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Prevalence of *Dirofilaria immitis* in domestic canines from two municipalities in the tropic of Guerrero, Mexico.

Prevalencia de *Dirofilaria immitis* en caninos domésticos de dos municipios del trópico de Guerrero, México.

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RESUMEN

El objetivo del presente estudio fue determinar la prevalencia de *Dirofilaria immitis* (DI) en caninos domésticos en los municipios de Cuauhtépec y Acapulco de Juárez, Guerrero, México. Se usó muestreo por conglomerados de acuerdo a los domicilios seleccionados. Se obtuvieron muestras sanguíneas solo de caninos mayores de un año de los dos municipios. En el estudio se encontró en el municipio de Cuauhtépec el 15.68% y en Acapulco de Juárez el 7.44% de caninos domésticos positivos a microfilarias. El mayor porcentaje de animales positivos se encontró en machos, en animales >3 años de edad y animales de talla chica y mediana en el municipio de Cuauhtépec ($P < 0.05$). Asimismo, en el municipio de Acapulco de Juárez se encontró un mayor porcentaje de hembras positivas, con edad >3 años de edad, de raza mestizo indefinido y que vivían en el exterior del hogar ($P < 0.05$). Se concluye que los caninos domésticos en los municipios de Cuauhtépec y Acapulco de Juárez, Guerrero se encontró una alta prevalencia de DI, y los machos tuvieron un mayor porcentaje en el municipio de Cuauhtépec y las hembras en Acapulco de Juárez.

Palabras clave: Nematodos gastrointestinales; infestación; corazón derecho; *Dirofilaria immitis*; sexo; muestras de sangre.

ABSTRACT

The objective of the present study was to determine the prevalence of *Dirofilaria immitis* (DI) in domestic canines in the municipalities of Cuauhtépec and Acapulco de Juárez, Guerrero, Mexico. Cluster sampling was used according to the selected addresses. Blood samples were obtained only from dogs older than one year of the two municipalities. In the study, 15.68 % were found in the municipality of Cuauhtépec and 7.44% in domestic canines positive for microfilariae in Acapulco de Juárez. The highest percentage of positive animals was found in males, in animals > 3 years of age and small and medium animals in Cuauhtépec municipality ($P < 0.05$). Likewise, in the municipality of Acapulco de Juárez, a higher percentage of positive females was found, with an age > 3 years of age, of an indefinite mestizo race and living outside the home ($P < 0.05$). It is concluded that domestic canines in the municipalities of Cuauhtépec and Acapulco de Juárez, Guerrero, found a high prevalence of DI, and males had a higher percentage in the municipality of Cuauhtépec and females in Acapulco de Juárez.

Keywords: Gastrointestinal nematodes, infestation, right heart, *Dirofilaria immitis*, sex, blood samples.

INTRODUCTION

Canine filariasis is the disease that gives name to the infestation by the parasite *Dirofilaria* in dogs, also known as heartworm, cardiac verminosis, heartworm disease, worm disease in the heart (Rawlings and Calvert, 1997, Montoya-Alonso *et al.* , 2011; Alho *et al.*, 2018). In the world there are two species of *Dirofilaria*, of interest for human and veterinary medicine (*D. repens* and *D. immitis*) (Liotta *et al.*, 2013). *D. repens* is a disease present in Europe, Africa and Asia; on the other hand DI or heartworm present in mammals, mainly carnivores and primates: dogs, cats, foxes, coyotes, ferrets and sea lions (Vezzani *et al.*, 2006, Orihel *et al.*, 1998, Genchi *et al.*, 2011; Alho *et al.*, 2018).

DI is a cosmopolitan nematode, originally considered of strict veterinary importance; subsequently it was recognized as zoonotic; in humans it causes skin and lung lesions. Cases of dirofilariasis have been reported in large mesenteric vessels, peritoneal vessels, spermatic cord and "right" heart (Chinapa *et al.*, 2004, Sánchez-Klinge *et al.*, 2011, Wang *et al.*, 2019).

Clinically ill animals show few signs of infestation; although these depend on the severity of the disease, the location of the filaria, the time that has been present (Knight, 1980, Ceribasi and Simsek, 2012, Dearsley *et al.*, 2019), and the amount of damage to the heart; as well as the lungs, liver and other organs; but always the affected animal will show less tolerance to exercise (Fox *et al.*, 1999, Ceribasi and Simsek, 2012, Wang *et al.*, 2019). The adult worms, in the canine, form a mass in the right ventricle causing a congestive heart failure in the pulmonary artery; while microfilariae circulate in the blood (Knight, 1980, Fox *et al.*, 1999, Montoya-Alonso *et al.*, 2011, Alho *et al.*, 2018).

