

Case Report

First report of *Dirofilaria repens* infection in a microfilaraemic cat from Romania

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ABSTRACT

The present study describes the first report of *Dirofilaria repens* infection with the presence of both microfilariae and adult nematodes in a cat from Northeastern Romania. Briefly, a 5-year-old male mixed breed cat was presented to a veterinary clinic in Iasi (Romania), for neutering, in early February 2020. During the surgery, two whitish worms were removed from the internal part of the scrotum. Two adult nematodes, one female and one male, were identified, on the basis of morphological features, as *D. repens* with whitish, cylindrical bodies, measuring 12.5 cm and 6.5 cm in length, respectively. At histology, the female nematode showed two cavitated structures containing myriads of variably arranged microfilariae. The male had a transversal diameter of 350 µm, a 10 µm thick cuticle and a ridge-period of 10 Mm. multiplex PCR confirmed the diagnosis of *D. repens* from both nematodes. The Knott's test revealed the presence of microfilariae of *D. repens*. Routine biochemistry panel was within range with one exception, urea serum level slightly increased. The haematology results revealed an increased number of neutrophils, lymphocytes and eosinophils. The cat had an infection with *Otodectes cynotis* as well. The cat was discharged with the following therapy recommended: oral doxycycline (10 mg/kg) for 30 days and topical moxidectin, monthly doses. After six months, the Knott's test gave negative results. Further studies should include new insights of *D. repens* infection in cats concerning its epidemiology, diagnosis and control.

1. Introduction

Dirofilaria repens and *Dirofilaria immitis* are parasites with dogs as the main host, causing subcutaneous dirofilariasis and heartworm disease, respectively. Both parasites, and particularly *D. repens*, have zoonotic potential, and are endemic in many parts of Europe, including Romania (Mircean et al., 2012; Ionică et al., 2015; Ciuca et al., 2016; Capelli et al., 2018). More importantly, *D. repens* is currently considered an emerging agent in Europe (Capelli et al., 2018). Compared to dogs, the cat is an unusual host for both *D. repens* and *D. immitis*, because the production of

microfilariae occurs only in 20% of cats with mature female and male worms and the microfilaria usually last only a few months and at low load (McCall et al., 2008; Simón et al., 2012). However, there is only scant information about the prevalence of *D. repens* infection in cats in Europe (Traversa et al., 2010; Bajer et al., 2016; Diakou et al., 2019; Genchi et al., 2019). In dogs, the infection with *D. repens* is often asymptomatic or induces non-specific signs, such as pruritus, dermatitis, dermal swelling, or subcutaneous nodules associated with the presence of adult nematodes (Venco et al., 2011; Albanese et al., 2013). In cats, there are few accidental reports of *D. repens* infection that included the

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presence of microfilariae in the blood in asymptomatic cats (Długosz et al., 2016; Bajer et al., 2016) or in cats with alopecia, erythema, papulae and crusting dermatitis (Tarello, 2011) without the identification of the adult parasites. Moreover, two reports from Ukraine and Italy described the isolation of the adult nematodes from the subcutis of the scrotum during a routine sterilization (Mazurkevich et al., 2004) or from multiple subcutaneous nodules, having computed tomography (CT) features of a fibrosarcoma (Manzocchi et al., 2017). To the authors knowledge, there are no published reports on natural *D. repens* infection in domestic cats in Romania, although a single clinical case of natural heartworm infection in a cat in Southern Romania has been recently described (Pană et al., 2020). Hence, the present study describes the first report of *D. repens* infection with the presence of both microfilariae and adult nematodes in a cat from Northeastern Romania.

2. Material and methods

2.1. Case history

A 5-year-old male mixed breed cat, originated from northeastern Romania from a rural area (Vorovesti, Miroslava) from Iasi county, was presented to a veterinary clinic in Iasi for neutering in early February 2020. The cat was regularly vaccinated and dewormed only once, at six months of age. The cat spent most of the time outdoors, and the owners did not report any significant travel history. Before the arrival at the clinic, the cat was missing from home for 2 weeks, but the owner reported that he frequently used to disappear for several days. Every time he returned home, he had fight wounds and sometime ear mite infections. Hence, the owner decided to neuter the cat, in order to reduce the territorial fighting and his absence from home. During the surgery, two whitish nematodes were removed from the internal part of the scrotum. The worms were fixed in 70% alcohol for parasitological examination and together with samples of blood in EDTA and serum was sent to the University of Naples, Department of the Veterinary Medicine and Animal Production, Laboratory of Parasitology.

