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Short communication

Prevalence of *Dirofilaria immitis* antigen and antibodies to *Leishmania infantum* in cats from southern Portugal

Carla Maia [a,b,*], Cláudia Ramos [a], Mónica Coimbra [c], Luís Cardoso [d,e], Lenea Campino [a,f]

[a] Unidade de Parasitologia Médica, Instituto de Higiene e Medicina Tropical (IHMT), Universidade Nova de Lisboa (UNL), Lisboa, Portugal
[b] Centro de Malária e outras Doenças Tropicais, IHMT-UNL, Lisboa, Portugal
[c] Clínica Veterinária Porto Seguro, Olhão, Portugal
[d] Departamento de Ciências Veterinárias, Escola de Ciências Agrárias e Veterinárias, Universidade de Trás-os-Montes e Alto Douro (UTAD), Vila Real, Portugal
[e] Parasite Disease Group, Instituto de Biologia Molecular e Celular, Universidade do Porto, Oporto, Portugal
[f] Departamento de Ciências Biomédicas e Medicina, Universidade do Algarve, Faro, Portugal

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ABSTRACT

Vector-borne diseases (VBD) are caused by a range of pathogens transmitted by arthropods and have emerged in recent years, showing a wider geographic distribution and increased global prevalence. In addition to their veterinary medical importance, cats play a central role in the transmission cycles of some VBD agents by acting as reservoirs, amplifying hosts or sentinels. The aim of this study was to determine the prevalence of *Dirofilaria immitis* antigen and of antibodies to *Leishmania infantum* in a sample of 271 cats from southern Portugal. Thirteen (4.8%) cats were positive to *D. immitis*, while antibodies to *L. infantum* were detected in 10 (3.7%) animals. The prevalence of *D. immitis* and *L. infantum* in the feline population from southern Portugal should alert for the need to implement control measures to protect animals and people from these zoonotic parasites. Furthermore, both parasitoses must be included in the differential diagnosis in feline clinical practice.
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1. Introduction

Vector-borne diseases (VBD), caused by a range of pathogens transmitted by arthropods, have emerged in recent years and currently show a wider geographic distribution and increased global prevalence [1,9]. In addition to their veterinary importance, dogs and cats play a central role in the transmission cycles of some zoonotic VBD agents by acting as reservoirs, ampliflying hosts or sentinels, a circumstance that requires a One Health approach [5].

Heartworm disease caused by the filarial nematode *Dirofilaria immitis* and leishmaniosis caused by the protozoan *Leishmania infantum* are endemic zoonoses in the Mediterranean basin, with transmission by mosquitoes mainly from genera *Culex*, *Aedes* and *Anopheles*, and by phlebotomine sand flies of genus *Phlebotomus*, respectively [10,13]. Albeit dogs are the primary reservoir hosts for human infection, both parasitoses have been also reported in other hosts, including cats [6,12].

Canine dirofilariosis and leishmaniosis are endemic in Portugal with an overall national seroprevalence of 3.6–8.9% [3] and 6.3% [4], respectively. Antibodies to *D. immitis* were reported in 15.0% of domestic cats from central and northern Portugal [15], while *Leishmania* seroprevalence in cats ranged from 1.3% in the region of Lisbon [8] to 2.8% in northern Portugal [2].

In the region of Algarve, the southernmost part of continental Portugal, the levels of *D. immitis* infection (antigen detection) ranged from 5.1% in apparently healthy dogs to 17.1% in animals with clinical signs compatible with a VBD [3]. Still in the Algarve, *Leishmania* seroprevalence in dogs ranged from 3.8% in randomly screened apparently healthy animals [3] to 40.6% in dogs that were clinically suspected of having leishmaniosis [7]. *Leishmania* DNA has recently been detected in 12.5% of the cats from the Algarve, with *L. infantum* revealed as the infective species by DNA sequencing [9].

In view of the scarcity of epidemiological data simultaneously targeting these two zoonotic parasites in the feline population, the aim of this study was to determine the prevalence of *D. immitis* antigen and of antibodies to *L. infantum* in cats from the Algarve, southern Portugal.

