



# Prevalence of *Dirofilaria immitis* Infestation in Dog in Agartala, Tripura, India

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## Authors' contributions

This work was carried out in collaboration among all authors. Author PB conceived and designed the study. Authors PB, S. De and S. Das collected the samples. Author DB did the statistical analysis. Authors PB and SS prepared the manuscript. All authors read and approved the manuscript.

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## ABSTRACT

**Aim:** The present investigation was aimed to study the prevalence of *Dirofilaria immitis* in dogs.

**Place and Duration of Study:** The present work was conducted for a period of one calendar year from February 2023 to January 2024 at different veterinary hospitals, dispensaries and private clinics in and around Agartala, Tripura.

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**Methodology:** Blood samples were collected from 824 numbers of dogs and they were subsequently examined by wet blood film method and modified Knott's technique.

**Results:** Overall prevalence of *D. immitis* was found 22.08%. Breed, sex, age, season and category wise prevalence of *D. immitis* was recorded highest in mongrels (28.41%), in male dogs (22.57%), in dogs more than 3 years age (22.94%), in pre-monsoon season (25.86%) and in stray dogs (34.02%).

**Conclusion:** The result shows a relative high prevalence of dirofilariasis in Agartala, Tripura and hence veterinarians should pay more concern to this zoonotic disease and take appropriate measures to prevent the disease to dog population as well as human beings.

**Keywords:** Agartala; *Dirofilaria immitis*; dog; modified knott's test; Tripura.

## 1. INTRODUCTION

Among all the helminths that cause disease to canines, dirofilariasis caused by *Dirofilaria immitis* (*D. immitis*) is considered as one of the most common and pathogenic. Though this parasite can infect any animal but its primary hosts were mainly dogs and cats. [1]. The disease is present in all around the world however, it is most common in tropical and subtropical areas, where it is regarded as one of the most significant zoonotic diseases of humans and animals [2]. Mosquitoes like *Culex*, *Anopheles* and *Aedes* were mainly act as vector of dirofilariasis where the infective third-stage larvae transmitted to the vertebrate hosts through their bite. The nematode mainly lives in the right ventricle, pulmonary artery, posterior venacava but can also present in right auricle, anterior chamber of eye, inter digital cysts and other parts of body. Heartworm disease due to *D. immitis* has been reported as an emerging zoonosis in India [3] and also increasing prevalence and low level of awareness about dirofilariasis among common peoples highlights the importance of public awareness especially to the pet owners. Various epidemiological factors like the distribution of the mosquito species, mosquito fertility, mosquito population density, animal behavior, environmental temperature, living conditions and the average age of the susceptible host etc. were the major factors for prevalence and distribution of the parasitic vector [4-6]. The cardiopulmonary form of dirofilariasis is a worldwide distributed vector-borne transmitted disease mainly affecting dogs and cats [7] and can be fatal if not treated in time [6]. Laboratory diagnosis of dirofilariasis in live animals is always made based on the presence of microfilariae in the tested blood sample [8].

In India, prevalence of *D. immitis* in dogs has been earlier reported by many workers like from Mizoram and Assam [9,10], Assam

(Bhattacharjee and Sarmah,2013) and from Goa [8]. In Agartala, the popularity of keeping a dog as pet is growing day by day very rapidly. However, no systematic study has been reported from Agartala in dogs so far in this aspect. Therefore, the present study was designed to explore the prevalence of *D. immitis* in dogs in Agartala, Tripura, India.

## 2. MATERIALS AND METHODS

### 2.1 Selection of Animals and Collection of Blood Samples

The present work was conducted in 824 numbers of dogs which were brought to veterinary clinical complex (College of Veterinary Science and Animal Husbandry, R.K. Nagar, Tripura), different veterinary hospitals, dispensaries and private clinics in and around Agartala city. The study was conducted for 1 calendar year starting from February 2023 to January 2024.

Dogs belonging to different breed, age groups (below 1 year, 1-2 years and more than 3 years age group), sex and categories (stray dogs, pet dogs and working dogs) were examined for detection of *D. immitis*. The seasons and months were divided as per Meteorological Centre, Agartala i.e pre-monsoon (March, April and May), monsoon (June, July, August, and September), post-monsoon (October, November and December) and winter (January and February). For collection of blood sample, approximately 4 ml of blood was drawn from the cephalic vein of dog and collected in a disodium salt of ethylene diamine tetra acetic acid (Na<sub>2</sub>EDTA) vacuum tubes and stored at 4°C until parasitological examination.

### 2.2 Examination of Blood Samples

Each blood samples were examined by two conventional microscopic examination

techniques i.e wet blood film method and modified knott's test.

#### A. Wet blood film method

A fresh drop of anticoagulated blood from each sample was placed on a clean glass slide, covered with a cover slip and examined under a compound microscope at 10X magnification for detection of microfilaria of *D. immitis*. Further, blood samples were examined under high and low power objective for morphological identification of the microfilariae on the basis of morphology and movement pattern [11].

