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HELMINTHOLOGY - SHORT COMMUNICATION

Canine and feline dirofilariosis in a highly enzootic area: first report of feline dirofilariosis in Greece

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Abstract

Dirofilaria immitis (heartworm) is enzootic in many areas of the world and quite prevalent in southern European countries. Although dogs are the main host of the parasite, cats may also be infected, and the prevalence of feline dirofilariosis is associated with the respective prevalence of canine infection in any given area. The aim of the present study was to investigate the proportion of *D. immitis* infection among dogs and cats that were not under any kind of prophylactic treatment and were living in a heartworm enzootic area. In total, 180 stray animals (148 dogs and 32 cats) living in a shelter in Northern Greece were examined for heartworm infection by the Knott's test and serology (antigen and in cats also antibody detection), and additionally echocardiography in the infected cats. Thirty-seven (25%, CI 18.7–32.5%) of the dogs and 3 (9.4%, CI 3.2–24.2%) of cats were found to be positive, by at least one of the tests applied. In 2 of the infected cats, the parasites were also detected by echocardiography. One of the positive cats died suddenly 1 year after diagnosis and at necropsy two decomposing *D. immitis* were found in the right ventricle and pulmonary artery. This is the first report of confirmed feline dirofilariosis in Greece. The detected proportion of infection in cats was 38% of the respective canine infection in the examined shelter. The results of the present study underline the high risk of infection of cats living in enzootic areas and the imperative character of preventive measures in such conditions.

Keywords *Dirofilaria immitis* · Canine dirofilariosis · Feline dirofilariosis · Heartworm · Prevalence · Stray animals

Introduction

Dirofilariosis (heartworm disease) represents one of the most serious cardiopulmonary diseases, with occasionally fatal outcome to its final hosts. It is caused by *Dirofilaria immitis*, a mosquito-transmitted, nematode parasite, with worldwide distribution, affecting primarily dogs, but also wild canids, the domestic cat and other felids (Genchi et al. 2007; Lee and Atkins 2010; Moudgil et al. 2015). Furthermore, *D. immitis* is considered a zoonotic parasite, as it can occasionally affect humans, causing pulmonary dirofilariosis (Simon et al. 2009).

In Europe, dirofilariosis is enzootic in many areas, mainly in the southern countries and around the Mediterranean basin (ESDA 2017), with a prevalence of infection that varies between 2% and > 50% in dogs (Diakou et al. 2016a). Feline dirofilariosis is also confirmed in Europe, by a number of publications, reporting antibody (Vieira et al. 2015), antigen (Diakou et al. 2017) or both antibody and antigen detection (Kramer and Genchi 2002; Montoya-Alonso et al. 2014; Maia et al. 2015), as well as echocardiographic and post-mortem findings (Genchi et al. 2008). However, case reports of clinical feline dirofilariosis, with descriptions of the signs, outcome of the diseases or gross and histopathological lesions are scant (Venco et al. 2008; Panopoulos et al. 2017).

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Greece is enzootic for *D. immitis*. In a recent survey, the prevalence of infection in dogs varied from 0 to 14% in the biggest urban centers of the country (Diakou et al. 2016a); however, it seems that it is even higher in some areas of Northern Greece (Founta et al. 1999). Indeed, heartworm disease is much more prevalent in Northern Greece and significantly lower in southern regions, showing a pattern of uneven distribution across the country (Diakou et al. 2016a). Although most pet owners apply sufficient heartworm prevention to their dogs (Diakou et al. 2016b), there is a high number of stray animals that do not receive any kind of prophylactic treatment, thus represent an important reservoir of the parasite. Interestingly, although dirofilariosis in dogs is common in many areas of Greece, confirmed feline dirofilariosis has never been reported in the country. However, *D. immitis* antigen was detected in 4.7% of the cats examined in various areas of Greece in a recent survey (Diakou et al. 2017).

Generally, it is estimated that feline dirofilariosis is present in enzootic areas with a prevalence that ranges from 5 to 20% of the prevalence in respective local canine population rate (Ryan and Newcomb 1995). In this context, the aim of the present study was to verify the hypothesis that stray animals act as a significant reservoir of *D. immitis* by investigating the proportion of dirofilariosis in dogs and cats that do not receive any prophylactic treatment and live in a hyperenzootic area of Greece, and also to estimate the occurrence of feline dirofilariosis under the high infection pressure in that particular environment.

Materials and methods

Animals

In total, 180 animals, i.e. 148 dogs (80 males and 68 females) and 32 cats (18 males and 14 females) were examined. These were all stray, adult animals living in a shelter located in Northern Greece (Sarakina, Macedonia, Greece, 40.58′ N, E 23.21′ E). Dogs were kept outdoors, in individual cages, while the colony of cats was kept in compartmentalised enclosures, indoors as well as outdoors. None of the animals, at the time of sampling, was under any kind of preventive treatment against heartworm disease.

