

## *Dirofilaria immitis* en caninos del medio Sinú: un posible riesgo para la salud pública

### *Dirofilaria immitis* in canines of Medio Sinú: a potential risk to public health

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#### **Abstract**

**Introduction:** *Dirofilaria immitis*, is a worldwide distribution parasite that causes progressive cardiopulmonary disease in dogs, which is located in the pulmonary arteries and right ventricle and produces endothelial injury and pneumonia. The parasite is transmitted by mosquito bites to canines, but it may be found in a variety of mammals, including humans living in endemic areas, which turns it into a zoonosis. The infection has been reported in different Colombian regions.

**Objective:** To determine antibodies against *D. immitis* in dogs of Cereté, Ciénaga de Oro, and San Carlos, municipalities of Medio Sinú (Córdoba).

**Materials and methods:** Blood samples of 105 crossbreed dogs were collected from the urban areas of these municipalities in order to conduct a descriptive cross-sectional study and stratified sampling. Antibody to *Dirofilaria immitis* was measured through the implementation of an enzyme-linked immunosorbent assay (ELISA). Descriptive analysis and Chi-square were used to determine whether there were significant differences with respect to *D. immitis* seropositivity and the parameters investigated (age, race and geographic region of origin).

**Results:** The seroprevalence to *D. immitis* was 5.7%, and the antibodies

were detected in the municipalities evaluated. No statistically significant differences were observed between the seropositivity of different age groups, races, sexes, and municipalities.

**Conclusions:** Due to the environment of humans in the municipalities of Medio Sinú, along with the detection of circulating antibodies in canines of *D. immitis*, the agro-ecological conditions of this region, the presence of mosquitoes and hosts, humans can be exposed to the transmission of pathogens and be a risk to public health; therefore, it is necessary to implement prevention and vector control measures.

**Keywords:** Antibodies, antigens, diptera, epidemiology, nematodes, zoonoses (Source: DeCS).

## Introduction

The objective was to determine antibodies against *D. Immitis* in canines of three municipalities of the Middle Sinú (Cerete, Ciénaga de Oro and San Carlos) in Córdoba (Colombia).

*Dirofilaria immitis*, is a parasite that produces progressive cardiopulmonary dirofilariasis in canines, which it is located in the pulmonary arteries and the right ventricle and produces endothelial damage and pneumonitis. It is transmitted by mosquitoes bite (vectors) to canines and other species such as: *Vulpes vulpes* or red foxes (2); *Canis latrans* or coyotes (3), *Canis aureus* or jackals (4); in *Canis aureus* or golden jackals (5), *Felis catus* or domestic cat, *Felis silvestris* or wild cat, *Ailurus fulgens* or red pandas, *Ursidae* or bears, *Otariinae* or sea lion, non-human primates (6) and humans, constituting it as a zoonosis (7).

The vectors of *D. Immitis* are mosquitoes of the gender *Aedes*, *Culex* (8), *Anopheles* (9), *Culiseta* (10), *Mansonia*, *Coquillettidia* and *Psorophora* (11), *Myzozhynchus*, *Armigeres* and *Taeniothyncus* (11). Currently, *Aedes koreicus* is an invasive species in Europe, and is likely to be a competent vector of *D. immitis* (13).

Dirofilariasis disease is of worldwide distribution except in the polar regions, the highest frequency occurs in tropical and subtropical regions where temperature, humidity and vegetation are factors for a higher density of vectors and by the presence of hosts the parasite completes its biological cycle (14). In Greece, mosquito population dynamics have been shown to play an important role in the distribution of *D. immitis* (15).

Humans have a high probability of becoming infected when they inhabit

endemic areas (16), usually they are asymptomatic and the lesions are discovered by radiological examinations when a malignant mass is suspected in the chest (17;18), or cases of unusual location such as in the spermatic artery (19), in hepatic nodules (20), at the intraocular (21), scrotal (22) or in the eyeball (23).

In Colombia *D. immitis* has been reported in different regions of the country; antibodies against *D. Immitis* were detected in an indigenous community name Tikuna in the Amazon, Colombia, (24). In Bucaramanga, Colombia, a seropositivity was reported to *D. Immitis* in humans and canines in the same area (25); cases of human pulmonary dirofilariasis have also been reported (26). The parasite has also been found in high-altitude cities and cold climates (27). By serological tests (28;29;30), case reporting (31), molecular techniques (32), combination of serology and molecular tests (25;32); these results can confirm the adaptability of vectors to different environmental conditions (33).

