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Prevalence and Risk Factors Associated with Microfilarias Infection in Dogs from Villahermosa, Tabasco, Mexico

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Abstract: The prevalence of microfilaremic dogs with >3 years old and the risk 2 factors (color hair coat, sleeping area and breed) associated with this infection were studied. A total of 86 blood samples were evaluated using the Thick Blood Smear Technique (TBS) and modified Knott test for the circulating microfilarias detection. For determining risk factors, a survey was applied to the owners. The prevalence of microfilaremic dogs was 24.41% (21/86). Two microfilarias species were detected *Dirofilaria immitis* (19.76%) and *Dipetalonema reconditum* (4.65%). The pure breed variable showed to be a risk factor of importance (OR = 2.32, 95% CI = 0.59-9.54, p = 0.16). A different prevalence was observed according to color hair coat: White 38.46% (OR = 2.78, 95% CI = 0.99-7.76, p = 0.03); black 22.22% (OR = 0.77, 95% CI = 0.29-2.08, p = 0.17); white with black 16.66% (OR = 110.6, 95% CI= 0.06-5.4, p = 0.36) and brown 0%. The sleeping area had not differences in this study because and indoor (OR = 0.98, IC = 0.69-1.39, p = 0.20) both outdoor (OR = 1.03, IC = 0.51-2.07, p = 0.20), the dogs were similarly microfilariae infected. The results indicated a high prevalence of microfilaremic dogs in the studied zone, the white hair coat and breed purity seemed to be risk factors of importance for acquiring the microfilarias infection.

Key words: Prevalence, *Dirofilaria immitis*, *Dipetalonema reconditum*, risk factors, Knott test, Mexico

INTRODUCTION

Ectoparasites, like mosquitoes, abound in tropical humid climates; they are vectors and in other times, intermediate host of various immature forms of endoparasites that affect mammals (Cancrini *et al*., 2003, 2007; Genchi *et al*., 2009; Otranto and Dantas-Torres, 2010). One of these associations between internal and external parasites presented in these regions are caused by mosquitoes of various genera such as the hematophagous nematode *Dirofilaria immitis* that affects mainly canines and in smaller amount, felines and other animal species including man (Labarthe and Guerrero, 2005; Wisely *et al*., 2008). Some studies (Sathyan *et al*., 2006; Pampiglioni *et al*., 2009) had presented microfilariae infections in humans. Hira *et al*. (2008) reported two cases of filariasis in humans from Kuwait; Poppert *et al*. (2009) reported a case of filariasis in subcutaneous tissue and concomitant meningoencephalitis. Kartashev *et al*. (2011) carried out a retrospective study for 9 years, finding 129 cases of subcutaneous dirofilariasis and two cases of pulmonary dirofilariasis in humans. These results showed the importance carrying out studies to know and control microfilariasis in canines and discovering the new endemics zones and the improvement of the diagnostic tests for microfilariae detection. Currently, there are several methods for detecting microfilariasis in dogs and cats such as the TBS, capillary tube detection, modified Knott test (Ko *et al*., 2007), antigenic detection for Enzyme Linked Immunosorbent Assay (ELISA), Polymerase Chain Reaction (PCR) (Ranjbard *et al*., 2007) and Quantified Blood Cells (QBC) (Wang, 1998; Mylonakis *et al*., 2004). Dirofilariasis affect directly to the circulatory system of the infected animals (Niwetpathomwat *et al*., 2007) after a long incubation period, the adult nematodes migrate the cardiac cavities and the blood vessel lumen, causing various cardiovascular problems as pulmonary embolism, hypoattenuating round filling defects in pulmonary arteries, arterial dilations with straight and abrupt cut-off appearances in the pulmonary embolism regions, pulmonary infarctions, a cavity formation and spontaneous pneumothorax and emboli migration which spoil the animal health and subsequently can cause death (Mupanomunda *et al*., 1997; Jung *et al*., 2010). Various risk factors have an important role in the disease

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epidemiology according to which it was reported by Bolio *et al.* (2007) that the dogs <3 years old present higher risk than the younger ones; the breed is not an important factor and gender is a controversial factor because some studies indicate a higher prevalence in males and other studies indicate a higher prevalence in females. According to the above, the aim of this study was to determine the prevalence of microfilariae in dogs in Villahermosa, Tabasco, Mexico and for determining if the hair coat color, breed and the place where the dogs sleep (indoors or outdoors) are risk factors of epidemiologic importance.

MATERIALS AND METHODS

Study area: The present study was carried out in Villahermosa city, Tabasco, Mexico. The city is located at 17°59'N and 92°56'W, altitude of 10 m above sea level. The climate is damp tropical with rains throughout the year (AF), the average temperature in the area is 26°C with a minimum of 15°C and a maximum of 44°C and the relative humidity is 80%.

Determination of the sample size: The sample size (n) was determined according to Mateu and Casal using the following statistical equation:

$$n = \frac{Z^2 pq}{E^2}$$

The confidence limit Z was 95% (1.96), the positive prevalence p was 6% in agreement with previous studies carried out in tropical areas of Mexico (Bolio *et al*., 2007), the negative prevalence q was 94% and the standard error E was 5%. The sample size was 86 animals.

