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OCCURRENCE OF DIROFILARIA IMMITIS MICROFILARIAE IN STRAY DOGS OF DURG, CENTRAL INDIA

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ABSTRACT: *Dirofilaria immitis*, a nematode spread by insects, is the cause of dirofilariasis, a zoonotic parasitic disease that affects people all over the world. This parasite primarily infects dogs and cats. Thus, the purpose of this study was to ascertain the incidence of *D. immitis* in stray dogs of Durg Chhattigarh. Transmission of *D. immitis* occurs in its larval stage by over 70 species of mosquitoes of the genera *Aedes*, *Ochlerotatus*, *Anopheles* and *Culex.* Blood samples from thirty two stray dogs were examined for microfilariae using modified Knott's technique, blood smear, and direct wet smear microscopy. Using direct blood smear microscopy and modified Knott's approach, the infection incidence ranged from 6.25% to 12.5%, respectively. Among four positive cases observed 3 were male and 1 was female therefore no statistically significant relationship was seen in the occurrence of *D. immitis* with age and gender. The rhythmic periodicity was also confirmed by examining the positive cases in six hours interval and maximum microfilaraemia was observed during 18.00 hours and minimum during 06.00 hours. Result of this study revealed the presence of dirofilariosis in dogs, though the sample size is small and exhaustive study needs to be done for knowing the actual prevalence status of this disease. Considering the zoonotic nature of the disease this information could be utilized by veterinarians and public health workers to take appropriate measures for its control.
Keywords: *Dirofilaria immitis*, stray dogs, incidence, rhythmic periodicity, zoonotic

Dirofilaria immitis is the parasite that causes canine heartworm infection, a disorder that is recognised worldwide (Tahir *et al*., 2019) and has pertinent veterinary care implications (Lee *et al*., 2010) This parasite primarily infects domestic animals, particularly dogs and cats (Atkins 2003). Over 70 mosquito species belonging to the genera Aedes, Ochlerotatus, Anopheles, and Culex are known to transmit *D. immitis* (Dantas-Torres and Otranto 2020). *D. immitis* is present in India, particularly in the northeastern states, where its prevalence ranges from 4.7% to 29.5% (Megat Abd Rani, 2010). Prevalences of dirofilariosis are impacted by vector abundance and presence, which are controlled by climate variables including humidity and temperature (Simón 2012). As it is a zoonotic disease humans can become intermediate hosts for the larval stage of infection; to date, reports of human infections have come from Iran, Japan, and the United States (Ettinger *et al*., 2005).

The life cycle of *D. immitis* encompasses a reservoir and a susceptible host, which are biologically linked through a competent arthropod vector (Bowman and Atkins 2009). While feeding on a naïve dog, infected mosquitoes of the genera Culex, Aedes, Ochlerotatusor Anopheles (Capelli *et al* 2013), can transmit *D. immitis* infective third-stage larvae (L3) to the definitive host. Larvae migrate into the skin through the insect bite wound, developing in L4 and reaching the pulmonary arteries (Otranto and Deplazes, 2019). Parasites continue their development and growth for 4–5 months, becoming adults.

Due to the adult worms' localisation in the pulmonary arteries and right heart chamber, infected dogs may experience a potentially lethal clinical syndrome that includes cardiorespiratory changes such pulmonary hypertension, dyspnoea, and ascites (Bowman and Atkins 2009).

Diagnosis of adult *D. immitis* is done in dead dog by post-mortem examination while the larval stages can be diagnosed by morphological observation of circulating larvae by stained blood smears, direct wet smears, modified Knott's technique and the Wylie's filtration technique (Irwin and Jefferies, 2004). Histochemical or immuno-histochemical staining of circulating microfilariae has also been performed (Ananda *et al*., 2006). Detection of circulating antigen with commercial test kits is currently available and widely used for *D. immitis* (Ranjbar-Bahadori *et al*., 2007). Molecular diagnostic approaches are also increasingly utilised for research and surveillance purposes (Rishniw *et al*., 2006).

Overall, the prevalence of dirofilariosis in dogs is quite significant and necessitates collaboration between veterinary and public health authorities. This includes a continuous requirement for current epidemiological data on the parasite's dissemination (Bowser and Anderson, 2018).

In the present study, the occurrence of *D. immits* microfilariae was investigated in stray dogs of Durg, Chhattisgarh central part of India.

MATERIALS AND METHODS

Blood samples were collected from 32 stray dogs. Vials containing ethylene diamine tetra acetic acid (EDTA) were used to collect blood samples. Light microscopy examination of wet blood smear and Giemsa-stained thin blood smears and the concentration method by modified Knott's test were used to evaluate blood samples for circulating microfilariae. Using the wet blood smear technique, a drop of blood (about 20 µl) was put onto a sterile microscope slide with a cover

