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Epidemiological aspects of *Dirofilaria immitis* infection in dogs from Assam of Northeast India

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PEER REVIEW

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Comments

The authors have evaluated all the possible host related risk factors and some of the environmental risk factors related to the epidemiology of *D. immitis* in dogs. The results are worth publishing in the interest of companion animals' welfare as well as from zoonotic point of view.

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ABSTRACT

Objective: To analyze the epidemiology of *Dirofilaria immitis* (*D. immitis*) infection in different categories of dogs in Assam.

Methods: Microscopy of blood in wet film preparation was conducted in two dog populations during 2009 and 2010, and data obtained were analyzed to investigate the infection pattern according to season, living condition, breed, sex and age of dogs.

Results: Examination of 424 clinically ill dogs (hospital population) presented at the referral Teaching Veterinary Clinical Complex, Guwahati, Assam revealed 5.42% cases microfilaraemic. Clinical cases of *D. immitis* infection were recorded throughout the year with 9.10% as the highest record during pre-monsoon and 1.12% as the lowest in the winter. Pet dogs staying indoor showed 4.76% microfilaria positive compared to 7.95% record among working dogs with restricted outdoor activity. Breed wise, Dalmatian dogs representing 3.53% of the study population were negative to microfilaria while among the positives, Doberman showed 30.76% infection rate as the highest and 2.94% lowest record in the Labrador. Rate of infection was slightly higher in the males (5.81%) than the females (4.81%). Dogs below 2 years of age were negative to microfilaria while it was 3.70% in 2–4 years age group with a rising trend reaching 28.00% as the highest in 6–8 years age group. Examination of non-descript (street dog population) adult dogs living outdoor showed 29.54% microfilaraemic, the rate of infection being higher in males (37.50%) than the females (25.00%). Similar non-descript dogs living indoor as pets however showed only 11.11% microfilaria positive.

Conclusions: The study shows endemicity of *D. immitis* in dogs of Assam with living condition and age as the host related important risk factors, which have significant role in its epidemiology.

KEYWORDS

Dirofilaria immitis, epidemiology, dog, Assam, India.**1. Introduction**

Dirofilaria immitis (*D. immitis*) is a filarial nematode which resides in the pulmonary arteries and right ventricle of heart in dogs. Mosquitoes under several genera including *Culex*, *Aedes*, and *Anopheles* are the competent vectors of the parasite. Dogs with heavy infection suffer from cardio-respiratory disturbances, resulting in multiple organ dysfunctions. In India, the parasite has been sporadically

reported in dogs and wild captive carnivores from the north and northeastern region^[1–3]. A case of human dirofilariosis was also reported from Mumbai in Central India^[4]. Despite the strong belief that the parasite is confined to India's northeastern region^[2,5], the epidemiological situation here is far from clear. Reports available so far on the prevalence of *D. immitis* in dogs from this region are mainly based on postmortem study in non-descript street dogs^[2,3]. The present study was undertaken to analyze the risk factors of

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D. immitis infection in different categories of dogs in Assam.

2. Materials and methods

The present study was conducted for a period of two years (2009 and 2010) in three categories of dogs *viz.* pet, working and street dogs from Assam. A total of 336 pets belonging to private owners and 88 working dogs owned by the paramilitary forces stationed in different areas of Assam are brought to the Teaching Veterinary Clinical Complex, Guwahati for diagnosis, and treatment of different clinical illness were selected for blood examination. Similarly, 44 adult dogs of non-descript breed and living on the street captured by a non-governmental organization– Just Be Friendly for sterilization under birth control programme sponsored by Animal Welfare Board of India were subjected to blood examination. Anticoagulated blood samples obtained from all these animals were examined by microscopy in wet film preparation for the detection of microfilaria. Identification of microfilaria in positive cases was done on the basis of morphology and subsequent confirmation of the presence of heartworm antigen in the respective dog blood using a commercial kit SNAP 3DX (Idexx Lab, USA). Informations pertaining to the age, breed, sex and living condition of the dogs were collected and the influence of host related factors in the epidemiology of *D. immitis* infection was determined.

Data on age, breed, sex and living condition of dogs were subjected to statistical analysis using Statistical Package for Social Science version 17.0 (SPSS Inc. Chicago, IL, USA). Chi-square test of association was used to establish the significance.

3. Results

Clinical cases of *D. immitis* infection characterized by the presence of microfilaria in blood were detected in all seasons of the year (Table 1). The rate of infection varied from 1.12% as the lowest record during winter season to a maximum of 9.1% during pre-monsoon season and the difference within season was non-significant ($P < 0.05$).