#### B. Modified knott's test

About 1 ml of well mixed anticoagulated blood was added to 9ml of 2% formalin in a centrifuged tube. It was then centrifuged at 1500 rpm for 5 minutes. The supernatant was then discarded and a drop of sediment was taken on a clean glass slide then mixed with a drop of 0.01% of methylene blue, covered with a coverslip and examined under high and low power objective for detection of microfilaria of *D. immitis*.

### 2.3 Statistical Analysis

Results were expressed as the percentage. A difference with value  $p < 0.05$  was considered statistically significant. Chi-square test was performed to determine presence or absence of significant difference in parameters among the different groups using the Statistical Package for

Social Sciences, Version 17.0.1 software (SPSS Inc., Chicago, IL, USA).

### 3. RESULTS AND DISCUSSION

The present study revealed that, out of 824 dogs examined 182 dogs were found positive for microfilaria of *Dirofilaria immitis* with overall prevalence of 22.08% (Figs. 1 & 2). The present prevalence was higher than the findings of (Bhattacharjee and Sarmah, [12] and Borthakur et. al., [9] in Assam, India and less than the finding of Borthakur et. al., [10] from Mizoram, India. This variation of prevalence may be due to the fact that occurrence of dirofilariasis is dependent upon the presence of sufficient numbers of infected, microfilaraemic dogs, susceptible mosquitoes, and a suitable climate which favour the breeding of mosquitoes [13,14].

The breed, sex, age, season and category wise prevalence of *D. immitis* are presented in Table 1. Breed-wise, prevalence of *D. immitis* was significantly ( $p < 0.05$ ) higher in Mongrels (28.41%) followed by labrador retrievers (25.43%). Earlier, Ananda and D'souza [15] also reported highest prevalence of dirofilariasis in non-descript dogs. Higher prevalence of *D. immitis* was encountered in small breed like Pug, German spitz as well as large breed like Labrador retriever, German shepherd and Doberman pinscher which implies that exposure to mosquitoes play a vital role in occurrence of dirofilariasis in dogs rather than any particular breed of dog were susceptible to it.

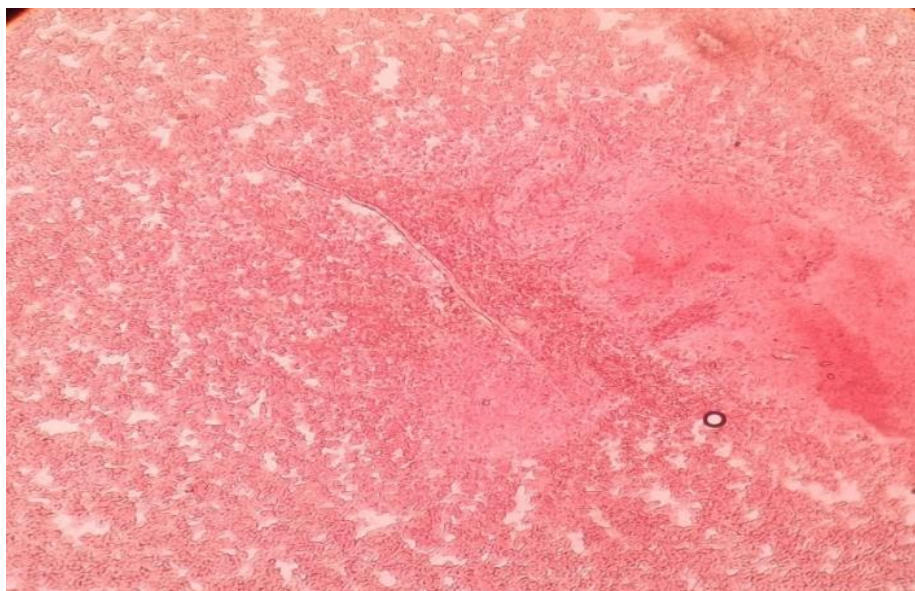


Fig. 1. *Dirofilaria immitis* in wet film method (10X)

**Table 1. Breed, sex, age, season and category wise prevalence of *Dirofilaria immitis* in dogs**

Breeds	No. examined	No. positive for <i>Dirofilaria immitis</i>	Prevalence (%)
Mongrel	272	82	30.14
Cross breed	145	29	20.00
Labrador retriever	114	26	22.80
German shepherd	96	17	17.70
German Spitz	89	14	15.73
Lhasa apso	35	02	5.71
Pug	24	05	20.83
Golden retriever	11	1	9.09
Dalmatian	6	1	16.67
Doberman pinscher	6	1	16.67
Rottweiler	6	1	16.67
Cocker spaniel	5	1	20.00
Beagle	5	1	20.00
Pomeranian	5	1	20.00
Siberian husky	5	0	00.00
P<0.001*			
<b>Sex</b>			
Male	505	114	22.57
Female	319	68	21.31
P>0.05 <sup>NS</sup>			
<b>Age Group</b>			
Upto 1 year	96	10	10.41
1-3 years	326	66	20.24
More than 3 years	462	106	22.94
P<0.001*			
<b>Season/Month</b>			
Pre monsoon	259	67	25.86
Monsoon	238	49	20.58
Post monsoon	198	47	23.73
Winter	129	19	14.72
P>0.05 <sup>NS</sup>			
<b>Categories</b>			
Pet dogs	454	69	15.19
Stray dogs	241	82	34.02
Working dogs	129	31	24.03
P<0.001*			

