Current situation of the presence of the zoonotic nematode *Dirofilaria immitis* in dogs and humans in Bucaramanga, Colombia

CURRENT STATUS: UNDER REVIEW

**Parasites & Vectors**  ☛ **BMC**

M.Victoria Esteban-Mendoza  
Universidad Cooperativa de Colombia

Víctor Arcila-Quiceno  
Universidad Cooperativa de Colombia

J. Albarracín-Navas  
Universidad Cooperativa de Colombia

Isabel Hernández  
University of Salamanca

C. Flechas Alarcón  
Universidad Cooperativa de Colombia

Rodrigo Morchón  rmorgar@usal.es  
Universidad de Salamanca  
*Corresponding Author*  
*ORCID: 0000-0003-2699-1482*

**DOI:** 10.21203/rs.2.19256/v1

**SUBJECT AREAS**  
*Parasitology*  *Epidemiology*

**KEYWORDS**  
*Dirofilaria immitis*, dog, heartworm, human dirofilariosis, prevalence, seroepidemiology, Wolbachia* pipiens*
Abstract

Background: Cardiopulmonary dirofilariosis caused by Dirofilaria immitis is a zoonotic, vector-borne infection, whose main hosts are both domestic and wild canids and which can be transmitted to humans. It mainly occurs in tropical and subtropical climates, and temperature and humidity are the main factors that favor the presence and proliferation of Culicidae mosquitoes. There have been few reports of this disease in dogs and humans in Colombia, a region with favorable climatic conditions for the development of this disease. Therefore, this research aimed to study its current prevalence in dogs and the risk of human exposure to the disease in Bucaramanga, one of the most populated areas in Colombia located at the center of the country. Furthermore, its demographic and environmental characteristics could be useful as a study model for other similar locations and neighboring countries.

Method: Serum samples from 351 dogs and 506 humans from the Bucaramanga metropolitan area were analyzed. All dog samples were tested with a commercial immunochromatographic test kit to detect the presence of circulating antigens of D. immitis. Human samples were analyzed using a non-commercial ELISA test kit to detect IgG against the somatic antigens of adult D. immitis and Wolbachia. Positive results were further confirmed using a Western blot analysis.

Results: Dirofilaria immitis prevalence was 10.82% (95% CI) in dogs and 5.12% (95% CI) in microfilaremic dogs. Seroprevalence in humans was 6.71% (95% CI) and was significantly higher in individuals aged 16–34 years and in women than in men.

Conclusions: This study describes seropositivity to D. immitis for the first time in a Colombian human population located in the same area as that of dogs infected with
D. immitis, which represents a potential threat to public health. In humans, age and sex can be considered risk factors for exposure to D. immitis.

Background

Cardiopulmonary dirofilariosis, caused by Dirofilaria immitis, is a vector-borne disease that occurs worldwide, and its main hosts are domestic and wild dogs and cats [1]. Different species of mosquitoes, such as the genera Culex spp., Aedes spp., and Anopheles spp., are involved in the transmission of this parasite. These species represent a constant risk of infection because they feed on both animal and human hosts [2]. Seroprevalence studies have been conducted in regions where infected dogs have been found, which indicated previous contact with the parasite and cases with pulmonary nodules. For this reason, dirofilariosis is considered an emerging public health problem because of its zoonotic potential [3, 4].

Cardiopulmonary dirofilariosis is a chronic, progressive, and life-threatening disease. Adult worms are lodged in the pulmonary artery and the right ventricle of a dog's heart. Mosquitoes ingest the microfilariae after the females produce them, and after two successive molts, stage-3 larvae are inoculated into the definitive host during the next blood draw [2]. In humans, immature worms are embolized in the pulmonary microarteries, leading to the formation of benign lung nodules (pulmonary dirofilariosis), most cases of which are asymptomatic [1, 2]. Moreover, D. immitis harbors endosymbiotic bacteria of the genus Wolbachia, which participate in the parasite’s molting and embryogenesis and play a key role in the immune and inflammatory response to the disease [5, 6].

Dirofilaria immitis is primarily located in tropical and subtropical climates and depends mainly on environmental factors, including temperature and humidity, in
addition to human behavior such as implantation of irrigation systems, taking pets on trips, and new urban developments, that favor the presence and proliferation of its transmission vectors. However, the number of reports in areas with less warm climates has increased, which indicates that the disease is expanding [1, 2, 7, 8]. The South American continent is one of the most biodiverse areas on the planet, with a combination of factors such as intensification of agricultural practices, landscape modification, poor ecosystem protection, and potentially unstable economies, which potentially lead to the spreading of the disease and its vectors [9]. On this continent, the disease has only been reported in Argentina, Colombia, Peru, and Brazil, with relatively few studies in domestic dogs; very few sporadic cases of pulmonary dirofilariosis have been described [1, 10, 11, 12]. The disease has been reported in dogs from different areas of Colombia, even in high-altitude areas with cold weather, with mean prevalence values of 0.91–16.12% according to different methodologies [10, 13]. Only one case of human pulmonary dirofilariosis has been described [14], and two seroepidemiological studies were conducted in an area within the Colombian Amazon where infected dogs were also found [15, 16].

