HELMINTHOLOGIA, 57, 1: 49 – 56, 2020

Case Report

First report on clinical feline heartworm (*Dirofilaria immitis*) infection in Romania

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Summary

*Dirofilaria immitis* (Nematoda; Filarioidea) is the causative agent of heartworm disease (HWD), a severe and potentially fatal condition in dogs. Although cats are considered more resistant to infection than dogs, they are also susceptible to heartworm infection. Moreover, the clinical importance of feline dirofilariosis has increased in recent years, especially in heartworm endemic areas. In contrast to dogs, definitive antemortem diagnosis of heartworm infection in cat is difficult to achieve and a combination of testing methods must be used for clinical confirmation. Here we describe a clinical case of heartworm infection in a 12-year-old male mixed breed cat, originated from Southern Romania, which was referred to a veterinary clinic with a history of vomiting and clinical signs of respiratory distress. The thoracic radiograph showed a diffuse bronchointerstitial pattern of the pulmonary parenchyma. The serological test for *D. immitis* circulating antibody was positive and heartworms were visualized by echocardiography in the main pulmonary artery. In conclusion, the present study clearly shows that cats are at risk for *D. immitis* infection in heartworm-endemic areas in Romania. Additionally, the findings highlight the urgent need for increased awareness among veterinary practitioners of the existence of feline heartworm diseases and for adequate prophylactic measures to be applied. To our knowledge, this is the first report on clinical evolution and radiographic and echocardiographic features of a naturally heartworm-infected cat in Romania.

Keywords: *Dirofilaria immitis* infection; respiratory distress; thoracic imaging; cat; Romania

Article info

Received October 9, 2019
Accepted December 16, 2019

Introduction

Heartworm disease (HWD) is a severe and potentially fatal cardio-pulmonary disease caused by the mosquito-borne filarioid *Dirofilaria immitis*, primarily infecting dogs, but also other carnivores, including domestic and wild felids. Adult worms are located in the pulmonary arteries and occasionally in the right heart. The dog is considered the main reservoir, although *D. immitis* can also affect cats and other mammal species, and in rare cases, humans (McCall et al., 2008). HWD is endemic in Mediterranean countries but currently an increase in the geographical range and its spreading towards central and eastern European areas are reported (Genchi et al., 2011). 

Cats are also susceptible to heartworm infection, although they are considered more resistant to infection than dogs. There are some indications based on which the cat is considered an imperfect or not ideal host for heartworms, such as: low adult worm burden (1 – 6 worms) (Dillon et al., 2007), their short life span (2 – 3 years) (Genchi et al., 2008), lack or short duration of microfilaremia, and a prolonged pre-patent period (8 months) (Dillon et
al., 2007; Atkins et al., 2008). Therefore, most cats seem to tolerate heartworm infection for a prolonged period without or only with transient clinical signs (González-Miguel et al., 2010).

Moreover, clinical signs associated with HWD in cats are usually nonspecific, comprising predominantly respiratory signs (intermittent coughing, dyspnea, tachypnea, ataxia) but also gastrointestinal disorders (e.g. vomiting, diarrhea, weight loss, anorexia), as well as the frequent asymptomatic course of feline dirofilariosis in which the sudden death of the animal is sometimes the only clinical sign shown (Dillon et al., 1984; McCall et al., 1994; Lee & Atkins, 2010). Some spontaneously self-cure as a result of natural death of parasites in infected cats without showing clinical signs or a suddenly acute respiratory syndrome may arise without warning (Atkins et al., 2000; McCall et al., 2008). All these facts make the diagnosis of HWD in cats more difficult and underestimated (Lee & Atkins, 2010).

Dirofilariosis is usually diagnosed by detection of the circulating microfilariae in the peripheral blood, immunological tests (ESDA, 2017) and molecular approaches (Liu et al., 2005; Nuchprayoon et al., 2006). Because of the specific features that characterize HWD in cats, reflected by the relatively low adult worm burden, low intensity and transitory microfilaraemia, low circulating antigenemia, the diagnostic approach to feline heartworm infection must incorporate a combination of testing methods (Lee and Atkins, 2010). Of these, heartworm serology (antigen test, antibody test), thoracic radiography and echocardiography are considered the most useful tools of clinical confirmation (Atkins et al., 2000; ESDA, 2017).

