



Original Article

Serosurvey of Canine Dirofilariasis in a Population of Southern Guatemala

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ABSTRACT

Introduction: Canine dirofilariasis, is a vector-borne disease caused by the parasitic filarial nematode named *Dirofilaria immitis*. Cardiopulmonary dirofilariasis is a worldwide distributed disease affecting domestic and wild canines as well as felines, causing pulmonary or cutaneous infections in humans. In recent years, scientists have reported the importance of investigating human cases of dirofilariasis since it can be easily confused with lung disease. Guatemala has a high-density of the canine population. Few studies have reported a high prevalence of canine dirofilariasis; nevertheless, knowledge in Guatemala of *Dirofilaria immitis* is scarce and practically inexistent. Given this, the current study aimed to explore the circulation of antibodies against *D. immitis* in dogs in the southern part of Guatemala.

Materials and methods: Data were recollected from 110 male and female dogs aged more than a year from various breeds in the village "El Brito", Escuintla, Guatemala. One milliliter of blood was collected from each dog. The samples were analyzed using ELISA test to examine the presence of antibodies in adult worms.

Results: The prevalence determined for antibodies against *Dirofilaria immitis* of sampled canines was 2.7%, and the most frequent age of the canines was 1-3 years old (85%).

Conclusions: According to the obtained data, the prevalence of canine dirofilariasis in a southern region of Guatemala was low.

1. Introduction

Canine dirofilariasis, also known as heartworm disease, is due to an infection by *Dirofilaria immitis* (*D. immitis*). Its importance, concerning the well-being of pets, has increased in recent years since it is one of the most common parasitic diseases in dogs and an emerging zoonosis¹. This disease is distributed worldwide, with a prevalence ranging from 7.57% in Africa and 22.68% in Australia (Figure 1)². This prevalence is influenced by climate and topography, but most importantly, by the presence of the mosquito vectors, including ubiquitous *Culex* spp., *Aedes* spp., and *Anopheles* spp.³.

The adult worm is typically situated in the pulmonary artery and right ventricle of the heart of canines and felines, and in some cases, humans. Clinical signs in canines include dyspnea, hemoptysis, and right-sided congestive heart failure symptoms, such as

ascites, pulmonary edema, and death (Figure 2). Another severe complication of canine heartworm is caval syndrome in which displacement of filaria from pulmonary



Figure 1. World map, the prevalence of *Dirofilaria immitis* in dogs per continent (Source: Anvari et al.²)



Figure 2. Macroscopic view / chronic infection with heartworm disease in a dog (source: Carretón et al.⁸)

arteries into the right atrium and ventricle causes tricuspid valve insufficiency, increased pressure in the caudal vena cava, and subsequent hepatic congestion⁴.

Studies in wild carnivores on *D. immitis* have gained importance due to their likely transmission to domestic dogs. Foxes, coyotes, wolves, sea lions, harbor seals, laboratory ferrets, bears, muskrats, raccoons, and bobcats are wild carnivores susceptible to this disease⁵. Serbia had its first findings of dirofilariasis in wild carnivores in 2014⁶. In Romania, 3.24% of the tested population gave a positive result, including a red fox, golden jackals, and a wildcat⁷. Latin America has scarce studies on wild carnivores.

In recent years, scientists have reported the importance of investigating human cases of dirofilariasis since it can be easily confused with lung cancer, and most of the nodules are discovered accidentally. Human dirofilariasis is commonly asymptomatic. However, in some cases, it presents cough, chest pain, eosinophilia, hemoptysis, and fever⁹. In 2015, the Russian Federation reported a case of human dirofilariasis in a 14-month-old child for the first time¹⁰. Another study reported 1,782 cases in humans (116 distributed in the United States and approximately 50 cases in South America and Costa Rica)¹¹. In Central America, some epidemiological data has been generated in Costa Rica¹². However, no data has been published for the rest of the countries¹³.

Like other Latin American countries, Guatemala has a high-density of canine population. Poverty, lack of water, and sanitation issues in southern Guatemala¹⁴ increase the probability of vectors of *D. immitis* in the area. Few studies have been conducted in Guatemala on this parasite. Several villages in three departments of Guatemala reported a 29.7% prevalence of *D. immitis* using the same method used in this study¹⁵. Nevertheless, knowledge in Guatemala of *D. immitis* is scarce and practically inexistent. Therefore, the current research aimed to explore the circulation of antibodies against *D. immitis* in dogs in the southern part of Guatemala.