The purpose of this investigation was to determine the presence of D. immitis in dogs and their possible contact with the human population in the metropolitan area of Bucaramanga, Colombia.

Methods

Sampling area

The metropolitan area of Bucaramanga, which belongs to the capital city of the Department of Santander in Colombia, includes the municipalities of Bucaramanga, Floridablanca, Piedecuesta, and Girón (Fig. 1), located near the capital of Colombia,
Bogotá. It extends to an area of 1,479 km\(^2\), and the municipal area occupies 165 km\(^2\); it is located at 959 m above sea level. It includes two sectors of different geographical conformations: one formed by a plateau and the other by a valley. Its climate is tropical, with a mean annual temperature of 23.4 °C and significant precipitation levels with an approximate mean of 1,159 mm [17]. It has an estimated population of 1.2 million people, and there are 32,000 censored dogs in the city of Bucaramanga alone. In addition, there are numerous uncensored vagrant dogs throughout this area [18].

**Samples used**

This study included samples from 351 dogs and 506 humans collected during the months of February–June 2018. The dog and human samples were collected by members of the veterinary staff of different clinics and associations and the Higuera Escalante Laboratory’s health care staff, respectively. For the canine population, signed informed consent from the owners was considered the inclusion criterion. Variables considered for the analysis were age, sex, municipality of residence, socioeconomic status, and whether they lived inside or outside a house. For the human population, being of legal age and signing the informed consent were the inclusion criteria. Variables for the analysis were age, sex, municipality of residence, socioeconomic status, living with pets, type of pet (dog or other), and presence of water sources at < 200 meters. Confidentiality of patient information was always maintained, and all study participants gave their written consent.

Regarding the dog population (Table 1), 132 (37.61%) were male and 219 (62.39%) were female, and their average age was 5.12 years (age range, 9 months–15 years). The number of samples collected by age groups of < 1, 1–3, 4–6, 7–10, and 11-
15 years was 31 (18%), 109 (19%), 119 (17%), 67 (39%), and 25 (16%), respectively. The number of samples collected by zone was 144 in Bucaramanga (41%), 73 in Floridablanca (20.8%), 73 in Girón (19.9%), and 64 in Piedecuesta (18.2%). In terms of breed, most of the sampled dogs were mongrels (n = 194, 55.3%), followed by Labrador (n = 35, 10%), Golden Retriever (n = 14, 4%), and Pinscher (n = 16, 4.6%); other breeds constituted 15.3% (n = 92). According to socioeconomic status, the number of samples collected was 139 in stratum 1 (39.60%), 57 in stratum 2 (16.23%), 79 in stratum 3 (22.50%), 75 in stratum 4 (21.36%), and 1 in stratum 5 (0.28%); no dogs were sampled in stratum 6. Socioeconomic stratification was carried out taking six strata the hierarchical socioeconomic difference from misery (1), poverty (2), poverty with some economic resource (3), middle class (4), upper middle class (5) to upper class (6) [19]. Based on whether they lived inside or outside a house, the number of sampled dogs living inside a house corresponded to 187 (53.3%) and outside a house corresponded to 164 (46.7%).

Regarding humans (Table 2), 159 (31.42%) were men and 347 (68.55%) were women, with an average age of 36 years (age range, 18–89 years). The number of samples collected by age groups of 18–35, 36–50, 51–65, and 66–90 years was 294 (58.1%), 118 (23.32%), 73 (14.42%), and 21 (4.16%), respectively. The number of samples collected by zone was 189 in Bucaramanga (37.35%), 176 in Floridablanca (34.78%), 64 in Girón (12.64%), and 77 in Piedecuesta (15.23%). The number of samples collected by socioeconomic status was 30 in stratum 1 (5.92%), 144 in stratum 2 (28.45%), 193 in stratum 3 (38.14%), 121 in stratum 4 (23.91%), 16 in stratum 5 (3.16%), and 2 in stratum 6 (0.39%). The number of samples collected according to water sources located at < 200 meters was 232 for “yes” (45.84%) and
for “no” (54.15%).

Immunological tests

Dogs and human blood samples were collected in 3 mL tubes and centrifuged. The resulting serum was stored at −20 °C until further processing. Blood samples were also collected with EDTA for the study of microfilaremia in dogs.

Dog serum samples were tested for the presence of D. immitis antigens using a commercial immunochromatographic test kit (Uranotest Dirofilaria®, Urano Vet SL, Barcelona, Spain) according to the manufacturer’s instructions. Microfilaria counts were performed in blood samples with EDTA by applying the Knott technique [20].

