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# Clinical and pathological effects of *Dirofilaria repens* and *Dirofilaria immitis* in a dog with a natural co-infection

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ABSTRACT

Canine dirofilarioses are mosquito-borne zoonotic diseases with a continuous expansion of their geographical distribution, as a consequence of different climatic and ecological factors. *Dirofilaria immitis*, the aetiological agent of heartworm disease, has gained a major veterinary interest, mainly due to its severe clinical implication. In the last decades, *D. repens*, despite of being regarded as a less pathogenic species, regained attention due to its recognized zoonotic potential. Romania has been traditionally regarded as a non-endemic country, but recent epidemiological surveys are highlighting the presence of both *D. repens* and *D. immitis*. The present case report describes the clinical and pathological features of a natural co-infection with *D. repens* and *D. immitis* in 5 year old male Boxer from north-western Romania. Based on clinical and laboratory investigations, a diagnosis of kidney failure and chronical cystitis was established. The parasites were identified by morphology and confirmed by PCR and sequencing. To the best of our knowledge this is the first worldwide report of the ectopic presence of *D. repens* adults in the pelvic cavity and mesentery by natural infection and first report of the ocular localization of this parasite for Romania. The clinical and paraclinical findings suggest the implication of *D. repens* microfilariae in the aetiology of histopathological lesions. Further investigations are needed in order to establish the actual pathogenic potential of *D. repens*, a frequently neglected parasite.



Canine dirofilarioses are mosquito-borne diseases undergoing a continuous expansion of their geographical distribution area, due to several factors, including the climate change, the introduction of new competent vector species, traveling and relocation of infected dogs [1]. The female nematodes release blood-circulating larvae (microfilariae) which become infective after being ingested by mosquitoes (genera *Culex*, *Aedes*, *Anopheles*), which act as intermediate host and vector [2].

The main veterinary concern is focused on *Dirofilaria immitis*, which causes a severe and life-threatening cardio-pulmonary condition [3]. This species originates in Mediterranean basin, but during the last decades, the infection has spread towards northern, central and eastern countries [1,2]. A second species that has recently regained attention is *D. repens*, due to its zoonotic importance in Europe [2]. In dogs, this species causes subcutaneous dirofilariosis, which is generally asymptomatic or associated with various dermatological conditions [4]. Despite the similarity of development requirements and favouring factors, this species seems to be spreading more rapidly than *D. immitis*, currently having a larger distribution range in Europe [2].

Until recently, Romania has been traditionally regarded as a non-endemic country and only some reports on the occurrence of canine dirofilarioses from the first half of the 20th Century were available. The first comprehensive epidemiological studies were conducted few years ago, highlighting the presence of both *D. repens* and *D. immitis* and showing a relatively high frequency of co-infections [5].

While the pathological effects of the *Dirofilaria* spp. adults have been intensively studied, the pathogenicity of microfilariae has not been completely elucidated.

The present case report describes the clinical and pathological features of a natural co-infection with *D. repens* and *D. immitis* in a dog from Romania, revealing new possible erratic migration paths for *D. repens*.

A 5 year-old male Boxer was referred to the Faculty of Veterinary Medicine Cluj-Napoca, at the Small Animal Internal Medicine Clinic, for haemodialysis treatment, after being diagnosed with chronic kidney failure in a private medical practice in Satu-Mare town (north-western Romania). The dog had a history of progressive weight loss, irregular mild cough, poor appetite, polydipsia, dysuria, pollakiuria, sporadic vomiting and lethargy. The physical examination revealed: a score 2 body condition, poor coat condition (Fig. 1), two cutaneous swellings on the right foreleg and one at the lateral surface of the thigh,

