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Unusual localization of *Dirofilaria repens* (Spirurida: Onchocercidae) infection in the testicle of a dog

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ABSTRACT

This report describes an uncommon localization of *Dirofilaria repens* in the testicle of a nine-year-old dog from southern Italy. The dog underwent neutering and three adult nematodes were found in the *tunica vaginalis*. At gross anatomy, no pathological changes were observed on the tunica, in the testicle or epididymis. The parasites, one female and two males, were morphologically and molecularly identified as *D. repens*. This is the first report of *D. repens* in the canine testicle in Italy. This case report highlights the asymptomatic nature of *D. repens* infection, suggesting that dogs living in endemic areas may act as silent carriers. Careful screening and an effective chemoprophylaxis should be ensured for all animals potentially exposed to *D. repens* infection in order to reduce the risk of infection to humans in areas where the parasite is endemic.

1. Introduction

Dirofilarioses are vector-borne parasitic diseases caused by spirurid nematodes belonging to the genus *Dirofilaria*, being *Dirofilaria immitis* and *Dirofilaria repens* the most important species causing canine dirofilariosis [1]. The former species is the agent of a severe disease known as canine heartworm disease, while the latter is usually associated with a non-pathogenic subcutaneous localization of adult worms. In spite of its low pathogenic significance in dog, *D. repens* causes relevant public health concern. It is in fact considered the principal agent of human dirofilariosis in the Old World [2–4].

In the last decades the distribution of *D. repens* has significantly spread throughout Europe [5] and the parasite has colonized new geographical areas with new clinical cases increasingly reported in both dogs and humans, thus becoming a paradigmatic example of an emergent pathogen [6,7].

In the definitive hosts (e.g. dogs, foxes, wolves and coyotes), the adults of *D. repens* localize mainly in the subcutaneous connective tissues [6]. The lack of clinical symptoms in most of the dogs infected by *D. repens* coupled with the difficulties in diagnosing the infection (e.g., absence of serological tests) account for the low awareness of the disease by veterinary practitioners. In addition, the invasion of alien mosquito species that may act as competent vectors for *D. repens* (e.g. *Stegomyia albopicta*) has led to the spreading of *D. repens* infection all

over Europe, especially in eastern countries. In addition to the subcutaneous localization, uncommon localizations of adults of *D. repens* in dogs have been reported in the pelvic and mesentery cavities [8], in the eyes and subconjunctive [8–11] and a few reports in the gonads [12–14].

In the present report, we describe the presence of three adults of *D. repens* in the testicle of a stray dog from Calabria (southern Italy). This case represents one of the few descriptions of testicular localization of *D. repens*, the first in Italy. While reporting this uncommon localization, we stress on the importance of a proper inclusion of this parasitosis in the diagnostic and preventive protocols of dogs at risk towards a better control of the infection in canine and human patients.

2. Materials and methods

A nine-year-old male, mixed-breed medium size (25 kg) dog, kept in the shelter of Taurianova (a municipality nearby the Aspromonte National Park, province of Reggio Calabria, Calabria region), was presented for neutering on 23 January 2019 in compliance with the Italian law on stray animals (DL 281/91). During the surgery, the presence of whitish worms on the surface of the testicle was noticed. Because of the presence of worms, the testicles were preserved refrigerated (+4 °C) and sent to the unit of Parasitology of the Department of Veterinary Sciences (University of Messina) for speciation. The dog underwent

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physical examination and faecal and blood samples were collected. The faecal sample was analysed using the Baermann-Wetzel technique [15] and flotation technique, using a solution of sodium nitrate and sugar (1.300 N/m³ SPG) for the detection of endoparasites [16]. The presence of circulating microfilariae was assessed using a modified Knott's test [17], and a complete cell blood count (CBC) and blood chemistry were also performed using automated analysers (Benesphera **H32VET, Avantor Performance Materials Inc., Center Valley, PA, USA and Chem 7, Catalyst DX, IDEXX laboratories, Inc., Westbrook, Maine, United States, respectively). Additionally, the presence of specific antibody anti *Leishmania infantum* was checked using a commercial ELISA test (ID Screen[®] Leishmaniasis indirect, IDvet, Grabels, France).

