Acta Scientific Veterinary Sciences (ISSN: 2582-3183)

Volume 7 Issue 11 November 2025

Research Article

Prevalence and Molecular Identification of *Dirofilaria Repens* in Dogs from the Suburban Region of Mumbai, India - A Retrospective Survey

Sundarachelvan S*, Nishith Gokharn, Sanjana Karwe, Vaishnavi Degloorkar, Mansi Tulpule, Komal Gaikwad and Sunil Kanauliya

The Progressive Pet Clinic, Vasai West, Maharashtra, India

***Corresponding Author:** Sundarachelvan S, The Progressive Pet Clinic, Vasai West, Maharashtra, India.

Received: October 08, 2025

Published: October 30, 2025

Abstract

Dirofilaria repens is a vector borne zoonotic filarial nematode that typically inhabits the subcutaneous tissues of canine. In this study, blood smear examination of 80 dogs were examined. Microfilaria were incidentally detected in the smears of five dogs. Polymerase Chain Reaction (PCR) was performed on five samples and to identify the microfilariae- and all the five samples were identified as D. repens. The prevalence of D. repens in the suburban region obtained in this study is 6.25%. Dogs are the potential mode of transmission for the spread of D. repens in humans, and thus this study attempts to throw light on the zoonotic significance of occurrence of D. repens and the necessity for essential prevention and control measures.

Keywords: *Dirofilaria repens*; Zoonoses; Dirofilariasis; Dogs

Introduction

Dirofilariasis is a severe disease condition in dogs, mainly caused by Dirofilaria immitis, which is transmitted through the bite of mosquitoes of Anopheles, Aedes and Culex species. Another species of the same genus, *Dirofilaria repens* is a helminthic zoonotic parasite, which resides in the subcutaneous connective tissues, mainly of dogs. Transmission of the infective stage of *Dirofilaria repens* occurs when an infected mosquito vector feeds on dogs or other hosts, including humans. The parasite exhibits a complex life cycle, with dogs serving as the definitive host and mosquitoes as intermediate hosts [8]. In dogs and humans, adult worms inhabit the subcutaneous connective tissues [15]. Female worms release microfilariae into the bloodstream of the host [20]. In humans, however, the parasite fails to develop to the adult stage and remains as an immature form, sometimes causing visceral larva migrans and the formation of subcutaneous nodules [4].

Historically, adult worms found in canine subcutaneous tissue were often misidentified as *D. immitis* until the parasite was properly classified in 1911 [1]. *D. repens* is the only recognized filarial worm infecting dogs in Sri Lanka, whereas both *D. repens* and *D. immitis* have been reported in India [2]. Cases of *D. repens* infection in dogs have been documented in Southern India [6,20] as well as

in Assam [17]. To date, there have been no published reports of canine dirofilariasis in Maharashtra. In a study conducted in Kerala, peripheral blood smear examination revealed *D. repens* infection in 7% of dogs examined [5].

With regards to human cases, the clinical signs of *D. repens* was observed in a 3-year old girl in the year 1566 [4]. More than 1,700 human cases of dirofilariasis have been documented worldwide till date, suggesting that wherever canine dirofilariasis is present, humans are at risk of infection [18].

There are previous reports of *Dirofilaria immitis* in Maharashtra, and the first report was from Mumbai by Badhe and Sane, in 1989 [21]. However, as per the literature, so far there has not been any documented literature of occurrence of *Dirofilaria repens* in Maharashtra, especially in Vasai-Virar region. In this article, we document five cases of zoonotic subcutaneous dirofilariasis in a dog caused by *D. repens in* Vasai-Virar region in the outskirts of Mumbai.

Materials and Methods

Blood samples from dogs submitted to the author's private service (The Progressive Pet Clinic, Vasai West, Mumbai, India) were examined over a period of two years from May 2023 to May 2025. Approximately 2 ml of blood was collected from the cephalic vein of 80 dogs exhibiting various clinical signs. The blood samples were not collected from healthy animals, but only collected from animals showing clinical signs such as vomiting, anorexia and abdominal distension. Details of the animals which showed positive results for microfilaria are summarized in Table 1. The sampling method employed in this study is convenience sampling as all the samples were collected only from the dogs presented to The Progressive Pet Clinic, Vasai West, Maharashtra.

Blood smears were prepared on clean glass slides and stained using Field's stain. Field's stain, a Romanowsky-based technique, utilized two dyes: Field's stain A (a dark purple solution containing methylene blue and azure in phosphate buffer) and Field's stain B (an orange solution of eosin Y in phosphate buffer). Each smear was immersed in Field's stain B for 5 to 6 seconds, gently rinsed with tap water, then stained with Field's stain A for 10 to 30 seconds, followed by air drying. The slides were examined microscopically under 100x magnification, following the protocol described by Mbassi., *et al*. [3].

