

## Granulomatous metritis caused by suspected *Dirofilaria immitis* in a dog: a case report

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**ABSTRACT:** Here we describe a unique uterine mass in a dog with granulomatous lesions caused by filarial larvae from the family Onchocercidae. An 8-year-old female Maltese was presented to a local animal hospital with anorexia, depression, and vaginal discharge. A markedly distended uterus was observed on lateral abdominal radiographs, leading to a clinical diagnosis of pyometra or uterine mass of an unknown origin. During surgery, the left uterine horn contained a 5 cm diameter mass adhered to adjacent soft tissue. On gross inspection, the mass contained numerous white nematodes. Microscopically, this mass was characterised by a granulomatous inflammation of the myometrium and endometrium. Because all of the nematodes were dead, definitive species identification was not possible. However, based on the histologic appearance, these nematodes were tentatively identified as *Dirofilaria immitis* larvae.

**Keywords:** dog; filarid; granuloma; larva; nematode; uterus

Larval parasitic migration into various host organs is an important component of the life cycle of many parasites. Various larval parasites inhabit a variety of organs, including the heart, lung and intestines, where they complete their normal life cycle and sexual maturation. However, aberrant parasite migration into atypical sites such as the nervous, reproductive, urinary, or skeletal systems can also occur and can induce inappropriate tissue injury. Several previous publications in the veterinary medical literature have reported such aberrant parasite distribution affecting the spinal cord (Gomez et al. 2010; Johnson et al. 2010), brain (Tanabe et al. 2007), kidney (Aresu et al. 2007), eye (Dantas-Torres et al. 2009) and joint spaces (Hodges and Rishniw 2008). From these reports, a variety of important differential diagnoses have evolved concerning the clinical signs and pathologic lesions that can develop due to aberrant parasitic life cycles. To the best of our knowledge, the present case represents the first report of granulomatous metritis in a dog caused by aberrant migration of nematode larva interpreted to be *Dirofilaria immitis*.

### Case description

An 8-year-old female Maltese was presented to a private animal hospital with anorexia, depression, and vaginal discharge. Serum biochemistry analysis was within normal limits, but the com-



Figure 1. Lateral abdominal radiograph showing the markedly distended uterus (arrows)

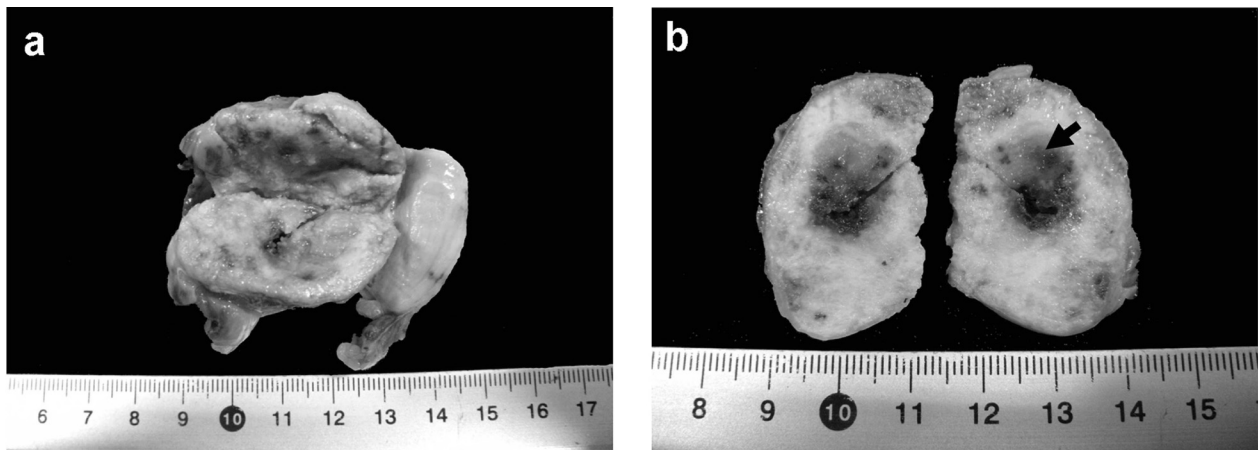


Figure 2. (a) Images of the left uterine horn. (b) Cut surface showing the necrotic centre (arrow) surrounded by a dense fibrous connective tissue capsule

plete blood count revealed leucocytosis (31 600 cells/ $\mu$ l, reference range: 6000–17 000 cells/ $\mu$ l). A *Dirofilaria immitis* antigen detection test result was negative. Based on the initial findings including lateral abdominal radiographies showing a mark-

edly distended uterus (Figure 1). Since pyometra was strongly suspected, abdominal surgery was performed. During surgery, a 5 cm mass with multifocal haemorrhage was found in the left uterine horn. Adhesions were found to the adjacent soft