2. Material and methods

2.1. Study area

The Algarve is the southernmost region of mainland Portugal. It has a Mediterranean climate, with warm weather (annual average temperature of 18 °C) and low rainfall nearly year-round (annual average precipitation of 500 mm). Summer (June–September) is the driest and

* Corresponding author at: Unidade de Parasitologia Médica, Instituto de Higiene e Medicina Tropical, Rua da Junqueira 100, 1349-008 Lisboa, Portugal.
E-mail address: CarlaMaia@ihmt.unl.pt (C. Maia).

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warmest season with average temperatures ranging from 16 °C to 30 °C (www.meteo.pt).

2.2. Animals and samples

From November 2011 to May 2014, a total of 271 cats from the Algarve were studied. Domestic cats were randomly included after the owner's informed consent. In the case of stray cats, which were captured to be neutered and then released, a written consent for enrolment was obtained from the legal detainer, i.e. the person in charge of the rescue association. Whenever available, data on gender, breed, age, fur length, living conditions, deworming and use of insecticides were registered for each cat (Table 1). Blood samples (1–2 ml) were collected by cephalic or jugular venipuncture. Serum was separated by centrifugation and stored at −20 °C until use.

This study was ethically approved by the board of the Institute of Hygiene and Tropical Medicine (IHMT-UNL) as complying with the Portuguese legislation for the protection of animals (Law 92/1995).

2.3. Detection of D. immitis antigen

Antigen from the ovary of mature *D. immitis* female worms was detected by using a commercial enzyme-linked immunosorbent assay (ELISA) kit (PetChek Canine Heartworm Antigen Test®, IDEXX Laboratories, Westbrook, Maine, USA), following the manufacturer's instructions listed in the product package insert. Colour development indicates the presence of heartworm antigen in the sample.

2.4. Detection of antibodies to L. infantum

The direct agglutination test (DAT) for titration of specific antibodies was performed using a standard freeze-dried *Leishmania* antigen as previously described [2]. Briefly, cat sera were two-fold diluted from 1:25 to 1:25,600 in saline solution (0.9% NaCl) containing 0.1 M β-mercapto-ethanol. Fifty microlitres of DAT antigen (1×10^7 promastigotes/ml; KIT Biomedical Research, Amsterdam, The Netherlands) was subsequently added. Results obtained with DAT are expressed as an antibody titre, i.e. the reciprocal of the highest dilution at which agglutination (large diffuse blue mats) is still clearly visible after 18 h incubation at room temperature. The DAT cut-off was established at a serum dilution of 1:100 [2].

Table 1
Prevalence of *Dirofilaria immitis* antigen and antibodies to *Leishmania infantum* in cats from southern Portugal.

Independent variable/category	No. (%) of cats tested	*D. immitis*		*L. infantum*	
		% of positive	95% CI	% of positive	95% CI
Gender	271	$p = 0.371$		$p = 0.503$	
Female	180 (66.4)	3.9	1.6–7.8	4.4	1.9–8.6
Male	91 (33.6)	6.6	2.5–13.8	2.2	0.3–7.7
Breed	109	$p = 1.0$		$p = 0.164$	
ESH	72 (66.1)	4.2	0.9–11.7	6.9	2.3–15.5
Other	37 (33.9)	5.4	0.7–18.2	0.0	0.0–9.5
Age	89	$p = 0.151$		$p = 0.276$	
[6–11 months]	35 (39.3)	0.0	0.0–10.0	0.0	0.0–10.0
[1–17 years]	54 (60.7)	7.4	2.1–17.9	5.6	1.2–15.4
Fur length	187	$p = 1.0$		$p = 1.0$	
Short	176 (94.1)	5.1	2.4–9.5	3.4	1.3–7.3
Medium or long	11 (5.9)	0.0	0.0–28.5	0.0	0.0–28.5
Lifestyle	271	$p = 0.761$		$p = 0.730$	
Domestic	86 (31.7)	3.5	0.7–9.9	4.7	1.3–11.5
Stray	185 (68.3)	5.4	2.3–9.7	3.2	1.2–6.9
Deworming	238	$p = 0.623$		$p = 0.333$	
No	210 (88.2)	4.3	2.0–8.0	3.8	1.7–7.4
Yes	28 (11.8)	7.1	0.9–23.5	7.1	0.9–23.5
Insecticides	231	$p = 0.189$		$p = 0.504$	
No	214 (92.6)	4.2	1.9–7.8	3.7	1.6–7.2
Yes	17 (7.4)	11.8	1.5–36.4	5.9	0.2–28.7
Total	271	4.8	2.6–8.1	3.7	1.8–6.7

ESH: European Shorthair.