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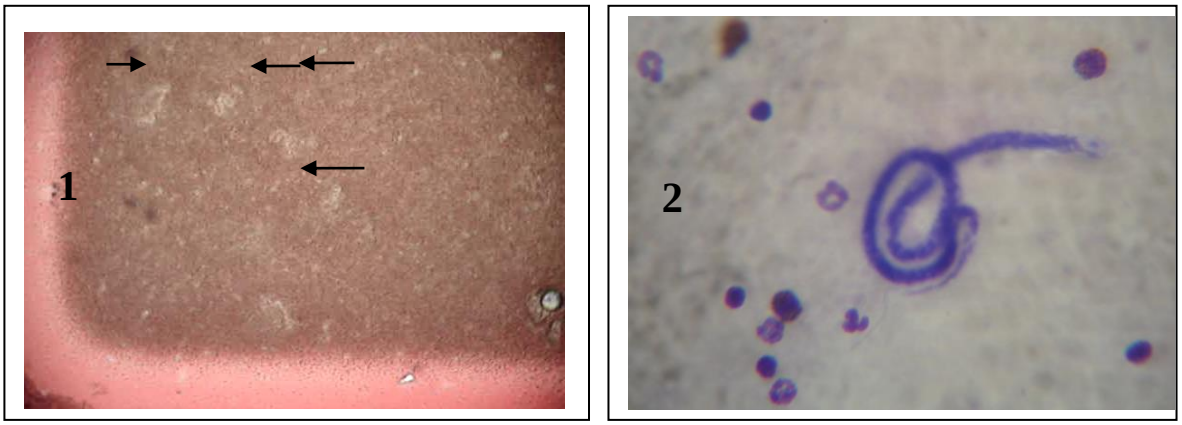
slide and viewed under a microscope. Samples that showed faint, undulating larval movements were deemed positive (Rimal *et al*., 2021). In modified knott procedure, 1 millilitre of blood and 9 millilitres of 2% formalin were centrifuged for five minutes at 1500 rpm. After that, the tube's top solution was gradually drained, one or two drops of Methylene Blue were added to the precipitate, and the slides were pipetted onto a microscope equipped with 40X and 10X lenses to check for Dirofilaria immitus microfilariae. On thick blood slides, the samples were also stained using the Giemsa's staining technique (Anvari *et al*., 2019). Based on the morphometry and morphology of the anterior and posterior extremities, microfilariae were identified at the species level (Soulsby, 1982). Based on the microfilaraemia periodicity for *D. immitis* (Ionică *et al*., 2017) blood collections were performed from 06.00 p.m. to 06.00 a.m.

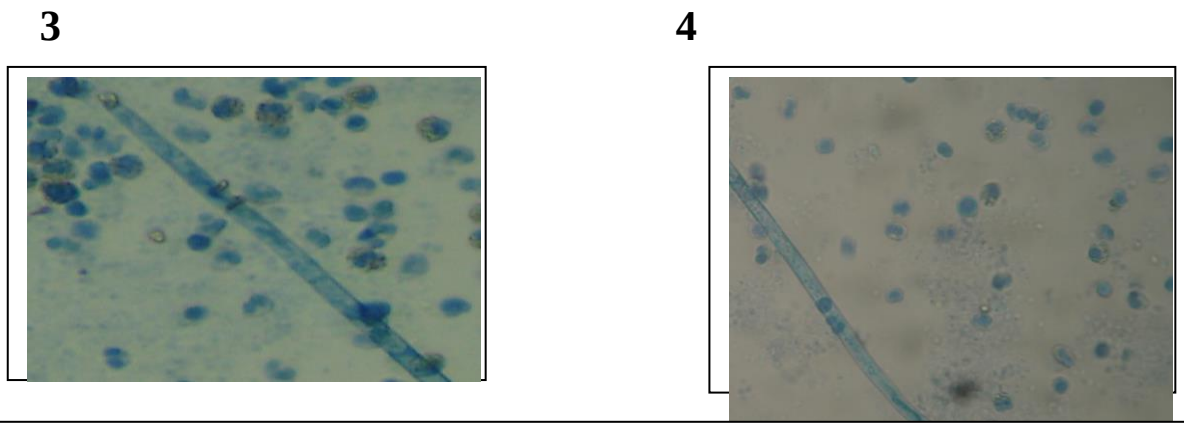
RESULTS AND DISCUSSION

The parasitological examination of 32 live dog blood samples revealed that 2 (6.25%) of the animals tested positive for microfilariae by wet smear (Fig.1) and giemsa staining (Fig.2). Samples examined using the modified Knott's approach had a greater diagnostic sensitivity (12.5%) than those examined using wet smear microscopy or geimsa staining (6.25%). The length of the larvae ranges from 309 µm to 317 µm, had a conical front end and a narrow, straight back end, which is very suggestive of *D. immitis* (Fig. 3 and 4). The prevalence of male is 9.38% (3/32) and female is 3.13% (1/32). Based on the scant number of research it is revealed that *D. immitis* is indigenous to northeastern India and D. repens to southern India. Prevalence of *D. immitis* ranges from 3% to 57% (Megat Abd Rani *et al*., 2010a; Megat Abd Rani *et al*., 2010b; Borthakur *et al*., 2015) and *D. repens* was from 7% to 21% (Malatesh *et al*., 2019; Ananda *et al*., 2006). In a study by Rimal *et al*., (2021) reported 19.3% microfilaraemia of *D. immitis* from Nepal. Numerous researchers (Ionică *et al*., 2016; Hosseini *et al*., 2022) indicated that the kinetics of microfilaremia exhibited comparable patterns for *Dirofilaria* species. All four of the positive cases had *D. immitis* at all sampling periods, with multiple peak microfilaremia values of which one was common for all dogs between11 pm to 1 am, while minimum counts occurred between 6 am to 8 am.

CONCLUSION

Considering that *D. immitis* is a zoonotic parasite, the application of ectoparasiticides and repellents is highly recommended for dogs. Strict hygienic measures including sanitation and waste management, control programs of stray dogs and adequate control of mosquitoes are urgently demanded to reduce the potential threat represented by *D. immitis*





Recovered microfilariae of *Dirofilaria immitis*. **1.** The whole microfilariae indicated by the arrows in wet blood smear. **2 .**The whole microfilaria with × 10 stained by giemsa stain. **3** The Anterior extremity of the microfilaria stained with modified Knott's method **4**The posterior extremity of D. *immitis* microfilaria.

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