Sampled animals: Dogs >3 years old were randomly sampled (n = 86). Only, dogs >3 years old were used in this study according as reported by others studies where they have demonstrated this variable is a risk factor in the disease epidemiology (Bolio *et al*., 2007; Yildirim *et al*., 2007). These dogs differed in morphological characteristics, breed, purity of breed, color hair coat, feeding habits, indoor or outdoor sleeping, handling and sanitary status. The animals were sampled in their houses where they lived and a survey was applied to dog owners about risk factors under study was which were recorded to determine epidemiological associations with microfilariae infections.

Blood sampling for the microfilariae identification: Blood samples were taken from the cephalic vein between 06:00 and 09:00 h with 4 mL Vacutainer® tubes with EDTA. The samples were identified by a file number for each sampled animal and were preserved at 4°C.

Processing of the blood samples: Blood samples were processed in the Clinical Diagnostic Laboratory of the Veterinary hospital of the Universidad Juarez Autonoma de Tabasco. The blood was evaluated 1 h after it was obtained from the animals by means of the TBS and modified Knott test for microfilariae identification according to Knight (1987).

Risk factors studied: The color hair coat (white, white and black, black and brown), indoor or outdoor sleeping area and breed (pure and no pure) were studied as the possible epidemiologic risk factors which can directly affect the amount of microfilariae infected dogs.

Statistical analysis: Prevalence was determined by using the following equation:

$$P_t = \frac{C_t}{N_t}$$

Where:

P_t = The estimated prevalence in the right time when the study was carried out

C_t = The total number of cases resulting positive during that period of time (positives to TBS Technique the modified Knott test)

N_t = The total number of studied dogs (86) during that period of time

The evaluation of variables association to determine the potential risk factors was realized using statistical test Chi-square (χ^2) by means case control study, the positive results were used as the dependent variable and the color hair coat, breed and sleeping area were used as the independent variables. Odds Ratio (OR) were determined for each independent variable as epidemiological association measures for this purpose, it was used a contingency (Table 1) χ^2 by means PROC FORMAT and PROC FREQ procedures of the statistical program SAS (2003).

Table 1: Evaluation of sleeping area and breed as risk factors associated to the microfilariae infection in dogs

Variables	Positives	Nagetives	OR	CI	p-values
Sleepng area					
Indoor	7	21	1.03	0.51-2.07	0.20
Outdoor	14	44	0.98	0.69-1.39	
Breed					
Pure	3	4	2.32	0.59-9.54	0.16
No pure	18	61	0.91	0.75-1.09	

OR: Odds Ratio of the association between studied factor and risk for infection (>1 risk factor); CI: Confidence Interval

RESULTS AND DISCUSSION

The detected microfilariae prevalence was of 24.41% in the peripheral blood of canines that were >3 years of age from the studied zone (21/86). According to their morphology, two different species of microfilariae were identified, *Dirofilaria immitis* (19.76%) and *Dipetalonema reconditum* (4.65%). From a total of the analyzed samples, the TBS technique detected 17 positive cases and the modified Knott test 21 cases (detected the same 17 and 4 more).

The risk factors evaluation of Table 1 indicated that the sleeping area (indoors or outdoors) is not considered as a risk factor (OR = 1.03, 95% CI = 0.51-2.07, p = 0.20 y OR = 0.98, 95% CI = 0.69-1.39, p = 0.20, respectively). The pure breed variable showed to be a risk factor (OR = 2.32, 95% CI = 0.59-9.54, p = 0.16) for the infection acquisition, compared as no pure breeds (OR = 0.85, 95% CI = 0.75-1.09, p = 0.16). The association among the color hair coat and the risk for the infection acquisition showed to be an important factor in the epidemiology of microfilariae infection (Table 2).

Of the four colors hair coat studied, only the white color was associated with microfilariae infection in dogs (OR = 2.78, 95% CI = 0.99-7.76, p = 0.03). Table 2 shows that dark colors statistically are not considered as risk factors (OR<1). It was observed that in dogs with brown hair coat have not statistical associations because it was not found either microfilariae positive dog with this color hair coat. The prevalence found in the study was >15.6% which has been reported by Samano in blood samples of dogs from Cunduacan, Tabasco. Labarthe and Guerrero (2005) reported that the prevalence of *Dirofilaria immitis* in dogs is stable between 7.3 and 7.5%, according to what was mentioned above, in other tropical zones of Mexico some studies had reported a prevalence between 6 and 7% (Bolio *et al*., 2007) which differs from the data found in this study however the low control of canine population growth and the evaluation of new endemic zones could be the cause of the increasing prevalence in this parasitic infection. The prevalence of *Dipetalonema reconditum* (4.65%) was >3.8%, reported by Samano.