For molecular analysis, blood samples that detected microfilariae were submitted to Elango Labs for PCR-based detection and characterization of the parasites. Each PCR reaction comprised 5 µL of 2x Promega GoTaq Green Master Mix, 1 µL each of 10 µM forward and reverse primers (IDT), 1 µL of template DNA (normalized to 50 ng/µL), and 2 µL of nuclease-free water. Initial testing for *Dirofilaria immitis* involved cycling conditions of 95°C for 5 minutes (initial denaturation), followed by 29 cycles of 95°C for 30 seconds, 58°C for 30 seconds, and 72°C for 45 seconds, concluding with a final extension at 72°C for 5 minutes. After negative results for *D. immitis*, further PCR for dirofilarial species was performed under similar cycling, with the annealing temperature set at 54°C. PCR products were separated on 2% agarose gels (SeaKem® LE Agarose, Lonza) prepared in in-house TAE buffer and run using a Bio-Rad Wide Mini-Sub Cell GT Cell electrophoresis unit. Gels were stained and visualized with the GELSTAN gel documentation system (Medicare), and a 100 bp DNA Ladder RTU (GeneDirex, Inc.) was used as a molecular size marker [19].

Results

From May 2025 to August 2025, a total of 80 blood smear examinations from dogs were performed in The Progressive Pet Clinic, Vasai west. Out of this, filarial worms were detected in the blood smears of 5 dogs (6.25%) in the locality of Vasai west, Maharashtra. Based on the morphology observed in the microscopic view, *Dirofilaria repens* was suspected. Furthermore, molecular confirmation of the parasite was done by Polymerase chain reaction.

Table 1: Details of dogs included in the study with their clinical presentation.

Case	Details of the dogs					Anamnesis and Clinical presentation	Location	Microfilaria detection
	Name	Age	Sex	Breed	Vaccination status			
1.	Dog 1	8 years	M	Doberman	Not vaccinated	Presented for an episode of vomit for three days, lethargy, inappetence and stiff gait noticed.	Sonarbhat Papdy, Vasai west	Yes
2.	Dog 2	3 years	F	Cross breed	Not vaccinated	Presented for growth inside vagina, which was diagnosed as Canine Transmissible Venereal Tumour (CTVT). Chemotherapy treatment was provided.	Thane	Yes
3.	Dog 3	10 years,2 months	F	Labrador	Not vaccinated	Presented for anorexia and abdominal distension for the past 4 days. Ultrasonography showed severe splenomegaly.	Bhabila, Vasai west.	Yes
4.	Dog 4	12 years, 2 months	M	German Shepherd	Vaccinated	Came in with abdominal effusion. Fine needle aspiration cytology of splenic mass showed the presence of *Dirofilaria.*	Tarakhol kohli, Vasai west.	Yes
5.	Dog 5	6 years, 5 months	M	Cocker spaniel cross	Non- vaccinated	The dog came in for inappetence in the past 3 days. Had ticks on body. Suspected for tick borne infection.	Near D'mello house.	Yes

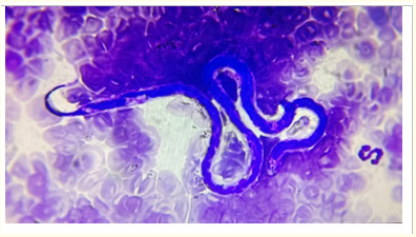

Figure 1: Detection of *D. repens* in blood smear of Dog 2.

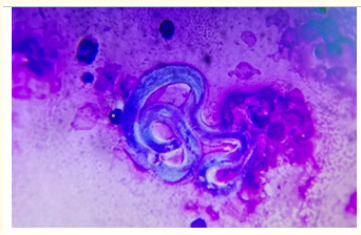

Figure 2: Detection of *D. repens* in blood smear of Dog 3.

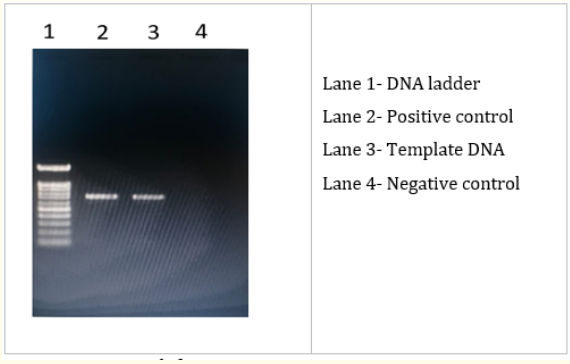

Figure 3: PCR result for Dog 4.

