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Prevalence of Canine Heartworm in Dogs from Monterrey, Mexico

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Abstract: Canine dirofilariosis is a vector-borne disease caused by *Dirofilaria immitis* and causes severe clinical signs. A high prevalence has been reported in the USA and other countries while in Mexico it has been lower. In the present study 391 dogs were sampled at random at the city of Monterrey, Mexico and were tested by the ELISA method using a canine commercial kit. Only 28 animals resulted positive (7%) and included different dog breeds and mixed blood animals. According to sex, 18 were males and 10 females while the prevalence was higher in animals with short hair. According to exposition to pest control and place of stay, animals that have been exposed to insecticides and were kept outside the house had a higher prevalence of *D. immitis*. These results are in agreement with previous studies that have also performed random sampling in the same city.

Key words: Canine, hearth-worm, dirofilariosis, vector borne, animal, Mexico

INTRODUCTION

Canine dirofilariosis is an endemic disease of dogs caused by *Dirofilaria immitis* although, it has also been reported in a great number of other animal species such as cats, coyotes, ferrets, foxes, leopards, ocelots, wolves and humans (Liu *et al*., 2005; Ranjbar-Bahadori *et al*., 2007; Icen *et al*., 2011; Gholami *et al*., 2011).

The parasite is transmitted by indirect way trough hemathophage mosquitoes. At least 70 species of mosquitoes are capable of transmitting the disease as intermediate hosts; *Aedes*, *Anopheles* and *Culex* are the genus that more often play the role of vectors (Dingman *et al*., 2010; Reifur *et al*., 2004).

Hearth worm infection can have several effects in both dogs and cats. Clinical signs normally are asymptomatic whereas some animals have cardiac insufficiency with skin and in some cases nervous manifestations, depending of the parasite localization (Knight, 1987). Dogs often have microfilaremia while this condition is very rare in cats.

It is easier to diagnose the infection in dogs than in cats. In dogs, clinical signs include cuffing, exercise intolerance, disnea, hepatomegalia, sincope and ascitis (Dingman *et al*., 2010) while in cats are lethargy, cuffing, anorexia and vomiting. Commonly cats have disnea or respiratory diseases similar to asthma while dogs show right cardiac failure (Nelson *et al*., 2005; Nelson, 2008). Clinically the disease is a non-contagious infestation caused by the parasite presence at the right ventricle of the heart and right pulmonary artery in dogs and other canines; this is translated into cardiac insufficiency due to pulmonary vascular obstruction with skin and nervous manifestations (Morgan *et al*., 2003).

Some researchers report prevalence of 50% in areas where no preventive treatment against canine filariasis exists while in felines is present in 5-20% of the population (Atkins *et al*., 1998; Bowman *et al*., 2007; Carleton and Tolbert, 2004; Hermesmeyer *et al*., 2000; Miller, 1998; Nogami and Sato, 1997; Patton and McCracken, 1991).

In the United States and Canada there are reports that consider this disease as endemic being reported at 50 states of the USA (Soll and Knight, 1995; Nelson *et al*., 2005). In Mexico there are studies related to *Dirofilaria immitis* frequency in several cities of the country where from 1,028 canines sampled was observed a prevalence that changed in each city from 0.4-15.6%.

A study performed at the state of Yucatan in 2007 reported a prevalence of 7% in canines sacrificed searching the parasite at the hearth in other Latin-American studies the prevalence has been found to be from 7-50% or even higher (Gonzalez *et al*., 2007).

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In order to achieve a correct diagnosis, it is necessary to demonstrate the presence of microfilariae in the blood stream and the right identification to establish its pathogenicity, since there are microfilariae belonging to other species of non-pathogen filarial nematodes that can be misidentified as *Dirofilaria immitis* (Kelly, 1973). The Knott test or membrane filtration can detect microfilariae in blood stream. Antigen tests for *D. immitis* detect proteins in the parasite capsule but are only for the adult female. False negatives occur when the concentration of antigen circulating in blood is low which can happen when a minimal parasite presence, parasite immaturity or the absence of female nematodes (Dingman *et al*., 2010; Montano *et al*., 2002). Blood tests detect if the animal has been exposed to the hearth-worm but not necessarily that it has the infection. The Enzyme Linked Immune Assay (ELISA) can be used to detect *D. immitis* antigens (Dingman *et al*., 2010).

On the basis of previous studies performed at Monterrey, Nuevo Leon, Mexico in 1993 by Franco-Molina and in 1994 by Hinojosa-Hernandez where a low prevalence of the disease was found and because of the importance of this disease in dogs, we carried out a study to estimate *D. immitis* prevalence in dogs by the ELISA test using the canine SNAP 4Dx kit (IDEXX Labs inc. USA).

MATERIALS AND METHODS

Blood samples were obtained from 391 dogs of different breeds in the city of Monterrey using as inclusion factor only animals with fixed address, age over 6 months. It was decided to sample only one animal per house in case of having more than one dog. The examination of the dogs started with physical evaluation followed by blood sampling. All dogs showed no symptoms of any disease.

This study was carried out in the city of Monterrey, Nuevo Leon located in the Northeast of Mexico with a territorial extension of 451.30 km^2. Location coordinates are 25°40'17"N, 100°18'31"W. Altitude is 530 m above sea level.

