; most specimens are immature with mild inflammation and nodule formation around the worm with high eosinophilia (Belding, 1965). The infection of *D. immitis* in carnivores in Mymensingh Municipal Area, vectors and (3) correlated its prevalence in the infection in carnivores with that vectors in the area studied.

MATERIALS AND METHODS

Animal ethical statement

The study plan was approved by the animal welfare and experimentation ethical committee (AWEEC/BAU/19).

Euthanasia and Post-mortem

The study was carried out in Mymensingh Municipal Area of Bangladesh and 25 dogs, 15 cats and 7 foxes mostly adults and belonged to local breeds (street dogs, community cats and wild foxes) were examined. The age of the animals were determined based on teeth of the dead /euthanized carnivores. The animals were euthanized by direct intra-cardial injection of saturated solution of Magnesium Sulphate (MgSo₄. 5 H₂O) between the 4th or 5th intercostal space. The carcasses were following the standard procedure of post-mortem examination as described by Cable (Cable, 1953) and the heart, right ventricle, pulmonary artery, aorta and lungs were removed, and examined thoroughly petridish/glass jars. The chambers of the heart, aorta and pulmonary artery were opened carefully.

Collection and identification of parasites

The parasites were collected with the help of curved needles and were put on the petridish containing normal saline. Collected parasites were cleared off blood preserved in glycerine alcohol (Glycerine 5 parts and 75% alcohol 95 parts). Parasites were identified by preparing temporary slides by adding one drop of luke-warm lactophenol following the keys and description published elsewhere (Belding, 1965; Soulsby, 1982).

Detection of microfilariae from blood

The peripheral blood from trapped carnivores was collected in clean vials premedicated with anticoagulants (Ca-EDTA). A drop of blood was taken on the slide and mixed with a drop of normal saline and wet smears were examined under microscope for detecting living microfilariae made and stained. Giemsa's stain slides were examined under microscope using 100X objective by adding oil immersion blood was preserved by adding 2% formalin for further use.

Examination of mosquitoes and identification of microfilariae

For vector studies, 1910 mosquitoes of *Culex* sp. *Anopheles* sp. and *Aedes* sp. and about 23 *Ctenocephalides canis* fleas and examined (Chandler and Read, 1961). Vectors were identified using the descriptions (Belding, 1965; Soulsby, 1982). With intention At least 10 mosquitoes from each collection was taken on a slide and dissected in a few drops of saline using needles and examined under the microscope using objectives 40x. Microfilariae those showing movement were examined under oil immersion using objectives up to 100x. Similarly 23 fleas were examined and identified. Microfilariae were identified following the keys description published previously (Belding, 1965; Soulsby, 1982).

Statistical analysis

Statistical analysis was made to determine the number of infected dogs, cats, and foxes within their respective population. Similarly, the number of mosquitoes infected from the population of each genera were determined, correlation coefficient was determined to relate infectivity of microfilarial prevalent in different genera of mosquitoes using standard methods (Chi-square Test) (Swaroop et al., 1966).

RESULTS

D. immitis was detected in the right ventricle of 14 (56%) dogs, 5 (71.4%) foxes and none of 15 cats (Table 1). A maximum of 18 parasites (10 females and 8 males) were recorded in a dog where the parasites were also found in the pulmonary artery and the minimum number recorded was 3 (2 females and 1 Male). Likewise maximum number of parasites collected from fox was 13 (9 Females) and 4 Males) and minimum 2 (1 Female) and 1 Male), respectively (Figures 1 and 2). We found that 61.5% male dogs 75.0% male foxes were infected with parasite (Table 3). First stage microfilariae (mf1) were detected in (Figure 3) 44.0% dogs and 57.14% foxes (Table 4).

Table 1. Prevalence of *D. immitis* in carnivores in Mymensingh Municipal area

Animals	Number of Animals Examined(N)	Percentage of infections (%)
Dog	25	56
Cat	15	0
Fox	7	71.42

Table 2. Age wise frequency distribution of *D. immitis* in carnivores in Mymensingh Municipal area

Animals	Animals up to 2 years examined (N)	Percent of Infection (%)	Animals above 2 years examined	Percentage of infection (%)
Dog	13	46.1	12	66.6
Cat	8	0	7	0
Fox	3	66.6	4	75.0

Dog = Probability 0.5293, P>0.05 Non significant

Foxes = Probability 0.5460, P>0.05 Non significant

Table 3. Sex wise distribution of *D. immitis* in carnivores in Mymensingh Municipal area

