

Research Note

Dirofilaria repens in scrotum of dogs

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Received 12 March 2016; received in revised form 10 July 2016; accepted 12 July 2016

Abstract. Out of 150 apparently healthy male dogs from Kerala, India examined, five revealed single worm in the scrotum. They were found protruding out from beneath the tunica vaginalis when it was incised during the castration operation. Genomic DNA isolated from these worms amplified *D. repens* specific 246 bp PCR products (KP050552, KP050553). Thickened and enlarged epididymis along with interductal fibrosis, congestion of veins and widening of the cavernous spaces of testes and epididymis were observed in the worm infected dogs. Semeniferous tubules were intact and showed various developmental stages of sperms. The infection resulted in less impact on spermatogenesis. The present communication forms the first report from India on the presence of *D. repens* in the peritoneal location, which is frequently observed in the subcutaneous locations.

Dirofilariosis is a mosquito borne infection caused by parasites of the genus *Dirofilaria*, which includes many species, viz., *Dirofilaria repens*, *D. immitis*, *D. tenuis*, *D. ursi* etc. *D. repens* is a filarial nematode usually observed in the subcutaneous connective tissue of dogs and other carnivores (Soulsby, 1982). Cases of zoonotic dirofilariosis were reported from different regions of world including Kerala, India (Pampiglione *et al.*, 2001, Sabu *et al.*, 2005). This parasitic infection resulted in subcutaneous, subconjunctival and ocular tumors in human beings (Mukherjee *et al.*, 2012). Mosquitoes of the genera *Culex*, *Anopheles*, *Aedes* and *Armigera* are considered as suitable vectors for the parasite (Anyanwu *et al.*, 2000). Species specific detection of different filarial worms based on morphology is difficult, especially for those under the genus *Dirofilaria*. The present study describes the occurrence and

molecular confirmation of the filarial nematode, *D. repens* in the scrotum of dogs. The pathological changes associated with the presence of the worm in the scrotum which can affect the reproductive performance of the adult male dogs were also investigated.

Castration operations were performed in a total of 100 apparently healthy male stray dogs as a part of the Animal Birth Control programme at Veterinary Hospital, Pettah, Thiruvananthapuram during the period August-September, 2013. Similarly, 50 operations performed at Veterinary Hospital, Puthiyedam, Kanjoor, Ernakulam during the same period were also included in the study. Out of 100 dogs examined at Thiruvananthapuram, worms were encountered in the scrotum of three animals during the castration operations. Similarly, out of 50 dogs examined at Ernakulam, two revealed the presence of worms in the

scrotum. Worms were collected for morphological identification and molecular confirmation. The genomic DNA from the whole worm was isolated (Sambrook & Green, 2012). Amplification of 246 bp repetitive fragment specific for *D. repens* (Vakalis *et al.*, 1999) using primers [Forward: 5'-CCGGTAGACCATGGCATTAT-3' and Reverse: 5'-CGGTCTTGGACGTTTGGTTA-3'] was performed. The PCR assay was carried out in 25 µL volume containing 2.5 µL 10X PCR buffer (Genei, Bangalore), 1 µL (0.25 mM) dNTP, and 20 pmol of each primer, 1.5 U *Taq* polymerase and 5 µL of template DNA. Reaction conditions were as follows: Initial denaturation at 94°C for 5 minutes followed by 40 cycles each with 94°C for 30 seconds, 55°C for 30 seconds and 72°C for 5 minutes. After sequencing of the PCR products, homology search was made using NCBI BLAST. Tissue specimens were collected from testes and epididymis and preserved in 10 per cent buffered neutral formalin for histopathological examination (haematoxyline-eosin staining) after making paraffin sections of 5 µm thickness.

Worms (>7 cm long) were single and noticed protruding out from beneath the tunica vaginalis when it was incised during the surgical procedure (Fig. 1). They were morphologically identified as *Dirofilaria repens*. Two PCR products out of five positive cases were sequenced and the data submitted to GenBank (KP050552, KP050553). Homology search performed using NCBI-BLAST revealed ninety per cent homology among these accessions. Previously reported *D. repens* isolates from Kerala (JN830762, JQ706073) also revealed 90 per cent identity in comparison to these isolates. Also, 89 per cent identity was observed with an already published sequence of *D. repens* (L15323). Grossly, the epididymis of testis was thickened and enlarged. Histopathological examination of testes and epididymis revealed interductal fibrosis, congestion of veins and widening of the cavernous spaces. However, semeniferous tubules were intact and showed various developmental stages of sperms (Fig. 2).

