Dirofilarioses in two cats in southern Italy

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Abstract
Two cats infected by *Dirofilaria immitis* and *Dirofilaria repens*, respectively, were taken to two different private practitioners for a clinical examination. The analyses conducted on the first cat revealed a microfilaraemia due to *D. repens* of 66 mfs/mL by a modified Knott's test. No clinical signs of *D. repens* infection were observed in the cat. The animal was euthanised because of a lymphoma condition, and two adult females of *D. repens* were found in the subcutaneous tissue of the lumbar and left scapular regions at the post-mortem examination. The second cat showed severe abnormalities in the white blood cells, including eosinophil count. Microfilariae of *D. immitis* were detected in the blood smear, with an average length (n = 2) of 296.2 μm. These clinical cases represent the first reports of feline dirofilarioses in southern Italy and are indicative of a common occurrence of dirofilarial infection in the local canine population.

Keywords Dirofilariosis · Heartworm disease · Subcutaneous dirofilariosis · Cat · Case report · Feline dirofilariosis

Introduction

Dirofilaria immitis and *Dirofilaria repens* are zoonotic mosquito-borne filarial species causing heartworm disease (HWD) and subcutaneous dirofilariosis (SCD) in canine, feline and human hosts (Simón et al. 2012). Both filarial nematodes are distributed worldwide in tropical and temperate regions (Otranto et al. 2009; Dantas-Torres and Otranto 2020) and are transmitted by mosquitoes of the genera *Aedes*, *Anopheles* and *Culex* (Eldridge and Edman 2000). Cats are considered less susceptible hosts than dogs to *D. immitis* infection (Miller 1998; Venco et al. 2011; European Society of Dirofilariosis and Angiostrongylosis (ESDA) 2018; Genchi et al. 2019), showing a clinical presentation of the HW infection characterised by a dichotomous outcome: self-cure of the animal or dramatic, acute and, in some cases, fatal syndrome (i.e. HW-associated respiratory disease (HARD)) (Miller 1998; ESDA 2018; Pennisi et al. 2020). On the other hand, as with the infection in dogs, cats affected by SCD are asymptomatic or paucisymptomatic (Tarello 2011; Pennisi et al. 2020). The diagnosis of *D. immitis* infection in cats is challenging compared with that in dogs, due to the low and transient microfilaraemia, which makes the concentration methods (i.e. modified Knott's test) less reliable than antigenic or serological ones (Donahoe 1975; Miller 1998). As the diagnosis is difficult, data on feline HW prevalence and distribution is scarce and limited to few studies carried out in northern Italy (Genchi et al. 1992; Genchi and Kramer 2020). Likewise, *D. repens* reports in cats are poor and fragmentary, with a lack of data from southern Italy (Genchi et al. 2019). However, the estimated feline HW prevalence is 5–20% of that of the whole infected canine population living in the same geographical area (ESDA 2018). Thus, considering the distribution of HW infection in the canine population throughout Italy (Mendoza-Roldan et al. 2020), an increase of HW cases in cats also should be expected in southern regions. This study reports the first two cases of *D. immitis* and *D. repens* infections in cats living in southern Italy, describing the clinical features of these little-known infections.

Case report 1

On the 28th of April 2020, a 3.5-year-old female spayed domestic shorthair cat, European breed, was taken to a private

practice in the Province of Foggia (Apulia region, southern Italy) with a 1-month history of respiratory clinical signs (i.e. dyspnoea, nasal discharge, upper respiratory noise; Video (Supplementary file)), anorexia, weight loss and lethargy. After the first physical examination, the cat was evaluated by a baseline assessment consisting of a complete blood count, serum biochemistry, urinalysis and retroviral screen using a point-of-care immunochromatographic test SNAP for the detection of feline leukaemia virus (FeLV) and feline immunodeficiency virus (FIV) infections. The analysis revealed mild abnormalities in the red blood cells (i.e. presence of macrocytes, toxic neutrophils, Döhle bodies, cytoplasmic foaming), while the eosinophil count was normal. In addition, some biochemical parameters were increased (i.e. CPK (768 IU/L), AST (56 IU/L), ALT (124 IU/L), GGT (1.4 IU/L), total bilirubin (0.49 mg/dL), amylase (1307 IU/L), anion gap (28.3) and serum amyloid (33.6 μL/mL)). The presence of microfilariae (mfs) was revealed in the blood smear, and the patient scored FeLV positive.