Though microfilaraemia is seldom present in infected cats, because patency is short, and performing a modified Knott test for detecting circulating microfilariae is rarely successful, when present, microfilaraemia is considered a definitive diagnosis (ESDA, 2017). However, serological tests are typically the initial screening tool for feline dirofilariosis and have been used for large-scale studies (Kramer & Genchi, 2002) and also to assess clinical infections (Atkins et al., 2000). Currently, commercially available antigen tests, that target a protein in the reproductive tract of adult females, are considered highly specific and sensitive (100 % and 99.9 %, respectively) methods for infection screening or for confirming a clinical suspicion (Genchi et al., 2018). However, sensitivity can be lower in case of low burden or single worm/sex infection (ESDA, 2017). Antibody tests are able to detect infection by both male and female worms and are the only tests that can be positive as early as 2 months post infection (Morchon et al., 2004) but do not confirm a current infection or the presence of adult worms (Snyder et al., 2000).

In cats with either immature or adult heartworms, significant parenchymal and airway disease is manifested radiographically and histologically (Atkins et al., 2000; Dillon et al., 2007). Therefore, additionally, and independent of serologic test results, thoracic radiography may provide strong evidence of feline HWD and is valuable for assessing the severity of disease and its monitoring (ESDA, 2017). Also, echocardiography is a useful adjunctive test in cats with clinical suspicion of HWD and, in almost all cases, is the only definitive way to diagnose infection antemortem (Atkins et al., 2008).

During the last years, several different Polymerase-Chain-Reaction (PCR)-based protocols have been developed for single species or simultaneous detection of different Dirofilaria species in cats using nested and multiplex PCR and PCR-RFLP (Restriction Fragment Length Polymorphism), respectively assays, as useful diagnostic tools to detect the *D. immitis* low infection in feline HWD (Liu et al., 2005; Nuchprayoon et al., 2006).

The clinical importance of HWD in cats has increased in recent years, especially in heartworm endemic areas, mainly do to increased awareness of this disease, as even a small number of worms are potentially life-threatening (McCall et al., 1994). In the last decade the dynamics of canine HWD in Romania has been changed from sporadic to emerging (Ionita et al., 2012) and currently an alarming increased prevalence is reported, with endemic foci in South-Eastern areas (Mircean et al., 2012; Gîrdan et al., 2015; Pana et al., 2018). However, to our knowledge, there are no reports about feline heartworm disease in Romania. Here we describe a clinical case of natural heartworm infection in a cat in Southern Romania.

**Material and Methods**

**Case presentation**

A twelve year-old male mixed breed cat was referred, in October 2018, to a veterinary clinic in Bucharest with a history of vomiting and difficult breathing. The cat originated from a rural area from Giurgiu county (44°24′54″N 25°49′28″E) (Southern Romania), at about 30 km far by Bucharest. The cat, with a mixed indoor and outdoor life style, lived with other 2 cats and 24 dogs in the same household and she had not received heartworm prevention prior to the visit, as the owner stated. The history of cat, as recalled by the owner, did not include any travel outside the home city limits. Cat spent most of the time outdoors.

**Laboratory investigations**

Cat blood samples were collected and submitted for routine hematological and biochemical investigations and for parasitological testing. In order to assess the cause and the severity of the disease, the cat was also subjected for imagistic investigations consisting of thoracic radiography (by using a Roentgen Examinion Maxivet DR apparatus) and cardiac echocardiography (by using a General Electric Logiq 9 ultrasound system). Parasitological evaluation included a modified Knott test (Knott, 1939), for detecting circulating microfilariae of *D. immitis* and two commercial immuno-chromatographic rapid tests for detecting circulating *D. immitis* antigen (Ag) (SNAP®4DX® Plus, Idexx) and antibody (Ab) (FeliCheck-3, VetExpert), respectively. The modified Knott test was applied for the concentration, detec-
tion and identification of microfilariae, as briefly described here: 1.0 ml of EDTA venous blood was mixed in a 15 ml conical centrifuge tube with 9.0 ml of 2 % buffered formalin and centrifuged for 5 minutes at 1500 rotation per minute (rpm). The supernatant was decanted, and a drop of 0.1 % methylene blue was added to the sediment and stained for 2 minutes. A drop of the sediment was transferred to a glass slide and covered with a cover slip. The slide was examined under the microscope at 10x to detect the presence of microfilariae and then at 40x to observe the morphological features. The whole sediment was analyzed.

**Results**

**Clinical and laboratory findings**

On physical and clinical examinations, the cat was in a good body condition but showed intermittent coughing, cyanotic mucous membranes and dyspnea, without murmur and arrhythmia. The hematological examination revealed increased number of the white blood cells (WBC 25.99 K/µL) characterized by lymphocytosis (13.6 K/µL), mild neutrophilia (NEU 11.09 K/µL) and monocytes (0.54 K/µL). The biochemical analysis showed increased hepatic enzymes (alaninaminotranspherase 67 UI/L; alkaline phosphatase 118 UI/L; total protein 8.7 g/dl) (Table 1).

