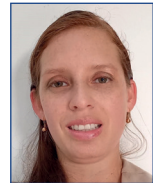


Seroprevalence of *Dirofilaria immitis* in canines from animal shelters in the Colombian coffee region (Eje Cafetero)



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Abstract

In Colombia, there are reports of approximately 6.4 million pets, making this the fourth country in Latin America and leading the pet sector with an annual growth of 13%. This study aimed to determine the seroprevalence of *Dirofilaria immitis*, a parasitic nematode, in 170 canines from the municipalities of Pereira, Santa Rosa de Cabal, and La Virginia, located in the department of Risaralda and the municipalities of Calarca and Armenia in the department of Quindío. A cross-sectional study was carried out on canines from animal shelters during the period 2021–2022. Blood samples were taken, and in vitro immunochromatography was performed to detect specific lesions for *Dirofilaria immitis*. A peripheral blood smear confirmed

positive patients, and a chest x-ray was performed to show changes in the morphology of the heart and blood vessels. Seroprevalence was determined by calculating proportions with the 95% confidence interval determined by the exact or Clopper-Pearson method for a proportion based on sample size and the number of positive cases. The seroprevalence of the test for *Dirofilaria immitis* was 0.62% (95% CI = 0.016–3.42%). This parasite is a nematode of low distribution in areas of the coffee region. However, surveillance programmes that help control and reduce its transmission should be established.

Key words: *dirofilariasis; heartworm; insect vector; nematodes*

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Introduction

Dirofilaria immitis or heartworm is a nematode-type parasite considered an emerging pathogen with zoonotic potential that can affect canines as definitive hosts (Genchi et al., 2019). However, its presence has been reported in wild canids, felines, and even humans, and they act as accidental hosts (Dantas-Torres and Otranto, 2020). This agent is the cause of canine dirofilariasis, a chronic, progressive, and fatal disease whose adult worms can affect pulmonary arteries and lung tissue, ending in heart failure (Montoya-Alonso et al., 2015). *D. immitis* is the most important life-threatening parasitic infection in domestic canines (Laidoudie et al., 2020). This disease is considered zoonotic and includes nematodes of the genus *Dirofilaria*, which includes more than 40 species (Bamorovat et al., 2017). The larvae in their third stage can be transmitted between hosts by the bite of mosquito species *Anopheles spp*, *Culex spp*, and *Aedes spp*, which are considered vectors of the disease (Nguyen et al., 2016). In turn, more than 70 different species of mosquitoes have been reported that allow microfilariae to develop into infective third-stage larvae (L3) *in vitro* (McCall et al., 2017). These microfilariae reside in pulmonary arteries and right ventricles. They can be found circulating in blood; however, since this parasite can hide, determining antigens is considered the most sensitive method (Wang et al., 2018). This parasite is distributed worldwide in tropical and subtropical regions and some temperate climate regions (Torres-Chable et al., 2019). Its distribution depends on environmental factors such as temperature and humidity, the distribution, fertility, and population density of the mosquito, and the age of the definitive or accidental host, among others (Anvari et al., 2020). The presenta-

tion of heartworm disease ranges from asymptomatic patients to symptoms such as congestive heart failure, bronchial collapse, pulmonary infarction, pulmonary hypertension, acute or chronic respiratory distress, or even death of the animal (Al-Salihi et al., 2019).

Studies of *D. immitis* prevalence in South America are scarce and fragmented, and given its chronic nature, the frequency rates may be higher than those reported. In Colombia, the presence of this agent has been reported in Bucaramanga, Bogotá, Valle de Aburrá, Barranquilla, and Cartagena. However, to date, no studies have reported this agent's presence in the coffee region (Muñoz et al., 2020). This disease is of great economic importance. The impact on animal health and welfare has led to the investment of millions of dollars in its prevention, diagnosis, and treatment each year (Wolstenholme et al., 2015). Serological tests such as ELISA and immunochromatography for detecting antigens are highly specific and sensitive tests against *Dirofilaria immitis* that are used in reference diagnostic laboratories (Panarese et al., 2020). This study aimed to determine the presence of *Dirofilaria immitis* in canine shelters in metropolitan areas of the departments of Risaralda and Quindío.