On the 8th of May 2020, the cat was brought to the Department of Veterinary Medicine, University of Bari (Italy), for further analyses. Blood and serum samples were collected from the cat, which, in the meanwhile, had not been treated with microfilaricidal drugs. An aliquot of blood in EDTA (1 mL) and serum (10 μL) was tested to evaluate the presence of mfs by modified Knott's method and to detect circulating antibodies anti-*Leishmania infantum* by an in-house immunofluorescent antibody test (IFAT), respectively. Thoracic radiography and abdominal ultrasound were performed to elucidate the physical abnormalities. Thoracic radiography revealed enlargement of the cranial mediastinum and sternal lymph node and mild pleural effusion. Abdominal ultrasonographic findings showed echo-structural alterations with heterogeneous patterns in the right renal parenchyma and the liver, without focal nodular lesions. No other changes were detected in the gastrointestinal tract or in other abdominal organs, as well as the cardiac ultrasound examination, that was within normal limits. During the clinical procedures, the patient demonstrated severe respiratory distress. Computed tomography (CT) diagnostic imaging showed a nasopharyngeal tumour partially occluding the larynx and confirmed the mediastinal involvement (Fig. 1). A fine-needle aspiration cytology test was performed on the pharyngeal and mediastinal mass, confirming the presence of lymphoma. The clinical and diagnostic findings were directed towards a differential neoplastic diagnosis with suspicion of lymphoma. Given the patient's clinical condition and the owner's refusal for other diagnostic-therapeutic modalities, the cat was euthanised. At the modified Knott's test, the cat was positive for *D. repens* infection with 66 mfs/mL of blood. The average body length of 5 mfs was 339.59 μm, determined by a digitally captured image software LAS V4.5 (Leica Microsystems). To confirm the species, genomic DNA was extracted from 100 μL of whole blood using the QIAampDNA Minikit (Qiagen, Germany) and, then, screened by conventional PCR (cPCR) using generic primers targeting 12S rDNA and *cox*1, set for filarial nematodes (Otranto et al. 2011). The purified amplicons were sequenced and compared with those available in GenBank by Basic Local Alignment Search Tool (BLAST-http://blast.ncbi.nlm.nih.gov/Blast.cgi). The BLAST analysis revealed the highest nucleotide identity (i.e. 100%) with reference sequences of *D. repens* in GenBank database (i.e. MT:012529 and KX:265047). By the serological examination, the cat was positive for antibodies anti-*L. infantum* with a titre of 1:80.

The post-mortem examination confirmed gross morphology alteration on mediastinal lymph nodes and pharyngeal mass. The histopathological examination of formalin-fixed tissues revealed CD3-positive and CD20-negative, atypical lymphoblastic lymphoid neoplastic cells predominantly infiltrating the cortex of the right kidney, hepatic and splenic tissues. Thus, the cat was placed at a stage of 4, due to the liver and kidney involvement. At the skinning of the carcass, two nematodes were found free in the subcutaneous tissues of the lumbar and the left shoulder regions (Fig. 2a, b). The nematodes were stored in 70% ethanol until the morphometrical analysis. The measurement of each nematode was determined as well as their sex after clarification in Amman's solution for light microscopy observation. Measurements and photographs were taken using a DM-LB2 microscope and Leica Las version 4.5.0 software (Leica Microsystems, Wetzlar, Germany). The identification was carried out using morphological keys (Euzeby 1961, 1981; Soulsby 1982; Bain and Chabaud 1986). The body was whitish with fine longitudinal