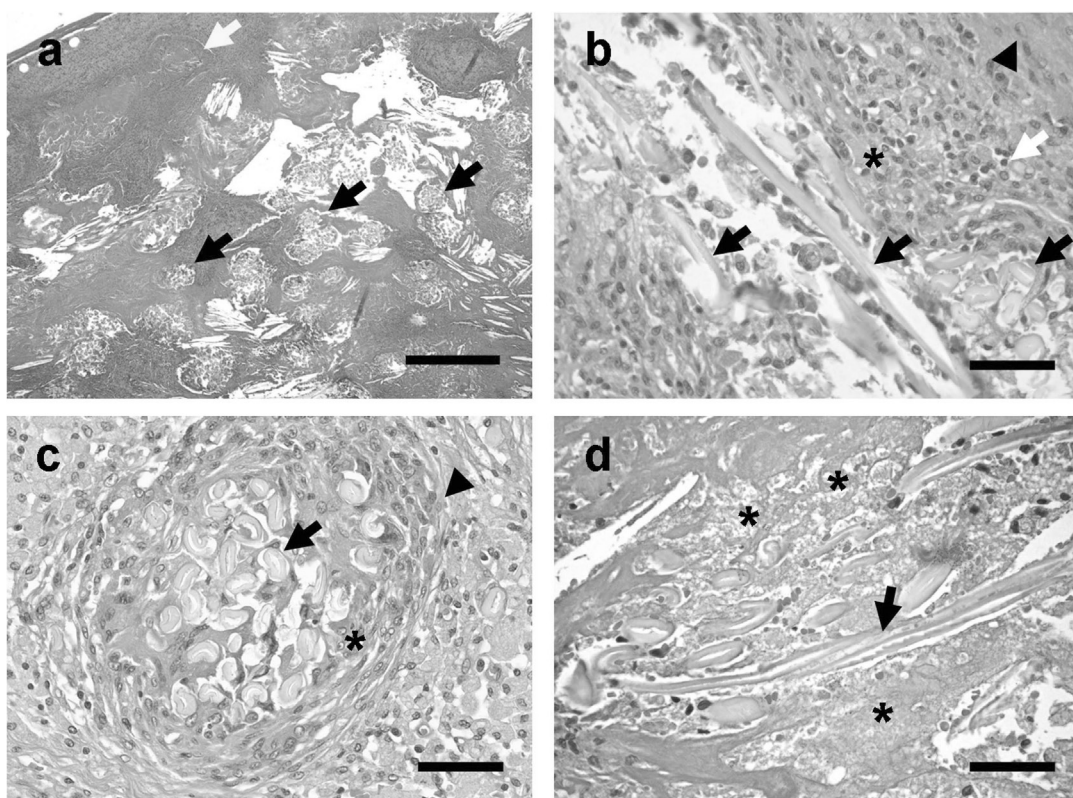


Figure 3. (a) Numerous granulomas within the endometrium and myometrium of the affected uterine horn. Bundles of tangled filarial nematode larvae (black arrow) and a newly formed granuloma (white arrow) are shown; H&E staining, scale bar = 400  $\mu$ m. (b, c) Longitudinal and cross-sections of filarial nematode larvae (arrows) surrounded by epitheloid macrophages (asterisks), lymphocytes (white arrow), and a fibrous capsules (arrow heads); H&E staining, scale bar = 50  $\mu$ m. (d) Granulomatous inflammation (asterisks) in the endometrium exhibiting extensive caseous necrosis and remnant cuticles (arrow) of the filarial nematode larvae; H&E staining, scale bar = 50  $\mu$ m

