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Molecular detection of *Dirofilaria immitis* in dogs and mosquitoes in Tabasco, Mexico

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ABSTRACT

Background & objectives: Dirofilaria immitis is a filarial nematode that causes heartworm disease in domestic as well as wild canines and felines; and cutaneous or pulmonary infections in humans. The purpose of the study was to estimate the prevalence of *D. immitis* in domestic dogs in Tabasco, Mexico and to assay mosquitoes temporally and spatially associated with dogs for evidence of infection.

Methods: Blood was collected from 1050 dogs in 1039 houses during a random household survey performed in 2016 and 2017. Genomic DNA was extracted and assayed by polymerase chain reaction (PCR) using pan-filarial primers and various species-specific primers. Dog owners were interviewed using a structured questionnaire designed to collect information on factors that may impact the occurrence of filarial infection. The association between canine dirofilariasis prevalence and factors likely to impact infection was determined by univariate logistic regression analysis, followed by multivariate binomial logistic regression analysis. Indoor and outdoor resting mosquitoes were collected from houses by manual aspiration. Mosquitoes were identified according to species, homogenized and tested by PCR for filarial nematodes.

Results: A total of 84 (8%) dogs were positive for *D. immiti*s DNA, while 3 (0.3%) dogs contained *Acanthocheilonema reconditum* DNA. Several factors were significantly associated with *D. immiti*s infection. For example, dogs that lived ≤100 m from a large source of open standing water were significantly more likely ($p = 0.002$) to become infected with *D. immiti*s than other dogs. Additionally, dogs with infrequent or no anthelmintic treatment were significantly more likely ($p = 0.0$) to become infected than dogs that were regularly treated. The entomologic investigation yielded 2618 female mosquitoes from 14 species. Four pools of *Culex quinquefasciatus* were positive for *D. immiti*s DNA and the minimum infection rate, calculated as the number of positive pools per 1000 mosquitoes tested, was 2.9.

Interpretation & conclusion: The study identified several factors positively associated with an increased risk of *D. immitis* infection in domestic dogs in Tabasco and provides evidence that *Cx. quinquefasciatus* is potentially an important vector in this region. This information can be used by local veterinarians and dog owners to reduce the burden of *D. immitis* on canine health.

Key words *Culex quinquefasciatus; Dirofilaria immitis;* dog; Mexico; mosquito; Onchocercidae; vectors

INTRODUCTION

Dirofilaria immitis (Order: Spirurida and Family: Onchocercidae) is a filarial nematode, common in tropical, subtropical and some temperate regions of the world. It is the causative agent of heartworm disease in domestic and wild canines and, to a lesser extent, felines[1–3]. It is also an occasional cause of pulmonary dirofilariasis in humans[1, 4–5]. In canines, heartworm disease is characterized by chronic cough, exercise intolerance, reduced appetite and weight loss and can progress to congestive heart failure and death[1–2]. The principal reservoir hosts of *D. immitis* are domestic and wild canines[2]. Felines and humans are usually incidental hosts. The pathogen is transmitted to susceptible hosts by haematophagous mosquitoes, particularly those in the *Culex*, *Aedes* and *Anopheles*[6–7]. Transmission occurs when a mosquito ingests first-stage larvae which migrate to the proboscis as

infective third-stage larvae (L3). Larvae are transmitted to susceptible vertebrate hosts upon mosquito blood feeding and reach sexual maturity in the main pulmonary arteries and right ventricle.

Studies designed to monitor canine populations in Mexico for evidence of *D. immitis* infection have historically relied on serological assays or microfilariae examination using the thick blood smear (TBS) or modified Knott's tests[8-11]. Few studies performed in Mexico have reported the use of molecular assays[12], however, no study has been performed in Tabasco or any of its neighbouring states. Information on the vector host range of *D. immitis* is also limited in Mexico[13–14] and entomologic-based surveillance for *D. immitis* has never been performed in Tabasco. To address these gaps, this study was carried out to estimate the prevalence of *D. immitis* and other filarial nematodes in domestic dogs in Tabasco, southern Mexico, and identify likely vector species responsible for transmitting the disease in the region.