Physical examination revealed skin lesions on the face, ventral neck and tail characterized by alopecia, and erythema. The cat presented, as well, an old mass-like lesion below the ear, covered with antibiotic spray (administered by the owner). The lesion was firm without soft pockets, already drained and in the process of healing (Fig. 1). In addition, the cat had bilateral excessive dark brown ceruminous exudates attached to the ear canal and inner surface of pinnae and ear scratching. The mucous



Fig. 1. Cat, 5-year-old-male with fight wounds and an old mass-like lesion below the ear, covered with antibiotic spray (administered by the owner). The lesion was firm without soft pockets, already drained and in the process of healing.

membranes were normal. No other abnormalities or nodules on the surface of the body were identified. The cat did not present fever or other abnormalities.

Based on the presence of the nematodes recovered from the internal part of the scrotum, an infection with *Dirofilaria* spp. was suspected. Therefore, the following laboratory investigations were performed: histopathological examination and molecular analysis on adult nematodes; and modified Knott's test for identification of the microfilariae in the blood. Moreover, routine biochemistry and haematology as well as smears of ear discharge by cotton swabs for parasitological examinations were performed.

2.2. Laboratory investigations

2.2.1. Histology, molecular analysis and Knott's test

Both nematodes were fixed in alcohol for 24 h and subsequently in formalin for other 24 h. Samples were dehydrated in ascending ethyl alcohol and then embedded in paraffin. Different transversal and longitudinal sections at 4 μm were stained with hematoxylin and eosin and were examined and photographed with a light microscope (Nikon eclipse E600) associated to a microphotography system (Nikon digital camera DMX1200). For molecular determination, genomic DNA was extracted from 25 mg of tissue from the worms, using the DNeasy® Blood and Tissue kit (Qiagen, Germany), following the manufacturer's instructions. Molecular analyses were performed following two protocols of multiplex PCR: one described by Gioia et al. (2010) (12S region) and the other one described by Rishniw et al. (2006) (5.8 + ITS2 region) for simultaneous detection of *D. immitis* and *D. repens*.

A modified Knott's test was used for the detection of circulating microfilariae of *D. repens* (Knott, 1939; Magnis et al., 2013) as follows. One mL of EDTA blood was mixed with 9 mL of formalin 2% and centrifuged for 3–5 min at 170g. The supernatant was removed from the tube and the content was stained with 1–2 drops of 1% methylene blue. A drop was placed on a microscope slide covered with a cover slip and observed under an optical microscope at 100 \times . Moreover, a microfilarial count (mff/mL) and measurements and morphological assessments were made with a calibrated ocular micrometer at a magnification of 400 \times . Length and width were recorded for 10 mff.

3. Results

Two adult nematodes, one female and one male, were identified, on the basis of morphological features, as *D. repens* with whitish, cylindrical bodies, measuring 12.5 cm (Fig. 2) and 6.5 cm in length, respectively (Mazurkevich et al., 2004; Venco et al., 2011). At histology, the female nematode had a transversal diameter of 650 μm and a 15 μm thick multilayered amphophilic hyaline cuticle with 4–6 μm wide periodic ridges spaced at about 15 μm intervals. The male had a transversal diameter of 350 μm , a 10 μm thick cuticle and a ridge-period of 10 μm (Fig. 3A) The cuticle lay on a palisade of large eosinophilic cells (coelomyarian musculature) that delimitate an optically empty space (pseudocoelom). A digestive tract was evident in the pseudocoelom of both nematodes; additionally, the female nematode showed two caviated structures containing myriads of variably arranged microfilariae (Fig. 3B).