Human samples were analyzed using a non-commercial ELISA test kit to detect the specific responses of anti-D. immitis and anti-Wolbachia IgG antibodies with some modifications [4, 7, 21, 22]. D. immitis adult worm extract (DiSA) and 1:100 serum dilutions were used to detect the presence of anti-D. immitis IgG antibodies. A recombinant form of the Wolbachia Surface Protein (rWSP) and 1:40 serum dilutions were used to detect the presence of anti-Wolbachia IgG antibodies. Goat anti-human IgG (H + L) conjugated to horseradish peroxidase (Sigma-Aldrich, Spain) was used at a 1:4000 dilution in both cases. Optical densities (OD) were measured at 492 nm with an Easy Reader (Bio-Rad laboratories, USA). The cut-off point (OD = 0.8 for DiSA and 0.5 for rWSP) was established by calculating the mean value + 3 standard deviations (3SD) of 30 serum samples obtained from dogs and clinically healthy humans (negative controls) who belonged to an area free of D. immitis. Human sera were considered positive when both non-commercial ELISAs were positive for the same serum sample. These results were additionally confirmed using Western blot analysis performed according to a previously described methodology [23, 24]. Both antigenic extracts were subjected to SDS-PAGE in 12% gels under reduced
conditions, and proteins were transferred onto nitrocellulose. Human sera were analyzed at a 1:40 dilution and anti-conjugates at a 1:500 dilution.

Geospatial analysis

A geospatial analysis was performed on the population of dogs and humans from the metropolitan area of Bucaramanga through a spatial overlay of positive cases using the SatScan software v.9.6. and the Bernoulli’s model with a 95% significance level (999 replications with p < 0.05) based on the Monte Carlo statistical significance test. Further, we established clusters, which are areas with a relative risk of infection in dogs and humans, with a maximum size of 50% of the exposed population, based on population census and positive cases. Clusters were imported into the QGIS software (3.8.0 version) to be visualized on the study area map.

Statistical analysis

Data was analyzed using SPSS 20.0 software for Windows (SPSS Inc./IBM, Chicago, IL, USA). This is a descriptive study with a univariate analysis for the determination of frequencies and a bivariate analysis through chi-square and OR estimation, based on which a statistical analysis was performed for the determination of the association between variables. In all cases, the level of significance was established with p < 0.05.

Results

Mean prevalence of D. immitis was 10.82% (38/351, 95% CI) in dogs and 5.12% (18/351, 95% CI) in microfilaremic dogs (Table 1). Regarding sex, a prevalence of 10.6% (14/351) was observed in males and 10.95% in females (24/351). The prevalence of D. immitis in the age groups of < 1, 1-3, 4-6, 7-10, and 11-15 years was 6.45% (2/31), 10.09% (11/109), 9.24% (11/119), 17.9% (12/67), and 8% (2/25).
Regarding municipality, a prevalence of 7.6% was observed in Bucaramanga (11/144), 13.7% (10/73) in Floridablanca, 12.9% (9/70) in Girón, and 12.5% (8/64) in Piedecuesta. Prevalence by socioeconomic status was as follows: 11.51% in stratum 1 (16/139), 10.5% in stratum 2 (6/57), 12.6% in stratum 3 (10/79), and 8% in stratum 4 (6/75); no positive or microfilaremic canines were observed for strata 5 and 6. Prevalence of 9.09% (17/187) and 12.8% (21/164) was observed in dogs living inside a house and those living outside a house, respectively.

No statistically significant differences were observed between the variables sex, age groups under 7 and over 7 years, socioeconomic status, municipality, and sleeping inside or outside a house. However, a statistically significant relationship was observed between municipalities and presence of microfilaremia (p < 0.01). No association was observed between the variables sex, socioeconomic level, and municipality, but age of dogs and whether they lived inside or outside a house can be considered risk factors for exposure to D. immitis (OR = 1.75 and 1.48, respectively). No statistically significant differences were found between exposure to D. immitis and the other variables, and no association was observed between them (p < 0.05).

Mean seroprevalence in humans was 6.71% (34/506, 95% CI) (Table 2) (Fig. 3). All samples positive for IgG-ELISA were confirmed using Western blot analysis, and D. immitis positive samples showed similar patterns in all cases (Fig. 2). Municipalities had seroprevalence values of 6.9% (13/189) in Bucaramanga, 5.7% (10/176) in Floridablanca, 3.1% (2/64) in Girón, and 11.7% (9/77) in Piedecuesta. In terms of socioeconomic status, prevalence was 20% (6/30) in stratum 1, 8.3% (12/144) in stratum 2, 5.7% (11/193) in stratum 3, and 4.1% (5/121) in stratum 4; no positive population was found in strata 5 and 6. A prevalence of 7.4% (28/377) was observed.
in the population living with pets, 7.8% (26/335) in the population living with 
canines, and 4.7% (8/171) in the population living with other species; those who do 
not live with pets had a prevalence of 4.7% (6/129). The prevalence in the age 
groups of 18–35, 36–50, and 51–65 years, which were established by finding the K 
number of intervals, was 5.8% (17/294), 8.5% (10/118), and 9.6% (7/73), 
respectively; the other group showed no positivity. A prevalence of 6.5% (15/232) 
was observed in the population that reported having water sources at < 200 meters 
from their house, and a prevalence of 6.9% (19/274) was observed in the population 
that did not report having water sources at < 200 meters from their house. 
There were no statistically significant differences between the variables and 
exposure to the parasite. There was no association between the variables of living 
with pets or not, especially dogs, but age and sex can be considered risk factors for 
exposure to D. immitis in humans (OR = 1.29 and 1.75, respectively).