Laboratory diagnosis

Whole blood samples in EDTA and serum samples were collected from each animal and were transferred to the Laboratory of Parasitology and Parasitic Diseases of the Veterinary Faculty of Aristotle University of Thessaloniki. All whole blood samples were examined for dirofilariosis using the modified Knott's method for the detection and morphological identification of microfilariae (Lindsey 1965; McCall et al. 2008). Serum samples were subjected to serological tests for the detection of antigen or/and antibodies. More precisely, dog sera were examined by the commercial test SNAP® Heartworm RT Test (IDEXX, USA), a snap test for canine dirofilariosis, with 84% sensitivity and 97% specificity for parasite burdens of < 4 female worms, according to the manufacturer whereas cat sera were examined by the commercial test DiroCHEK® (Synbiotics, USA) an ELISA test kit suitable for the detection of feline heartworm infections with sensitivity 100% and specificity 100% for infections with > 3 female parasites, according to the manufacturer. In addition, all cat sera were sent to the private Veterinary Diagnostic Laboratory "San Marco" in Padova, Italy for the detection of specific antibodies by the commercial ELISA kit for feline heartworm, Solo Step® FH (HESKA, USA) that detects infections with immature worms, adult male and female, one worm, single-sex male or female heartworm infections, according to the manufacturer.

Clinical evaluation, treatment and follow-up

All heartworm positive dogs were subjected to therapeutic treatment according to the guidelines of the American Heartworm Society (American Heartworm Society 2014a). Cats found positive for dirofilariosis (Table 1) were additionally subjected to standard clinical and laboratory evaluation (complete blood count, routine biochemical examination, urinalysis), along with cardiological examination that included electrocardiography, echocardiography and diagnostic imaging (thoracic radiographs). One of the positive cats showed clinical signs and was treated with doxycycline 10 mg/kg BID for 30 days and monthly administration of milbemycin oxime, while corticosteroids were avoided due to concurrent diabetes mellitus.

Results and discussion

Out of 148 dogs, 37 (25%, Wilson confidence interval, CI 18.7–32.5%) were found to be positive, by at least one of the tests applied (Knott's test or serology). More precisely, 34 were antigen seropositive and in 19 of them *D. immitis* microfilariae were detected. In addition, 3 dogs were positive in the Knott's test but negative in serology. Moreover, out of the 32 cats, 3 (9.4%, CI: 3.2–24.2%) cats (A, B and C, Table 1) were positive both in antigen and antibodies detection, but no circulating microfilariae were found. In two of the positive cats (cats A and C), 2–3 adult worms were detected during echocardiography in the main pulmonary trunk and right atrium (Fig. 1), without significant dilatation of the pulmonary artery branches. Apart from a mild enlargement of the right ventricle, secondary changes were not observed. Biochemical and haematological analysis results were within normal range in all cats. Cats A and B were asymptomatic and

Table 1 Diagnosis and management of *Dirofilaria immitis* positive cats found in a shelter in Northern Greece

Cat	Diagnosis	Signs	Treatment	Outcome
A	Knott: − Ag: + Ab: + ECHO:+ x-ray: no findings	No	No	Death 1 year later: necropsy (decomposing nematodes in the pulmonary artery)
B	Knott: − Ag: + Ab: + ECHO: − x-ray: no findings	No	No	Death few days later (no further investigation)
C	Knott: − Ag: + Ab: + ECHO: + x-ray: EPA	Loud breathing Coughing Anorexia Weight loss	Doxycycline (10 mg/kg BID × 30 days) Milbemycin oxime (16 mg monthly)	5 months after treatment, serology and ECHO were negative

Ag = antigen detection, Ab = antibody detection, ECHO = echocardiography, EPA = enlargement of pulmonary arteries

did not receive any kind of treatment. Cat B, however, died suddenly soon after serological diagnosis and before any other examinations could be performed.

Cat A was not re-presented to the clinic for follow-up examinations and died suddenly 1 year after diagnosis. During necropsy, two decomposing *D. immitis* were found in the right ventricle and pulmonary artery. The posterior end of the nematodes was missing, and the internal organs were not conspicuous, leaving an almost empty cuticle. The cause of death was determined as pulmonary embolism, eosinophilic granulomatous inflammation, pulmonary infarcts and bronchial collapse associated with feline heartworm disease.

Cat C showed loud breathing, coughing, anorexia, and weight loss. The thoracic x-rays showed enlargement of the pulmonary arteries, without tortuosity, as well as a mild diffuse interstitial pattern (Fig. 2). Five months after the initial diagnosis and the administration of doxycycline and milbemycin oxime, this animal tested seronegative both for antigen and antibody detection and the nematodes were no longer visible by echocardiography.