In laboratory, the testicle was examined under a stereomicroscope and the nematodes gently removed and classified at species level using morphometrical keys [18,19]. Also, in order to support morphological identification, a central fragment of the body of the nematode was analysed with a specific quantitative PCR test developed for *D. immitis* and *D. repens* detection [20].

3. Results

The dog was hosted in the shelter of Taurianova, where it shared the pen with other 4 dogs, and had never been treated against endo- or ectoparasites since its arrival in May 2013. Upon physical examination, the nutritional status was poor (body condition score of 3/9), vital signs and others clinical parameters (heart/ pulse rate, respiratory rate, CRT and temperature) were within the physiological standards of the species and neither clinical signs nor skin alterations (e.g., dermatitis or cutaneous/subcutaneous nodules) were detected. The parasitological examinations on the faecal samples yielded negative results. Circulating microfilariae with an estimated load of 12 microfilariae per ml of blood were detected. Microfilariae (Fig. 1) measured $369.4 \pm 10.7 \mu\text{m}$ in length and $8.87 \pm 0.58 \mu\text{m}$ in width, and were classified as *D. repens* [21].

The dog tested seronegative for *Leishmania*; CBC and blood chemistry values were within the species ranges, though the number of white blood cells was slightly increased (Table 1).

Upon gross anatomy, three elongated nematodes were found in the left testicle (Fig. 2A), in particular two parasites were observed in the cavity of *tunica vaginalis*, while a third nematode was observed within the parietal layer (Fig. 2B). No degenerative or flogistic change of the *tunica vaginalis*, testicle and epididymis was observed. The nematodes were two males and one female. The males were 6.2–6.5 cm long and 1–1.2 mm wide, while the female was 14.3 cm long and 1.5 mm wide (Fig. 2C). Both males and female were characterized by a rounded whitish body with the cuticle featured by distinct longitudinal ridges on

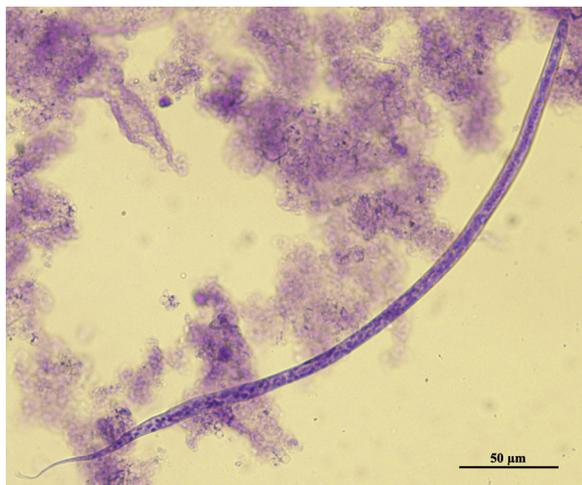


Fig. 1. Microfilaria of *Dirofilaria repens*.

Table 1

Haematological and biochemical parameters of the dog infected with *Dirofilaria repens*. The parameter out of the reference range for the specie was indicated with *.

Parameter	Value	Reference Range
WBC	19.9 K/ μl *	6.0–16.0
RBC	6.25 M/ μl	5.50–7.90
Hgb	13.2 g/dL	12.0–18.0
Hct	42.1 %	37.0–55.0
MCV	67 fL	60–76
MCH	21.0 Pg	20.0–27.0
MCHC	31.2 g/dL	32.0–38.0
RDW	16.2 %	12.0–16.0
Plt	333 K/ μl	240–400
PDW	13.8%	
CREA	0.8 mg/ dL	0.5–1.8
BUNALT	19 mg/ dL	7–27
BUN/CREA	24	
ALT	92 U/L	10–125
AST	43 U/L	0–50
ALKP	37 U/L	23–212

the surface (Fig. 3A); the cephalic region was rounded and furnished by a rudimentary buccal capsule with small cephalic papillae. The oesophagus was differentiated into muscular and glandular regions, with no distinction between them; the digestive tract was constituted by a single tube divided into anterior (oesophagus) and posterior (intestine) parts joined by a simple junction. The caudal region of the males had a spiral, conical shape. In the anterior part of the males it was possible to observe the sexual organ composed of a single tube coiled in a spiral (Fig. 3B). The caudal region of the males was characterized by the presence of two asymmetric spicules (Fig. 3C), 0.45 mm and 0.17 mm for the left and right, respectively, and by the presence of the six caudal papillae, five pre-cloacal and 1 post-cloacal. In the female, the vulvar aperture was located immediately behind the oesophagus-intestine junction. The morphometrical features of the parasites were consistent with the species *D. repens*.