MATERIAL & METHODS

Study area

The State of Tabasco in southern Mexico (global positioning system coordinates 18°39'–17°19' N and 90°57'– 94°8' W) has a hot, tropical climate and an average elevation of 10 m[15]. Tabasco is officially divided into five distinct ecological regions: Centro, Chontalpa, Pantanos, Rios and Sierra (Fig. 1). The Centro is an urban area, Chontalpa is covered by extensive savannah, Pantanos contains numerous marshes, Rios is a stony, dry region with a small jungle and Sierra is characterized by mountains and dense jungle.

Calculation of sample size

A total of 261930 domestic dogs are estimated to live in Tabasco according to an unpublished canine census performed in 2015 (Diego Quiroga Iduarte, The Secretaria de Salud del Estado de Tabasco, personal communication). Using a statistical formula for finite and known populations[16], it was calculated that a sample population of ≥1302 dogs would be representative of the entire population. Stratified sampling with proportional allocation was performed to determine the number of dogs needed to be sampled in each ecological region[17]. The analysis revealed that the minimal numbers of dogs needed from each ecological region to perform a robust statistical analysis are as follows: Chontalpa–572; Centro–332; Pantanos–191; Rios–140 and Sierra–67. Sufficient numbers of dogs to perform a robust statistical analysis were sampled in Centro and Sierra but this could not be achieved in Chontalpa, Pantanos and Rios because the field workers were concerned about their safety while traveling through these regions and therefore, terminated the sampling earlier than originally planned.

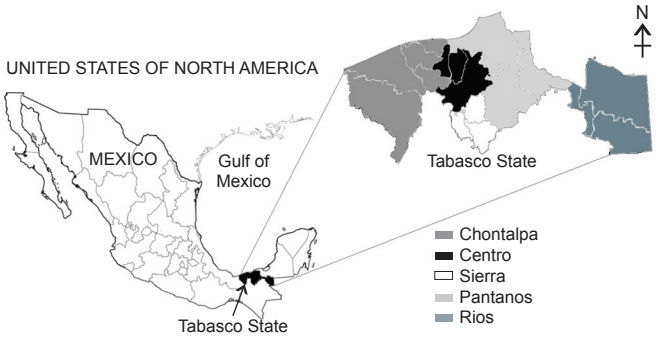

Fig. 1: Geographic location of the five ecological regions in Tabasco, Mexico.

Random household survey

Dogs were sampled during a random household survey with verbal consent obtained from their owners. Demographic information was collected by interviewing dog owners using a structured questionnaire. The survey was also designed to collect information on other factors likely to impact the occurrence of filarial infection.

Information collected is as follows: Age of dog (≤2 or >2 yr), gender (male or female), breed (purebred or non-purebred), size (small/medium or large), hair colour (light or dark), hair length (short or long), body score (<3 or ≥3), distance of house from closest source of open standing water (*i.e.* a swamp, lagoon, marsh or artificial reservoir; ≤100 m or >100 m), application of anthelmintic treatment at least once every six months (yes or no), vaccinations up-to-date (yes or no), veterinary care at least once a year (yes or no), *Cx. quinquefasciatus* collected at the house (yes or no), primary sleeping area (indoors or outdoors), primary food type (commercial or homemade food) and excessive garbage at the house (yes or no). None of the houses were located within 50 m of each other. If six or more dogs were present at one house, only two dogs were sampled.

Blood collections

Whole blood was collected from the cephalic vein of each animal using tubes containing ethylenediamine tetraacetic acid (EDTA) as an anticoagulant (Vacutainer, BD Biosciences, Franklin Lakes, NJ). Blood samples were placed on wet ice in an insulated container, transported to the laboratory and stored at –80°C until required.

Mosquito collections

Indoor and outdoor resting mosquitoes were collected

using modified CDC backpack aspirators (model 1412; John W. Hock Company, Gainesville, FL). The houses of all dog owners who granted us permission to perform mosquito collections were inspected. Each house was searched once, typically for 10–15 min. Indoor collections primarily focused on bedrooms, living rooms and bathrooms with a thorough examination of walls, ceilings, furniture, curtains and hanging clothes.

