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Research paper

The impact of the climate on the epidemiology of *Dirofilaria immitis* in the pet population of the Canary Islands

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ABSTRACT

Cardiopulmonary dirofilariosis (heartworm) is a zoonotic vector borne disease caused by *Dirofilaria immitis* which affects domestic dogs and cats. Two of the seven Canary Islands are historically hyperendemic areas of dirofilariosis, although no epidemiological study has ever been carried out which includes the other islands. The aim of the study was to complete the epidemiological status of cardiopulmonary dirofilariosis in the canine and feline population throughout all the Canary Islands. 1643 client-owned dogs and 707 client-owned cats were tested for *D. immitis* antigens (dogs), and anti-*D. immitis* and anti-*Wolbachia* antibodies (cats). The prevalence of canine dirofilariosis in the Canary Islands was 15.7%, and the seroprevalence of feline dirofilariosis was 18.1%. A remarkable disparity was found when evaluating the results by island separately, which ranged from from 0% in Lanzarote and El Hierro, low prevalences and seroprevalences in Fuerteventura (1.8% and 2.5% in dogs and cats, respectively), to higher prevalences on the other 4 islands; ranging between 15.7% (dogs) and 14.3% (cats) in La Palma 22.5% (dogs) and 24.1% (cats) in Tenerife. In addition, prevalences and seroprevalences were very variable within each island, these differences being associated to local climate conditions. The distribution and prevalence of dirofilariosis in the Canary Islands is heterogeneous and related to climate, demographic factors and management of pets in the studied areas. Dirofilariosis remains hyperendemic in 4 of the 7 Islands. Since *D. immitis* is a zoonosis, veterinary and health authorities should be aware of the current prevalence and seroprevalence of animal dirofilariosis. The results show the need for awareness raising campaigns to promote the implementation of prophylactic measures in pets, in order to achieve a decrease in the prevalence of animal dirofilariosis in the Canary Islands.

1. Introduction

Cardiopulmonary dirofilariosis (heartworm) is a vector-borne disease caused by *Dirofilaria immitis*. It mainly affects domestic dogs and cats, although it has also been described in other pets, like ferrets, and in a variety of wild carnivores. It is transmitted by the species of culicid mosquitoes of the genera *Culex*, *Aedes* and *Anopheles*. The prevalence of the infection, therefore, is influenced by climatic conditions: those regions with high temperatures and humidity benefit the perpetuation and reproduction of the mosquito vectors and, hence, the transmission (Carretón et al., 2012; Simón et al., 2012). Canine dirofilariosis constitutes a risk for the human population since *D. immitis* can also be transmitted to humans, in which benign pulmonary nodules are produced; these are often confused with lung cancer in X-ray and thoracic CT scans (Simón et al., 2009).

Europe currently presents high prevalences of canine dirofilariosis in the southern Mediterranean countries; in addition, the spread of the disease towards non-endemic areas and countries previously considered free of dirofilariosis has been reported (Genchi et al., 2009; Morchón et al., 2012).

Although, the Canary Islands belong to the European Union politically, they are geographically located near to the Western Saharan coast. Previous studies revealed the existence of dirofilariosis on the two most populated islands, showing prevalences from 41.8% in 1987 to 21% in 2003 in dogs from Tenerife (Valladares et al., 1987; Guerrero et al., 1989; Stenzernberger and Gothe, 1999; Montoya et al., 2006) and a decrease from 67% in 1994 to 19% in

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2011 in dogs from Gran Canaria (Montoya et al., 1998; Montoya-Alonso et al., 2010a,b, 2011). Regarding cats, a seroprevalence of 33% was found in Gran Canaria in 2011, this being the first time that a higher seroprevalence in cats over prevalence in dogs has been described in the same endemic area. Since the techniques used detected antibodies, these data showed the risk of infection rather than the actual prevalence of dirofilariosis in the feline population (Montoya-Alonso et al., 2011).

A geo-environmental model for the prediction of potential transmission risk of *Dirofilaria* in Spain predicted the highest number of generations in the SouthWestern quadrant of the Iberian Peninsula, Mediterranean regions and the Canary and Balearic islands; furthermore, the longest period of transmission of *Dirofilaria* appeared in the Canary Islands where it occurs throughout the year (Simón, 2014). Despite this worrying evidence, no epidemiological study has ever been carried out that includes all the Canary Islands. For this reason, the aim of the present study was to complete the epidemiological status of cardiopulmonary dirofilariosis in the canine and feline population of the Canary Islands, and compare the results by the different climates present in the islands.

2. Methods

2.1. Location and climate of the Canary Islands

The Canary Islands are situated 97 km (60 miles) from the African coast of the Sahara, between the meridians 13° and 19° W and the parallels 27° and 30° N. The Archipelago consists primarily of seven islands, all of which are volcanic in origin. The islands are Tenerife, Fuerteventura, Gran Canaria, Lanzarote, La Palma, La Gomera and El Hierro (listed from largest to smallest). The Archipelago also includes a number of small islets most of which are uninhabited except for the islet belonging to Lanzarote called La Graciosa. Further demographic and geographic data are included in Table 1.