Materials and methods

Ethics statement

All people gave their informed consent prior to inclusion in the study. All procedures with animals were carried out under the guidelines of the Bioethics Committee of the Corporación Universitaria Santa Rosa de Cabal, Risaralda, Colombia (22 June 2022).

Study type and sampling locations

This study was a cross-sectional type carried out from 2021-2022, to determine the prevalence of *Dirofilaria immitis* in several Colombian municipalities from the Quindío and Risaralda departments (Colombian coffee region). This study was carried out in dog shelters located in the municipalities of Pereira, Santa Rosa de Cabal, and La Virginia, in the department of Risaralda, a region in central-western Colombia. In this area, rainfall volumes are between 1500 and 5000 mm per year. The average air temperature of 22°C registers at altitudes between 1000 and 2500 m (Carder, 2019). In turn, dogs were sampled in animal shelters in the municipalities of Calarcá and Armenia in the department

of Quindío. This region is at an elevation between 1180 and 4500 m, with an average air temperature of 20°C and precipitation between 1427 and 3037 mm per year (CRQ, 2020). According to the 2019 anti-rabies vaccination census (Minsalud, 2019), the dog population is approximately 85,620 and 137,131 in the Quindío and Risaralda departments, respectively (Fig. 1).

Sampling

In this study, 170 samples were taken at convenience from canines located in animal shelters. Veterinarians took the samples with the prior informed consent of owners caring for these animals. The animals were immobilised to facilitate handling and to avoid trauma during sample



Figure 1. Study locations in Risaralda and Quindío departments in Colombia. Graphic design: Arias-Montoya Juliana 2023

collection. From each animal, 1 mL whole blood was collected in tubes with EDTA as an anticoagulant for making peripheral blood smears, and 1 mL whole blood in tubes with clot-activating serum/separator gel additive to obtain serum. Samples were obtained by puncture with a hypodermic needle from the cephalic or jugular vein after disinfection of the puncture site. The age of the dogs was classified into three groups: a group of puppies between 1 and 12 months of age, an adult group between 1 and 7 years of age, and a group of geriatricians older than 7 years; this information was provided by the manager of each animal shelter. The variables considered in the study were age, sex, breed, dog origin, animal shelter location, presence of rivers, wetlands, lakes near the house, and wastewater disposal. The exclusion criteria were canines with incomplete information in the data form or those without a signed informed consent by the owner.

Laboratory analysis

For the diagnosis of heartworms in the canines of this study, Uranotest® *Dirofilaria* rapid heartworm antigen tests were used, based on a one-step in vitro immunochromatographic technique, for the qualitative determination of *Dirofilaria immitis* antigen, following the manufacturer's instructions. Positive results obtained through the test were verified by direct visualisation of the parasite through a peripheral blood smear using Romanosky staining. Haemolysed and coagulated samples older than 15 days after collection were excluded. Positive patients underwent a complete blood count and chest X-ray as complementary tests. The samples were analysed in the microscopy laboratory of the Veterinary Medicine and Zootechnics program of the Faculty of Health Sciences, Technological University of Pereira.

Statistical analysis

The *D. immitis* prevalence was determined by calculating proportions with the 95% confidence interval (CI), determined by the exact or Clopper-Pearson method for a proportion based on sample size and the number of positive cases. Likewise, true prevalence (TP) was calculated using the following equation (Eqn. 1) (18).

$$TP = \frac{AP + Sp - 1}{Se + Sp - 1}$$

Equation 1. TP: true prevalence, AP: apparent prevalence = (positive cases x 100 samples) Sp: test specificity; Se: test Sensitivity.