The modified Knott's test revealed the presence of microfilariae of *D. repens* with the following morphologic features: unsheathed with conical cephalic end and the tail like the handle of an umbrella (Fig. 4). Minimum and maximum measurements regarding length and width ranged from 352 to 390 μm and 7.5–9.2 μm . The average number of microfilariae/10 μl of EDTA blood was 325. Routine biochemistry panel was within range with one exception, urea serum level slightly increased (40.5 mg/dl; reference (ref.) value: 12.0–32.0 mg/dl). The haematology results revealed an increased number of neutrophils (14,890 mm^3 ; ref. value: 29.50–74.50 mm^3), lymphocytes (1580 mm^3 ; ref. value: 20.0–61.20 mm^3) and eosinophils (340 mm^3 ; ref. value: 3.40–11.40).

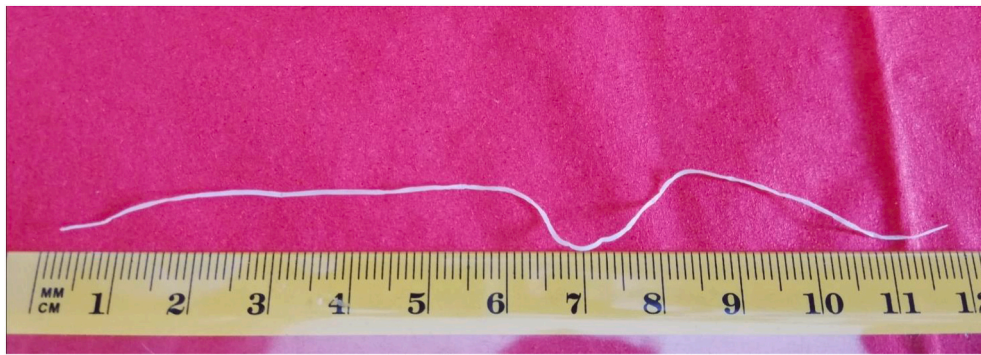


Fig. 2. *Dirofilaria repens*, adult nematode, female (length =12.5 cm), recovered from the internal part of the scrotum during a sterilization of a cat from Romania.

Multiplex PCR of Gioia et al. (2010) amplified a fragment of 327 base pairs, specific for *D. repens* and multiplex PCR of Rishniw et al. (2006) amplified a fragment of 484 base pairs, specific for *D. repens*. The sequence of the PCR product obtained following the protocol described by Gioia et al. (2010) is available in GenBank database with the accession number: MW242630. Moreover, the sequence analysis of the PCR product obtained following the protocol described by Rishniw et al. (2006) is available in GenBank database with the accession number: MW242631.

The sample tested negative for *D. immitis*. On microscopic examination of ear cerumen six parasites characteristic of adult *Otodectes cynotis* were found.

4. Treatment and follow-up

The cat was discharged with the following therapy recommended: oral doxycycline (10 mg/kg) for 30 days and topical moxidectin, monthly doses. Moreover, the modified Knott's test was performed after six months to monitor infection status (European Society of Dirofilariosis and Angiostrongylosis, 2017). Following one month post therapy, the cat's ears became normal without exudation and irritation. No parasites were identified by re-examination. The cat had a normal response toward sound and was interpreted as normal hearing ability. After six months, the modified Knott's test gave negative results.

5. Discussion

Over the recent decades infection with *D. repens* in cats has gained the attention of the scientific community with significant differences between the infection in cats and the better known canine subcutaneous dirofilariosis recognized. These include different aspects in microfilaria load, the length of the adult nematodes, ectopic localizations of the adults and clinical presentation (Pennisi et al., 2020). Therefore, a continuous awareness of veterinarians of its presence is of pivotal importance, knowing that *D. repens* is considered an emerging zoonosis in Europe. Herein, we describe the first report of the presence of *D. repens* adult nematodes in the internal part of the scrotum, in a microfilaraemic cat from northeastern Romania, showing an increased number of neutrophils, lymphocytes and eosinophils. To the authors knowledge, no epidemiological surveys have been conducted to investigate the presence of *D. repens* in cats in Romania where high prevalence of infection is reported in dogs and humans (Popescu et al., 2012; Arbune and Dobre, 2015; Ioničă et al., 2015; Mircean et al., 2017; Ciuca et al., 2016, 2018). In contrast to subcutaneous dirofilariosis in dogs, the prevalence of *D. repens* in cats is not well defined, because of the low number of L3 larvae that manage to develop into the adult stage and also due to scant information about its life cycle in cats (Pennisi et al., 2020). Moreover, the ectopic localization of the adult nematodes, in the internal part of the scrotum in the case presented here, is more frequent in