Discussion

The prevalence of filariasis due to various species of *Dirofilaria* is steadily increasing in different parts of India. As per the literature, there is no previous documentation of occurrence of *D. repens* in Maharashtra. In the present study, *D. repens* has been accidentally detected in five out of the 80 dogs presented to The Progressive Pet Clinic, Vasai, Maharashtra. In this study, the prevalence of *Dirofilaria repens* was 6.25%. Filariasis in dogs has been reported with the prevalence of 7.00% (n = 160) and 21% (n = 400) respectively in Kerala and Karnataka, due to the causal agent *D. repens* [16].

The microfilariae of *D. repens* were significantly greater ($P < 0.001$) in all measured dimensions except for the length of the cephalic space, which was significantly shorter ($P < 0.001$) than that of *D. immitis*. The cephalic space of *D. repens* was characterized by being short and routinely being terminated by a distinct pair of nuclei that were separate from the remaining somatic nuclei of the microfilaria. The cephalic space of the smaller microfilaria of *D. immitis* was longer and did not have the distinct nuclei separated from the somatic column nuclei near the anterior end. The character of the cephalic space seems to be a criterion that could be routinely used for the easy differentiation of these 2 microfilariae in stained blood films [14]. The external longitudinal ridges of the cuticle are the feature that readily differentiates between the *D. immitis* from *D. repens* [22].

Dogs can serve as reservoirs for humans, leading to the spread of the parasite through infected dogs, with the associated infections in humans in areas where the parasite was not previously native. The reasons for the parasite's rapid spread may include global warming and globalization, which have resulted in an expansion of the mosquito vector range, as well as the importation of pets from and the travel of pets to southern countries [4].

Sabu., *et al*. [5] have thrown light on the zoonotic importance of *Dirofilaria repens* infection and detected it in subcutaneous tissue of 12 humans as well as in blood smears of 11 out of 160 dogs from Mannuthy, Kerala. Kotigadde., *et al*. [6] have reported a case of solitary subcutaneous dirofilariasis of the eyelid due to *D. repens* in a 47-year-old woman in Karnataka.

The present study throws light on the occurrence of sub clinical *D. repens* infection in dogs and its potential to serve as transmission risk to humans. The diagnosis of *D. repens* in dogs is difficult because the dog doesn't show any specific symptoms and clinical signs pertaining to the presence of infection. Alongside, even in humans, the symptoms of *D. repens* infection is very non-specific depending upon the location of the worm, thus averting the effective diagnosis. Subcutaneous nodules are produced in humans, and ocular lesions may lead to damage to eyesight. Thus, there is a need for an effective surveillance of *D. repens* in dogs, which act as a potential reservoir for the spread of worms to humans.

Zoonotic cases of dirofilariasis have increased in the last decades paralleling the presence in endemic areas and also correlating with the new foci of infection in previously non endemic areas. This indicates that in areas where canine dirofilariasis is present, humans are also exposed to the infection, suggesting the importance of One Health approach in diagnosing, treating and controlling this zoonotic parasitosis [7]. The prevalence of *D. repens* in Vasai-Virar region could be attributed to the increased stray dog population in the region in recent years, as dogs are the potential reservoirs of the parasite and they can transmit the agent to other dogs and humans. Furthermore, unplanned land use and water stagnation enhance the mosquito breeding sites. Changes in local ecology facilitate higher rates of vector-borne parasitic diseases such as dirofilariasis in Vasai-Virar and similar regions. Increasing urbanization may have also exacerbated vector spread, raising transmission risks in new regions.

The prevention of the filarial infections is crucial as the treatment of infected animals is expensive and cumbersome. Alongside, the zoonotic potential of *D. repens* infection and the sub-clinical cases in pets observed in this study underscore the importance of the prevention and control of spread of the filarial worms. In countries endemic for dirofilaria, there acts a protocol according to which all dogs that are 8-month-old (and not older than that) undergo thorough screening and, in case the infection is not detected, they go through the prophylactics with macrocyclic lactones on a yearly basis during their whole life [13]. Macrolytic lactone drugs

have been widely used in the past three decades for the prevention of D. immitis, but most of the filarial worms are now resistant to these drugs [12]. Many species of mosquitoes can serve as vectors of the parasite, and at least one species of vector mosquitoes in any suitable climate can live in even a small amount of water, either polluted or clean [9]. The American Heartworm Society canine guidelines recommend that environmental control measures, including treatment of standing water sources with insect growth regulators (IGR) combined with mosquito adulticidal measures should be strictly followed in order to effectively control the spread of microfilarial worms. In addition to mosquito control, keeping pets inside during peak mosquito hours and/or the use of mosquito repellents on pets may also reduce the risk of infection. Furthermore, adequate preventive treatment for pets covering the whole of the transmission season is crucial for effective prevention, and a year-round approach is often the best option because of climatic differences in humidity levels having a great impact on the risk of exposure for susceptible hosts [11].