Overall, the climate is subtropical and desert, moderated by the sea and in summer by the trade winds. The climate varies from mild and wet to very dry, according to the position of the islands in relation to the north-east trade winds and to the altitude of each island, Indeed, different climates can be found when travelling on an island and ascending in altitude from the coast to the central peak. According to the Köppen Climate Classification (State Meteorological Agency of Spain, 2012), the Canary Islands present hot desert (BWh) and cold desert (BWk) climates, characterized by evaporation which exceeds precipitation on average but is less than half the potential evaporation, with an average temperature of >18 °C (BWh) and <18 °C (BWk). The hot steppe (BSh) and cold steppe (BSk) climates are characterized by evaporation which exceeds precipitation on average but is less than the potential evaporation; the difference between these two measures is less than that found in a BW climate. Average temperature is >18 °C (BSh) and <18 °C (BSk). The temperate climate with hot and dry summers (Csa) is characterized by hot summers with the average temperature of > 22 °C in the warmest month. The temperate climate with dry and warm summers (Csb) is characterized by warm summers with the average temperature in the hottest month of ≤22 °C, along with 4 months or more of the average temperatures > 10 °C. The areas in which the described climates are present on each island can be observed in Fig. 1 .

2.2. Sample collection

1643 client-owned dogs, presented to 88 veterinary clinics for routine health examinations between May 2014 and February 2015, were included in the study. Of these, 884 were female and 759 were male; 434 were mixed-bred dogs and 1209 were pure-bred dogs; of the latter, 492 were Canary Hounds and 1151 included a total of 55 different breeds recognized by the Fédération Cynologique Internationale (FCI).

707 client-owned cats, presented to 87 veterinary clinics for routine health examinations over the same time period, were included. Of these, 368 were female and 339 were male; 159 were pure-bred cats (including 9 different breeds recognized by the FCI) and 548 were mixed-bred cats.

The participating veterinary clinics represent 29.4% of the overall clinics registered in the Canary Islands. The criteria for inclusion of dogs and cats were: being over 6 months of age, never having received treatment for heartworm disease, no previous history of heartworm infection, and owner consensus to participation in the survey. A complete record was kept for each animal, including identification by age, sex and breed; clinical history, and demographic data. The distribution by islands of dogs and cats included in the study are enumerated in Table 1.

The study was approved by the ethical committee of Veterinary Medicine Service of Las Palmas de Gran Canaria University and was carried out in accordance with the current European legislation on animal protection.

2.3. Laboratory analysis

Blood samples were collected from the cephalic or jugular vein. Samples were then placed in 3 ml serum tubes and centrifuged. Serum was kept at −20 °C until tests were performed.

All canine serum samples were analysed for circulating *D. immitis* antigens using a commercial immunochromatographic test kit (Urano test Dirofilaria®, Urano Vet SL, Barcelona, Spain) according to manufacturer's instructions.

Feline *D. immitis* infection was identified using serological techniques for anti-*D. immitis* and anti-*Wolbachia* antibody detection (Morchón et al., 2004) with some modifications. Briefly, the plates were coated with 0.8 mg of *D. immitis* somatic antigen and *Wolbachia* surface protein (WSP). Serum samples were prepared at 1/100 for anti-*D. immitis* serum antibodies and 1/40 for anti-WSP antibody detection. Horseradish peroxidase-labelled anti-feline IgG antibody (Kirkegaard and Perry Laboratories, Gaithersburg, Maryland, USA), was applied at 1/4000 dilution. The optical densities were measured in an Easy-Reader (Bio-Rad Laboratories, Hercules, California, USA) at 492 nm. Cut-off points of enzyme-linked immunosorbent assay (ELISA) *D. immitis* 0.8 and ELISA WSP 0.6 were obtained as arithmetic mean optical density ±3 standard deviations of sera of clinically healthy cats. As stated in previous epidemiological studies, seropositivity was considered when animals were positive to both tests (Montoya-Alonso et al., 2011, 2015; Vieira et al., 2015).

2.4. Statistical analysis

Data were analysed using SPSS Base 20.0 software for Windows (SPSS Inc.,/IBM, Chicago, Illinois, USA). Descriptive analysis of the considered variables was carried out considering the proportions of the qualitative variables. Chi-square and Fischer exact tests to compare proportions were performed. In all cases, the significance level was established at $P < 0.05$.

3. Results

The prevalence of canine dirofilariosis in the Canary Islands was 15.7%, with a greater prevalence of 17.1% in females compared with 14.1% in males ($p < 0.05$). By age, prevalence was 16.4% for dogs <3 years, 17.1% for dogs aged 3–6 years and 12.3% for those >6 years. There were no statistically significant differences among the three