In the spatial exploration of dogs positive for D. immitis within the study area 
(Fig. 3), no significant clusters were found with p < 0.05. However, cluster 1 showed 
a relative risk of 7.90 in dogs. However, three significant clusters were detected 
taking into consideration the magnitude and distribution of seropositive humans: 
cluster 1, located in the rural area of the municipality of Piedecuesta comprising 
83.3% of expected cases with a relative risk of 0.84 (p < 0.01) compared with 
individuals living outside that cluster; cluster 2 located in the southeast part of the 
municipality of Bucaramanga comprising 93.2% of expected cases with a relative 
risk of 0.94 (p < 0.01) compared with individuals living outside that cluster; and 
cluster 3 located in the municipal capital of Girón comprising 98.9% of expected 
cases with a relative risk of 0.99 (p < 0.01) compared with individuals living outside 
that cluster.
Discussion

Cardiopulmonary dirofilariosis is a zoonotic and vector-borne disease. The occurrence of dirofilariosis in humans depends mainly on the presence of infected dogs and vectors for transmission within a given area. At the same time, other factors, such as increase in temperature and humidity owing to climate change; emergence of new disease-transmitting species; transportation of infected hosts; modification of the environment owing to human activities; agricultural practices and irrigation areas; deficiency and economic instability; and adverse meteorological events such as hurricanes or tropical rains in the area, affect the development of the disease. [1, 4, 25]. Further, it is important to note that although it is a vector-borne disease, not all L3 that come into contact with the host develop into adults, neither in dogs nor in wild hosts [9].

Although there are numerous studies that report the presence of D. immitis in dogs worldwide, most of the information regarding humans comes from clinical cases and retrospective reviews. In these cases, there are only data from the affected population that showed some type of clinical manifestation, excluding the infected population that does not have symptoms related to the disease or any clinical manifestation, making its study even more difficult. Seroepidemiological studies show the complementary part of the problem because they detect contact with the parasite by analyzing the anti-Dirofilaria and anti-Wolbachia immune response and are excellent tools to analyze the risk of infection among the human population residing in an endemic area (1,4,7,22,26,27,28).

In Colombia, dirofilariosis is a very poorly studied disease. Few studies have addressed this problem during the last 20 years [10, 13, 15]. Furthermore,
prevalence values in dogs vary (0.91–16.12%) and have been obtained using different methodologies. In humans, there is only one clinical case of a patient from whom an adult worm identified as Dirofilaria sp. was extracted from the lung [14], and there are two studies that warn of the existence of human infections caused by D. immitis in communities from the Colombian Amazon where infected dogs have been found [15, 16].

In this study, we analyzed the presence of D. immitis in dogs and humans in the metropolitan area of Bucaramanga, Colombia by analyzing the presence of circulating antigens of D. immitis within the canine population, and the response of anti-D. immitis and anti-Wolbachia IgG antibodies in the human population as a study model for other places in Latin America.

The mean prevalence in dogs was 10.82%. The highest prevalence was found in the Floridablanca area (13.7%), followed by Girón (12.9%), Piedecuesta (12.5%), and Bucaramanga (7.6%). These areas are surrounded by vegetation and are characterized by an average annual temperature of 24 °C, high humidity levels, several gullies in their proximities or even in their interior that accumulate water in the rainy season, and the presence of two rivers (Oro and Surata in the areas of Girón and Bucaramanga, respectively). These conditions could promote breeding of these mosquitoes and disease transmission in said areas. Furthermore, prevalence in microfilardemic dogs was 5.12%, which was heterogeneously distributed across both sexes, within the different age groups, in the four municipalities, regardless of the different socioeconomic status of their owners, and the dogs’ lifestyle; thus, none of the analyzed factors are primarily responsible for transmission in dogs. This situation is similar to that observed in other endemic areas where the disease has been reported (2,4,7,8,10,27,28,29).
Regarding human infections, mean seroprevalence was 6.71% and the highest prevalence was 11.7% in Piedecuesta, followed by 6.9% in Bucaramaga, where prevalence in dogs reached similar values (12.5% and 7.6%, respectively); moreover, we observed slightly lower seroprevalence values of 5.7% in Floridablanca and 3.1% in Girón. where the prevalence observed in dogs was 13.7% and 12.9%, respectively. These data are related to the geographical location of the samples, and humans with positive serology have been reported in the same location as infected dogs. In addition, there are spatial clusters in these areas with a relative risk of < 1 for humans, suggesting a positive association between the variables studied and a higher frequency of contact with the parasite. Thus, these data suggest a direct relationship between the presence of dogs and humans infected by D. immitis. This is similar to what occurs in other European areas such as Spain, Portugal, Romania, and Russia, where the risk of infection among humans has been studied [4, 7, 8, 27, 28, 29].