The climate of the region has an average of 21°C but because of annual thermal oscillation of 18°C with important contrast among seasons. In Summer time temperatures above 30°C are common with an average in July and August of 34°C. In Winter, cold air arrive constantly to the region, often accompanied of humidity from the coast, making the temperature descend drastically and every year at least 2-3 days are recorded with 0°C or less. The average annual precipitation is of 600 mL spread mainly in Summer with September as the rainiest month.

The city was divided in quadrants in accordance with its cartographic plan. From this map, the 15 most urbanized quadrants were chosen since, the others belonged to non well developed neighborhoods and few human population. Sampling was performed according the dog population density and owner cooperation, sampling only one animal per city block and only one animal per house.

To determine the sample size, calculations were made in basis of the population's representative sample (infinite) with precision level of 5%, confidence level of 95% and a power of statistical test of 80% in order to ensure reliability of the results and that they could be translated to the population under study using a 16% prevalence according to previous studies in the country. Sample size was determined using Epidat 3.1. Blood was extracted from the jugular vein with Vacutainer vacuum tubes and sterile needles, drawing close to 5 mL from each animal. No anesthetic or tranquilizer was used. The samples were carried in a container with refrigerant material to the laboratory and kept al 4°C until they were centrifugated at 3000 rpm for 5 min to separate the serum after which were processed to determine the presence of antibodies against lyme disease.

For the *in vitro* diagnosis for detection of antibodies against *Dirofilaria immitis* in the samples, a commercial kit canine SNAP*4Dx (IDEXX labs inc., USA) was used. Before starting the procedure samples must be at room temperature. The sera either fresh or refrigerated were utilized after no more than a week from the sampling. Sensibility and specificity of the kit for the disease are reported with a minimum of 98.8 and 100%, respectively.

RESULTS AND DISCUSSION

A total of 28 animals of 391 resulted positives. Dog breeds with positive animals were Chihuahua, Schnauzer, Basset Hound, Fox Terrier, West Highland, White Terrier, Pomerania, Golden Retriever, Bull Terrier, German Sheppard and mixed blood animals. Due to the low level of prevalence found (7%), no risk factor could be associated that indicated an influence for the disease. Regarding to sex, 18 males and 10 females were positives (Table 1) indicating prevalence of 10% for males and of 4% for females. Positive animals were divided according to hair length (Table 2). Prevalence found were 56, 31 and 11% for short, medium and long hair, respectively. Prevalence of positive animals regarding to the presence of pest control were 78 in exposed animals while non-exposed animals were 21%. According to place of stay animals located inside the house had a prevalence of 39% while animals kept outside showed 53%.

Table 1: Distribution of positive animals to *Dirofilaria immitis* by sex

Sex	Sample	Positive	Prevalence
Female	218	10	4
Male	173	18	10

Table 2: Positive animals to *Dirofilaria immitis* according to hair length

Hair length	Total	Female	+	%	Male	+	%
Short	222	127	7	5	95	10	10
Medium	125	71	2	3	54	6	11
Large	44	20	1	5	24	2	8
Total	391	218	10	-	173	18	-

The prevalence found in this study is similar to the reported by Gonzales *et al.* (2007) which were of 7% using the wide-drop procedure, 8.3% with the Knott technique and necropsy analysis showed that 7% of animals were infected with *D. immitis*.

The low prevalence in some studies can be explained by the fact that the antigen detectable with serological tests are only produced by adult females and no antigen is detected when only male or young parasites are present furthermore, these techniques can not show the presence of the antigen when microfilariae are eliminated by immune-mediated reactions due to the preventive use of microfilaricide in a monthly basis as well as the immaturity of the adult parasite (Dingman *et al.*, 2010; Montano *et al.*, 2002).

Some evidence exists that in areas where no preventive treatment against canine filariosis is in place, the estimated prevalence raises to 50% while in felines can be from 5-20% (Dingman *et al.*, 2010). Because no such prevalence were found in this study, it is possible to assume that in the area sampled preventive pest control measures are in effect.

In the United States and Canada this disease is considered as endemic having been reported at 30 states of the USA (Soll and Knight, 1995). This result is in agreement with the ones presented here. In Mexico, the frequency of positive animals for *D. immitis* was shown to be diverse according to the city in which the animals were located; the prevalence was 0.4% in Cuernavaca, 2.7 in Mexico city, 3.8% in Guadalajara, 9.2% in Veracruz, 13% in Ciudad Victoria and 15.6% in Villahermosa. This indicates that prevalence changes according to the geographical location and the prevalence found in the present study lies around the average of the ones reported at the different areas of the country that have been analyzed.

CONCLUSION

Regarding to previous studies in the city of Monterrey, we can inform that the results found in this study are in disagreement with the reported by Franco-Molina where the prevalence was of 26% in that study the results could be skewed because sampling was performed only in animals that were suspects of having the disease whereas in the present study sampling was totally at random. On the other hand, a different research conducted by Hinojosa-Hernandez where sampling was also performed at random showed prevalence of 14% with the wide-drop technique and of 7% with the modified Knott method. These last results are closer to the results found in this study and probably more in concordance with the actual prevalence of *D. immitis* infection in the studied area.

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