Results

Of the 170 canines sampled in the different animal shelters, 75% were male, and 35% were female; 80% of the sampled canines belonged to the group of adult canines, 97% of the canines were of the mixed breed, 70% of the sampled sites were in urban areas with a sewage system. A single positive canine was obtained for *Dirofilaria immitis* antigen measurement immunochromatographic test. The prevalence of the test, or apparent prevalence (AP), was 0.58% (95% CI = 0.015–3.23%). However, using the AP, Se, and Sp of the test, the true prevalence (TP) was calculated using equation 1 (18). Thus, it was possible to establish that the true prevalence of *Dirofilaria immitis* was 0.62% (95% CI = 0.016–3.42%), based on the above; specifically, at the upper limit of the 95% CI, it could be indicated that the TP of the disease in the canine population would be ≤ 3.4%.

The positive case corresponds to an adult mestizo female canine rescued while pregnant from the streets of the department of La Guajira in the Caribbean region. In the animal shelter, the canine have

Table 1. Haematological affected parameters in a female dog positive to *D. immitis*

Parameters	Results	Reference values
HB (g/dL)	12.00 =	12.00 – 18.00
MCH (pg)	19.20 ↓	19.50 – 24.50
MCHC (g/dL)	29.10 ↓	31.00 – 39.00
RDWC %	15.10 =	14.00 – 20.00
RDWS (fL)	38.30 ↓	
PLT (10 ⁹ /L)	79.00 ↓	165.00 – 500.00
MPV (fL)	11.80 ↓	3.90 – 11.10
PCT %	0.09 ↓	
PDWC %	40.90 ↓	
PDWS (fL)	23.10 ↓	

been living the last ten month of her life. The animal shelter is located in a rural area of Santa Rosa de Cabal in the department of Risaralda. This refuge has a septic tank and no nearby water source, river, lake, or stream. When the sample was taken, the dog did not present heartworm-related symptoms such as cough, dyspnoea, exercise intolerance, abnormal lung sounds, or altered heart sounds. The patient had good body condition, bright pink mucosa, and a normal coat. The white cells paraclinical tests taken as a blood count were within normal ranges; however, parameters such as haemoglobin (12 g/dL), MCH (19.20 pg), MCHC (29.10 g/dL), and platelets (79.00 (10⁹/L) were below the lower limit, showing the red series affected by the parasitism (Table 1). The peripheral blood smear showed decreased platelets, and parasitic forms compatible with *Dirofilaria* sp. were observed (Fig. 2).

The chest X-ray showed a slight diffuse increase in interstitial radiopacity. The trachea was normal, bronchi walls without alterations, no patterns of fluid and/or pulmonary oedema were observed, arterial and venous vessels without changes in their diameter, pleura without alterations

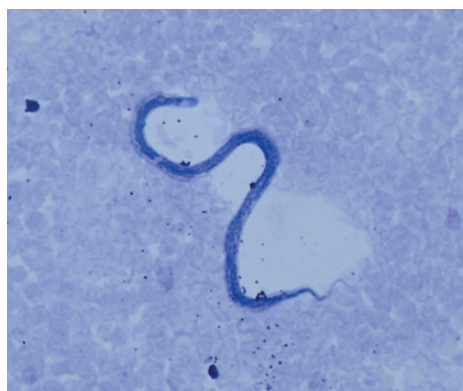


Figure 2. Female dog peripheral blood smear, showing microfilariae compatible with *Dirofilaria* sp. Wright's stain, 40 x magnification.

in the ventrodorsal view, some pleural fissures were slightly highlighted; however, the increase in diffuse radiopacity in the interstitium is not a sign of a specific finding of any entity, there was no evidence of cardiomegaly or marked vascular pattern (Fig. 3).

The patient was treated with doxycycline x 100 mg orally for 4 weeks, prednisolone x 5 mg orally for 8 days, and Advocate 2.5 mL (imidacloprid 250 mg + moxidectin 62.5 mg) for dogs 10-25 kg