cats than in dogs due to the consequence of unpredictable migration of larval stages (McCall et al., 2008; Simón et al., 2012). So far, all the reported cases or epidemiological studies regarding *D. repens* infections in cats were either asymptomatic cats or symptomatic cats presenting microfilariae in the blood, but without the isolation of the adult nematodes, or asymptomatic cats, or mimicking other disorders, such as dermatitis, fibrosarcoma with the isolation of the adult nematodes from the lesions or from multiple subcutaneous nodules (Mazurkevich et al., 2004; Traversa et al., 2010; Tarello, 2011; Manocchi et al., 2017; Bajer et al., 2016). To the authors' knowledge, cases of infection with *D. repens* in cats with both adults and microfilariae in the blood have not been reported until now. However, there is a case reported with only one similarity with our case, of a cat with *D. repens* adult nematodes recovered from the subcutis of the scrotum during a sterilization but without identification of microfilariae in the blood. The cat was apparently healthy, a three-year-old male European cat from Kiev, which lived in an apartment with outdoor access, without clinical signs or other disorders reported (Mazurkevich et al., 2004). Our case is the second report of the extraction of adult nematodes from the subcutis of the scrotum, during a sterilization, but with the presence of microfilariae in the blood. Moreover, we can consider that the cat had high microfilarial counts, taking into account the microfilariae loads of *D. repens* reported in dogs (Miterpáková et al., 2016; Ioničă et al., 2017) *Dirofilaria repens* infection in dogs very often runs asymptotically, but a variety of skin alterations has been reported such as skin nodules, pruritus and itching (Albanese et al., 2013); however, no inflammatory reactions are surrounding the living parasite. Usually, inflammatory and painful nodules may be associated with localizations such as the scrotum (Demiaszkiewicz et al., 2009). Interestingly, in humans there are more cases reported with *D. repens* worms located in the scrotum, than in dogs (Soussi et al., 2004; Fleck et al., 2009; Bertozzi et al., 2015; Boldiš et al., 2020). Perhaps, this depends on the fact that humans are unusual hosts and more exposed to unpredictable adult nematode localizations, similar to the cats. However, our cat did present with some abnormal haematological parameters such as increased numbers of eosinophils, lymphocytes and neutrophils. We cannot hypothesize that all these blood disorders are due to the presence of *D. repens*, because the cat had an ear mite infection and a recent abscess, with a collection of pus that included redness, pain, warmth, and swelling, that might justify the increased numbers of eosinophils, lymphocytes and neutrophils. Moreover, we do not know if the cat was positive for other parasites, because we could not perform a coproparasitologic examination. With regards to the high number of eosinophils, we might conclude that the presence of *D. repens* and *Otodectes cynotis* could be the cause. Furthermore, the predominance of eosinophils was also indicated in a case of a cat presenting with large and multiple subcutaneous nodules containing adult and larval parasites and mimicking the clinical presentation and CT features of a fibrosarcoma (Manocchi et al., 2017). In contrast, our case did not present a chronic granulomatous inflammatory process or a