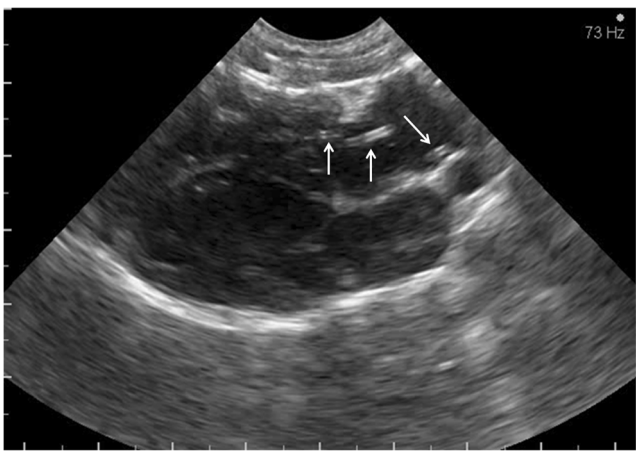

Fig. 1 Echocardiographic (right parasternal short-axis, using a microconvex probe for improved near-field resolution) view of the heart of an infected cat (cat A), showing adult worm(s) in the right atrium as hyperechoic parallel lines (arrows)

Dirofilariosis is endemic in many areas of Europe. Most infections have been recorded in southern countries such as Italy, Portugal, Spain, Greece and the south of France (Genchi et al. 2005; Diakou et al. 2016a). The present study was conducted in a highly enzoonotic area for dirofilariosis (Diakou et al. 2016a) and reveal high percentage (25%) of canine heartworm infection among stray dogs, i.e. animals living permanently outdoors and receiving no or minimum veterinary care. More importantly, the first confirmed feline dirofilariosis cases in Greece are reported herein. The percentage of infection in cats (9.4%) was found to be 38% of the respective canine infection rate (25%) in the same shelter. However, examination of a higher number of cats would be necessary in order to draw any definite conclusion about the actual proportion of feline vs canine dirofilariosis in the area. This proportion was estimated 5% to 20% of the dog prevalence in the USA (Ryan and Newcomb 1995).

Although cats are potential hosts for *D. immitis*, they are generally more resistant to infection and thus considered less suitable hosts than dogs (Venco et al. 2011). Mosquito feeding preference is also possibly a contributing factor to the lower prevalence of feline infection (Genchi et al. 2007). Some of the main mosquito species serving as vectors are not attracted by cats to the same extent as they are by dogs. However, it has been found that *Aedes albopictus*, one of the most common vectors in some areas of the world, shows no feeding preference between these two hosts (McCall et al. 2008). In fact, a study conducted in the hyperendemic area of Po River Valley

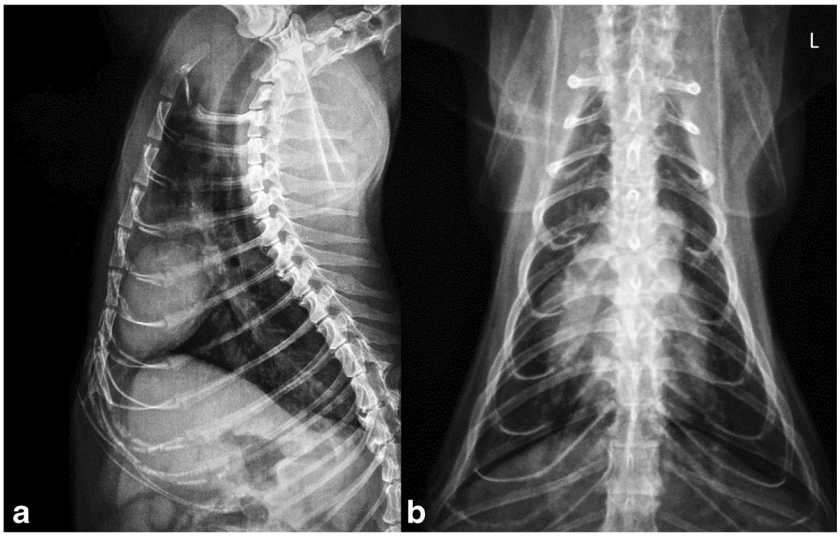

Fig. 2 Lateral (**a**) and ventrodorsal (**b**) x-rays of cat C. Enlargement of the pulmonary arteries is observed, along with a mild diffuse interstitial pattern

in northern Italy demonstrated a significantly high rate of infection in the cat population examined, estimated to be 10% of the dog prevalence (Venco et al. 2011). In addition, studies from the island of Gran Canaria in Spain, where dirofilariosis occurs in over the half of the canine population, showed a notable increase in the feline infection rate in the time period between 2004 and 2011, resulting in a higher prevalence (determined by antibody detection against combined *D. immitis* and its symbiotic bacterium *Wolbachia pipientis* antigens) in feline than in canine population (Morchon et al. 2004; Montoya-Alonso et al. 2011).