### **Materials and Methods**

**Type of study:** Descriptive study of cross-sectional cut.

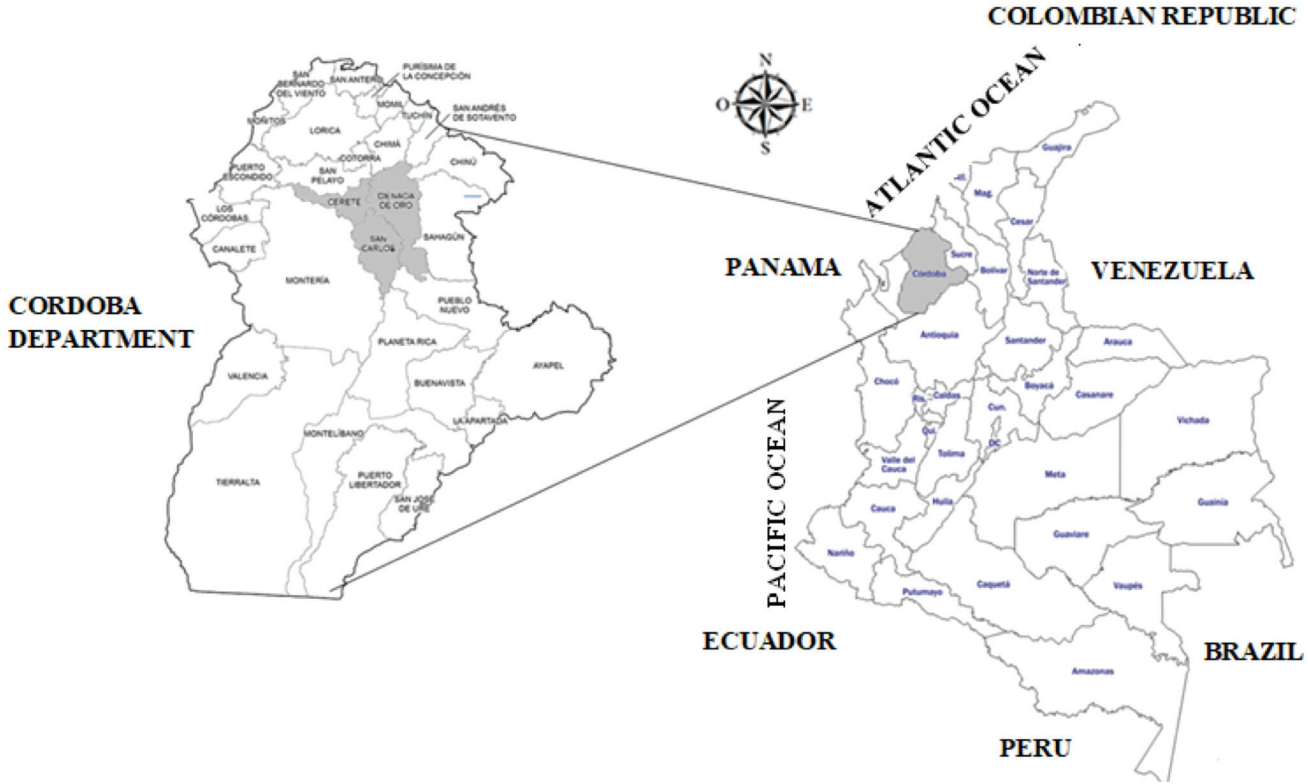
**Study area:** It was carried out in the urban area of the municipalities of Cereté, Ciénaga de Oro and San Carlos (Córdoba), this are in the geographi-



*In Colombia *D. immitis* has been reported in different regions of the country; antibodies against *D. Immitis* were detected in an indigenous community name Tikuna in the Amazon, Colombia.»*

cal region of Medio Sinú (Figure 1).

**Figure 1.** Location of the municipalities where antibodies against D. Immitis in canines were determined: Cereté, Ciénaga de Oro and San Carlos,



**Sample size:** The sample size were 105 canines of different ages according to canine vaccination data from these urban areas which was 14,393 canines. A stratified sampling was carried out with proportional allocation in each municipality according to Table 1.

