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Research Note

Stray dogs of Sofia (Bulgaria) could be an important reservoir of heartworm (*Dirofilaria immitis*)

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Summary

Dirofilaria immitis (heartworm) is a zoonotic and an emerging disease, expanding in Europe. In Bulgaria, the presence of the parasite has been described in many regions. However, canine heartworm has hardly been evaluated in the capital of the country and, therefore, the aim of this study was to evaluate the prevalence and distribution of canine heartworm in Sofia. Eighty stray dogs from the city of Sofia and the metropolitan area were analysed for circulating *D. immitis* antigens. The prevalence was 31.25 %, being 34.7 % in the metropolitan area and 25.8 % in the city of Sofia. The current results are among the highest reported in the country. This could be due to the lack of prophylactic measures against infection in these dogs, but also to the spread of *D. immitis* into non-endemic countries. Stray dogs may act as an important reservoir of heartworm being a risk for client-owned animals and for the development of pulmonary dirofilariosis in inhabitants. The results show the need to establish further epidemiological studies and prophylactic campaigns for stray and client-owned animals, as well as to create awareness campaigns about the severity and importance of this disease for both animals and humans.

Keywords: *Dirofilaria immitis*; zoonosis; heartworm; dirofilariosis; epidemiology; seroprevalence

Introduction

Dirofilaria immitis is a parasitic nematode that causes heartworm infection. It is a vector-borne disease transmitted by culicid mosquitoes and that mainly affects dogs and cats. Furthermore, *D. immitis* is a zoonotic parasite that causes pulmonary dirofilariosis in infected humans (McCall *et al.*, 2008; Simón *et al.*, 2012). Heartworm is a worldwide distributed infection. Due to the humidity and temperature required by mosquito vectors, the disease is mainly present in tropical and subtropical areas. In Europe, *D. immitis* is endemic in southern countries (Genchi *et al.*, 2009); however, heartworm is considered an emerging disease and is spreading toward eastern, central and northern Europe, affecting countries or

areas previously considered free of the parasite (Morchón *et al.*, 2012). Several factors have been reported as possible causes of this spread, such as climate change, the development of human activity for agricultural or urban uses in new areas, the growing movement of microfilaricidal dogs throughout Europe or the introduction of new species of mosquitoes able to act as vectors. Also, infected wild animals can be reservoirs of the disease (Morchón *et al.*, 2012; Simón *et al.*, 2012).

Bulgaria is located in southeastern Europe. The country has mainly humid continental and oceanic climates (Kottek *et al.*, 2006; Penin, 2007). Sofia is the capital of the country and is located in western Bulgaria, in the Sofia Valley, surrounded by the Balkan Mountains to the north, and Lyulin, Vitosha and Lozenska mountains to the

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southwest. The average altitude of the valley varies from 500 to 2290 meters and, unlike most of the European capitals, Sofia has no large rivers crossing it, but several small ones. Sofia has a humid continental climate, with cold, snowy winters and warm, sunny summers. The average annual temperature within the city is 10.6°C (Kottek *et al.*, 2006; Penin, 2007). There are 1.5 million people living in the city and 1.7 million people living in its metropolitan area (Eurostat 2018a; 2018b).

Previous publications have shown presence of heartworm in Bulgaria. The first cases in dogs were documented in 1997 – 1999 and, since then, the presence of *D. immitis* has been described and studied in some regions of the country (Pantchev *et al.*, 2015). Georgieva *et al.* (2001) reported a canine prevalence of 7.4 % in the Stara Zagora region. Between 2001 and 2006, microfilaremia was detected in 8.62 % of dogs from different Bulgarian districts (Kirkova *et al.*, 2007) and there were 87 registered cases of canine heartworm in Plovdiv and surrounding regions (Kostadinov, 2007). Between 2012 and 2014, 34.3 % of the samples sent to a parasitology laboratory tested positive for *D. immitis* (Iliev *et al.*, 2017) and a study determined 15 % of prevalence in 33 dogs from a shelter in Sofia in 2013 – 2014 (Radev *et al.*, 2016). In 2015, a canine prevalence of 16.2 % in the Stara Zagora region was described by Pantchev *et al.* (Pantchev *et al.*, 2015). The disease has also been observed and studied in wild carnivores, being described high prevalences in foxes and jackals (Kirkova *et al.*, 2007; Mirchev *et al.*, 2013; Panayotova-Pencheva *et al.*, 2016). These studies show the presence of heartworm in Bulgaria. However, there is a lack of current epidemiological data of canine *D. immitis* in Sofia. Therefore, the aim of this study was to obtain the prevalence and distribution of *Dirofilaria immitis* in dogs in the capital of Bulgaria and its metropolitan area.

Materials and Methods

The present study included 80 stray dogs from different parts of the city of Sofia and its metropolitan area. The animals were captured between December 2017 and February 2018 for routine neutering campaigns that take place in Sofia. A complete record was kept for each animal, including identification, age, sex, breed and location. The inclusion criteria were being dogs over 7 months of age and had not been treated with macrocyclic lactones. Of the included dogs, 45 % were female and 55 % were male. The age ranged from 1 to 19 years old (mean: 7.1 years). Animals were further divided into 3 groups of age, from 1 to 4 years (n=25), from 5 to 9 years (n=29), and from 10 to 19 years (n=26). There were 60 mongrel dogs and 20 pure-bred dogs. According to the distribution, 38.75 % dogs were living in the city of Sofia while 61.25 % dogs were living in the metropolitan area. Blood samples were collected from the cephalic vein of every studied animal and all the dogs were tested for circulating *D. immitis* antigens using a commercial immunochromatographic test kit (Uranotest *Dirofilaria*®, Urano Vet SL, Barcelona, Spain). The

tests were performed according to the manufacturer's instructions. The data were analyzed using the SPSS Base 25.0 software for Windows. The descriptive analysis of the variables considered was carried out studying the proportions in the qualitative variables. The chi-square test was performed to compare proportions. In all the cases, the significance level was established at $p < 0.05$.