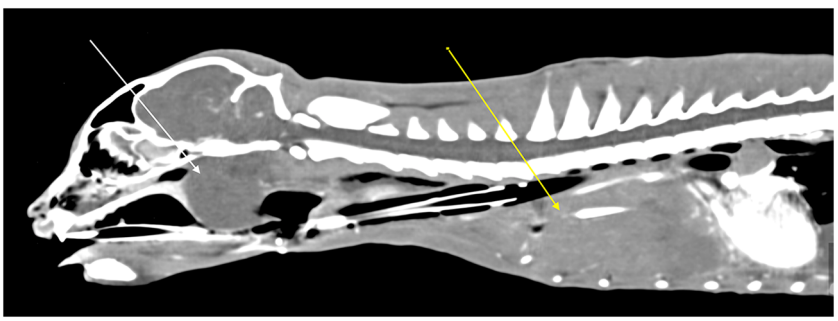

Fig. 1 Oblique sagittal MPR CT scan images of case 1 showing the pharyngeal (white arrow) and mediastinal mass (yellow arrow)

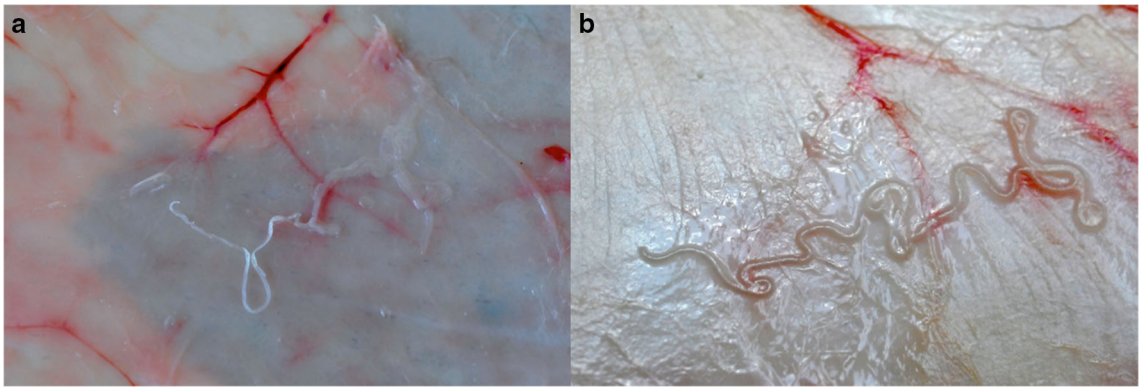

Fig. 2 Adults of *Dirofilaria repens* found in the subcutaneous tissues of the lumbar (**a**) and left shoulder (**b**) regions, respectively

cuticular ridges (Fig. 3d). The nematodes were 117.0 mm and 114.0 mm long. The anterior and posterior ends were rounded. The anterior end was 618.8 μm wide at the nerve ring level with a smooth head cuticle (Fig. 3a, b). The nerve ring and the vulva were located at 246 μm and 2077 μm, respectively, from the anterior end (Fig. 3b, c). The tail was 69 μm long. The two nematodes were identified as females of *D. repens*, and mfs were present in the uterus of both specimens. Uterine mfs were 150.7 μm long and 5.9 μm wide. Examination of the intestinal content revealed the presence of *Taenia taeniaeformis* cestodes which were identified on morphological characters (Euzéby 1982).

Case report 2

On the 27th of April 2020, a 13-year-old, European breed, entire female shorthair cat living in a colony from Taranto (Apulia region, southern Italy), was taken to a private veterinary practice, because of nasal discharge and a history of