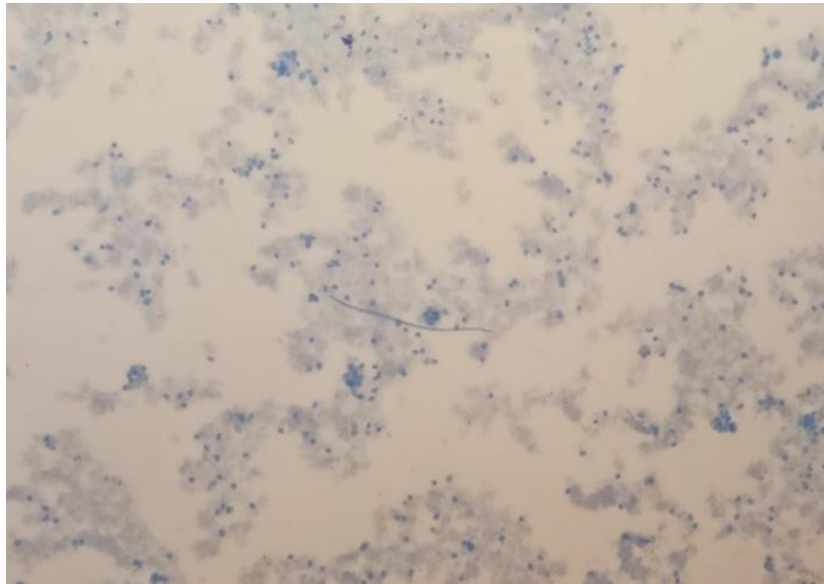
\*Denotes statistically significant p value ( $p < 0.001$ ); <sup>NS</sup> Denotes statistically non-significant p value ( $p > 0.05$ )

Sex-wise the prevalence of *D. immitis* was non-significantly ( $p > 0.05$ ) slightly higher in males (22.57%) than female dogs (21.31%). This correlates with Malatesh et al., [16] who also recorded higher prevalence in male dogs than female dogs. Higher prevalence in males might be due to the fact that male dogs have more scavenging and wandering habit which might enables them to get more proximity to mosquitoes resulted in higher infestation to them.

The prevalence of *D. immitis* was significantly ( $p < 0.05$ ) higher more than 3 years age group (22.94%) as compares to 1-3 years age group

(20.37%) and less than 1 year age group (10.41%). Higher prevalence observed in more than 3 years age group due to prepatency period of the parasite [17,2]. However, longer exposure time period in older dogs to the vector i.e. mosquitoes also considered as one of the risk factor for occurrence of Dirofilariasis in older dogs [18].

Seasonally, the prevalence rates of *D. immitis* were recorded non-significantly ( $p > 0.05$ ) higher in pre-monsoon (25.86%) followed by post-monsoon (23.73%) monsoon (20.58%) and winter (14.72%) season. Earlier, Bhattacharjee



**Fig. 2. *Dirofilaria immitis* in modified Knott's technique (10X)**

and Sarmah, [12] and Malatesh et al., [16] also found higher prevalence *D. immitis* in pre-monsoon season. The high prevalence in pre-monsoon might be due to the favourable environmental conditions for breeding of mosquitoes leading to higher *D. immitis* infection.

Category-wise, the prevalence higher prevalence rate of *D. immitis* was significantly ( $p < 0.05$ ) higher in stray dogs (34.02%) followed by working (24.03%) and pet dogs (15.19%). Highest prevalence of the *D. immitis* in stray dogs corroborated with the findings of Barthakur et al., [9] and Bhattacharjee and Sarmah, [12]. The highest prevalence of *D. immitis* observed in stray might be due to the fact that stray dogs mainly live in outdoor, roaming here and there because of which they are more prone to being easily bitten by mosquitoes. However, pet and working dogs were usually maintained in a better hygiene condition and proper cares were taken by their owner/attendants which lower the rate of infection to them.

#### **4. CONCLUSION**

Tripura is a north-eastern state in India with its capital at Agartala. The State shares international border with Bangladesh in its three sides and also national border with Assam and Mizoram states. The meteorological aspects of the state like average rainfall, humidity and vast forest area were ideal for breeding of mosquitoes. In our study considerably high rate of *Dirofilaria immitis* in dogs was observed in

Agartala, Tripura. Present study revealed that male dogs of more than 3 years age group are more prone to dirofilariasis in pre-monsoon season. Stray dogs showed higher infection rate than pet and working dogs. Though both large and small breed of dogs found to be affected with dirofilariasis, the infection rate was higher mongrel dogs. Since dirofilariasis has a zoonotic importance so it is necessary to take immediate attention regarding controlling of the vectors as soon as possible to reduce possible human infection. To best of our knowledge, our study is the first study related to *D. immitis* from Tripura but further in depth detailed molecular study needed.

#### **DISCLAIMER (ARTIFICIAL INTELLIGENCE)**

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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#### **COMPETING INTERESTS**

Authors have declared that no competing interests exist.



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