Short Communication

Occult Dirofilariosis in Dogs of North Eastern Region in India

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Abstract

Background: The North Eastern Region in India is endemic for canine heartworm disease but in clinics accurate diagnosis is some times difficult. The aim of the present study was to determine the prevalence of occult infections for heartworm disease in canine in two geographical regions of North Eastern India.

Methods: A total of 782 numbers of three categories of dogs namely, working dogs of military and paramilitary forces, pet dogs and stray dogs were screened for the presence of heartworm infection from August 2011 to July, 2012 in Guwahati (Assam) and Aizawl (Mizoram). Conventional, immunological and molecular techniques were followed for this epidemiological study. The criteria to determine the occult heartworm cases were based on the differences between heartworm positive cases in PCR test and antigen ELISA test.

Results: The findings revealed an overall 22.69 percent occult case. The working dogs had highest prevalence (60%) followed by pet (29.16%) and stray dogs (17.75%).

Conclusion: The highest percentage of occult heartworm infection was present in working dogs maintained under military or paramilitary forces.

Keywords: *Dirofilaria immitis*, Occult infection, India

Introduction

India's dog population is estimated over 25 million and 80 % of this population are either partially restricted or community (Stray), or feral (unrestricted) dogs (Menezes 2008). Again, 17 % of Indian households were reported to own a pet or domesticated dog (Sudarshan et al. 2006).

Among the disease producing agents in canines, "helminths" are one of the predominating group of which *Dirofilaria immitis* is established as the most pathogenic filarial nematode in terms of disease pathogenicity. The nematode releases unsheathed microfilariae (Mf) into blood. Mosquitoes like *Culex*, *Aedes*, *Anopheles*, *Mansonia* etc are incriminated as intermediate host of this parasite (Bemrick and Sandholm 1966, Ludlam et al. 1970, Lok 1988, Arellano et al. 2002). In India, prevalence of this parasite in dogs has been earlier reported (Borthakur et al. 2006, Megat Abd Rani et al. 2010, Bhattacharjee 2011, Borthakur et al. 2011).

Another filarid, *Dirofilaria repens*, a parasite of subcutaneous tissue of dogs and other canids. This parasite has been reported from many parts of the world including India (Megat Abd Rani et al. 2010). *Dirofilaria repens* accidentally affects humans and several human cases have been reported from India, mostly from South India (Senthilvel and Pillai 1999, Sekhar et al. 2000, Nadgir et al. 2001, Sabu et al. 2005) and also reported from Assam (Nath et al. 2010).

Laboratory diagnosis of dirofilariasis in live animals is always in forefront in terms of simple demonstration and identification of microfilariae, serology and molecular techniques. Several commercial ELISA based test kits are available to diagnose heartworm in dogs but these kits are not widely used in

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our country where heartworm test in every dog is not mandatory. DNA based techniques provide an alternative approach which is very sensitive and accurate for identification of the filarial parasites (Favia et al. 1996, Nuchprayoon et al. 2005, Rishniw et al. 2006). Sometimes microfilariae in circulating blood of heartworm infected dogs are not seen and such condition is termed as "occult infection". The reason for occult infection of *D. immitis* in dog with no microfilariae in blood is due either to male or female worm infection, single worm infection, presence of immature females during pre-patent stage of development, geriatric infection and ectopic infection. Numerous side effects associated with occult infections are severe cough, dyspnoea and crackles. Immune mediated allergic pneumonitis sometimes associated with occult infections produces eosinophilia besides pneumonic sign and symptoms.

The present communication is based on the reports of epidemiological study of heartworm disease in North Eastern states of India.

Materials and Methods

Study areas

The study was undertaken systematically for a period of one calendar year from August, 2011 to July, 2012, in dogs from Guwahati and Aizawl. Guwahati, a city of Assam is located at the latitude of 26°11'0" N and longitude of 91°44'0" E having annual rainfall of 1500–2600 mm with an average altitude of 52 mts msl and Aizawl, the capital city of Mizoram State of north east located at 23°43'27" N and 92°43'2" E having annual rainfall of 2400–2962 mm with an average altitude of 1132 mts msl. Both the cities are separated by surface distance of 550 km.

Selection of dogs

Three categories of dogs were selected for the epidemiological study, working dogs of military and paramilitary force, pet dogs and stray dogs. Pet dogs of different breeds and paramilitary dogs mostly of Labrador and German Shepherd breeds brought to the Teaching Veterinary Clinical Complexes (TVCC) of the College of Veterinary Science, Assam Agricultural University, Khanapara and the College of Veterinary Sciences and AH, Central Agricultural University, Selesih, Aizawl, Mizoram during the study period were taken for the study. The stray dog population consisted of local non-descript street dogs of either sexes captured from different parts of the city for sterilization by local non-governmental organization like Peoples for Animal (PFA) and Just be friendly (JBF). Additionally stray dogs brought for slaughter at Aizawl were examined during slaughter.

Altogether 782 dogs were examined. Dogs exhibiting specific clinical signs of heartworm diseases were also recorded. Three categories of dogs like working (103), pet (266) and stray (413) totaling 782 numbers were examined. Dogs under the study were of either sexes or a total of 488 dogs from different localities of Guwahati and 294 dogs from Aizawl formed the entire base of study during the programme.

Blood sampling

Blood sampling from hospital dogs was done at clinics soon after presenting the animals by the owners for clinical investigation. In case of stray dogs, dog shelters were visited time to time and samples were collected. Approximately 5 ml of blood was drawn from the cephalic vein collected in disodium salt of ethylene diamine tetra acetic acid (Na_2EDTA) vacuum tubes and stored at 4 °C until further use.