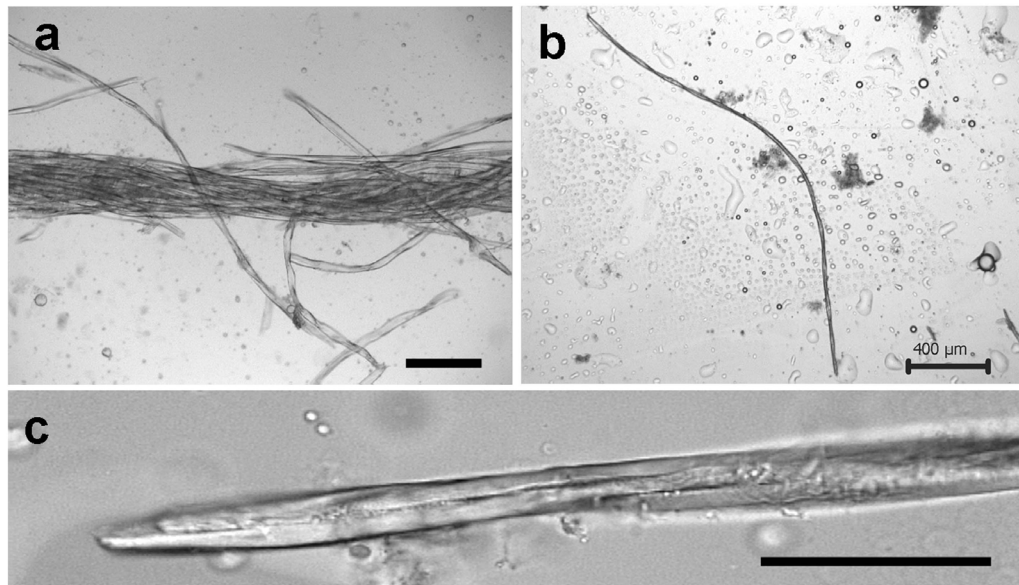


Figure 4. (a) Bundle of tangled nematode larvae isolated from the cut surface of the uterine mass; light microscopy at 100× magnification, scale bar = 200 µm. (b) Image of the filarial nematode larva characterised by a sharp, gently tapered cephalic end and a straight sharp tail; light microscopy at 50× magnification, scale bar = 400 µm. (c) The head of a filarial nematode larva showing the absence of a sheath along with sharp and gently tapered cephalic ends; light microscopy at 400× magnification, scale bar = 50 µm

tissues, including the large intestine. Due to the extensive adhesions, an ovariohysterectomy was performed. The uterus and mass were then placed in 10% neutral buffered formalin for fixation. Fixed tissue was sent to the Department of Veterinary Pathology, Kyungpook National University (Republic of Korea), and processed routinely for histopathology.

Grossly, the uterine mass was approximately 5 cm in diameter (Figure 2a) and it completely replaced the left uterine horn. On the cut surface, the uterine mass was surrounded by a fibrous connective tissue capsule encircling a necrotic centre that also contained bundles of numerous whitish tangled filarial larvae (Figure 2b). The nematodes were easily removed from the uterine mass. Histologically, the uterine mass was characterised by randomly scattered regions of granulomatous inflammation and necrosis. The uterine mass contained a marked inflammatory infiltrate of epithelioid macrophages, foreign body giant cells, Langhans giant cells, lymphocytes, plasma cells, neutrophils and fibroblasts, typical features of granulomatous inflammation (Figure 3a). Granulomas were observed in the endometrium and inner myometrium of the left uterine horn along with numerous bundles of tangled filarial larvae (Figure 3a). Cross and longitudinal sections of the nematodes were found surrounded by

a zone of necrosis and inflammation characterized by degenerate cells, cellular debris, neutrophils, and macrophages including epithelioid macrophages and multi-nucleated giant cells (Figure 3b, c). In general, the regions of necrosis were associated with the presence of degenerate and dead nematodes (Figure 3d). Fragments of degraded filarial nematodes were found within macrophages and multi-nucleated giant cells throughout the affected uterine horn.

Although the filarial larvae were in various stages of degeneration, cuticle remnants were available for identification. Individual larvae had a width of 8–15 µm and length of 1.6–2.0 mm (Figure 4a, b). The larvae had sharp, gently tapered cephalic ends and straight sharp tails (Figure 4b). A sheath was not present and transverse striation was observed on the thick cuticles. The cephalic space was short and no cephalic hook was observed (Figure 4c). These histologic features allowed us to identify the white filarid nematodes as members of the Onchocercidae family.

## DISCUSSION AND CONCLUSIONS

In the present study, we describe severe granulomatous metritis caused by filarial nematode migration in a dog. The filarial nematodes were

identified as immature larvae belonging to the family Onchocercidae based on morphological evidence such as lack of a sheath, sharp and gently tapered cephalic ends, and straight sharp tails. Onchocercidae, a family of nematodes included in the superfamily Filarioidea, contains 70–80 genera (Anderson 2000). Among the genera Onchocercidae, *Brugia malayi*, *Brugia pahangi*, *Cercopithifilaria grassi*, *Dipetalonema reconditum*, *Dipetalonema dracunculoides*, *Dirofilaria immitis*, and *Dirofilaria repense* are known to infect dogs (Anderson 2000). Moreover, several reports showed that various species of nematodes including *Dirofilaria repense*, *Dipetalonema reconditum*, *Dipetalonema dracunculoides*, *Cercopithifilaria grassi*, *Brugia malayi*, *Brugia ceylonensis*, and *Brugia pahangi* are characterised by microfilaremia with negative dirofilaria antigen assay results (Fischer et al. 2002; Genchi et al. 2005). Therefore, the filarial larvae identified in the present study should be differentiated from other species of larvae.