Regarding the variables evaluated, sex may be a risk factor for human exposure to D. immitis. In our study, we found that seropositive individuals were mostly women. There are several studies that suggest that serological screening in humans should be carefully interpreted [7, 8, 28, 29]. Further, we observed that age can be a risk factor. In our study, the population with the highest seropositivity was that from the age group of 16–34 years. However, other studies report that the risk of infection increases with age [4, 7, 8, 27, 28, 29]. Humans within this age group perform more activities outside their homes and in areas with vegetation. However, it is necessary to analyze this fact more closely, and more data and optimization of serological screening are required in future studies.

Furthermore, not only did this study allow us to address the problem from a
biological point of view but also from a socioeconomic point of view in case of humans. The highest seroprevalence was observed in stratum 1, where sanitary hygiene conditions are not adequate (20%), followed by stratum 2 (8.3%), stratum 3 (5.7%), and stratum 4 (4.1%). Seropositive individuals were not detected in the last two strata where the sanitary hygiene level is optimal. Socioeconomic status has been associated with mortality and the use of health services, which indicates that a lower income reduces the application of prophylactic and preventive measures to vectors and canines that live with humans (González de Haro, 2006). Environmental sanitation elements, such as water; sewage, garbage, and waste disposal; sanitary landfills; and garbage treatment, influence the prevalence of parasitosis. These data allow us to associate the lack of sanitary hygiene with the development of dirofilariosis, which may become a socially determinant public health factor, as in the case of other vector-borne diseases in Colombia such as malaria, leishmaniosis, Chagas disease, and human ascariosis [9, 30, 31].

Conclusions

In conclusion, this study describes, for the first time, seropositivity to D. immitis and WSP in a human population in Colombia, specifically in one of the most populated areas of the country, the metropolitan area of Bucaramanga, with a high presence of dogs infected by D. immitis, which are a potential threat to public health. The corresponding authorities should take measures to monitor and control this emerging zoonotic disease to reduce prevalence in canines, while including human pulmonary dirofilariosis in the differential diagnosis of pulmonary nodules. However, it is necessary to perform further studies in Colombia regarding vectors and reservoirs of infection using a larger sample size, as well as epidemiological
studies in humans to clarify the risk of this infection.

Abbreviations

3SD: 3 standard deviations; CI: confidence interval; DiSA: D. immitis adult worm extract; EDTA: ethylenediaminetetraacetic acid; ELISA: enzyme-linked immunosorbent assay; IgG: Immunoglobulin G; rWSP: recombinant Wolbachia surface protein; OD: optical densities; SDS-PAGE; sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

Declarations

Ethics approval and consent to participate

The sampling process complied with the Helsinki code of ethics and animal welfare and was approved under resolution by the Ethics Committee of Universidad Cooperativa de Colombia.

Consent for publication

Not applicable.

Availability of data and materials

The datasets supporting the conclusions of this article are included within the article.

Competing interests

The authors declare that they have no competing interests.
Funding

This study has been supported by Universidad Cooperativa de Colombia (INV2213) and Agencia de Desarrollo Económico de Castilla y León, Spain (cofinanced with FEDER funds).

Authors’ contributions

MVEM, RM and VAQ designed the study and wrote the manuscript. LAN, IH and CFA performed the fieldwork and collected the data. MVEM, RM and VAQ participated in the discussion of the results and corrected the manuscript. All authors read and approved the final manuscript.

Acknowledgments

We would like to thank the staff of the Higuera Escalante Blood Bank and veterinarians from the companion animals’ clinic of Universidad Cooperativa de Colombia, Biovet veterinary clinical diagnosis.

Author details

1GRICA Group, Universidad Cooperativa de Colombia, School of Veterinary Medicine and Zootecnhics, Master’s in Animal Health and Production, UCC, Calle 30 No. 33-51, Bucaramanga, Colombia. 2Group of Animal and Human Dirofilariosis, Parasitology Area, Faculty of Pharmacy, University of Salamanca, Campus Miguel Unamuno s/n, 37007, Salamanca, Spain. 3Research Group from Higuera Escalante Clinical Laboratory and Blood Bank, Bucaramanga, Colombia
References

[1] Simón F, Siles-Lucas M, Morchón R, González-Miguel J, Mellado I, Carretón E, Montoya-Alonso JA. Human and animal dirofilariasis: the emergence of a zoonotic mosaic. Clin Microbiol Rev. 2012;25(3):507-44.

[2] Morchón R, Carretón E, González-Miguel J, Mellado-Hernández I. Heartworm disease (*Dirofilaria immitis*) and their vectors in Europe - new distribution trends. Front Physiol. 2012;3:196.