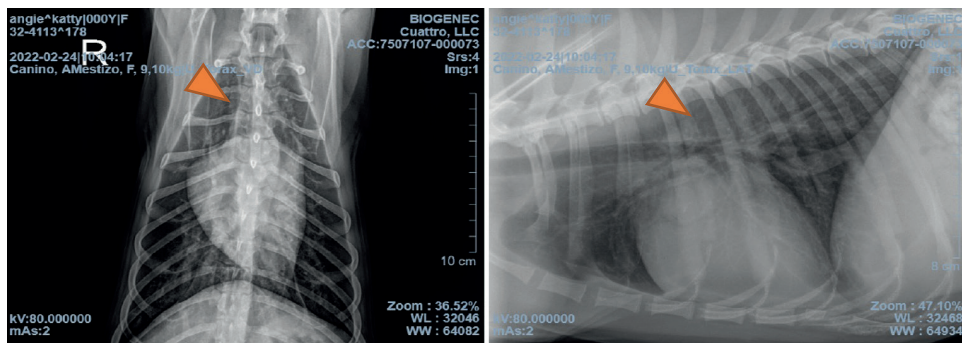


Figure 3. Chest X-ray. Canine female positive for *D. immitis*. Views: ventrodorsal (left) and lateral (right). Arrowheads show mild diffuse interstitial radiopacity.

administered on the animal's back. After treatment, blood samples for paraclinical tests were taken, but no parasitic forms compatible with *Dirofilaria immitis* was observed in the blood smear. However, the serological test was positive. The patient was treated again with the same pharmacologic treatment, and the result was negative.

In this study, the animal caretaker was sampled to examine the peripheral blood smear, thick blood smear and direct observation of the red blood cells. Fortunately, no forms compatible with *Dirofilaria immitis* were observed. In addition, the patient is in good health and reports feeling well.

Discussion

This study is the first report on the prevalence of this parasite in the Colombian coffee region; however, similar studies have been carried out in Columbia in cities such as Medellín, Barranquilla, Cartagena (McCown et al., 2015), and Bucaramanga (Muñoz et al., 2020). The prevalence of this parasite in this study was 0.62%, similar to other studies, such as the Valle de Aburrá case with a prevalence of 0.35% (Orozco et al., 2006), and lower than other studies in Colombia, where the prevalence

was 10.82% in Bucaramanga (Muñoz et al., 2020), 11.30% in Barranquilla, Puerto Colombia, and Atlántico (Otalora et al., 2022), and 20.8% in Cartagena (Labarthe et al., 2018). This suggests a low distribution of this parasite in certain regions of Colombia. *Dirofilaria immitis* is widely distributed worldwide in Africa, Asia, Australia, Latin America, and Mediterranean countries and is considered an emerging parasitic zoonotic disease in Europe (Ogbaje et al., 2016).

The transmission of this parasite can vary according to region and the type of landscape, demonstrated by higher infection rates in canines from tropical and subtropical coastal regions since the development of the vector depends on adequate environmental conditions such as water, air temperature, and the presence of wetlands (Dantas-Torres and Otranto 2013). However, it has been reported that climate change and higher temperatures recorded in South American countries influence heartworm transmission, along with the displacement of positive cases without treatment, the introduction of vectors, and urbanisation (Cuervo et al., 2013). *Dirofilaria immitis* and *D. repens* can affect humans, and is considered an emerging zoonosis, and is considered endemic in some areas

worldwide (Mendoza-Roldan et al., 2021). Italy is the country with the highest prevalence in humans (Capelli et al., 2018). In humans, it can cause benign pulmonary nodules that are often confused with lung carcinomas (Morchon et al., 2022). In this study, the animal caretaker was not positive for *Dirofilaria sp.*. However, the possibility of zoonotic infection due to the close coexistence with the infected canine cannot be ruled out.

In this study, it is unknown if the heartworm-positive animal had the parasite before arriving at the animal shelter or was infected during its stay at the shelter; however, being positive for heartworm, it can be considered a possible fomite of transmission since they are important for the maintenance of the infection and the spread of the parasite to new areas (Morchón et al., 2012). The heartworm antigen only appears in the blood of infected canines some 6–9 months post-infection. Therefore, a positive heartworm antigen test generally indicates an infection acquired sometime within the previous year (Brown et al., 2012). Due to the previous origin of the infected animal (La Guajira, Caribbean region), this patient likely contracted the infection in the Colombian Caribbean.