Outdoor collections were made in the backyards of houses and surrounding areas (defined as outdoor areas within 10 m of the house). Mosquitoes were transported alive to the laboratory, euthanized at –80°C and identified to species and sex on a chill table using published identification keys[18]. Mosquitoes were sorted into pools of up to 10 according to species, sex, study site and collection date.

Extraction of genomic DNA

Genomic DNA was extracted from canine blood and mosquitoes using different protocols. Canine blood was processed using the salting-out method of Miller *et al*[19] with several modifications.

Briefly, anti-coagulated blood (100 µl) was suspended in 180 µl of erythrocyte lysis buffer (10 mM Tris-HCl pH 8.0, 1% Triton X-100, 11% saccharose), incubated for 5 min at room temperature and centrifuged (18,000 × g; 15 min, 4 °C). Pellets were resuspended in 60 µl of leucocyte lysis solution (10 mM Tris-HCl pH 8.0, 400 mM NaCl, 2 mM EDTA) supplemented with 2 µl of 20% sodium dodecyl sulfate (SDS) and 10 µl of proteinase K solution (1 mg/ml proteinase K, 1% SDS, 2 mM EDTA) and incubated for 1 h at 65 °C. Samples were mixed with 30 µl of 3 M potassium acetate, incubated for 30 min at 4°C and centrifuged (18,000 × g; 15 min, 4°C). Supernatants were collected, mixed with 200 µl of isopropanol, incubated overnight at 4°C and centrifuged (18,000 × g; 15 min; 4°C). Pellets were washed with 70% ethanol and resuspended in TE buffer (10 mM Tris-HCl pH 8.0, 1 mM EDTA).

Female mosquitoes were homogenized in 200 µl of maceration buffer (0.1 M NaCl, 0.2 M saccharose, 0.1 M Tris, 0.05 M EDTA, 0.05% SDS) using mortars and pestles. Homogenates were centrifuged (18,000 × g; 30 sec; 4°C) and supernatants were collected and incubated at 65°C for 30 min. After the addition of 21 µl of 8 M potassium acetate, samples were incubated for 30 min at 4°C and centrifuged (18,000 × g; 15 min; 4°C). Supernatants were collected, mixed with 200 µl of 100% ethanol, incubated overnight at 4°C and centrifuged (18,000 × g; 15 min, 4°C). Pellets were washed in 70% ethanol and resuspended in TE buffer.

Polymerase chain reactions (PCRS)

PCRs were performed using pan-filarial primers and positive samples were further analyzed using various species-specific primers, including primers specific for *D. immitis* and *A. reconditum*. The pan-filarial primers, DIDR-F1 (5'-AGTGCGAATTGCAGACGCATTGAG-3') and DIDR-R1 (5'-AGCGGGTAATCACGACTGAGTTGA-3'), amplify a 430–664 bp region of ribosomal DNA[20]. The *D. immitis*-specific primers, DI-COI-F1 (5'-AGTGT AGAGGGTCAGCCTGAGTTA-3') and DI-COI-R1 (5'-ACAGGCACTGACAATACCAAT-3'), amplify a 203 bp region of the cytochrome oxidase subunit 1 (COI) gene. The *A. reconditum*-specific primers, AR-COI-F1 (5'-GTGTTGAGGGACAGCCAGAATTG-3') and AR-COI-R1 (5'-CCAAAACTGGAACAGACAAAACAAGC-3'), amplify a 208 bp region of the COI gene.

Reactions were performed using 2.5 µl of genomic DNA (50–200 ng), 2.5 U of Taq polymerase, 10 pM of each primer and 250 µM of each dNTP in 1× PCR buffer (20 mM Tris-HCl pH 8.4, 50 mM KCl, 1.5 mM $MgCl_2$) in a final volume of 25 µl.