[3] Montoya-Alonso JA, Carretón E, Morchón R, Silveira-Viera L, Falcón Y, Simón F. The impact of the climate on the epidemiology of *Dirofilaria immitis* in the pet population of the Canary Islands. Vet Parasitol. 2016;216:66-71.

[4] Fontes-Sousa AP, Silvestre-Ferreira AC, Carretón E, Esteves-Guimarães J, Maia-Rocha C, Oliveira P, Lobo L, Morchón R, Araújo F, Simón F, Montoya-Alonso JA. Exposure of humans to the zoonotic nematode *Dirofilaria immitis* in Northern Portugal. Epidemiol Infect. 2019;147, e282, 1-5.

[5] Simón F., Kramer LH, Román A., Blasini W., Morchón R., Marcos-Atxutegi C., Grandi G., Genchi C. Immunopathology of *Dirofilaria immitis* Infection. Vet Res Commun. 2007;31:161-71.

[6] Genchi C, Kramer LH, Sassera D, Bandi C. Wolbachia and its implications for the immunopathology of filariasis. Endocr Metab Immune Disord Drug Targets. 2012;12(1):53-6.

[7] Ciuca L, Simòn F, Rinaldi L, Kramer L, Genchi M, Cringoli G, Acatrinei D, Miron L, Morchon R. Seroepidemiological survey of human exposure to *Dirofilaria* spp. in Romania and Moldova. Acta Trop. 2018;187:169-74.

[8] Kartashev V, Afonin A, González-Miguel J, Sepúlveda R, Simón L, Morchón R,
Simón F. Regional warming and emerging vector-borne zoonotic dirofilariosis in the Russian Federation, Ukraine, and other post-Soviet states from 1981 to 2011 and projection by 2030. Biomed Res Int. 2014;2014:858936.

[9] Maggi RG, Krämer F. A review on the occurrence of companion vector-borne diseases in pet animals in Latin America. Parasit Vectors. 2019;12(1):145.

[10] Labarthe N, Guerrero J. Epidemiology of heartworm: What is happening in South America and Mexico? Vet Parasitol. 2005;133:149-56.

[11] Dantas-Torres F, Otranto D. Dirofilariosis in the Americas: a more virulent Dirofilaria immitis? Parasit Vectors [Internet]. 2013;6(1):288.

[12] de Argôlo EGG, Reis T, Fontes DAT, Gonçalves EC, Giese EG, Melo FTV, Dos Santos JN, Furtado AP. Canine filariosis in the Amazon: Species diversity and epidemiology of these emergent and neglected zoonoses. PLoS One. 2018;13(7):e0200419.

[13] McCown ME, Monterroso VH, Cardona W. Surveillance for Ehrlichia canis, Anaplasma phagocytophilum, Borrelia burgdorferi, and Dirofilaria immitis in Dogs From Three Cities in Colombia. J Spec Oper Med. 2014;14(1):86-90.

[14] Beaver PC, Orihel TC LG. Pulmonary dirofilariosis: restudy of worms reported gravid. Am J Trop Med Hyg [Internet]. 1990;43(2):9-167.

[15] Vieira C., Montoya M., Agudelo S., Simón F. Human antibody response to a 56-kDa purified excretory/secretory product of Dirofilaria immitis. Trop Med Int Heal. 2000;5(12):855-9.

[16] Vieira C, Vélez ID, Montoya MN, Agudelo S, Alvarez MI, Genchi C, Simón F. Dirofilaria immitis in Tikuna Indians and their dogs in the Colombian Amazon. Ann Trop Med Parasitol. 1998;92(1):123-5.

[17] Climate-Data.org / AM OP / OpenStreetMap contributors. Clima Bucaramanga:
Temperatura, Climograma y Tabla climática para Bucaramanga - Climate-Data.org [Internet]. [cited 2019 Jun 24]. Available from: https://es.climate-data.org/america-del-sur/colombia/santander/bucaramanga-5923/

[18] Albarracín Navas J, Cala FA. Primer censo canino y felino del área urbana del municipio de Bucaramanga. Rev Colom Cienc Pecua [Internet]. 2011.

[19] DANE. Clasificación socio-económica Colombia. 2019. [cited 2019 Dec 14]. Available from: https://www.dane.gov.co/files/geoestadistica/Preguntas_frecuentes_estatificacion.pdf

[20] Acevedo RA, Theis JH, Kraus JF, Longhurst WM. Combination of filtration and histochemical stain for detection and differentiation of *Dirofilaria immitis* and *Dipetalonema reconditum* in the dog. Am J Vet Res. 1991;42:537-40.

[21] Simón F, Muro A, Cordero M, Martin J. A seroepidemiologic survey of human dirofilariosis in Western Spain. Trop Med Parasitol. 1991;42(2):106-8.

[22] Simón F, Prieto G, Morchón R, Bazzocchi C, Bandi C, Genchi C. Immunoglobulin G antibodies against the endosymbionts of filarial nematodes (*Wolbachia*) in patients with pulmonary dirofilariasis. Clin Diagn Lab Immunol. 2003;10(1):180-1.

