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Prevalence and seasonal variations of canine dirofilariosis in Portugal



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ABSTRACT

Dirofilariosis is a severe vector-borne emergent disease that is spreading worldwide and becoming a serious threat to human and veterinary public health. Portugal, a Mediterranean country, has favorable climate conditions for mosquito development and survival. At present, accurate data on the prevalence and epidemiological pattern of dirofilariosis in Portugal is scarce and outdated. To study these trends, a project was developed to assess the current prevalence of *Dirofilaria immitis* infection in shelter dogs as well as the prevalence of *Dirofilaria* species present in three coastal regions in central Portugal: Coimbra, Santarém, and Setúbal. Blood samples were collected from 696 shelter dogs during three consecutive years: 2011, 2012, and 2013. A rapid immunomigration technique was performed to detect female *D. immitis* antigens. Concurrently, to detect and identify circulating microfilariae, a modified Knott's technique and acid phosphatase histochemical staining were also performed. Of the 696 dogs sampled, 105 were positive for *D. immitis*, with an overall prevalence of 15.1%. Forty of the 105 dogs were antigen negative but were positive for *D. immitis* microfilariae. Three animals were co-infected with *D. immitis* and *Acanthocheilonema dracunculoides*, and there was also one dog infected only with *A. dracunculoides*, all confirmed by polymerase chain reaction. The highest prevalence of canine heartworm during the 3-y period was in Setúbal (24.8%), followed by Coimbra (13.8%), and Santarém (13.2%), with significant inter-district differences. Our results demonstrate a higher prevalence of dirofilariosis compared with findings of previous studies and show an increasing rate of infection in the southern areas of Portugal attributed, at least in part, to bioclimatic and ecological factors. The present study updates the epidemiological situation and correlates the risk of dirofilariosis transmission within each region. These findings are highly relevant to both human and veterinary public health, contributing to the general awareness of pet owners and veterinarian practitioners and reinforcing the need for effective control measures against vectors and preventive therapy in companion animals.

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1. Introduction

The growing incidence of human and animal dirofilariosis in endemic Mediterranean countries as well as the emergence of autochthonous cases in central and northern European regions (traditionally considered as non-endemic) provide clear evidence that this zoonotic disease is becoming a serious threat to human and veterinary public health. Several factors may account for this tragic epidemiological scenario, namely global warming, colonization by new competent vectors species, pet traveling, and human environmental interactions (Colwell et al., 2011; Genchi et al., 2011; Morchón et al., 2012; Simón et al., 2012).

Canine dirofilarial infections are vector-borne diseases caused by nematode species, specifically *Dirofilaria immitis* and *Dirofilaria repens*. Adult *D. immitis* reside in the pulmonary arteries and right heart chambers, inducing lung, vascular, and heart damage that may culminate in heart failure. Adult *D. repens* inhabit subcutaneous tissues, inducing dermatitis and subcutaneous nodules (McCall et al., 2008). In urban and rural areas, dogs are the main reservoirs for *D. immitis*, whereas cats may serve as major reservoirs for *D. repens* (Tarello, 2002). Moreover, many wild animals can also act as reservoirs for *Dirofilaria* spp., playing an important role in the perpetuation of this parasite to companion animals as a consequence of their peridomestic interactions. In Portugal, the prevalence of *D. immitis* in red foxes ranges from 3.23% in north central locations, such as Coimbra (Eira et al., 2006) to 11.8% in south central districts, such as Santarém and Setúbal (Carvalho-Varela and Marcos, 1993; Carvalho-Varela et al., 1993). Additionally, *D. immitis* also has been reported in three European otters *Lutra lutra* in Portuguese natural freshwater ecosystems (Torres et al., 2004; Saraiva et al., 2012).

Humans are accidental hosts, and the resulting pathological lesions, such as ocular, subcutaneous and pulmonary disorders, are usually asymptomatic. For this reason, infections may go undiagnosed, resulting in underestimated prevalence (Simón et al., 2012).

D. immitis and *D. repens* are transmitted by common Culicidae mosquitoes of the genera *Aedes*, *Culex*, and *Anopheles* carrying the L3 infective larval stage. In Portugal, only *Culex theileri* was found to be naturally infected with *Dirofilaria* spp. larvae. The low rate of filarial infection in this species (4.76%) (Ribeiro et al., 1983) suggests the involvement of other Culicidae mosquitoes in dirofilariosis transmission.

Despite the known endemicity of *D. immitis* in mainland Portugal, accurate data on the epidemiologic incidence are scarce and outdated. Canine dirofilariosis is endemic in mainland Portugal as well as in Madeira Island. Based exclusively on microfilariae detection, without species differentiation, prevalence rates of 16.7% in Ribatejo, 16.5% in Alentejo, 12% in Algarve, and 30% in Madeira were found (Araújo, 1996). In 2011, Balreira et al. reported a 2.1% seroprevalence of canine *Dirofilaria* infection in northern and north central Portugal, with higher rates in Aveiro (6.8%) and Coimbra (8.8%).

Recent data from Cardoso et al. (2012) using a commercial ELISA kit (SNAP® 4Dx®, IDEXX Laboratories, Inc.,

Westbrook, Maine, USA) reported the national average prevalence of *D. immitis* in apparently healthy dogs to be 3.6% and 8.9% in dogs suspected to have clinical signs associated with canine vector-borne disease.

Other concomitant dog filarial infections may occur in Portugal, namely *Acanthocheilonema reconditum* and *A. dracunculoides* (Gomes and Grácio, 2003). Since their pathogenic potential and public health profile is less significant than that of *D. immitis*, the correct identification of their microfilariae is critical to permit initiation of a differentiated therapeutic approach (Pantchev et al., 2011).

There are no published data on seasonal dirofilariosis transmission trends in Portugal. Therefore, an update on the epidemiological pattern and transmission dynamics is required to clearly identify which *Dirofilaria* species are currently present in the country, aiming to determine the best treatment schedule according to the species and seasonal distribution. The present paper reports the regional *Dirofilaria* findings in canine populations in different ecological areas during three consecutive years in mainland Portugal.

2. Materials and methods

2.1. Survey areas

Three estuarine areas of known endemicity for canine dirofilariosis and high-density mosquito population in central Portugal were evaluated: Coimbra (north central region, 40°12'47" N 8°27'7" W), Santarém (central region, 39°14'24" N 8°40'59" W) and Setúbal (south central region, 38°31'19" N 8°53'9" W). The same nine animal shelters were tested each year (October/November 2011, April/May 2012, May/June 2013) to determine trends in the prevalence of *Dirofilaria* spp. in these regions.

2.2. Animal sampling

The number of animals required was estimated using WinEpi program (stratified sample approach), taking into account the global prevalence of canine dirofilariosis in each district as well as the average number of animals in the shelters.

A total of 696 shelter dogs, including 307 males (44.1%) and 389 females (55.9%), were randomly selected (Table 1). Dogs younger than 6 months were excluded due to the long life cycle of *D. immitis*. Only dogs admitted to the kennels in the previous 6 months were included in the survey to preclude evaluating the same animal in subsequent years.

2.3. Data records

A complete record was kept for each sampled dog, including the animal's age, gender, breed, and hair coat length (short or long) as well as a photograph to confirm animal's identity. All animals sampled were strays; thus, there was no information available regarding previous heartworm preventive treatments. Additionally, deworming or heartworm preventive treatments were not routinely implemented in the surveyed shelters.

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