**Tabl2 1.** Canine determination according to a stratified sampling with proportional allocation by municipality

| Municipality   | Target population | Percentage | Number of Samples |
|----------------|-------------------|------------|-------------------|
| Cereté         | 8729              | 61         | 64                |
| Ciénaga de Oro | 3938              | 27         | 28                |
| San Carlos     | 1726              | 12         | 13                |
| <b>Total</b>   | <b>14393</b>      | <b>100</b> | <b>105</b>        |

**Sample collection:** After disinfection of the forearm area and by cephalic or saphenous vein venipuncture, a 5 ml blood sample was taken in a vacu-tainer tube without vacuum anticoagulant (red cap); each sample was labeled with the respective identification of the animal and kept in refrigeration

between 4o C and 8o C in an polystyrene refrigerator, until it was taken to the laboratory of the Julio E. Cuervo clinic from the University of Córdoba Berástegui headquarters, where the serum was obtained by centrifugation at 3500 rpm for 5 minutes, which was kept in freezing (-70o C) in Eppendorf tubes until processing.

**Sample processing:** Commercial SNAPMR test kit was used, which is an enzyme immunoassay for semi-quantitative antigen detection D. Immitis canine in serum, plasma, or whole blood of canines and felines. The sensitivity of the test is 98%, the specificity is of 100% (LC from 85, 96 to 100%) and with a Kappa value of 0.98%. This technology allows the conjugated and the sample to be mixed and then this mixture is added to the device that is activated by releasing the reagents stored inside the device; the interpretation of the results was made by the display of the colors in the window and the manufacturer’s instructions were followed.

**Statistical analysis:** A database in Excel format was developed, the information on the variables of the evaluated canines and the results obtained in the laboratory was recorded. Chi-square was used to determine whether the variables age, race and origin were independent of the seropositivity for antigen D. Immitis, and descriptive statistics were calculated using the SAS software.

### Results

The seroprevalence determined for antigen D. Immitis in the 105 canines in the urban areas of Cereté, Ciénaga de Oro and San Carlos (Córdoba) was 5.7% (6/105). In each of the municipalities evaluated (Table 2) the presence of the antigen D. immitis was determined and by the Chi-square test (value=3.159; p=0.790) it was shown that there are no statistical differences (P>0.05) between the seropositivity, and the municipalities evaluated

**Table 2.** Seroprevalence by municipality of canine evaluated in the Medio Sinú region.

| Town/ City     | Evaluated Canines |       | Seropositives |      | Seronegatives |       |
|----------------|-------------------|-------|---------------|------|---------------|-------|
|                | n                 | %     | n             | %    | n             | %     |
| Cereté         | 64                | 60.95 | 2             | 1.90 | 62            | 59.1  |
| Ciénaga de Oro | 28                | 26.67 | 2             | 1.90 | 26            | 24.76 |
| San Carlos     | 13                | 12.38 | 2             | 1.90 | 11            | 10.44 |
| Total          | 105               | 100   | 6             | 5.70 | 99            | 94.30 |

In Table 3, the seroprevalence by age is presented, where, by the Chi-square (value=1.286; p=0.733) it was found that there are no statistical

differences ( $P>0.05$ ) between seropositivity and age.

**Table 3.** Seroprevalence by age of canines evaluated in the Medio Sinú

| Age (years) | Evaluated Canines |       | Seropositives |      | Seronegatives |       |
|-------------|-------------------|-------|---------------|------|---------------|-------|
|             | n                 | %     | n             | %    | n             | %     |
| 1 - 2       | 55                | 52.30 | 3             | 2.85 | 52            | 49.50 |
| 3 - 4       | 22                | 20.90 | 2             | 1.90 | 20            | 19.00 |
| 5 - 6       | 15                | 14.20 | 1             | 0.95 | 14            | 13.30 |
| > 7         | 13                | 12.30 | 0             | 0    | 13            | 12.40 |
| Total       | 105               | 100   | 6             | 5.70 | 99            | 94.20 |

The largest population evaluated corresponded to canines of creole or crossbreed, where it was obtained that for each pure-bred canine three canines of creole or crossbreed were sampled (Table 4); despite this proportion, no statistical differences were found per Chi-square (value=0.403;  $p=0.525$ ), ( $P>0.05$ ) for this variable.