Microfilaria circulates in the bloodstream, but cannot develop adult worms without passing through an intermediate and transmitting host, the "hematophagous" mosquito that belong to the Phylum Arthropoda, Insecta Class, Diptera Order, Nematocera Suborder, Culicidae Family (Christensen, 1978; Liotta *et al.*, 2013; Alho *et al.*, 2018) and the genera: *Aedes*, *Anopheles*, *Culex* and *Taeniohynchus*; the family has 3,000 species included in 34 genera, receptive as intermediate hosts and biological DI vectors (Sánchez-Klinge *et al.*, 2011, Urquhart *et al.*, 2001, Simón *et al.*, 2012, Dearsley *et al.*, 2019) .

For the biological cycle to occur in the mosquito, it is necessary that in the infected mosquito a series of transformations take place in larval stages (L1, L2 and L3), all this happens between 13 to 16 days (Kittleson and Kienle, 2000; Lynn, 1999; Montoya-Alonso *et al.*, 2011; Dearsley *et al.*, 2019). On the other hand, the development in the host mammal occurs after 2 days to 3 months, reaching lengths of 3.2 to 11 cm (L4 and 5; Bowman and Lynn, 1999; Liotta *et al.*, 2013; Wang *et al.*, 2019). The number of adult

worms harbored varies from 1 to more than 250 in the dog (Dillon, 2000; Dearsley *et al.*, 2019). The survival of DI in the dog is from 3 to 8 years (Atkins, 1994; Dillon, 2000; Newton, 1968; Liotta *et al.*, 2013; Dearsley *et al.*, 2019).

In Mexico epidemiological studies have been conducted to determine the presence of DI in dogs, and its prevalence varies according to the living conditions of the animals and the climatic variations of the region (Bautista-Garfias *et al.*, 2001). Indeed, the highest prevalences are found in tropical and subtropical regions (Labarthe and Guerrero, 2005, Liotta *et al.*, 2013, Wang *et al.*, 2019). In a prevalence study conducted in the 11 municipalities of Nayarit state, they found a prevalence range of 2.5 to 33.33 % (González-Morteo *et al.*, 2015, Wang *et al.*, 2019). Rodríguez-Vivas *et al.* (1994), conducted a study in Mérida city, Yucatán, and found a prevalence of 12.5 %.

The blood test is the most practical and simple method for the diagnosis of DI; although it is a qualitative test, it does not indicate the number of microfilariae and the severity of the lesions. In fact, the diagnosis is usually established from regular blood tests (Paras, 2011, Wang *et al.*, 2019), such as blood count, cases of chronic cough in individuals living in endemic areas and to detect worms adults in the heart through radiographic plates, especially in cases of vena cava syndrome (García *et al.*, 2011; Alho *et al.*, 2018). In sick animals, a clinical diagnosis is made by several methods for the identification of microfilariae of infected dogs: a) method of direct observation of serum, b) serum concentration method; c) immunological methods to detect antigens and d) modified Knott technique (Ferrer -Montaño *et al.*, 2002; Fernández *et al.*, 2017; Dearsley *et al.*, 2019).

In Guerrero state and particularly in the municipalities of Acapulco de Juárez and Cuauhtémoc, there is no information on the prevalence of DI in domestic canines. Therefore, the objective of the present study was to determine the prevalence of *Dirofilaria immitis* in domestic canines in the municipalities of Cuauhtémoc and Acapulco de Juárez, Guerrero, Mexico.

MATERIAL AND METHODS

Study area

The present epidemiological study was conducted in the municipalities of Cuauhtémoc and Acapulco de Juárez, Guerrero, Mexico. This region of the state belongs to the tropics and is located between the coordinates 16 ° 51'48 "of Latitude North and 99 ° 53'24" of West Longitude, with an altitude of between 0 and 2,000 m a.s.l. The highest average temperature and humidity (39.5 °C and 76 %) occur during the summer months and the lowest months (16 °C and <70 %) during the winter months (García, 1974).