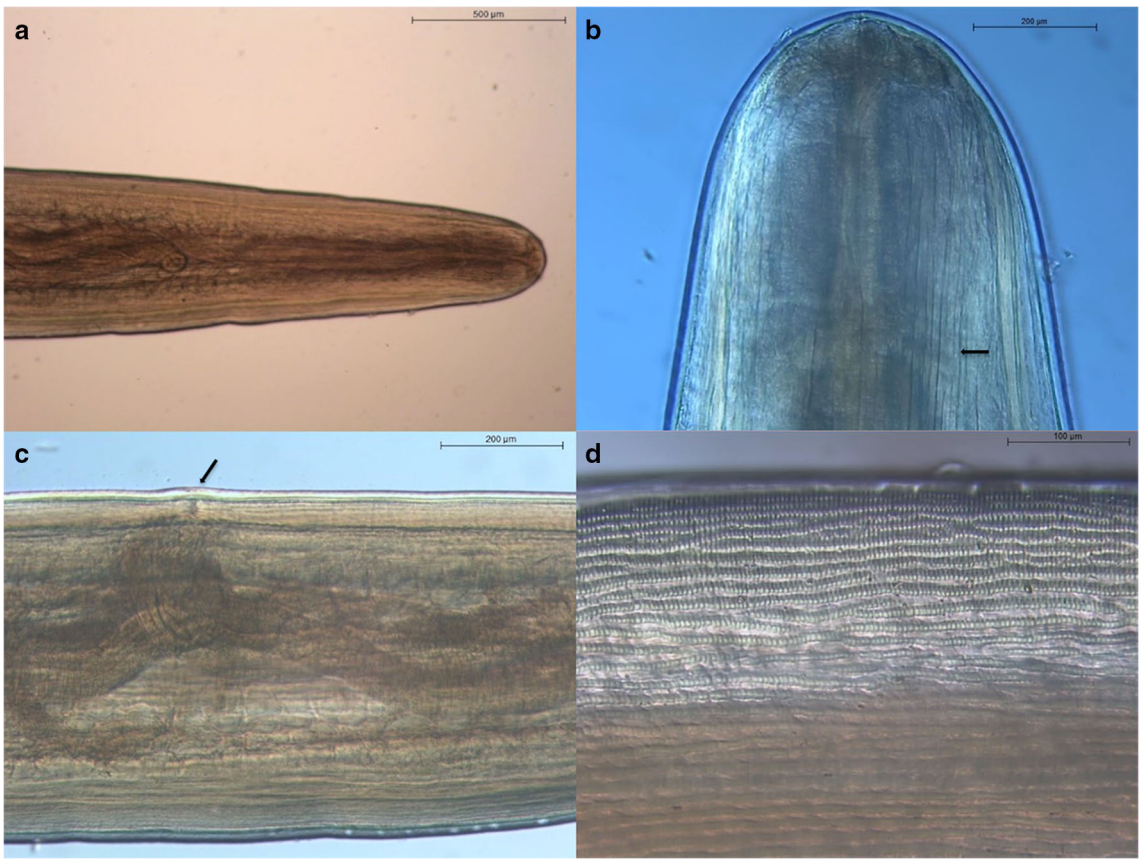

Fig. 3 *Dirofilaria repens* female observed at light microscopy. **a** Lateral view of the cephalic extremity; **b** ring nerve (arrow) in the anterior end; **c** lateral view of the nematode's vulva region and the terminal part of the genital system (arrow); **d** presence of longitudinal ridges on the nematode's cuticle along all the body length

scratching of the ears. During the physical examination, the veterinarian assessed only the presence of mites (i.e. *Otodectes cynotis*) in the ears of the animal, through the observation by otoscope, while no other abnormalities were observed. The cat was treated with selamectin (45 mg; i.e., 6mg/Kg) spot-on (Stronghold®, Zoetis, Belgium). The day after treatment, the cat showed respiratory distress and coughing, thus it was immediately treated with corticosteroids. After two days since the corticosteroid treatment, the animal was taken again to the veterinarian, without clinical signs related to the respiratory tract. The echocardiography showed no abnormalities, although at the thorax ultrasound examination, there was a pulmonary retraction. Blood and serum samples were collected and then sent to a private laboratory for haematological and biochemical analyses, revealing mild abnormalities to severe abnormalities in the white line of blood cells (i.e. WBC (20.8×10^3/μL), early-stage neutrophils (1040/μL), old-stage neutrophils (13,520/μL), eosinophils (1664/μL), basophils (208/μL)), plus the presence of Rouleaux. The eosinophil and basophil counts were higher compared to the reference levels (i.e. 100–1000/μL and up to 100/μL, respectively). The serum biochemical parameters showed some alterations (i.e. albumin (2.0 g/dL), globulin (5.2 g/dL), amylase (1292 IU/L), calcium (7.6 mg/dL), potassium (5.2 mEq/L), total iron (32 μg/dL), saturation (14.3%), serum amyloid (37.7 μL/mL)). The laboratory performed a Diff-Quick-stained blood smear, revealing 2 mfs. The stained blood smear was sent to the Department of Veterinary Medicine, University of Bari (Italy), for morphological identification. The average length was 296.2 μm (i.e. 277.1 and 315.4 μm, respectively), and the mfs were morphologically identified as *D. immitis* (Euzeby 1961). The cat was treated monthly with selamectin (45 mg) for 6 months. After the treatment period, a blood sample was tested by modified Knott's test and no mfs were detected. The cat completely recovered from a clinical point of view.