The reason why information on feline dirofilariosis prevalence and distribution is insufficient mainly lies in the difficulties in establishing the diagnosis for cats (Venco et al. 2011). In comparison to dogs, definitive ante-mortem diagnosis in cats is challenging because clinical signs and radiographic findings are ambiguous, non-specific and misleading (Venco et al. 2008). Clinical presentations vary widely, from asymptomatic infections to chronic respiratory distress or acute death. In a study of 50 cases by Atkins et al. (2000), 28% of the cats examined were diagnosed with dirofilariosis although they presented without any symptoms of infection. Cats that do not develop symptomatic heartworm disease for a long time are likely to automatically self-cure when the parasites' natural death occurs (Dillon 1998). The complicated definite diagnosis of feline dirofilariosis indicates the possibility of an underestimation of the real prevalence and distribution of the infection in the total feline population.

It has been shown that in cats the majority of immature parasites are destroyed soon after their arrival to the pulmonary arteries, a fact that triggers an inflammatory reaction. In a few cats the parasites reach maturity but generally have a shorter life span than in dogs (2–4 years vs 5–7 years) (McCall et al. 2008; American Heartworm Society 2014b). On the other hand, there is evidence that cats develop pulmonary disease in the presence of immature *D. immitis*, i.e. in the prepatent phase, regardless if the parasites eventually reach maturity. This pathogenesis of the parasite in feline hosts is described with the term heartworm-associated respiratory disease (HARD) (Nelson 2008).

Even if suspicion of feline dirofilariosis has been raised, the diagnosis remains demanding. The maximum number of adult worms is seldom higher than five so they may be neglected even during a post-mortem examination (Ryan and Newcomb 1995). Moreover, female worms are predominant and since only few worms usually develop in cats, they are often all female, which makes the majority of cases amicrofilaremic (negative Knott's test) (Ryan and Newcomb 1995). Antigen tests are very likely to provide false negative results due to low worm burdens and low antigen concentrations, therefore antigen testing alone is not considered a sufficiently reliable diagnostic method and it is suggested to be paired with at least specific antibody detection (Lee and Atkins 2010).

On the other hand, echocardiography is often more helpful in the diagnosis of feline than of canine heartworm disease, as the relatively long adult *D. immitis* are more easily detected in the shorter pulmonary arteries of the cats than of the dogs (American Hearworm Society 2014b). Thus, echocardiography in combination with serological testing and thoracic radiography is the recommended approach for clinical confirmation of feline heartworm disease.

In the present study, all positive cats were amicrofilaremic. Having taken this eventuality into consideration, all cats' blood samples were also submitted to serological examination. As a result, in 3 out of 27 cats both antigen and antibodies for *D. immitis* were detected. The result was then confirmed by echocardiography for only two of them because the third infected cat died before any other examination could be performed.

From a clinical point of view, two of the infected cats were asymptomatic and only one (cat C) showed non-specific signs, compatible with heartworm disease. Often, infected cats that remain asymptomatic develop dramatic acute signs that include

respiratory distress and sudden death after the natural death of the worms. Such an event could possibly be the cause of death of cat A, in which 2 decomposing nematodes were found in the right ventricle and pulmonary artery at necropsy. Similarly, such an event cannot be excluded as the cause of sudden death of cat B. In some cases, feline heartworm disease may be presented with chronic signs such as coughing, vomiting, diarrhoea and weight loss (Dillon 1998; McCall et al. 2008).

The treatment attempt of feline heartworm disease is usually based on the existence of clinical signs. Adulticide treatment in cats is generally avoided because it may lead to significant complications. As cats usually self-cure, when no clinical signs and radiographic evidence of lesions are present, waiting for the parasites to be spontaneously eliminated is the best choice (McCall et al. 2008). Although the benefits of doxycycline administration for the elimination of the *Dirofilaria*-symbiotic bacterium *W. pipientis* and consequently its pathogenesis to the cat have not been evidenced to date (American Hearworm Society 2014b), a 30-day administration of doxycycline and monthly administration of milbemycin oxime was considered the best choice of treatment for cat C. However, the evidenced elimination of the parasites in cat C (negative antibody and antigen test, no parasites in echocardiography) cannot be unequivocally attributed to the treatment applied.

The high rate of infection found in dogs and cats of the present study emphasises the important role of stray animals as reservoirs of *D. immitis* in enzootic areas. Dogs with owners are generally under a certain level of preventive treatment; however, cats receive chemoprophylaxis less often (Venco et al. 2011; Diakou et al. 2016b). The results of the present study emphasise the fact that cats in enzootic areas are in risk of infection and should be under preventive treatment (Venco et al. 2011; American Hearworm Society 2014b).

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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