Animals	Sex	Total Number of Animal examined (N)	Percentage of infections (%)
Dog	Male	13	61.53
	Female	12	50
Cat	Male	9	0
	Female	6	0
Fox	Male	4	75
	Female	3	66.66

Dog = Probability 0.8592, $P > 0.05$ Non significant

Foxes = Probability 0.5460, $P > 0.05$ Non significant

Table 4. Percentage of microfilariae (mf) in the carnivore's blood

Species of carnivores	Number of carnivores Examined (N)	Percentage of positive cases (%)
Dog	25	44
Cat	15	0
Fox	7	57.14

Dog = Probability 0.0000, $P < 0.01$ Significant

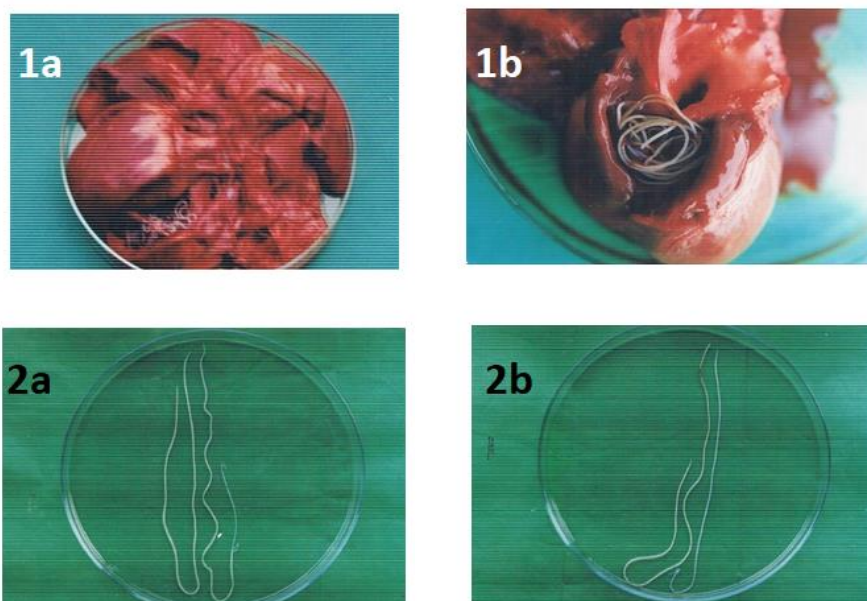
Foxes = Probability 0.0000, $P < 0.01$ Significant

Table 5. Identification of Mosquitoes

Species of Mosquitoes Examined	Number of Mosquitoes Collected	Number of Examined	Number of Infected Cases	Percentage of positive cases (%)
<i>Culex sp</i>	1030	115	13	11.3
<i>Anopheles sp</i>	670	49	3	6.1
<i>Aedes sp</i>	210	20	0	0

Culex sp = Probability 0.0000, $P < 0.01$ Significant; *Anopheles sp* = Probability 0.0000, $P < 0.01$ Significant

Of the 1910 mosquitoes belonging to the genera *Culex sp.*, *Anopheles sp.* and *Aedes sp.*, only 184 female mosquitoes; *Culex* (115), *Anopheles* (49) and *Aedes* (20); were examined, and mf3 were detected in 11.3% 6.1% and 0.0% mosquitoes, respectively (Figure 4). In all cases mf were detected in the thoracic muscles and proboscis (Table 5). Further none of the *Ctenocephalids canis* were found positive to mf.



Figures: 1a: Adult *Dirofilaria immitis* within the right ventricle of the heart of Dog; 1b: Adult *Dirofilaria immitis* within the right ventricle of the heart of Fox; 2a: Adult *Dirofilaria immitis* of Dogs in Petridish; 2b: Adult *Dirofilaria immitis* of Fox in Petridish

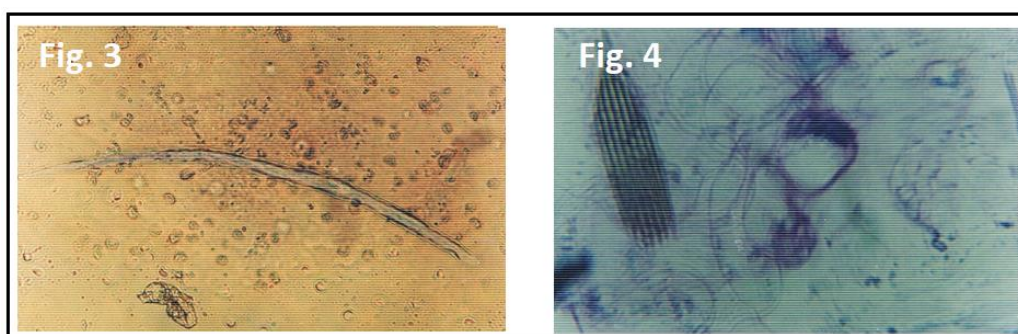


Figure 3. Microfilariae (mf) in the blood smear slide of Dog under microscope; **Figure 4.** Microfilariae from haemocoel of *Culex* sp (Female) under microscope