Animals

196 domestic dogs were randomly sampled, 102 in the municipality of Cuautepéc (41 males and 61 females) and 94 Acapulco de Juárez (41 males and 53 females). In both municipalities, the sampling criterion for animals was from north to south and from east to west, taking as a reference the center of each municipality. Of the animals found by domiciled visits, only those older than one year were sampled, because the biological cycle of the parasite is long and for the diagnostic method used it is required to have microfilariae in its plasma, about 7 months after the infestation (González -Morteo *et al.*, 2015). All the procedures of sampling and management of the animals reported in the present work were according to the Official Mexican Standard with technical specifications of the production, use and care of laboratory animals (SAGARPA, 2001).

Collection and preparation of samples

The blood samples were taken in the hours between 18:00 - 20:00 h, by the nocturnal periodicity. Prior to the extraction of blood, the animal was subjected to depilating the area; with the respective antiseptics, 3 ml of blood with anticoagulant (EDTA) were extracted from the cephalic vein and the samples were kept at 4 to 6 °C for 4 days for further analysis.

Subsequently, blood smears were performed in order to identify dead *dirofilaria* larvae. For the Thick Drop technique, a drop of fresh blood was placed on a slide, extending it to cover a double surface of the original; finally, the preparation was allowed to dry (in the air or in an oven at 37 °C) and traces of larval motility were observed under the microscope. The modified Knott technique was performed by mixing 1 ml of blood with 9 ml of 2 % formaldehyde in a glass tube; the mixture was centrifuged for 8 minutes at 1500 r.p.m. ; the supernatant was removed, and the 0.1 % methylene blue pellet was added, and the pellet was then examined under a microscope.

Variables of study

Sex, age (1, 3 and > 3 years), height (girl, medium, large), race (pure, mestizo undefined), body condition (CC, scale: 1 to 5) (Laflamme, 1997), activity or work in the field, cattle herding, care of the home, location (north, south, east or west) and possible risk factors such as the degree of urbanization of the colony-housing where the dogs lived and exposure to mosquitoes.

Statistical analysis

The sampling system by municipality Cuauhtémoc and Acapulco de Juárez, was by conglomerates according to the location of the households sampled (dynamics: north to south and east to west). Contingency tests were carried out in 2x2 tables; the proportions between variables were analyzed with the Chi-square test to establish the correlation between sex and disease. All the results were analyzed with the statistical program SAS (2004).

RESULTS AND DISCUSSION

In the municipality of Cuauhtémoc, 15.68 % of positive canines were found in microfilariae. The highest percentage of positive animals was found in males than in females and animals > 3 years of age ($P < 0.05$). The canines that presented the highest percentage were those of CC of 4 and 5 that the minors on this scale. On the other hand, a higher percentage of positive animals was found in small and medium size canines ($P < 0.05$). A higher percentage was also found in canines of an indefinite mestizo breed, who lived outside the home, in coexistence with other congeners and who spent more time caring for the home ($P < 0.05$) (Table 1).

The results of the present study show that a high prevalence of ID in canines was found in Cuauhtémoc municipality. Results consistent with this study were similar to those found in the municipalities of Bahía de Banderas Nayarit (12.7 % prevalence) (González-Morteo *et al.*, 2015) and Chontalpa Tabasco (17.5 % prevalence) (Torres-Chable *et al.*, 2018); but different from those reported by Rodríguez-Vivas *et al.* (1994) in Yucatán state (6.54 % prevalence). These same investigations (Rodríguez-Vivas *et al.*, 1994, González-Morteo *et al.*, 2015, Torres-Chable *et al.*, 2018), mention not having found an effect between the sexes (males and females), age, height, race, place where they lived (inside or outside the house) and coexistence with other dogs.

However, in the present study the animals where the microfilaria were found were males, with high CC (3 and 5 points), small size and medium size. This can be explained as follows: working dogs are generally of an indefinite mestizo breed, medium size, they inhabit outside the home, they have the possibility of interacting more with other dogs and due to the location they are more exposed to mosquito bites host of the DI parasite.

Table 1. Frequency and percentage of positive canines to DI microfilaria in the municipality of Cuatepec.