Reaction conditions were as follows: 94 °C for 2 min, 32 cycles of denaturing (94 °C for 30 sec), annealing (58 °C for 1 min, species-specific primers; 60 °C for 1 min, universal primers) and extension (72 °C for 30 sec), followed by a final extension at 72 °C for 7 min. An aliquot of each PCR product was examined by 2% agarose gel electrophoresis and visualized with ethidium bromide.

Statistical analysis

Univariate logistic regression analysis was initially performed to assess the potential association between predictor variables collected during the random household survey and canine dirofilariasis prevalence. Predictor variables with p-values < 0.2 were subjected to multivariate binomial logistic regression analysis using the statistical software IBM SPSS version 22 (IBM Corporation, Armonk, NY). The software calculated the odds ratios, 95% confidence intervals and p-values. Variables with p- values ≤0.05 were considered significant.

Ethical statement

The study protocol was evaluated and approved by the Ethical Committee of Health Sciences University in State of Tabasco, Mexico, Universidad Juarez Autonoma de Tabasco (Approval No. 175/DACA, dated September 17, 2015). All animals were handled and treated in accordance with experimental protocols approved by the Animal Care and Use Committees at the participating universities.

RESULTS

A total of 1050 apparently healthy dogs in 1039 houses were sampled in Tabasco from January 2016 to January 2017 (Table 1). The numbers of dogs from each ecological region are as follows: Centro (n = 334), Chontalpa (n = 400), Pantanos (n = 124), Rios (n = 124), and Sierra (n = 68). Filarial DNA was detected by PCR in 87 (8.3%) dogs. In total 84 (8%) dogs were infected with *D. immiti*s and 3 (0.3%) dogs were infected with *A. reconditum*. The *D. immiti*s infected dogs were from Centro, Chontalpa and Pantanos and all *A. reconditum* infected dogs live in Pantanos. No coinfections were identified.

In the univariate logistic regression analysis 12 of the 15 predictor variables produced *p*-values < 0.2 (Table 2). Of these, five variables were found to be positively associated with *D. immiti*s infection in the multivariate binomial logistic regression model (Table 3). Not surprisingly, a positive association between age and *D. immiti*s prevalence was observed. There was also a positive association between breed status and prevalence; non-purebred dogs were significantly more likely to contain *D. immiti*s DNA than purebred dogs. Additionally, dogs infrequently or never given anthelmintic treatment were five-fold more likely to be infected than regularly treated dogs. Dogs living in houses, where at least one *Cx. quinquefasciatus* was collected, were approximately four-fold more likely to be infected with *D. immitis* than other dogs. Another factor positively associated with prevalence was close living to open standing water.

A total of 325 (31.3%) houses were inspected for resting adult mosquitoes. The numbers of houses inspected in each ecological region are as follows: Centro (n = 83), Chontalpa (n = 126), Pantanos (n = 63), Rios (n = 32), and Sierra (n = 21). A total of 4622 mosquitoes from 15 species and seven genera were collected (Table 4). Of these, 2618 (56.6%) mosquitoes from 14 species were female. The most common species was *Culex quinquefasciatus* which made up 65% of the entire sample population, followed by *Cx. nigripalpus* (18.5%). Females were assayed by PCR for filarial DNA and four pools of *Cx. quinquefasciatus* were found positive for *D. immitis* DNA. Filarial DNA was not detected in any other mosquito spp. The *D. immitis* minimum infection rate for female *Cx. quinquefasciatus*, calculated as the number of positive mosquito pools per 1000 mosquitoes tested, was 2.9. Positive pools of *Cx. quinquefasciatus* were from Chontalpa.

Table 1. Prevalence of filarial nematodes in domestic dogs in Tabasco, Mexico (January 2016–January 2017)

Ecological region	Number (%) of dogs positive for filarial DNA	
	D. immitis	*A. reconditum*
Centro	6/334 (1.8%, CI = 0.31–3.08%)	0/334 (0)
Chontalpa	70/400 (17.5%, CI = 13.7–21.2%)	0/400 (0)
Pantanos	8/124 (6.5%, CI = 2.12–10.77%)	3/124 (2.4%, CI = – 0.28–5.10%)
Rios	0/124 (0)	0/124 (0)
Sierra	0/68 (0)	0/68 (0)
Total	84/1050 8% (CI = 6.3–9.64%)	3/1050 (0.28%, CI = – 0.03–0.6%)

CI—Confidence interval.