2.5. Data analysis

The exact binomial test established confidence intervals (CI) with a 95% confidence level. The chi-square and Fisher's exact tests were used to compare percentages of positivity among categories of the same independent variables and also the total prevalence of each agent. A p value < 0.05 was considered as statistically significant. Analyses were performed with Stemstat and SPSS® 21 software for Windows.

3. Results and discussion

The present study represents the first serosurvey of *D. immitis* and *L. infantum* carried out in the feline population living in southern Portugal. *D. immitis* antigen was detected in 13 (4.8%) animals, while antibodies to *Leishmania* were found in 10 (3.7%) cats at the dilutions of 1:100 (n = 1), 1:200 (n = 4), 1:800 (n = 3) and 1:1600 (n = 2). Two stray cats were positive to both agents.

Although in previous feline and canine studies higher positivities to *D. immitis* and *Leishmania* have been observed in adults [2,4,9,14] and in animals with short fur length [9,14], in the present study no significant differences were detected between the positivity to *D. immitis* or *L. infantum* among categories of the same variables (Table 1). This situation suggests that both infections are more or less uniformly distributed among the screened feline population. Furthermore, the prevalence of both infections was not significantly different from each other ($p = 0.523$) and no association was found between the positivity to *D. immitis* and the positivity to *L.infantum* ($p = 0.077$).

The prevalence of *D. immitis* antigen obtained in this study was lower than the detection of antibodies to the parasite (15.0%) in cats from northern and central areas of the country [15]. However, the presence of antibodies only indicates that an infection occurred and does not provide a guarantee that it still exists, while a positive antigen test result is indicative of an active adult infection [11]. The rapid ELISA test used in the present study has shown to be highly specific, but sensitivity may decline in dogs with worm burdens of two female heartworms or fewer [2]. Under these circumstances, the positivity of *D. immitis* antigen may underestimate the true prevalence of infection with the heartworm.

The overall seroprevalence of *Leishmania* found in the present study was apparently higher than the ones obtained in domestic cats from Lisbon (1.3%; [8]) and northern Portugal (2.8%; [2]), which might be related with the fact that most of the animals from the present study were stray cats and thus lived outdoors. An increased contact with the vectors might also be the reason for the higher prevalence of *Leishmania* infection in the surveyed cats living in a rural environment [4]. On the other side, by means of molecular studies, the detection of *Leishmania* DNA was higher in southern (12.5%; [9]) than in northern Portugal (0.3%; [16]). Altogether, these observations suggest that the climatic conditions in the southern region might be more favourable to the proliferation and abundance of vectors of *L. infantum* and even *D. immitis* [3]. Our results also seem to reinforce the assumption that conventional serology does not seem to be sensitive enough to detect *Leishmania* infection in cats. From an epidemiological point of view, serology may underestimate the real number of infected cats in endemic areas and should preferentially be used in combination with molecular techniques for the detection of DNA [6].

In the present study, the fact that no significant difference was detected between the positivity to *D. immitis* in cats positive or negative to *Leishmania* (and vice-versa) indicates a high specificity of both tests, i.e., apparently there were no serological cross-reactions between both parasites.

In conclusion, the detection of *D. immitis* and *L. infantum* in the feline population from southern Portugal completes and updates the mapping of feline infections with these two agents in continental Portugal, showing that both occur in cats throughout the country. The present study also reinforces the importance to alert the veterinary community, local cat owners and tourists from non-endemic countries (who bring their pets during vacations in Portugal) to the risk of infection by these two parasites and, consequently, to the need of implementing control measures to protect animals and people. In addition, our data strengthen the idea that, whenever appropriate, both parasitoses must be included in the differential diagnosis in feline clinical practice.

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