2. Materials and Methods

2.1. Ethical approval

This research was approved by the Bioethics Committee of the Graduate School, Veterinary Medicine and Animal Husbandry Faculty, University of San Carlos of Guatemala.

2.2. Study area

This research was conducted in the village "El Brito", located in the municipality of Guanagazapa, department of Escuintla, Guatemala, with coordinates 14°08'26.2 "N 90°39'38.3 "W. It is located at 315 meters above sea level with an average temperature of 18°C and 34°C; 13 millimeters of precipitation in the rainy season and 2 millimeters during the dry season; relative humidity of 81% average and 13 kilometers per hour of wind speed¹⁶. The collected data indicate that El Brito's total population is 1,840 people¹⁷.

2.3. Methodology

The Ministry of Public Health and Social Care (MSPAS for its acronym in Spanish) estimates a proportion of one dog for every five habitats, in which 368 canines represent the estimated population¹⁸. In the present study, due to some limitations, 110 dogs were sampled on August 2019 (equivalent to a 95% population, 8% margin of error, and assuming a 50% expected frequency), including 51 females and 59 males. Dogs were selected opportunistically during a vaccination campaign against rabies with the help of the president of Community Councils for Urban and Rural Development (COCODES) of the village. The sample was collected by including dogs of both sexes older than one year from different breeds.

2.4. Sample analysis

One milliliter of blood was collected from each dog, using a three-milliliter syringe with a 21-caliber needle; the extraction point was the antebrachial cephalic vein. The samples were introduced in anticoagulant EDTA K3 tubes. The latter is an alternative form of K2 EDTA, containing 3 potassium ions, which does not influence MCV at higher concentrations. All tubes were put in a cooler for transportation to the laboratory. The samples were analyzed in the Clinical Laboratory of the Veterinary Medicine Hospital at the Faculty of Veterinary Medicine and Animal Husbandry, University of San Carlos of Guatemala, in Guatemala City. A commercial kit of VetScan® Heartworm ELISA Rapid Test by Zoetis United States test was used. This test indicates the presence of antigens in the adult worm. The test was made according to the manufacturer's specifications.

2.5. Statistical analysis

The data collected was registered in Excel 2016. Categorical variables were analyzed with frequencies and

percentages. SPSS version 20.0 (USA) was used for analyzing the data. Descriptive statistics were used to demonstrate heartworm prevalence. T-test was used to compare data. Bonferroni test was chosen to determine the mean significant differences. The $p < 0.05$ was considered a significant difference.

3. Results

The average age distribution of sampled mixed-breed dogs over one year old in Guatemala showed in Table 1. The obtained results indicated that 85% of sampled canines were between 1-3 years old, followed by 6% between 3-5 years old, 6% between 5-7 years old, 2% between 9-11 years old, 0.5% between 7-9 years old, and 0.5% 13-15 years old.

Table 1. Average age distribution of sampled mixed-breed dogs over one year old in Guatemala

Age	Number of Samples	Percentage
1 - 3 years	94	85%
3 - 5 years	6	6%
5 - 7 years	6	6%
7 - 9 years	1	0.5%
9 - 11 years	2	2%
13 - 15 years	1	0.5%
Total	110	100%

Table 2 demonstrates the sex distribution of sampled mixed-breed dogs over one year old in Guatemala. Results showed that 46% of sampled canines were female and 54% were male.

The prevalence determined for antibodies against *Dirofilaria immitis* of sampled canines was 2.7%.

Table 2. Sex distribution of sampled mixed-breed dogs over one year old in Guatemala

Sex	Number of Samples	Percentage
Female	51	46%
Male	59	54%
Total	110	100%

4. Discussion

The prevalence found in the serosurvey was low despite the fact that dogs are susceptible to *D. immitis*¹⁹. This might be due to the fact that most of the sampled dogs were young, and infection rates increased with age²⁰. Although the climate is favorable for the existence of infectious vectors²¹, and the dogs were of varied ages and sexes, a considerably low prevalence was found.