Table 1

Season wise record of clinical *D. immitis* infection in hospital dogs.

| Season | Av. temp (°C) | Av. humidity (%) | No. examined | No. positive | Positive (%) |
|-------------------------------|------------------|---------------------|-----------------|-----------------|-----------------|
| Pre-monsoon (March, Apr, May) | 26.1 | 67.8 | 87 | 8 | 9.10 |
| Monsoon (Jun, July, Aug) | 29.5 | 81.5 | 136 | 6 | 4.41 |
| Post monsoon (Sept, Oct, Nov) | 26.8 | 80.3 | 112 | 8 | 7.14 |
| Winter (Dec, Jan, Feb) | 19.7 | 77.5 | 89 | 1 | 1.12 |

Results of blood examination in dogs according to their living condition are shown in Table 2. Pet dogs living indoor showed 4.76% microfilaraemia while working dogs with restricted outdoor activity were recorded 7.95% positivity.

The street dogs living outdoor showed 29.54% microfilariae positive which is significant ($P < 0.05$) compared to pet and working dogs.

Table 2

D. immitis infection rate according to living condition of dogs.

| Source | Category | Living condition | No. examined | No. positive | % positive |
|----------|----------|--------------------------|--------------|--------------|------------|
| Hospital | Pet | Indoor | 336 | 16 | 4.76 |
| | | Working Indoor & outdoor | 88 | 7 | 7.95 |
| Street | Street | Outdoor | 44 | 13 | 29.54 |

Breed wise, Dalmatian representing 3.53% of the hospital population examined were found microfilaria negative (Table 3). In other cases positive rate varied from 2.94% in Labradors to 30.76% in the Dobermans. The difference in infection rate was significant ($P < 0.05$). Out of 23 microfilaria positive hospital dogs, highest number of cases were seen in German Shepherd dogs (21.73%) followed by 17.39% each in Labrador, Doberman and non-descript dogs and 8.69% each as the lowest record in German Spitz and crossbreds. Non-descript local dogs kept as pet showed 11.11% microfilaria positive against 29.54% positivity in similar dogs living in the street (significant at $P < 0.05$).

Table 3

Breed wise *D. immitis* infection rate in dogs.

| Source | Breed | No. examined (% representation) | No. positive (% distribution) | % positive |
|----------|----------------------|------------------------------------|----------------------------------|------------|
| Hospital | Labrador | 136 (32.07) | 4 (17.39) | 2.94 |
| | German Shepherd dogs | 102 (24.05) | 5 (21.73) | 4.90 |
| | Cross-bred | 47 (11.08) | 2 (8.69) | 4.25 |
| | German Spitz | 42 (9.90) | 2 (8.69) | 4.76 |
| | Dalmatian | 15 (3.53) | 0 | 0 |
| | Doberman | 13 (3.06) | 4 (17.39) | 30.76 |
| | Others | 33 (7.78) | 2 (8.69) | 6.06 |
| | Non-descript (pet) | 36 (8.49) | 4 (17.39) | 11.11 |
| | Total | 424 (100) | 23 (100) | 5.42 |
| Street | Non-descript | 44 | 13 | 29.54 |

Male dogs from the hospital population showed 5.81% infection rate against 4.81% in the females (Table 4). Among the street dog population, infection in males (37.50%) was higher than the females (25.00%). In both the populations, difference was non-significant ($P < 0.05$).

Table 4

Sex wise *D. immitis* infection rate in dogs.

| Source | Sex | No. examined | No. positive | % positive |
|----------|--------|--------------|--------------|------------|
| Hospital | Male | 258 | 15 | 5.81 |
| | Female | 166 | 8 | 4.81 |
| Street | Male | 16 | 6 | 37.50 |
| | Female | 28 | 7 | 25.00 |

Age wise, microfilaria positive cases were detected in dogs above 2 years of age (Table 5). The infection rate increased from 3.70% in 2–4 years age group to a maximum of 28.0% in 6–8 years age group. However, in positive cases the difference in respect of age group was non-significant ($P < 0.05$). *Dirofilaria* infection showed a reducing trend after 8 years of age and 7.14% record was made in dogs of 10–12 years age group. Distribution of 23 recorded cases also showed similar increasing trend of infection after 2 years

of age with maximum case record (30.43%) in 6–8 year age group with a reducing trend thereafter.

Table 5

Age wise *D. immitis* infection rate in hospital dogs.

| Age | No. examined (% representation) | No. positive (% distribution) | % positive |
|------------|------------------------------------|----------------------------------|------------|
| > 6 month | 42 (9.90) | 0 | 0 |
| 6–12 month | 56 (13.20) | 0 | 0 |
| 1–2 year | 96 (22.64) | 0 | 0 |
| 2–4 year | 108 (25.47) | 4 (17.39) | 3.70 |
| 4–6 year | 60 (14.15) | 6 (26.08) | 10.00 |
| 6–8 year | 25 (5.89) | 7 (30.43) | 28.00 |
| 8–10 year | 23 (5.42) | 5 (21.73) | 21.73 |
| 10–12 year | 14 (3.30) | 1 (4.34) | 7.14 |
| Total | 424 (100) | 23 (100) | 5.42 |

4. Discussion

Situated on the foot hills of Himalayas, Assam and other 7 sister states of the northeastern region of India have mostly subtropical climate with deciduous rain forest. Environmental situations prevailing in this region with moderate temperature, high rainfall and humidity more than 60% throughout the year are ideal for breeding of mosquitoes and other vectors leading to high vector density and regular transmission of many diseases including malaria, lymphatic filariasis and visceral leishmaniasis of man[6]. Vector borne diseases like babesiosis, ehrlichiosis and different filarial infections are also known to occur in animals of this region[2,3,7,8].