DISCUSSION

Our study recorded the prevalence of *D. immitis* among dogs and foxes in Bangladesh. Age wise recording illustrated that 46.1% young dog and 66.6% adult dogs were infected and in foxes 66.6% young and 75.0% adult were infected (Table 2). These conform to the finding of *D. immitis* only in dogs in Bangladesh (Ahmed, 1976; Shaikh and Haq, 1984; Rahman, 1992). The prevalence of *D. immitis* in the Mymensingh Municipal Area was considered very high and commensurate with findings in other countries as recorded in Naha City Okinawa (Suenaga et al., 1976), in USA (Acevedo and Theis, 1981; Walters and Lavoipierre, 1984), in Japan (Hatsushika et al., 1992), in Canada (Slocombe and Villeneuve, 1993), in Italy (Ryan et al., 1995). All these above authors had denoted higher prevalence by 20.0% and above in dogs as a serious threat to health of man. None of these authors had studied prevalence of *D. immitis* in wild carnivores like foxes.

In Bangladesh, incidence of *D. immitis* in foxes was recorded in a report (Rahman, 1992) but none of *D. immitis* in jackals was reported in other reports (Shaikh and Haq, 1984). Whereas in the study areas, dogs and foxes had been found infected at a much higher incidence and these animals lived near the human habitations in the study areas. That is why it is considered a serious public health threat to man and may act as the principal reservoir of *D. immitis* in the area.

Prevalence of *D. immitis* in cats in many countries had been recorded (Deem et al., 1998; Smith et al., 1998; Saito et al., 1995; Roncalli et al., 1998; Genchi et al., 1995). One of the research succeeded in making stimulated transmission of *D. immitis* in cat through the bite of infected mosquitoes in USA (Monsour et al., 1995). In the study area none of 15 cats examined were found infected; reasons for absence of such infection status might be the vector mosquito species not normally biting the cats concerned mosquito species were infection free in the locality.

Age and sex wise infection in dogs/foxes were found insignificant ($P>0.05$) which indicated that dogs and foxes were equally susceptible to *D. immitis* infection perhaps due to their wild habitat.

Presence of mf1 in the peripheral blood was considered significant ($P<0.01$). This indicated that all dogs and foxes carried mf1 with increased probability for vector mosquitoes to pick up mf1. This statement was substantiated by the detection of mf3 in the hemocoel of mosquitoes such as 11.3 % in *Culex sp.*, and 6.1 % in *Anopheles sp.*

This detection of *D. immitis* M-3 in *Culex sp.* and *Anopheles sp.* was relevant to findings by several authors (Todaro and Morris, 1975; Lewandowski, 1977; Hansen and Grant, 1978; Buxton and Mullen, 1980; Pinger, 1982; Walters and Lavoipierre, 1982; Roberts, 1985; Wright and Boyce, 1989; Russel, 1985; Jones and Meisch, 1993) who implicated these two genera of mosquitoes in the transmission of *D. immitis*. But unlike the findings by some of the above authors, none of the 210 *Aedes sp.* of mosquitoes examined during this study were positive to microfilariae of *D. immitis*. This could probably due to collection of few *Aedes sp.* or collection time was not appropriate for abundance of *Aedes sp.* None of the authors had quantified the rate of infection by mosquitoes which were considered very important as a prelude by epizootiological point of view.

CONCLUSIONS

This study established the fact that the Mymensingh Municipal area is a highly endemic area for *D. immitis* infection in dogs and foxes. Besides suffering from dirofilariasis, these canines also act as reservoir of infection for man since *D. immitis* is transmitted to man by the vector mosquitoes like *Culex sp.* and *Anopheles sp.* with higher probability of developing pulmonary eosinophilia. Because of both veterinary and public health significance, further detailed studies on the prevalence of *D. immitis* in other region of the country are highly recommended.

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CONFLICT OF INTEREST

There is no conflict of interests.

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