Knott's test is widely known as an effective diagnostic tool for identifying microfilariae of several filarial nematodes (Mylonakis et al. 2004; Pantchev et al. 2009). In the present study, a Knott's test was not performed at the local animal hospital because the *Dirofilaria immitis* antigen detection test result was negative. Therefore, we could not confirm whether there were microfilariae in fresh blood samples. Although a Knott's test was not conducted, the numerous larvae that were easily isolated from the uterine mass allowed a morphological examination to be done.

Infections of *Brugia malayi*, *Brugia pahangi*, *Cercopithifilaria grassi*, or *Dipetalonema dracunculoides* were ruled out in our case based on sheath morphology (Sreter and Szell 2008; Ciocan et al. 2010; Kariuki et al. 2010). Therefore, infestation with *Dirofilaria immitis*, *Dirofilaria repens*, or *Dipetalonema reconditum* were considered. For more specific identification, we performed PCR genotyping using DNA from tissue samples taken from the formalin-fixed uterus, but all PCR reactions were negative for *Dirofilaria immitis*, *Dirofilaria repens*, and *Dipetalonema reconditum*. We believe that our assay could have produced false negative results since DNA samples isolated from formalin-fixed tissue are often unsuitable for PCR analysis (Reed et al. 2009).

Tarello reported that the differential diagnosis of *Dirofilaria immitis*, *Dirofilaria repens*, or *Dipetalonema reconditum* is mainly determined

via morphological characteristics of the microfilariae (Tarello 2003). Genchi et al. also reported on the morphological characteristics of microfilariae of *Dirofilaria* spp., *Dipetalonema reconditum*, *Dipetalonema dracunculoides*, and *Cercopithifilaria grassi* that infect dogs (Genchi et al. 2005). According to the study, *Dirofilaria immitis* microfilariae are characterised by the absence of a sheath, sharp cephalic ends, a straight tail with a sharp end, a width of 5–7 mm, and length of 290–330 mm. Several other groups have also identified the gently tapered head and straight sharp tail to be of diagnostic significance and to constitute unique characteristics of *Dirofilaria immitis* microfilariae (Genchi et al. 2005; Rishniw et al. 2006; Ciocan et al. 2010). In the present case, possible infection with *Dirofilaria repens* was ruled out based on the morphological characteristics of this organism including an obtuse cephalic end, filiform tail, and an umbrella handle-shaped end. *Dipetalonema reconditum* was also ruled out because of its blunt cephalic end with a protruding hook and button-hooked tail. The reported characteristics of *Dirofilaria immitis* were consistent with those observed in our present case. Based on previous reports of the diagnostic morphological features of larvae, we were able to differentiate the nematodes we isolated from other parasites in the family of Onchocercidae. Although we could not specifically identify the type of filarial nematode using molecular-based techniques, we concluded that these organisms were most likely *Dirofilaria immitis* larvae based on comparative morphologic characteristics.

The *Dirofilaria immitis* life cycle includes five larval stages (L1 through L5). The L3 larvae, which represent the infective stage of *Dirofilaria immitis*, molt and form L4 larvae in the subcutaneous, adipose, and skeletal muscular tissue of infected host animals (Atkins 2005). L4 larvae then molt into L5 immature adult *Dirofilaria immitis*, and migrate to the heart and pulmonary arteries (Atkins 2005). Based on previous reports and our microscopic findings including diameter and length of the filarial nematodes, we believe the filarial nematode larvae may have been in the L3–L4 stages.

To date, there have been no reported cases in the veterinary literature of granulomatous metritis induced by filarial nematode larvae infection. Moreover, only a few studies have described aberrant migration of parasites into unique anatomical sites in dogs including the eye (Dantas-Torres et al. 2009), articular joint (Hodges and Rishniw 2008),

and kidney (Aresu et al. 2007). Herein we report the first case of aberrant dirofilariasis in the uterus of a dog that resulted in granulomatous metritis. Based on this case, we suggest that aberrant filarial larvae migration should be included as a differential diagnosis in dogs with uterine masses.

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