Roth *et al*. (1993) carried out a study using different ELISA commercial kits finding different results in the studied dogs. Ranjbard *et al*. (2007) found 22% of prevalence in dogs from the province of North Iran using the Knott test, a commercial kit for *Dirofilaria immitis* antigens detection and adult parasites verification in the necropsy. Therefore, it is considered that prevalence can vary according to the diagnostic method used, QBC, TBS, Knott test or ELISA (Roth *et al*., 1993). The highest sensitivity obtained in this study by means the modified Knott test respect to TBS (21 vs. 17) according to what was reported by Bolio *et al*. (2007) who reported a higher sensitivity in modified Knott test compared with the TBS. Also, Mylonakis *et al*. (2004) found that TBS decreases more easily its sensitivity in direct relation with fewer amount circulating microfilariae in blood while modified Knott (1939) test and QBC have sensitivity more stable. Courtney and Zeng (2001) found that TBS technique decreases its sensitivity when circulating microfilaria concentrations are <50 microfilariae mL^{-1} of blood.

Yaman *et al*. (2009) and Razi *et al*. (2010) reported that dogs living outdoors have a higher prevalence of *Dirofilaria immitis* compared with dogs living indoors which differs from what was found in this study where there were no differences between the two sleeping areas. This could be due to different social and environmental factors such as the houses structure, dog own ers customs and education which impacts in the care of the dogs. It is important say that animals used in this study live outdoor during day and they are housed indoor only during night.

In this study, it was observed that despite mosquitoes (microfilariae vectors), they have a feeding behavior with higher activity during twilight hours; the dogs that stay sleeping outdoors in the nights are not more exposed than the ones that sleep indoors. This may be because of some insects that are considered vectors of diseases (to humans or domestic animals) are often around the houses and are attracted by light or the presence of their feeding sources (Reisenman *et al*., 2010) which could be the cause that keeps a similar risk level in both places.

Table 2: Evaluation of colour hair coat as risk factor for microfilariae infection in dogs

Colour hair coat	Nagetives	Positives	Total	Prevalence (%)	OR	CI	p-value
White	16	10	26	38.46	2.78	0.99-7.76	0.03
Black	35	10	45	22.22	0.77	0.29-2.08	0.17
W/black	5	1	6	16.66	0.60	0.06-5.40	0.36
Brown	9	0	9	0.00	-	-	-
Total	65	21	86	24.41	-	-	-

OR: Odds Ratio of the association between studied factor and risk for infection; CI: Confidence Interval of Chi-square (χ^2); (-): No calculated because absence of positive animals with this colour hair coat; W/black: White and black colours combinition in diverse proportions

The breed factor has been evaluated in various studies with diverse results. Gerber *et al.* (2007) mentioned that some breeds have higher sensitivity to certain infection types as Bernese Mountain and *Borrelia burgdorfferi* infection. Fontanarrosa *et al.* (2006) found that some protozoa affect more to pure breed dogs while some nematodes affect more to no pure dogs. Rapti and Rehbein (2010) did not find any differences between age, gender or breed of the *Dirofilaria immitis* infected dogs. However, Lefkaditis *et al.* (2010) reported that no pure breed dogs have a higher prevalence of *Dirofilaria immitis* than pure breed animals; other study reported that dogs of short hair coat, no pure breed and small size have a higher prevalence of *Dirofilaria immitis* compared as those have none of these characteristics (Lefkaditis *et al.*, 2010), in contrast as other reports that indicate that big breeds have higher possibility of infection acquisition (Yaman *et al.*, 2009), although some socioeconomic factors of the owners and their culture for taking periodically their dogs for veterinary revisions so, the use of prophylactic treatments (Merawin *et al.*, 2009; Jacso *et al.*, 2010) can play an important role in microfilariae prevalence both pure breed and no pure breed dogs on different geographic regions. The white hair coat showed be a risk factor (OR = 2.78, 95% CI = 0.99-7.76, p = 0.03) unlike dark colors.

Bentley *et al.* (2009) reported that mosquitoes can be captured using light sources with different intensity and color and they mentioned that female mosquitoes feeding with blood preferred free light zones while the ones that were not fed with blood were attracted by light sources, this is because it is possible that female mosquitoes not fed prefer clear zones and female mosquitoes fed prefer dark zones. The previously mentioned could explain what it was observed in Table 2 where white color obtained a risk index higher than dark colors (black and brown). Therefore, it is considered that color hair coat is important in the disease epidemiology, due likely to the attraction of mosquitoes to some colors according reported by Bentley *et al.* (2009) and illumination of the houses can be an attraction factor for female mosquitoes yet not fed. According to the above, the microfilariae infections epidemiology is complex and likely depends on many social, economic and cultural factors of their owners as other environmental factors of host and mosquitoes (microfilariae vectors) whereby, each endemic area should study the risk factors in their own geographical zone.

CONCLUSION

The prevalence of microfilaremic dogs that were >3 years old in the studied zone was of 24.41%, two species of microfilariae were found, *Dirofilaria immitis* (19.76%) and *Dipetalonema reconditum* (4.65%). The sleeping area was not a risk factor of importance in this study. The white hair coat and pure breed variables are risk factors for microfilariae infection acquisition.

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