Subsequently to the parasitological testing, circulating *D. immitis* microfilariae and Ag were not detected, but the serology was positive for feline heartworm Ab, as well as for FIV Ab (Fig. 1). The thoracic radiograph showed a diffuse bronchointerstitial pattern of the pulmonary parenchyma (more evident in the caudal lung lobes) (Fig. 2) and enlarged caudal lobar pulmonary arteries (Fig. 3).

During of cardiac ultrasonographic examination, adult heartworms were detected in the main pulmonary artery. The heartworm appeared as short, segmented strongly echogenic parallel lines (Fig. 4).

**Ethical Approval and/or Informed Consent**

All applicable national and institutional guidelines for the care and use of animals were followed.

**Table 1. Hematological and serum biochemical parameters of a 12-year old mixed breed cat with heartworm infection.**

| Parameter                        | Measure unit | Reference value   | Results (interpretation*) |
|----------------------------------|--------------|-------------------|---------------------------|
| White blood cells (WBC)          | K/µL         | 5.1 – 16.2        | 25.99 (↑)                 |
| Hematocrit (HCT)                 | %            | 27.7 – 46.8       | 38.9                      |
| Hemoglobin (HGB)                 | g/dL         | 8.1 – 14.2        | 13.1                      |
| Neutrophils (NEU)                | K/µL         | 2.3 – 10.7        | 11.09 (↑)                 |
| Lymphocytes (LYM)                | K/µL         | 1.2 – 6.8         | 13.6 (↑)                  |
| Monocytes (MON)                  | K/µL         | 0.1 – 0.4         | 0.54 (↑)                  |
| Platelets (PLT)                  | K/µL         | 156 – 626         | 216                       |
| Blood Uruine Nitrogen (BUN)      | mg/dL        | 15 – 35           | 27                        |
| Glucose (GLU)                    | mg/dL        | 61 – 120          | 162 (↑)                   |
| Alkaline phosphatase (ALKP)      | UI/L         | 12 – 65           | 118 (↑)                   |
| Total protein (TP)               | g/dL         | 5.4 – 7.9         | 8.7 (↑)                   |
| Alaninaminotranspherase (ALT)    | UI/L         | 8 – 53            | 67 (↑)                    |
| Creatinine (CRE)                 | mg/dL        | 0.5 – 2           | 1.5                       |
| Amylase (AMYL)                   | UI/L         | 370 – 1200        | 894                       |
| Total bilirubin (TBIL)           | µmol/L/dL    | 0.15 – 0.5        | 0.3                       |

*↑: increased

The SNAP®4DX® Plus test is a point-of-care ELISA-based immunochromatographic commercial kit for the detection of *D. immitis* antigen; additionally, it is able to simultaneous detect antibodies against *Anaplasma phagocytophilum/Anaplasma platys, Ehrlichia canis, Ehrlichia ewingii, and Borrelia burgdorferi* (IDEXX Laboratories, Westbrook, ME).

The FeliCheck-3 Ab test used is a chromatographic immunoassay for the simultaneously qualitative detection of feline heartworm Ab (FHW Ab), feline immunodeficiency virus antibody (FIV Ab), and feline leukemia virus antigen (FeLV Ag).

**Treatment and follow-up**

After diagnostic, a treatment protocol was designed for the infected
Fig. 1. Serologic (FeliCheck-3) test showing positive reaction for feline immunodeficiency virus (FIV) and feline heartworm (FHW) infection

Fig. 2. Thoracic radiograph showing a diffuse bronchointerstitial pattern in a 12-year old cat with heartworm infection (lateral view)
cat aiming firstly to stabilize the respiratory signs and to stop the progression of cardiac disease. A symptomatic treatment for the pulmonary hypertension (sildenafil, 1.0 mg/kg, every 12 hours, 30 days) was commenced. Additionally, supportive hepatic and immuno-stimulating therapy was recommended, for 30 and 60 days, respectively. After few days, the cat stopped vomiting, showing normal breathing and displayed no other suspicious clinical signs. Further heartworm chemoprophylaxis with monthly doses of either milbemycin oxime orally, or topical selamectin was recommended for the cat to avoid repeat infection (Litster & Atwell 2008), and serologic retesting at 6 – 12-month intervals for the purpose of monitoring infection status (ESDA, 2017).

As the positive cat lived in a house with other at risks’ animals, 2 cats and 24 dogs, we recommended to the owner to test all the co-habiting animals. The 2 cats were tested by serologic (Felícheck 3, VetExpert) for and by Knot tests, for circulating *D. immitis* Ab and microfilariae, respectively, and both were negative. The 24 co-habiting dogs were tested for circulating *D. immitis* Ag, by using the Ag test (Snap 4Dx) and microfilariae, by the modified Knott test. Of them, five dogs (5/24; 20.8 %) were positive for the both, circulating *D. immitis* Ag and microfilariae. For all the owned-cats and dogs, heartworm chemoprophylaxis was also recommended.