Antemortem examination conducted in the present study showed an overall 5.42% positivity for *D. immitis* infection in dogs with 4.76% as the lowest infection rate in the pets and 29.54% as the highest in the street dogs. Similar pattern in the infection rate varying from 26.50%–33.75% was recorded at post mortem in street dogs from another sister state, Mizoram, which is situated at an altitude higher than that of the present study area, Assam[2,3]. However, using most sensitive polymerase chain reaction method, only 1% prevalence of *D. immitis* was recorded in dogs of Sikkim, another hilly state of the northeast by Megat Abd Rani *et al*[5]. Higher level of infection rate recorded in the present study similar to other reports[9] might be due to situation of Assam and Mizoram at an altitude lower than that of Sikkim and the difference in mosquito population which remained constant upto an altitude of 800 m above mean sea level[10]. The present findings showed significantly higher infection rate in the street dogs than that of pet dogs similar to the earlier observations[11,12].

Clinical cases of *D. immitis* infection in hospital dogs were recorded in all seasons of the year and this varied from 1.12% detected in the winter to a maximum of 9.1% in pre-monsoon. However, differences were non-significant, similar to the records of Gadahi *et al*[13]. According to the number of dogs examined breed wise in the present study, Dalmatian and Doberman showed extreme negative and highest positive (30.76%) results respectively. Representations of these two breeds in the study population were the lowest among those

examined. Infection rate in the remaining specific breeds varied between 2.94%–4.90%. The breed wise significant variation observed is contradictory to the previous report[14]. Non-descript local dogs under two different living conditions showed wide variation in the infection rates thus suggesting that exposure to mosquitoes play an important role rather than the breed of dogs in the epidemiology of infection.

Sex wise, male dogs of both the populations showed non-significant but higher infection rate than the females. Many studies have also indicated similar pattern of higher infection in males[14–16].

A strong correlation was observed between the age of dogs and the rate of *D. immitis* infection. Animals up to 2 years of age were amicrofilaraemic, this finding is consistent with report of Byeon *et al*[17]. Although record of *Dirofilaria* infection in young animals below 2 years of age are available in the literature[18], highest infection rate in dogs of middle age groups have also been reported elsewhere[14,19,20]. Reduction in infection rate in dogs after 10 years of age observed in the present study is however in contradiction to earlier report[15].

The results of the present study imply that Assam is endemic for *D. immitis* with variable rates of infection in different categories of dog irrespective of breed and sex. Higher infection rate in the outdoor dogs reflect a conducive climate for the vectors and their potential to transmit filarial infections like other bacterial, viral and protozoal infections of man and animals.

Conflict of interest statement

The authors declare that they have no competing interests.

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Comments

Background

Heartworm disease in dog due to *D. immitis* is one of the most important vector borne parasitic diseases posing a threat to the companion animals. The disease is believed to be emerging in the face of global warming, climate change and also unabated movement of dogs from endemic to non-endemic places and vice-versa. In India, this infection is believed to be endemic in the northeastern region. However, detail information on its epidemiology and risk factors

in different categories of dogs including the high profile security dogs engaged by defence organizations in this region having international border with several countries remains to be elucidated.

Research frontiers

Through this paper, the authors have projected the status of *D. immitis* infection in different categories of dogs of Assam viz. street dogs, pet and working dogs with elucidation of different epidemiological risk factors such as geographical location relating to altitude level, seasonality of infection, living condition of dogs, age, breed and sex of dogs.

Related reports

Although no work was previously conducted on the epidemiology of *D. immitis* of dogs in India, the present findings on the level of infection in different categories of dogs are found to be comparable with the reports published from other parts of the world except certain minor variations depending on the geographical location.

Innovations & breakthroughs

The findings of the present paper have confirmed the previous belief on the endemicity of *D. immitis* in northeastern region of India including Assam. Evidence of infection in different categories of dogs has shown the high prevalence of potential mosquito vectors.

Applications

Informations obtained through this piece of research shall throw light on the measures to control the mosquito borne infections in dog and also possible risk of transmission to the human population in this region. Further, highly valuable and well trained dogs owned by different defence organizations will get priority towards getting rid of the infections through awareness programme.

Peer review

The authors have evaluated all the possible host related risk factors and some of the environmental risk factors related to the epidemiology of *D. immitis* in dogs. The results are worth publishing in the interest of companion animals' welfare as well as from zoonotic point of view.

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