[23] Perera L, Pérez-Arellano JL, Cordero M, Simón F, Muro A. Utility of antibodies against a 22 kD molecule of *Dirofilaria immitis* in the diagnosis of human pulmonary dirofilariasis. Trop Med Int Health. 1998;3(2):151-5.

[24] Santamaría B, Cordero M, Muro A, Simón F. Evaluation of *Dirofilaria immitis* excretory/secretory products for seroepidemiological studies on human dirofilariosis Parasite. 1995;2(3):269-73.

[25] AHS, 2019. [cited 2019 Dec 14]. Available from: https://www.heartwormsociety.org/.

[26] Morchón R, González-Miguel J, Mellado I, Velasco S, Rodríguez-Barbero A, Simón...
F. Adult Dirofilaria immitis excretory/secretory antigens upregulate the production of prostaglandin E2 and downregulate monocyte transmigration in an "in vitro" model of vascular endothelial cell cultures. Vet Parasitol. 2010;170(3-4):331-5

[27] Cabrera ED, Carretón E, Morchón R, Falcón-Cordón Y, Falcón-Cordón S, Simón F, Montoya-Alonso JA. The Canary Islands as a model of risk of pulmonary dirofilariasis in a hyperendemic area. Parasitol Res. 2018;117(3):933-6.

[28] Montoya-Alonso JA, Mellado I, Carretón E, Cabrera-Pedrero ED, Morchón R, Simón F. Canine dirofilariosis caused by *Dirofilaria immitis* is a risk factor for the human population on the island of Gran Canaria, Canary Islands, Spain. Parasitol Res [Internet]. 2010;107(5):1265-9.

[29] Montoya-Alonso JA, Carretón E, Corbera JA, Juste MC, Mellado I, Morchón R, Simón F. Current prevalence of *Dirofilaria immitis* in dogs, cats and humans from the island of Gran Canaria, Spain. Vet Parasitol. 2011;176(4):291-4.

[30] Caraballo L, Coronado S. 2018. Parasite allergens. Mol Immunol.100:113-9.

[31] Feachem RGA, Chen I, Akbari O, Bertozzi-Villa A, Bhatt S, Binka F, Boni MF, Buckee C, Dieleman J, Dondorp A, Eapen A, Sekhri Feachem N, Filler S, Gething P, Gosling R, Haakenstad A, Harvard K, Hatefi A, Jamison D, Jones KE, Karema C, Kamwi RN, Lal A, Larson E, Lees M, Lobo NF, Micah AE, Moonen B, Newby G, Ning X, Pate M, Quiñones M, Roh M, Rolfe B, Shanks D, Singh B, Staley K, Tulloch J, Wegbreit J, Woo HJ, Mpanju-Shumbusho W. Malaria eradication within a generation: ambitious, achievable, and necessary. Lancet. 2019;394(10203):1056-112.

[28] Montoya-Alonso JA, Mellado I, Carretón E, Cabrera-Pedrero ED, Morchón R, Simón F. Canine dirofilariosis caused by *Dirofilaria immitis* is a risk factor for the human population on the island of Gran Canaria, Canary Islands, Spain. Parasitol Res [Internet]. 2010;107(5):1265-9.
[29] Montoya-Alonso JA, Carretón E, Corbera JA, Juste MC, Mellado I, Morchón R, Simón F. Current prevalence of *Dirofilaria immitis* in dogs, cats and humans from the island of Gran Canaria, Spain. Vet Parasitol. 2011;176(4):291-4.

[30] Caraballo L, Coronado S. 2018. Parasite allergens. Mol Immunol.100:113-9.

[31] Feachem RGA, Chen I, Akbari O, Bertozzi-Villa A, Bhatt S, Binka F, Boni MF, Buckee C, Dieleman J, Dondorp A, Eapen A, Sekhri Feachem N, Filler S, Gething P, Gosling R, Haakenstad A, Harvard K, Hatefi A, Jamison D, Jones KE, Karema C, Kamwi RN, Lal A, Larson E, Lees M, Lobo NF, Micah AE, Moonen B, Newby G, Ning X, Pate M, Quiñones M, Roh M, Rolfe B, Shanks D, Singh B, Staley K, Tulloch J, Wegbreit J, Woo HJ, Mpanju-Shumbusho W. Malaria eradication within a generation: ambitious, achievable, and necessary. Lancet. 2019;394(10203):1056-112.