**Table 4.** Seroprevalence by breed of canines evaluated in the Medio Sinú region

| Breed      | Evaluated Canines |       | Seropositives |      | Seronegatives |       |
|------------|-------------------|-------|---------------|------|---------------|-------|
|            | n                 | %     | n             | %    | n             | %     |
| Crossbreed | 80                | 76.10 | 6             | 5.7  | 74            | 70.40 |
| Pure breed | 25                | 23.80 | 0             | 0.0  | 25            | 23.80 |
| Total      | 105               | 100   | 6             | 5.70 | 99            | 94.20 |

Related to sex, the number of males was slightly higher than the females; likewise, for the Chi-square (value=0.071;  $p=0.790$ ) no statistical differences were observed ( $P>0.05$ ) (Table 5).

**Table 5.** Seroprevalence by sex of canines evaluated in the Medio Sinú region.

| Sex    | Evaluated Canines |       | Seropositives |      | Seronegatives |       |
|--------|-------------------|-------|---------------|------|---------------|-------|
|        | n                 | %     | n             | %    | n             | %     |
| Male   | 58                | 55.20 | 6             | 5.7  | 74            | 70.30 |
| Female | 47                | 44.70 | 0             | 0.0  | 25            | 23.75 |
| Total  | 105               | 100   | 6             | 5.70 | 99            | 94.20 |

## Discussion

A seroprevalence to antigen *D. immitis* of 5.7% in three urban areas of Cereté, Ciénaga de Oro and San Carlos (Córdoba), municipalities located in the Middle Sinú region was determined, where the subhumid and humid tropical climate predominates with a monthly rainfall ranging between 110

and 182 mm (34); humid and sub-humid tropical climates are conducive to the presence of vectors (ticks and mosquitoes) that can transmit diseases (35). It has been determined that humidity and vegetation facilitate the development of mosquitoes (36).

A recent comparative study among Medellín (Andes region), Barranquilla and Cartagena (Caribbean region), the seroprevalence was 0% in Medellín and 3% in Colombian Caribbean cities where the humid tropical climate predominates (29). Previously in the Valle de Aburrá a seroprevalence of 0.35% was reported and it was concluded that in Medellín and the Valle de Aburrá *D. immitis* is not an endemic disease (28). A clinical case in a three-year-old German Shepherd born in Medellín and moved to the island of San Andrés since it was five months old, reflected an unknown if this archipelago is an endemic area (31).

On the other hand, a study conducted in Girardot (Cundinamarca) with a dry tropical climate, in the non-homeless urban canines, heart disease compatible with *D. Immitis* (27) was not diagnosed, since prophylactic treatments and better care can decrease exposure to vector-borne pathogens in endemic areas (37). Likewise, other studies have established that significant differences have also been found between homeless and non-homeless canines (38). Moreover, a comparative study among regions concluded that in Colombia no significant differences have been observed between the prevalence and the different geographical regions (39), but the habit of canine wandering the streets led to significant differences being established (40). In shelter canines from metropolitan areas of Bucaramanga (Colombia), a prevalence of 6.3% was determined in blood smear and 0.5% with an immunochromatographic test (30). Finally, another study in the same city determined a seroprevalence of 6.71% in humans living with canines, where the seroprevalence was 5.12% (25).

On this research, no statistical differences were found between seropositivity by municipality (Cereté, Ciénaga de Oro and San Carlos), adjoining municipalities of the Medio Sinú region, where canines have the same probability of becoming infected, because they live in the same region that offers favorable environmental conditions for the presence of vectors. However, the capture of mosquitoes of the gender *Aedes*, *Culex*, *Anopheles*, *Culiseta*, *Mansonia*, *Coquillettidia*, *Psorophora*, *Myzorrhynchus*, *Armigeres* and *Taeniothyncus* have been reported in The Medio and Bajo Sinú (40;41;42;43); although no studies have been carried out to show infection of *D. Immitis*

in these regions.

When the independent variable seropositivity and the dependent variables: race, sex and age were correlated, no statistical differences were determined, which indicates that canines of both sexes, of any race and age and who inhabit the urban areas where the study was implemented, have the same probability of infecting. Very similar results for age and sex have been reported in canines in Iran and other places (44) (45). Significant differences have been determined between pure and mestizo canines, but these differences may be due to prophylactic treatments and better veterinary care (46).

The habitat of humans in these municipalities of Medio Sinú, along with the detection of circulating antibodies in canines of *D. immitis*, the agroecological conditions of this region, the presence of mosquitoes and hosts can cause humans to be exposed to the transmission of pathogens and be a risk to public health; by which it is necessary to implement vector prevention and measures control.

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