In a cross-sectional study conducted (Rani *et al.*, 2010) to determine the prevalence and geographical distribution of canine filarial species in India, an overall prevalence of 9.3 per cent was observed for *Acanthocheilonema reconditum*, 6.7 per cent for *D. repens* and 1.5 per cent for *D. immitis*. They concluded that the most common filarial species of canines in India were *A. reconditum* and *D. repens*. Based on the available literature, it is accepted that *D. immitis* is geographically restricted to India's north-east and *D. repens* to south (Gogoi, 2002; Ananda *et al.*, 2006). Studies on prevalence rate of microfilariae in domestic dogs conducted at different regions of Kerala, a south Indian state (Sabu *et al.*, 2005; Saseendranath *et al.*, 1986; Radhika, 1997; Ravindran *et al.*, 2014) revealed values ranged between 7 to 42.68 per cent. Even though *D. repens* is considered as the most common cause for zoonotic dirofilariasis in Kerala (Sekhar *et al.*, 2000; Sabu *et al.*, 2005), very few cases of human subcutaneous dirofilariasis due to this parasite were reported from other Indian states (Nadgir *et al.*, 2001; Gautam *et al.*, 2002).

Little information is available on the pathogenesis of *D. repens* infection in dogs. *D. repens* infection in dogs is usually asymptomatic and adult parasite reside in painless subcutaneous nodules (Bredal *et al.*, 1998). Presence of adults and/or microfilariae in the skin produces apparent dermatological signs (Kamalu, 1986; Zivicnjak *et al.*, 2006). Clinical manifestation of *D. repens* infections in canines is classified under two syndromes *viz.*, nodular multifocal dermatitis (Scott & Vaughn, 1987) and development of several pruriginous papules (Halliwell & Gorman, 1989). In massive infection with adult worms and in cases of high microfilaremia, gross and histopathological changes in visceral organs (especially in spleen, liver, kidneys, lungs, heart and brain) were reported (Restani *et al.*, 1962; Mandelli & Mantovoni, 1966; Kamalu, 1991).

D. repens is frequently observed in the subcutaneous locations. However, in the present study, the occurrence of the *D. repens*



Figure 1. *Dirofilaria repens* protruding out from beneath the tunica vaginalis.

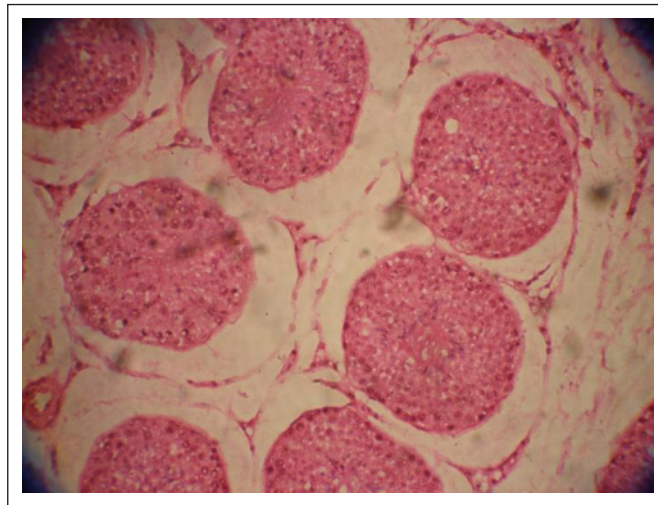


Figure 2. Cross section of testis showing spermatogenesis (H&E, 200x).

in the peritoneal cavity of dogs was unequivocally identified. Based on available literature the present communication forms the first report from India on *D. repens* in the scrotum of dogs. Moreover, based on the results of histopathology of infected testes, it was clearly identified that the infection produced less impact on spermatogenesis. However, fibrosis of epididymis resulted due to the presence of the parasite in the scrotum

can cause damaging effects for storage of sperms and other functions of epididymis.

The occurrence of *D. repens* in the human scrotum was first reported from Italy by Pampiglione *et al.*, 1982. Later, reports on the presence of the *D. repens* in the scrotum came from France (Nozais & Huerre, 1995; Leccia *et al.*, 2012), Sri Lanka (Dissanaike *et al.*, 1997), Tunisia (Soussi *et al.*, 2004; Fleck *et al.*, 2009) and India

(Singh *et al.*, 2010). The first canine scrotal dirofilariosis was reported from Poland (Demiaszkiewicz *et al.*, 2009). These dogs also did not show any pathological symptoms of infection.

Previously, two types of histochemical patterns in the microfilaria of *D. repens* were documented in dogs from Kerala (Ravindran *et al.*, 2014). A single red spot at anal pore region is the characteristic feature of *D. repens* microfilaria. However, a second pattern with local staining at the anal pore and diffuse staining in the central body was observed in 35.4 per cent of cases examined. The authors identified both forms as *D. repens* based on molecular techniques. It is not yet clear whether the parasites in the peritoneal locations show a different type of histochemical reaction for their microfilariae.

Acknowledgements. Financial support from Kerala State Council of Science and Technology and Environment (020/SRSAGR/2006/CSTE, 010-14/SARD/13/CSTE) is thankfully acknowledged.

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