Tables

**Table 1.** Prevalence in dogs in the Bucaramanga Metropolitan area in terms of gender, age, municipality, socioeconomic status, and their place of permanence

**Table 2.** Seroprevalence of human dirofilariosis in the metropolitan area of Bucaramanga, considering seropositivity is defined by the simultaneous positivity of anti-*D. immitis* and anti-rWSP antibody response

Figures
| VARIABLE                  | Sample (n) | + *D. immitis* | CI 95%       | Prevalence |
|---------------------------|------------|----------------|--------------|------------|
| GENDER                    |            |                |              |            |
| Male                      | 132        | 14             | .0474-.1511  | 10.6%      |
| Female                    | 219        | 24             | .0679-.1513  | 10.95%     |
| AGE GROUP (in years)      |            |                |              |            |
| <1                        | 31         | 2              | -.0271-.1561 | 6.45%      |
| 1–3                       | 109        | 11             | .0435-.1584  | 10.09%     |
| 4–6                       | 119        | 11             | .0338-.1357  | 9.24%      |
| 7–10                      | 67         | 12             | .0849-.2733  | 17.91%     |
| 11–15                     | 25         | 2              | -.0343-.1943 | 8.00%      |
| MUNICIPALITIES            |            |                |              |            |
| BUCARAMANGA               | 144        | 11             | .0276-.1122  | 7.6%       |
| FLORIDABLANCA             | 73         | 10             | .0562-.2178  | 13.7%      |
| GIRÓN                     | 70         | 9              | .0482-.2090  | 12.9%      |
| PIEDECUESTA               | 64         | 8              | .0417-.2083  | 12.5%      |
| SOCIODEMOCRATIC LEVEL     |            |                |              |            |
| Stratum 1                 | 139        | 16             | .0614-.1688  | 11.51%     |
| Stratum 2                 | 57         | 6              | .0231-.1874  | 10.5%      |
| Stratum 3                 | 79         | 10             | .0516-.2015  | 12.6%      |
| Stratum 4                 | 75         | 6              | .0090-.1261  | 8.00%      |
| Stratum 5                 | 1          | 0              | .000-.000    | 0.00%      |
| Stratum 6                 | 0          | 0              | .000-.000    | 0.00%      |
| PLACE OF PERMANENCE       |            |                |              |            |
| Indoors                   | 187        | 17             | .0493-.1325  | 9.09%      |
| Outdoors                  | 164        | 21             | .0718-.1736  | 12.8%      |
| **TOTAL**                 | **351**    | **38**         | **10.82%**   |            |
| Variable                   | Sample | Sample + D. immitis | CI 95%          | Prevalence |
|----------------------------|--------|---------------------|-----------------|------------|
| **GENDER**                 |        |                     |                 |            |
| Male                       | 159    | 9                   | .3192-.8808     | 5.7%       |
| Female                     | 347    | 25                  | .3939-6930      | 7.2%       |
| **AGE GROUPS (in years)**  |        |                     |                 |            |
| 18–35                      | 294    | 17                  | .2695-.6905     | 5.8%       |
| 36–50                      | 118    | 10                  | .2248-.7752     | 8.5%       |
| 51–65                      | 73     | 7                   | .2823-1.0510    | 9.6%       |
| 66–90                      | 21     | 0                   | -5.8531-6.8531  | 0.0%       |
| **MUNICIPALITIES**         |        |                     |                 |            |
| Bucaramanga                | 189    | 13                  | .4540-.9144     | 6.9%       |
| Floridablanca              | 176    | 10                  | .2286-.6805     | 5.7%       |
| Girón                      | 64     | 2                   | -.4187-1.4187   | 3.1%       |
| Piedecuesta                | 77     | 9                   | .3192-.8808     | 11.7%      |
| **SOCIOECONOMIC STATUS**   |        |                     |                 |            |
| Stratum 1                  | 30     | 6                   | .3016-.8010     | 20.0%      |
| Stratum 2                  | 144    | 12                  | .3406-.8023     | 8.3%       |
| Stratum 3                  | 193    | 11                  | .2093-.6055     | 5.7%       |
| Stratum 4                  | 121    | 5                   | .2630-1.1656    | 4.1%       |
| Stratum 5                  | 16     | 0                   | .000-.000       | 0.0%       |
| Stratum 6                  | 2      | 0                   | .000-.000       | 0.0%       |
| **WATER SOURCES LOCATED AT <200 METERS FROM THE HOUSE** | | | | |
| SI                         | 232    | 15                  | .3734-.7804     | 6.5%       |
| NO                         | 274    | 19                  | .3692-.7165     | 6.9%       |
| **LIVING WITH PETS OR NOT**|        |                     |                 |            |
| YES                        | 377    | 28                  | .4622-.7552     | 7.4%       |
| NO                         | 129    | 6                   | .1192-.6808     | 4.7%       |
| Canines                    | 335    | 26                  | .3760-.7668     | 7.8%       |
| Other species              | 171    | 8                   | .3889-.6808     | 4.7%       |
| **TOTAL**                  | 506    | 34                  |                  | 6.71%      |

**Figure 1**

Location of the metropolitan area of Bucaramanga, Colombia
Figure 2

Representative immunoblot analysis of the human sera: Negative control (1) and

Figure 3

Geospatial exploration of dogs and humans positive for D. immitis in the metropo

Supplementary Files

This is a list of supplementary files associated with the primary manuscript. Click to
download.

Graphical Abstract.tif