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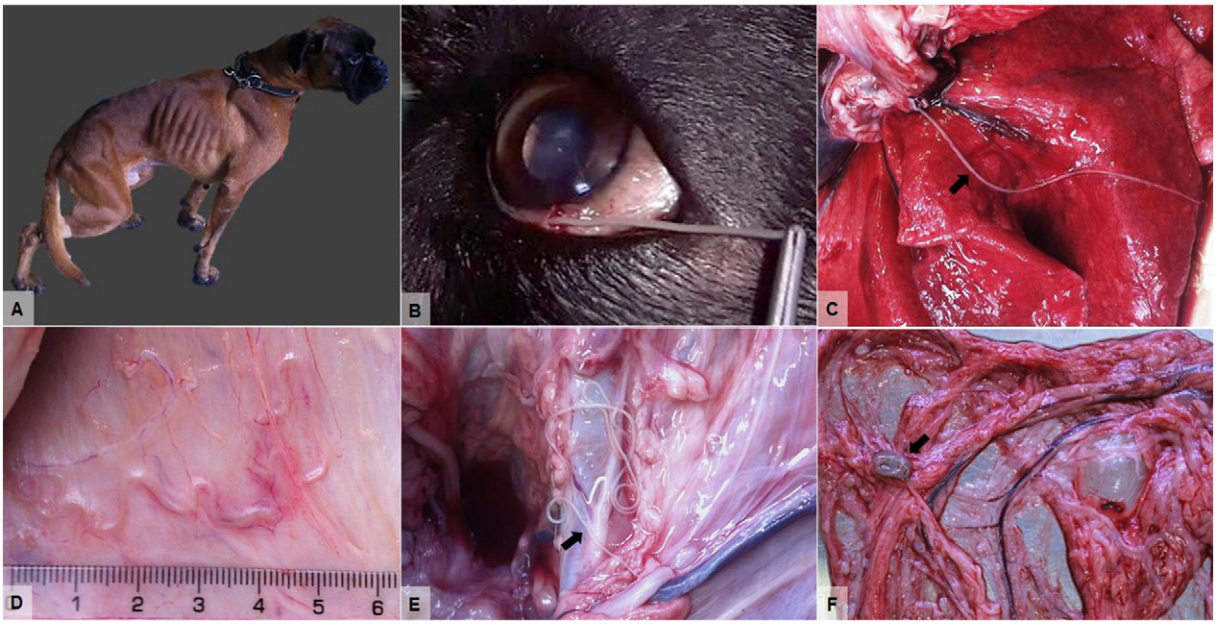


**Fig. 1.** Clinical and gross presentation of the *Dirofilaria repens* and *D. immitis* co-infection in a dog. A: Cachexia, with systemic muscle wasting and evident bony prominences; B: Intact *D. repens* adult live worm recovered from the bulbar subconjunctival space of the left eye; C: Dorsal view of the lung and great vessels, the presence of *D. immitis* (arrow) in the pulmonary artery; D: Serpentine adult *D. repens* in the subcutaneous tissue; E: adult *D. repens* in the retroperitoneal space of the pelvic cavity (arrow); F: Well demarked, coiled *D. repens* in the mesentery (arrow).

enlargement of prescapular and popliteal lymph nodes, mild congestion of conjunctival mucous membranes and vital signs within the normal ranges (rectal temperature = 38.4 °C, heart rate = 112/min, respiratory rate = 32/min). The inspection of the conjunctival mucous membrane revealed at the left eye, a small translucent mass. After surgical removal of the conjunctival mass, a 13 cm long, slender, white nematode was recovered (Fig. 1) and morphologically identified as an adult female of *D. repens* [6]. No treatments with antiparasitic drugs were specified in the dog's health card record.

A thoracic radiograph and echocardiogram were performed, but no alterations were observed. The ultrasound evaluation of the abdomen revealed a small accumulation of peritoneal liquid, a diffuse thickening of the urinary bladder and an increased echogenicity of the renal cortex.