**Discussion**

Over the recent decades feline dirofilariosis, caused by *D. immitis* heartworm, has gained the attention of the scientific community which recognized significant differences between feline and the classical, better known, canine heartworm disease, in all aspects: host response, patho-physiology, and clinical presentation (Lee & Atkins 2010; ESDA, 2017). Therefore, a conscious awareness of clinicians of its existence is critical. Here we describe the first survey carried out on the clinical evolution and radiographic and echocardiographic features of a naturally heartworm-infected cat in Southeastern Romania. To the authors’
knowledge, no epidemiological surveys were carried out to investigate the exposure of cats to *D. immitis* infection in Romania. In contrast to heartworm infection in dogs, the prevalence of feline heartworm infection is less well defined because definitive ante-mortem diagnosis is difficult to achieve. Additionally, according to the current opinion, most feline HW infections are asymptomatic, which support the idea that the true prevalence of HW infection in cats in HW-endemic areas is underestimated (Venco et al., 2008). However, it is generally estimated to be 5% to 20% that of the canine population in a particular area (Hermesmeyer et al., 2000). This estimation is supported by recent field surveys, such as epidemiological surveys in Italian endemic areas and the metropolitan area of Barcelona (Spain), that reported a prevalence of feline HW infection of approximately 10% and 10.4%, respectively of that of dogs in the same area (Venco et al., 2011; Montoya-Alonso et al., 2014). For Romania, recently, several studies have been reported on existing endemic foci for canine HWD in Southeastern Romania, the infection rate values varying from 13.4% to 23.0% (Ionita et al., 2012; Girdan et al., 2015; Anghel et al., 2016; Pană et al., 2018). The infected cat in the present study originated from such heartworm-endemic area in Southeastern Romania. Moreover, the cat was living with dogs of which 20.8% were confirmed to be heartworm infected. These demonstrate that cats from that area are at high risk for HW infection and it is very likely that many clinicians are not aware about the real risks of cats for HWD. Further epidemiological surveys will elucidate the current status of feline dirofilariosis in Romania. Moreover, it is suggested that cats that are living in the urban areas are also at risk to be infected with heartworm. Building construction and human activity increase the density of potential hosts and develop suitable environments for the proliferation of mosquito populations due to an increase in the provision of water sources and vegetation (Harrus & Baneth, 2005; Petric et al., 2014). The outdoor lifestyle of the infected cats in the present study appears as a risk factor for HW infection. It is states that outdoor lifestyle increases cat’s exposure to mosquito vectors in HW-endemic areas (Genchi et al., 2008). However, different studies showed that cats living strictly indoors are not fully protect against HW infection, as between 19% and 27% of confirmed HW-infected cats were indoor only (Atkins et al., 2000). The observed patho-physiology and clinical picture including history of vomiting, intermittent coughing, and increased respiratory efforts, are of the most common clinical signs heartworm-infection associated in cats (Venco et al., 2015). The echocardiography has proved the clinical suspicion of heartworm disease in the cat with positive serology (Ab test), but the Ag test for circulating *D. immitis* microfilariae was negative. These findings clearly support the re-

Fig. 4. Echocardiogram showing heartworms, seen as short, segmented, strongly echogenic parallel lines in the right pulmonary artery (arrow) of a 12-year naturally infected cat (Ao: aorta; PA: pulmonary artery) (right parasternal short axis view at the heart’s base)
commodation that multiple test diagnosis must be used for clinical confirmation of HWD in cats, some of which may need to be repeated, especially in endemic areas characterized by high index of suspicion (ESDA, 2017).

Recent evidence-based knowledge acquired from various field studies on the detrimental effects of the both larval and adult stages of D. immitis that can potentially have of any exposed cat, clearly demonstrate that prevention against infection in cats is the best approach to feline heartworm disease (Venco et al., 2015). For this, there are several highly effective heartworm preventive products commercially available, all from the macrocyclic lactone group. Therefore, a monthly feline heartworm chemoprophylaxis with either ivermectin (24 µg/kg) or milbemycin oxime (2.0 mg/kg) orally, or topical selamectin (6 – 12 mg/kg), moxidectin (1.0 mg/kg), or, or topical selamectin (0.48 mg/kg) is recommended at least during of the heartworm transmission season (Lee & Atkins, 2010; ESDA, 2017).

Conclusion

The present study shows that cats are at risk for D. immitis infection in heartworm-endemic areas in Romania. Additionally, this study highlights the urgent need for increased awareness among veterinary practitioners of the existence of feline heartworm disease and for adequate prophylactic measures to be applied.

Conflict of Interest

Authors state no conflict of interest.

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