The biomolecular analysis confirmed the morphological speciation, and the obtained sequence (Accession number: MF695085) displayed a homology of 99.85% with sequences of *D. repens* deposited in GenBank[®]. After the diagnosis of *D. repens* infection, the dog was treated with a monthly administration of moxidectin 2.5%/imidacloprid 10% (Advocate[®] spot-on per cani, Bayer S.p.A.) in order to eliminate the circulating microfilariae and to control its role as reservoir of the infection [22].

4. Discussion

This case report describes the accidental finding of three adults of *D. repens* localized in the *tunica vaginalis* of a dog testicle. This localization is quite uncommon, being one of the few documented in literature [12–14] and the first reported in Italy. Commonly, *D. repens* adults are localized in cutaneous and subcutaneous tissues without any preferential anatomical district, as adults of this filarial species have been detected in different areas, from temporal region to tarsus [23]. Conversely, in humans it seems that the parasite is mostly localized in the upper half of the body (74%), in particular the ocular region (eyelid, subconjunctiva, orbit) (35.3%) and also the upper limbs (11%) [2,24,25]. Other common sites in human patients are the scrotum and the testicles [6] probably due to the lower body temperature or which the patients might detect more frequently and/or the tropism of the parasite to higher concentrations of sexual hormones [2].

In dogs, when the parasite is localized in the subcutaneous tissues, the infection is generally asymptomatic or rarely associated with the presence of prominent subcutaneous nodules [23,26]. Skin disorders, such as localized alopecia, hyperkeratosis, crusting, acanthosis, eczema, pyoderma, edema or papular dermatitis have been seldom reported in

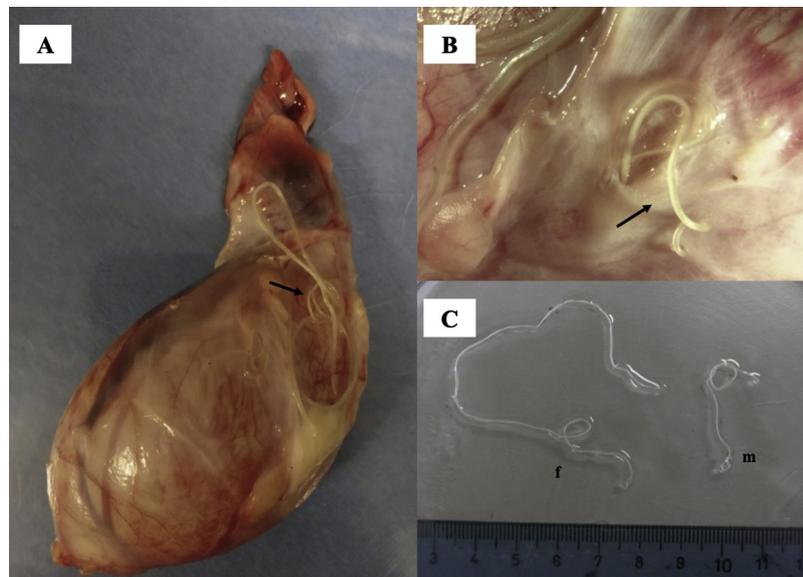


Fig. 2. A) Left testicle, note the protruding nematodes (arrow). B) Male of *Dirofilaria repens* in the thickness of the parietal layer of *tunica vaginalis* (arrow). C) Adults of *D. repens*: (f) female and (m) male.