The campaign was offered where infectious mosquitoes were present. However, a high percentage of the sampled canines spent most of their lives indoors, only going outside when necessary. The risk of being bitten by mosquitoes decreases in dogs kept indoors because the active time of mosquitoes is during dusk and dawn²². The presence of microfilariae in the blood of dogs is present until approximately 85 days after the infection, or it can be hidden¹¹. These factors can explain the lack of evidence of

D. immitis in the sampled dogs.

A second consideration would be the sex (Table 2) and size of the dogs. Of the three positive dogs, two were male, and all three were of medium size. Dogs of medium size are more likely to be used as work dogs and generally spend more time in the exterior of the house, increasing the exposure of being bitten by the vector²³.

As far as the author is concerned, this is one of the first research investigating the overall prevalence of *D. immitis* antibodies among dogs in Guatemala. The results reported only 2.7% of the dog population has antibodies. Furthermore, the obtained result revealed that clinical symptoms of *D. immitis* were not observed in any sampled canines.

A study conducted in Latin America, presented a prevalence of heartworm in three different cities of Columbia, 1.2% in Cartagena, 0% in Medellín, and 1% in Barranquilla²⁴, 2% prevalence in Brazil²⁵, and 1% in Cuiabá, Brazil²⁶ which is concordant with the results obtained in the current study. A survey in Mexico determined an 8.9% prevalence of *D. immitis*²⁷ and in Sucre, Venezuela of 8.7%²⁸, which is discordant with the present results. These results highlight the importance of further research in the current study area.

Wolbachia is present in every stage of the parasite lifecycle²⁸. The use of antibiotics, including doxycycline in combination with macrocyclic lactones (such as ivermectin, abamectin, and selamectin), has even been considered a treatment for heartworm disease in dogs because it interfered with *Wolbachia*²⁹. In adult worms, this protocol causes neuromuscular dysfunction and reduces microfilaria production, which leads to the degeneration of late-stage embryos³⁰. The combination of these antibiotics is widely used in Guatemala for dog infections. According to a study, a high percentage of the dogs sampled had recently been exposed to this protocol²⁹.

The accepted gold standard is the presence/absence of adult worms (pulmonary arteries and/or heart) in necropsy examination. However, necropsy as a gold standard for diagnostic assays presents difficult procedural and ethical challenges. This would be another justification for the use of the commercial kit of VetScan® Heartworm ELISA Rapid Test by the Zoetis United States.

According to a study, El Brito meets the climatic conditions for the presence of the vector³¹. Considering the results of this study, further research needs to be conducted to determine the presence of heartworm disease, adding a necropsy diagnosis in every deceased dog. Decreases in mosquito diversity due to urbanization alter vector-borne disease risk³². Regarding dog heartworm disease, this loss of mosquito diversity is associated with decreased heartworm prevalence within both the vector and the host. Although the response is likely different for diseases transmitted by one or a few species, mosquito diversity losses leading to the decreased transmission could be generalizable to other pathogens with multiple vectors.

Human dirofilariasis has been reported in countries,

such as Brazil, Cuba, Canada, Argentina, Ecuador, and the United States³³. No known case has been reported in Guatemala. However, more research needs to be done due to the public health issues of this parasite.

5. Conclusion

Results of this study indicated a prevalence of antibodies to *Dirofilaria immitis* of 2.7% in sampled canines. The majority (85) of the dogs sampled were between 1 and 3 years of age, and the majority (54%) were male. Of note, the findings of the current study suffer from some limitations, including the performance of the study in only one location and lack of medical histories of the sampled dogs. Given the findings of the present study (the first one in Guatemala) and considering that none of the canines exhibit symptoms, a small percentage of antibodies, and insignificant mortality rate in recent years, more research needs to be conducted to identify the prevalence of heartworm in canine population in El Brito, Guanagazapa, Escuintla.

Declarations

Competing interests

The authors have declared that no competing interest exists.

Author's contribution

The author wrote the manuscript and checked the final draft of the manuscript for publication.

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Availability of data and materials

The author granted the publisher the sole and exclusive license of the full copyright in the contribution. Consequently, the publisher shall have the exclusive right throughout the world to publish and sell the contribution in all languages and all other forms of electronic publication.

Ethical considerations

All participants in the following research were not subjected to any harm whatsoever. Full consent was provided by the owners of the dogs. The privacy and anonymity of participants have been ensured. The authors carefully examined all ethical issues concerning plagiarism, approval to publish, errors in fabrication, double publication, and submission.

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