Parasitological investigation

The prevalence study for *D. immitis* was conducted on the basis of conventional wet blood film method and Knott's Concentration Technique (KCT), immunological with a commercially available ELISA test kit

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(SNAP® 4Dx, IDEXX Labs. Inc., Westbrook, USA) and molecular techniques targeting to amplify the ITS-2 region of filarial worms rDNA developed by Rishniw et al. (2006) was followed.

Isolation of genomic DNA from blood was carried out using the DNeasy Blood and Tissue kit (Quiagen® Kit, Catalogue No 51104) and the protocols follows as per handbook provided by the manufacturer. The primer utilized was referred to as *pan filarial* primers, forward: DIDR F1 5'-AGT GCG AAT TGC AGA CGC ATT GAG-3' and reverse: DIDR R1 5'-AGC GGG TAA TCA CGA CTG AGT TGA -3' were utilized to amplify and differentiate *D. immitis, D. repens, Brugia malayi, B. pahangi, Acanthocheilonema* (*Dipetalonema*) *reconditum* and *A. dracunculoides*. To amplify the targeted ribosomal gene of *D. immitis* the PCR reaction mixture was consisted as 2.5 µl Taq polymerase buffer (10X), 01 µl dNTP (10mM), 0.5 µl MgCl2 (50mM), 0.75 µl of each forward and reverse primer (60pM), 0.5 µl Taq polymerase, 3.0 µl template DNA and by making the final volume upto 25.0 µl with NFW. The cycling condition used for amplifying the targeted product consisted of a initial denaturing step at 94 °C for 2 min and 32 cycles of denaturing (30 s at 94 °C), annealing (30 s at 60 °C) and extension (30 s at 72 °C), a final extension (7 min at 72 °C) and a soak at 4 °C in a Technee-5000 thermal cycler (Bibby Scientific). The confirmation of the amplified products were made by gel electrophoresis of the PCR product in 1.5 % agarose gel stained with Ethidium Bromide and visualized under gel doc (DNR Bio-Imaging System, MiniLumi).

Since PCR test can be utilized to detect up to one microfilaria per 250 µl of tested blood sample, hence, differences between heartworm positive cases in PCR test and antigen detection test (SNAP®4Dx) was considered as criterion for calculation of number of occult cases.

Results

Overall, 782 dogs were examined to detect *D. immitis* infection. The parasitological investigations were carried out by employing conventional, serological and molecular methods on 3 different categories of dogs like, working dogs maintained by military and paramilitary forces, pet dogs and stray dogs. The main objective of the study was to determine the occult dirofilariasis due to heartworm disease.

The study revealed an overall 22.69 percent occult case. The working dogs had highest prevalence (60%) followed by pet (29.16%) and stray dogs (17.75%) (Table 1).

Table 1. Occult cases of *Dirofilaria immitis*

Dog category	Numbers examined	Numbers found +ve by Ag ELISA (Snap 4Dx)	Numbers found +ve by Specific PCR (mff DNA)	Occult cases of *D. immitis**	% occult cases
		(1)	(2)	(3)	(4)
Stray dogs	413	107	88	19	17.75
Pet dogs	266	24	17	7	29.16
Working dogs	103	10	4	6	60.0
Overall	782	141	109	32	22.69

* Values in column (3) is obtained from values of column (1)-(2)

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Discussion

The present findings of higher occult cases recorded in working and pet dogs in comparison to stray dogs might be due to the fact that, owners of pet and working dogs are very much concerned about the health status of their animals. Hence, there is regularity in their health check-up by a professional often necessitated for anthelmitic medication with a preference for an endecticidal drug like ivermectin. This endecticide has microfilaricidal activity, thereby reducing circulating microfilariae. On the other hand, stray dogs are seldom taken care of with such type of medications.

Earlier, Borthakur et al. (2006) recorded 35.80 % dog affected with occult dirofilariasis in Mizoram. Their study was based on necropsy finding of confirmed heartworm cases and subjecting the blood sample from the same cases for KCT. Grieve et al. (1986) could record up to 61.9 percent occult dirofilariasis in infected dogs, who conducted the study with necropsy findings and ELISA test in Freeport, Grand Bahamas. Similarly, Lai et al. (2001) from Central Taiwan, Alves et al. (1999) from the city of Receife, Pernambae, Brazil, Labarthe et al. (1997) in the State Rio de Janerio, Brazil, and Yildiz et al. (2008) from Kirikkale, Turkey could record 25.53, 57.1, 34.64 (n= 127) and 27.46 (n=172) percent occult infection in dogs, respectively. These authors had their conclusion based on antigen detection test by ELISA and KCT. Further, they opined that abusive use of microfilaricidal agent contributed substantially to high rate of occult dirofilariasis. Recently, Malmasi et al. (2011) reported 17.7 % occult infections in southern coasts of the Caspian Sea based on 200 numbers of dogs surveyed by antigen test and modified Knott's test. In a more recent study, conducted in Guwahati, Bhattacharjee (2011) reported 7–37.5 percent occult cases of dirofilariasis. Dogs with 50–100 mature worms that received little exercise may never show signs of heartworm disease, also, one may not be able to find microfilaria in the blood of such subjects (Nayar 1990).

It is generally known that, an infection may be occult due to presence of either one sex of the parasite or due to longer pre-patent period (180 days) leading to amicrofilaremic.

Conclusion

The highest percentage of occult heartworm infection was present in working dogs maintained under military or paramilitary forces.

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