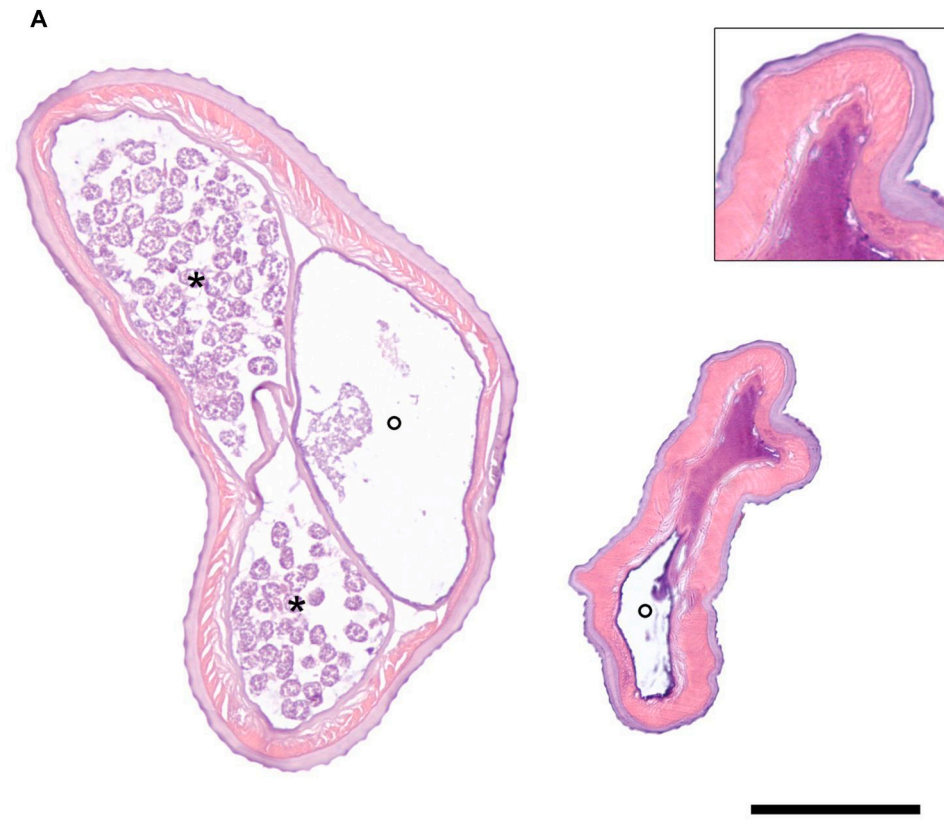


Fig. 3. A. Transverse section of mature female (left) and male (right) of *D. repens* isolated from the scrotum of the cat from Romania. Haematoxylin and eosin stain. Both nematodes showed an amphophilic hyaline cuticle with periodic ridges and laying on a tick eosinophilic coelomyarian musculature. Both nematodes had an evident digestive tract (circle) and, in the female *D. repens*, two uterine tubes engorged by myriads of variably arranged microfilariae were also present (asterisks). Inset: Higher magnification of the *D. repens* male. Note the multilayered cuticle and the periodic ridges. Scale bar = 150 μ m. B. Longitudinal section of mature female of *D. repens* isolated from the scrotum of a cat from Romania. Haematoxylin and eosin stain. The nematode showed an amphophilic hyaline cuticle laying on a tick eosinophilic coelomyarian musculature. In the pseudocoelom a digestive tract (circle) and a uterine tube (asterisks) containing myriads of variably arranged microfilariae (arrowhead) were evident. Scale bar = 50 μ m.