Discussion

The detection of both filarial nematodes in cats from southern Italy is a likely consequence of the spreading of the parasites in this geographical area. Indeed, considering the high prevalence recorded in the canine populations in southern Italy (Mendoza-Roldan et al. 2020; Panarese et al. 2020), an increase in the feline cases in the same geographical area could be expected. However, the high microfilaremia in the cat positive to *D. repens* and the presence of mfs in the positive one to *D. immitis* are a rather unusual record (Miller 1998; Tarello 2002), considering that only 50% of experimentally infected cats with *D. immitis* develops microfilaremia with a very low load (2–4 mfs/mL) (Miller 1998). Regarding the first cat, the subcutaneous dirofilariosis was characterised by the unusual and complete absence of clinical signs, such as nodules, pruritus and erythema (Pennisi et al. 2020). The critical health status of the animal was almost surely due to the systemic lymphoma, being the detection of filarial nematodes an accidental finding during veterinary laboratory practices. Besides, the cat was positive for FeLV and exposed to *L. infantum* probably since the cat was a stray individual for the first year of its life before being adopted, being potentially exposed to vector-borne pathogens and viral infections (Iatta et al. 2019). Therefore, the cat of the first clinical case, which was already compromised by several conditions (i.e. FeLV, *L. infantum* infection, lymphoma), became more susceptible to *D. repens* infection. This accidental finding suggests a spread of the pathogen also within the cat population living in this geographical area.

In the second cat, the respiratory condition may be related to the effect of macrocyclic lactones (i.e. selamectin spot-on), usually administered in the preventive treatment on the filarial worm (Dillon et al. 2017), or to the veterinary clinical practices, being too stressful for the patient that was already compromised with a lung condition. Despite the first episode of respiratory syndrome, the cat did not display any further respiratory distress following the monthly administration of selamectin. Therefore, it is unlikely that the respiratory episode could be related to the topical treatment, considering that, after the first administration, the adverse reactions should have been more intense due to the shown cat's hypersensitivity. The abnormalities in the white blood cells were not related to the HW infection, with the likely exception of the peripheral eosinophilia, which is reported as starting approximately 70 days after infection in experimental conditions (ESDA 2018). However, absolute eosinophilia cannot be considered a good marker of HWD, considering that is not a specific alteration of infection in cats but a common finding secondary to several parasitic and allergic diseases (ESDA 2018). In cats with HWD, the majority of the pathologic findings (i.e. cough, dyspnoea, weight loss and vomit, unrelated to food intake) are not frequent (Miller 1998; ESDA 2018), although acute-onset dyspnoea and an interstitial pattern on lung radiography could be observed as for this clinical case (Pennisi et al. 2020).

In this new scenario, veterinarians should be aware of dirofilariosis in cats that, as known for this species, may be even worse than in dogs, considering the rapid manifestation of the clinical signs. Furthermore, considering the challenging diagnosis and treatment of the infection, chemoprophylaxis should also be adopted for feline patients living in a canine dirofilariosis endemic area.

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Declarations

Conflict of interest The authors declare no conflict of interest.

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