In our study, the positive canine is female; however, studies report the tendency of male canines to be more exposed to the environment. Therefore, males are more predisposed to mosquito bites than females with house habits (Orozco et al., 2006). The dog positive in this study was sampled in rural areas, and it has been shown that canines in rural areas present a higher risk of infection since they stay outside the home longer and are more exposed to the vector (Boonyapakorn et al., 2008). At the same time, infectious diseases such as tropical diseases and those transmitted by vectors are directly relat-

ed to the socioeconomic conditions of the individual, particularly poverty, limited drinking water sources, and poor sanitation, which have repercussions on the health of companion animals (Bonilla-Aldana et al., 2020).

In this study, the presence of *Dirofilaria immitis* in dogs from animal shelters in two departments of Colombian territory was analysed. A routine in vitro qualitative technique was used to detect circulating antigens against *D. immitis*. Other studies have used this test due to its high percentage of sensitivity (94.4%) and specificity (100%), allowing rapid and accurate diagnosis (Montoya-Alonso et al., 2017; Esteban-Mendoza et al., 2020). However, despite the specificity of these tests, cross-reactions with antigens from other nematodes such as *Dirofilaria repens*, *Angiostrongylus vasorum*, and *Spirocerca lupi* may occur (Panarese et al., 2020). Other techniques can be used for diagnoses, such as the presence of microfilariae in fresh blood samples, modified Knott test, histochemical staining for microfilaria, or serological techniques such as lateral flow immunochromatography and ELISA. However, the latter can present false negatives in low parasite loads or scare antigenemia (Genchi et al., 2018). In recent years, molecular techniques such as PCR have allowed more precise identification of mosquitoes as vectors and the parasite in the affected animal, as these are more sensitive techniques than microscopic findings that also enable exact identification of the species involved (Vezzani et al., 2011).

Conclusions

In the Colombian coffee region, *D. immitis* prevalence is scare, however migrant positive cases can become a source of infection spread. Dirofilariasis is a zoonotic

disease whose most important reservoir is canines, affecting the cardiovascular and subcutaneous systems. Its diagnosis based on antigen tests constitutes a sensitive and specific tool against *Dirofilaria immitis*. However, due to the cross-reaction with other nematodes, it is recommended that antigen tests be complemented with molecular techniques to improve the diagnosis. This information can be used by institutions responsible for animal health and welfare in our region to better understand the epidemiological situation of this agent and direct plans to prevent this disease.

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Seroprevalencija *Dirofilaria immitis* u pasa iz skloništa za životinje u kolumbijskoj regiji kave (Eje Cafetero)

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Postoje izvješća o gotovo 6.400.000 kućnih ljubimaca u Kolumbiji i to je četvrta zemlja u Latinskoj Americi u sektoru kućnih ljubimaca, s godišnjim rastom od 13 %. Cilj je ove studije jest utvrditi seroprevalenciju *Dirofilaria immitis* u 170 pasa iz općina Pereira, Santa Rosa de Cabal i La Virginia, koje se nalaze u departmanu Risaralda i u općinama Calarca i Armenia u departmanu Quindío. Provedena je presječna studija na psima iz skloništa za životinje tijekom godine 2021.-2022. Uzeti su uzorci krvi i obavljena je *in vitro* imunokromatografija za detekciju lezija specifičnih za *Dirofilaria immitis*. Razmaz periferne krvi potvrdio je pozitivne

pacijente i obavljen je RTG prsnog koša da bi se vidjele promjene u morfologiji srca i krvnih žila. Seroprevalencija je utvrđena izračunom omjera uz interval pouzdanosti od 95% određen preciznom ili Clopper-Pearson metodom za omjer na temelju veličine uzorka i broja pozitivnih slučajeva. Seroprevalencija testa na *Dirofilaria immitis* bila je 0,62 % (95% CI = 0,016-3,42 %). Ovaj je parazit oblić u područjima regije kave niske rasprostranjenosti, ali predlaže se uspostaviti programe nadzora koji će pomoći kontrolirati i reducirati njegov prijenos.

Ključne riječi: *dirofilarioza*, srčani parazit, insekt prijenosnik, oblić