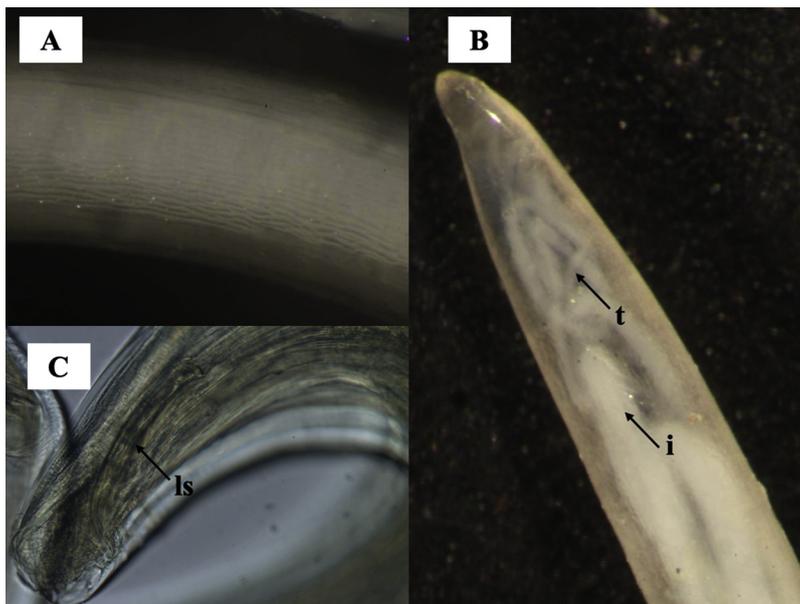


Fig. 3. *Dirofilaria repens*: A) External cuticle of *D. repens* with the typical longitudinal ridges on the surface. B) Cephalic region of the male with the sexual organ composed of a single tube that begins as testicle (t) coiled in a spiral and continuing as a seminal vesicle; (i) initial part of the intestine. C) Caudal region of the male with the asymmetric left spicule (arrow).

dogs infected by *D. repens* [27]. However, the presence of the parasite in other tissues or organs is commonly associated with alterations of the affected area. In fact, adult worms of *D. repens* have been detected in the eye of a dog reporting bulbar conjunctival mass associated with the eye and nasal mucopurulent discharge [11]; moreover, the presence of the parasite in a similar localization (i.e. dorsal bulbar conjunctiva) was associated with granulomatous nodule and conjunctivitis [10]. Other authors reported, together with the conjunctival localization, other uncommon localizations of the parasite in the pelvic and mesenteric cavity and in kidney associated with kidney failure and cystitis [8]. Similarly, in case of testicular localization, the presence of several alterations such as alopecia, erythema, pruritic rash in ventral abdomen and perineal region and nodules on the testicles were detected in infected dogs [14]. In addition, the parasite was also detected inside the testicular parenchyma associated with testicular enlargement, due to the presence of a three-centimetre-wide cyst containing the parasite and associated with a Leydig-cell tumor [12]. Another study carried out in Kerala, India, reported the presence of *D. repens* infection in the scrotum

and testicle in five out of one-hundred and fifty dogs examined [13]. In the above study, adults of *D. repens* were found in the tunical cavity following neutering surgery, and the presence of parasites was associated, in all the cases, with pathological changes of the testicles, such as thickening and enlargement of the epididymis, interductal fibrosis, congestion of veins and widening of the cavernous spaces. Despite the studies above, the localization in the *tunica vaginalis* but not in the testicular parenchyma herein reported was not associated with any pathological change. Moreover, no signs were detected in the scrotum or in the skin and subcutaneous tissues of the perineal region. This case suggests how *D. repens* infection can be completely asymptomatic in patients even if the parasite is localized in uncommon districts; thus, many animals living in endemic areas can be silent carriers of the parasitosis, though microfilaremic dogs are important reservoirs for human infection.

5. Conclusion

Data gained in this case suggest once more the fact that a careful screening for *D. repens* infection should be carried out on all animals potentially exposed, including those that have travelled to risky areas. In the same manner, efficacious chemoprophylaxis needs to be provided not only to animals that live in areas where the *D. repens* is endemic, but also to all dogs that had travelled to endemic areas, which, in turn, represents the best strategy to prevent the spread of disease and human transmission.

Conflict of interest

Nothing declared.

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