Variable	Category	Samples (n)	Positive to microfilaria (Knott)	
			Frequency	%
Sex	Female	41	3 ^a	7 ^b
	Male	61	13 ^b	21 ^a
Age (years)	1	36	4 ^a	11 ^b
	3	38	7 ^a	18 ^a
	>3	28	5 ^a	18 ^a
¹ CC	1 a 2	24	1 ^a	4 ^c
	3	49	8 ^b	16 ^b
	4 a 5	29	7 ^b	24 ^a
Size	Little	22	3 ^a	14 ^b
	Medium	77	13 ^b	17 ^b
	Large	3	0 ^a	0 ^a
Race	Pure	31	2 ^a	6 ^b
	Mestizo	71	14 ^b	20 ^a
Living place	Interior	16	0 ^a	0 ^b
	Exterior	86	16 ^b	19 ^a
Co-existence	Farm	49	2 ^a	4 ^b
	Canines	53	14 ^b	26 ^a
Activity	Home	38	1 ^a	4 ^b
	Field	64	5 ^b	23 ^a
Location	Norte	26	3 ^a	18 ^c
	South	22	7 ^b	19 ^c
	East	17	9 ^b	24 ^b
	West	37	7 ^b	11 ^a
Microfilaria	Negative	86	0 ^a	0% ^a
	Positive	16	16 ^b	15.65% ^b

¹Body condition (CC, scale 1 to 5)..

^{a,b,c} The literals inside column show significant difference between % (P<0.05).

On the other hand, in the municipality of Acapulco de Juárez, 7.44 % of domestic dogs positive for microfilaria were found. The highest percentage of positive animals was found in small animals, with CC from 1 to 3 and living with congeners in the home (P<0.05). Likewise, a higher percentage of positive females was found, with an age> 3 years of age, of an indefinite mestizo race and living outside the home (P <0.05, Table 2).

The results of the present study show that, in the city of Acapulco de Juárez, Guerrero, a low prevalence of DI was found in domestic dogs. Results similar to those of this study were reported by Rodríguez-Vivas *et al.* (1994) in the state of Yucatán (6.54% prevalence). Contrary to these results, they were different from those found in the

municipality of San Blas Nayarit by González-Morteo *et al.* (2015; 33.33% prevalence) and by Torres-Chable *et al.* (2018) in Chontalpa Tabasco (17.5% prevalence). These same investigations (Rodríguez-Vivas *et al.*, 1994, González-Morteo *et al.*, 2015, Torres-Chable *et al.*, 2018), mention not having found an effect between the sexes (males and females), age, height, race, place where they lived (inside or outside the house) and coexistence with other dogs.

However, in the present study the animals where the microfilaria were found were male, of low CC (1 to 3 points), small size and medium size; this can be explained as follows: most pets are of medium size, live outside the home, have the possibility of interacting more with other dogs and have a greater exposure to mosquito bites intermediate host of the parasite.

In the present study it can be mentioned that at least in the sampled municipalities, DI disease prevails; due to this and by some particular characteristics to consider it as an enzootia to the disease (Dirofilariasis). As the authors mentioned in their localities have considered it; It is possible to use this term because in this specific geographical area of the state of Guerrero the dog populations that live outside the home are continuously affected by mosquitoes and consequently by DI.

Table 2. Frequency and percentage of positive canines to DI microfilaria in Acapulco city.

Variable	Category	n	Positive to microfilariae (Knott)	
			Frequency	%
Sex	Female	53	6 ^a	11% ^a
	Male	41	1 ^b	2% ^b
Age (months)	8 a 12	40	3 ^a	8% ^a
	13 a 36	36	2 ^a	6% ^a
	>37	19	2 ^a	11% ^b
CC scale	1 a 3	50	6 ^a	12% ^a
	3 a 5	44	1 ^b	2% ^b
	small	36	6 ^a	17% ^a
Size	Medium	42	0 ^b	0% ^b
	large	16	1 ^b	6% ^b
	Defined	21	0 ^a	0% ^a
Race	Indefinite mestiza	73	7 ^b	10% ^b
Habitat	Interior	12	0 ^a	0% ^a
	Exterior	82	7 ^b	9% ^b
Living with animals	No	34	0 ^a	0% ^a
	Dogs	60	7 ^b	12% ^b
Microfilaria	Negative	87	0 ^a	0% ^a
	Positive	7	7 ^b	7.44% ^b

^{a,b}The literals inside column show significant difference between frequency and percentage (P<0.05).

CONCLUSION

The results of the present study allow us to conclude that, in the domestic dogs of the municipalities of Cuauhtepic and Acapulco de Juárez, Guerrero, a high prevalence of DI was found (average: 11.56 %), and the males had a higher percentage of infestation (21 %; Cuauhtepic). Likewise, it was found that canines of medium size and those with high CC were positive in the parasite detection tests.

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