Ethical Approval and/or Informed Consent

The design of 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.

Results

The prevalence of *D. immitis* in the canine population in the studied area was 31.25 %. By sex, male dogs showed a higher incidence (36.4 %) compared to females (25 %). By breed, the most affected dogs were mixed-breed dogs (33.3 %) against purebred dogs (25 %). There were no statistically significant differences by sex or breed.

Positive dogs were found from 3 to 19 years, with an average age of 8.7 years. When age ranges were considered, the highest seroprevalences were found in the oldest part of the population, with 42.3 % of positives in dogs from 10 to 19 years old, followed by 37.9 % in dogs between 5 and 9 years. The lowest number of positive cases (12 %) was represented by dogs from 1 to 4 years ($p < 0.05$).

The prevalence was higher in the metropolitan area of Sofia (34.7 %) than in the city of Sofia (25.8 %), although the differences were not statistically significant. The distribution of the positive cases of canine heartworm in the studied area can be seen in Figure 1.

Discussion

The present study reports the presence of heartworm in the stray dogs of Sofia and the metropolitan area. According to previous research, which reported prevalences from 7.4 % to 9.2 % in client-owned dogs in different regions of Bulgaria (Georgieva *et al.*, 2001; Kirkova *et al.*, 2007) and from 10 % to 15 % in stray dogs (Georgieva *et al.*, 1992; 2001; Radev *et al.*, 2016), the current results reported an increase in the prevalence of heartworm infections in the country. These results are consistent with studies that reported an increasing prevalence of *D. immitis* in Europe (Genchi *et al.*, 2009; Morchón *et al.*, 2012).

The institutions of Sofia are focused on decreasing the population of stray dogs, and there are no established control and prophylactic measures aiming to prevent the presence of different diseases, including heartworm. That is why the high prevalence reported in this study is probably influenced by the fact that all dogs were con-



Fig. 1. Map of the province of Sofia-city, where the distribution of the evaluated animals is shown. The urban part corresponding to the city of Sofia is shown as a darker color on the map. The blue dots correspond to negative dogs while the red squares correspond to heartworm-infected dogs (figures obtained and modified from a free media repository).

stantly exposed to mosquitoes and not receiving any prophylactic treatment. Probably, for the same reason heartworm infection was higher in the group of older animals. In 2015, it was estimated that 3844 ($\pm 10\%$) stray dogs lived in the city, being one of the greatest problems of the capital (Ivanova & Gechev, 2015). Although the presence of microfilariae was not evaluated in the studied dogs, it is estimated that between 75 – 95 % of the infected dogs that do not receive chemoprophylaxis have microfilaremia (Stogdale,

1984), so these stray dogs may act as an important reservoir for heartworm, increasing the risk of infection of client-owned animals and humans living in the city.

The abundant vegetation and water reservoirs (green parks, artificial lakes and water ponds) present in the city of Sofia and in the metropolitan area may offer a perfect environment for reproduction and proliferation of the mosquito vectors which could favor the spread of the infection; furthermore, in the city of Sofia high

levels of urbanization can cause the phenomenon called Urban Heat Islands (Gago *et al.*, 2013; Yang *et al.*, 2016) which retains heat and increase the temperature inside the city. This indirectly influences the development of *D. immitis* larvae in mosquito vectors during the colder months and thus enlarges the transmission season (Arnfield, 2003). In Sofia, the average annual temperature of the urban area is 0.5°C higher than that of the peripheral area; moreover, in winter the temperature in the center of the capital is 1° – 1.5° higher than that of the rural regions (Kovachev, 2005). Although the results of this work cannot be determined as definitive, given the small size of the sample studied, they are undoubtedly indicative of a widespread presence of the parasite in the city and indicative of the need for a broader study. In this regard, there is a lack of studies on heartworm in client-owned pets in Sofia and, according to the results obtained in this study, a high prevalence of *D. immitis* infection in these animals should be expected. This probably is due to the lack of knowledge of the general population about the disease, updated data on its epidemiology and prophylactic measures and campaigns aimed to avoid infection. Considering the growing trend of heartworm in different regions of the country, demonstrated by this and other recent studies (Pantchev *et al.*, 2015; Panayotova-Pencheva *et al.*, 2016; Iliev *et al.*, 2017), a nationwide study to learn the current distribution of heartworm disease in Bulgaria in pets should be done. There is a high risk of human dirofilariasis in areas of high canine prevalences (Simón *et al.*, 2012; Cabrera *et al.*, 2018). This can be observed in the increasing publication of cases of human infections by *D. repens* in Bulgaria (Harizanov *et al.*, 2014; Velev *et al.*, 2019). This is a zoonotic parasite that is increasing in Europe and the most frequent localizations are the eye region, subconjunctival or subcutaneous tissues in other body regions, forming nodules (Genchi and Kramer, 2017). Cases of pulmonary dirofilariasis by *D. immitis* have not yet been reported in the country; however, the apparent increase in the canine prevalence may increase the risk of infections in humans. Therefore, awareness of the disease should be promoted through the implementation of educational and prophylactic campaigns among veterinarians and owners. Also, competent authorities should be aware and alerted about the epidemiological situation in Sofia. Furthermore, being a zoonotic disease, health agencies and institutions of the country, as well as physicians, should consider the repercussions of this infection and include human dirofilariasis in the differential diagnosis of pulmonary nodules.

Conflict of Interest

Authors state no conflict of interest.

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