Blood and urine samples were taken for a complete laboratory evaluation. The haematological analysis revealed: non-regenerative anaemia [RBC 4.67 × $10^6$/μL, (reference interval 5.5–8.5 × $10^6$/μL), hemoglobin 10.7 g/dL (reference interval 12–18 g/dL), no reticulocytes (reference interval < 70.000)], lymphopenia [210/μL, (reference interval 1000–4800/μL)], neutrophilia [14,200/μL, (reference interval 3000–11,500/μL)] [7]. The serum biochemistry revealed increased values of blood urea nitrogen (BUN) [300 mg/dL (reference interval 10–28 mg/dL)], creatinine [10 mg/dL, (reference interval 0.5–1.5 mg/dL)], aspartate aminotransferase (ASAT) [129 U/L, (reference value 23–66 U/L)], alanine aminotransferase (ALAT) [334 U/L, (reference value 21–102 U/L)] and gamma-glutamyl transferase (GGT) [15.5 U/L, (reference value 1.2–6.4 U/L)]. A significant decrease in serum albumin was also recorded [1.37 g/dL, (reference interval 2.6–3.3 g/dL)] [8]. Urine analysis revealed proteinuria. Several microfilariae morphologically identified as *D. repens* [6] were present in the urinary sediment.

A rapid test (VetExpert Caniv-4) detecting antigens of adult female of *D. immitis* was performed and a positive result was registered. Following DNA extraction from a 0.2 mL whole blood sample, a duplex PCR amplifying a fragment of *cytochrome c oxidase* subunit 1 (*cox* 1) of *D. immitis* (169 bp) and *D. repens* (479 bp) was performed according to literature [9] and showed positivity for both species. The intensity of microfilaremia was evaluated by means of modified Knott's test and microfilariae were morphologically identified [6] and counted. Both *D. repens* and *D. immitis* were present and the obtained values were 7780 microfilariae/mL and 427 microfilariae/mL respectively.

Based on the clinical and paraclinical exams, a diagnosis of kidney failure, chronical cystitis and mixed infection with *D. repens* and *D. immitis* was established. In order to determine the primary cause of the kidney failure, an ultrasound guided kidney biopsy was performed. The histopathological examination of two needle core biopsies showed a severe and multifocal inflammatory process centered on the glomeruli and interstitial space, associated with atrophy and fibrosis of the renal parenchyma. Fragments of microfilariae were observed in the glomerular and peritubular capillaries. The histological findings were consistent with chronic and severe membranoproliferative glomerulonephritis and intralesional microfilariae. PCR evaluation of a third biopsy was positive only for *D. repens* DNA.

Taking into consideration the irreversible nature of the kidney lesions, the progressive degeneration of the animal's general status and the palliative nature of treatment, 30 days after the diagnosis was established, the owner requested euthanasia. During necropsy, the general examination revealed an emaciated body and anaemia. In the thoracic cavity, diffuse congestion of the lungs with multifocal proliferative pneumonia, mediastinal lymph node reactive hyperplasia and fibrous epicarditis affecting the left atria were observed. Diffuse liver fibrosis (moderate), severe visceral and parietal lymph node hyperplasia, multifocal ulcerative gastritis, splenomegaly (moderate) and follicular cystitis were noted in the abdominopelvic cavity. The kidneys were enlarged, diffusely pale, firm, with a striated medulla due to mineralisation. The three clinically observed swellings were investigated during the necropsy, but no parasites were found. However, over 300 adult *D. repens* were recovered from the subcutaneous tissues (Fig. 1) of the head, neck, thorax, limbs and testes. Two more adult nematodes morphologically identified as *D. repens* were recovered from the pelvic cavity (female) (Fig. 1) and the mesentery (male) (Fig. 1). The heart and pulmonary arteries were dissected and two gravid female *D. immitis* were recovered (Fig. 1).

As the localization of some specimens was quite unusual, in order to exclude the possibility of the occurrence of other *Dirofilaria* species, genomic DNA was extracted from adult nematodes of both morphologically identified species, which were further confirmed by means of