As this study involves only the dogs presented to The Progressive Pet Clinic, the sample size is less and the method of sampling is not random. But still this evidence is significant enough as a first report of prevalence of *D. repens* in dogs of Vasai-Virar region. As a future prospect of this study, an active surveillance screening can be done to know the exact prevalence of *D. repens* among the dogs of Vasai- Virar and adjoining regions. Additionally, the distribution of vector responsible for the transmission of disease can also be researched upon to trace back the entry of agent and formulate prevention and control strategies accordingly.

Bibliography

1. Perez-Sanchez R., *et al*. "Canine filariasis in Salamanca (northwest Spain)". *Annals of Tropical Medicine and Parasitology* 83 (1989): 143-50.

2. Dasanayake R., *et al*. "A study on canine dirofilariasis in selected areas of Sri Lanka". *BMC Research Notes* 15 (2022): 137.

3. Mbassi F A E., *et al*. "Performance of Field's stain compared with conventional Giemsa stain for the rapid detection of blood microfilariae in Gabon". *The American Journal of Tropical Medicine and Hygiene* 107.2 (2022): 383.

4. Capelli G., *et al*. "Recent Advances on Dirofilaria repens in Dogs and Humans in Europe". *Parasites Vectors* 11 (2018): 663.

5. Sabu L., *et al*. "Dirofilariosis in dogs & humans in Kerala". *Indian Journal of Medical Research* 121.5 (2005): 691.

6. Kotigadde S., *et al*. "Human dirofilariasis due to Dirofilaria repens in southern India". *Tropical Parasitology* 2.1 (2012): 67-68.

7. Mendoza-Roldan JA., *et al*. "Zoonotic Dirofilaria immitis and Dirofilaria repens infection in humans and an integrative approach to the diagnosis". *Acta Tropica* 223 (2021): 106083.

8. Kini R G., *et al*. "Human dirofilariasis: an emerging zoonosis in India". *Journal of Parasitic Diseases* 39.2 (2015): 349-354.

9. McCall JW., *et al*. "Shifting the paradigm in Dirofilaria immitis prevention: blocking transmission from mosquitoes to dogs using repellents/insecticides and macrocyclic lactone prevention as part of a multimodal approach". *Parasites and Vectors* 10.2 (2017): 525.

10. American Heartworm Society (AHS). "Current canine guidelines for the diagnosis, prevention and management of heartworm (Dirofilaria immitis) infections in dogs". (2014).

11. Pennisi MG., *et al*. "Dirofilarioses in cats: European guidelines from the ABCD on prevention and management". *Journal of Feline Medicine and Surgery* 22.5 (2020): 442-451.

12. Prichard R K. "Macrocyclic lactone resistance in Dirofilaria immitis: risks for prevention of heartworm disease". *International Journal for Parasitology* 51.13-14 (2021): 1121-1132.

13. Bespalova N., *et al*. "Modern Methods of Etiotropic Therapy and Preventive Measures in Dogs Suffering Dirofilariasis". In International scientific and practical conference" Agro-SMART-Smart solutions for agriculture" (Agro-SMART 2018) (2018): 90-94.

14. Liotta JL., *et al*. "Differentiation of the microfilariae of Dirofilaria immitis and Dirofilaria repens in stained blood films". *The Journal of Parasitology* 99.3 (2013): 421-425.

15. Megat Abd Rani P A., *et al*. "Canine vector-borne diseases in India: a review of the literature and identification of existing knowledge gaps". *Parasites and Vectors* 3.1 (2010): 28.

16. Das NS., *et al*. "First Case of Dirofilariasis in a Dog From Goa, India". *Indian Journal of Veterinary Sciences and Biotechnology* 17.2 (2021): 105-107.

17. Borthakur SK., *et al*. "Occult dirofilariosis in dogs of North Eastern region in India". *Journal of Arthropod-Borne Diseases* 10.1 (2015): 92.

18. Montoya-Alonso J A., *et al*. "Canine dirofilariosis caused by Dirofilaria immitis is a risk factor for the human population on the island of Gran Canaria, Canary Islands, Spain". *Parasitology Research* 107.5 (2010): 1265-1269.

19. Smith R., *et al*. "Nationwide molecular survey of Dirofilaria immitis and Dirofilaria repens in companion dogs and cats, United States of America". *Parasites and Vectors* 15.1 (2022): 367.

20. Chirayath D., *et al*. "Molecular characterization of 5S ribosomal spacer sequences of Dirofilaria repens microfilariae in dogs in Kerala, India". *Journal of Parasitic Diseases* 44.4 (2020): 864-868.

21. Badhe BP and Sane SY. "Human pulmonary dirofilariasis in India: a case report". *The Journal of Tropical Medicine and Hygiene* 92.6 (1989): 425-426.

22. Khedr S., *et al*. "Scanning electron microscopy as a confirmatory tool for morphological identification of Dirofilaria (Nochtiella) repens". *Parasitologists United Journal* 14.1 (2021): 22-28.