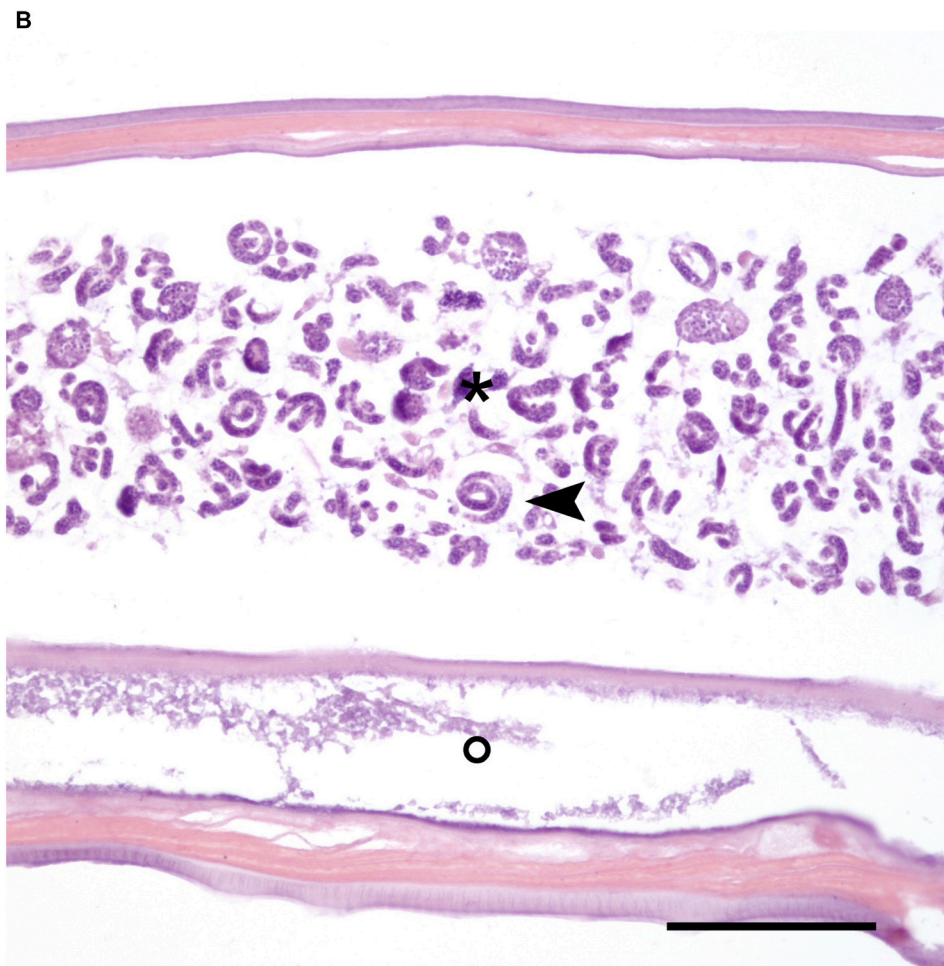




Fig. 4. Microfilariae of *D. repens* detected in the bloodstream of the cat by modified Knott's test.

marked inflammatory reaction surrounding the worms as described in the case mentioned above, that could be likely responsible for increased numbers of eosinophils, lymphocytes and neutrophils.

The cat presented in the case here was born in Romania and had never travelled abroad, indicating that the infection was acquired locally. Compared with canine cases, reports of feline subcutaneous dirofilariasis may provide a better indication of its epidemiology, thus allowing a better surveillance for human infection, because cats tend to be more solitary than dogs, so they have more territorial marking behavior (Tarello, 2003). So far, there is no adulticidal drug approved for the treatment of subcutaneous dirofilariasis in cats. The only report is describing a successful surgical removal of nodules containing adult nematodes (Manzocchi et al., 2017).

6. Conclusions

In conclusion, the authors present the first report of *D. repens* infection in a cat from Romania, that presented simultaneously microfilariae in the blood and adult nematodes in the internal part of the scrotum. Knowing that the cat is an unusual host for both *Dirofilaria* species, because the production of microfilariae occurs only in 20% of cats with mature female and male nematodes and the microfilarial production from the blood usually lasts only a few months and at low load (McCall et al., 2008; Simon et al., 2012), this case has a wide epidemiologic importance. Thus, veterinarians should be responsible for providing accurate information about the zoonotic transmission of parasite infections from pets, as well as gathering more data regarding the prevalence and pathogenicity of *D. repens* in this animal reservoir. Recently, a study has been provided valuable data for better prevention of *D. repens* infection in dogs, that included parasite biology and the immune response of the infected host (Ciuca et al., 2020). Further studies should include new insights of *D. repens* infection in cats as well, regarding the development of an immunological response to infection that could lead to application in epidemiological studies, and as an aid in the diagnostic approach, knowing that in cats, unlike dogs, the infections occur often without microfilariae. Indeed, other valuable data could be obtained by investigating the proportion of subcutaneous dirofilariasis in cats that do not receive any prophylactic treatment and live in endemic areas of Romania, in order to have some basis to being to estimate the potential exposure and occurrence of the disease in humans.

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Ethical standards

The study was performed in compliance with current national laws and regulations.

Declaration of Competing Interest

none.

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