Table 2. Univariate logistic regression of predictor variables

Variable	B	SE	Wald	df	OR	*p*-value	CI (95%)
Age (≤ 2 or >2 yr).	0.45	0.23	3.76	1	1.57	0.052*	0.99–2.48
Sex (Male or Female)	0.79	0.22	1.20	1	1.08	0.729	0.69–1.68
Breed (purebred or non-purebred)	1.79	0.40	20.22	1	6.04	0.000*	2.75–13.22
Size of dogs (small/medium or large)†	0.91	0.30	9.23	1	2.48	0.002	1.38–4.47
Hair colour (dark or light)†	0.65	0.27	5.52	1	1.92	0.019*	1.11–3.33
Hair length (short or large)	0.36	0.26	1.92	1	1.44	0.165	0.86–2.42
Body score (<3 or ≥3)	0.30	0.27	1.15	1	1.34	0.282	0.78–2.33
Standing water within 100 m (yes or no).	1.13	0.23	23.11	1	3.12	0.000*	1.96–4.96
Anthelminthic treatment every six months (yes or no)	2.12	0.30	47.41	1	8.32	0.000*	4.55–15.22
Vaccinations up-to-date (yes or no)	0.23	0.24	0.92	1	1.26	0.337	0.78–2.03
Veterinary care at least once a year (yes or no)†	1.84	0.35	26.50	1	6.31	0.000	3.13–12.74
Cx. quinquefasciatus collected at the house (yes or no)	1.44	0.28	26.60	1	4.25	0.000*	2.45–7.37
Sleeping area (primarily indoors or outdoors)	–1.41	0.72	3.81	1	0.24	0.051*	0.05–1
Food type (primarily commercial or homemade)†	2.08	0.42	23.74	1	8.06	0.000	3.48–18.65
Excessive garbage at the house (yes or no)†	0.72	0.22	10.37	1	2.06	0.001	1.32–3.21

*Variables included in the multivariate analysis; †Variables excluded from the multiple analysis because they were considered to be suppressor variables or presented multicollinearity with other variables; B—Regression coefficient; SE—Standard deviation; Wald—Wald test; df—Degrees of freedom; OR—Odds ratio; CI—Confidence interval.

Table 3. Factors significantly associated with *Dirofilaria immitis* prevalence in dogs in Tabasco, Mexico (January 2016–January 2017)

Variable	No. of dogs positive for *D. immitis* DNA	Odds ratio	95% confidence interval	*p*-value
Age (yr)				
≤2	28/476 (5.9)	0.55	0.33–0.92	0.023
>2	56/571 (9.8)	1.80	1.08–2.97	
Breed				
Purebred	6/339(1.8)	0.30	0.12–0.74	0.008
Non-purebred	78/708 (11)	3.25	1.35–7.82	
Regular use of anthelmintics				
Yes	13/585 (2.2)	0.19	0.10–0.36	0.0
No	71/462 (15.4)	5.21	2.77–9.81	
Cx. quinquefasciatus collected at the house				
Yes	21/88 (23.9)	3.20	1.73–5.89	0.0
No	63/959 (6.6)	0.31	0.17–0.57	
Large body of standing water within 100 m				
Yes	56/432 (13)	2.22	1.33–3.68	0.002
No	28/615 (4.6)	0.45	0.27–0.74	

Figures in parentheses indicate percentages. The three dogs positive for *A. reconditum* were excluded from the analysis.

Table 4. Summary of mosquitoes collected in Tabasco, Mexico, (January 2016–January 2017)

Species	Number of pools	Number of mosquitoes			Ecological region
		Female	Male*	Total	
Aedeomyia (*Aedeomyia*) *squamipennis*	0	0	4	4	C, Ch
Aedes (*Ochlerotatus*) *angustivittatus*	2	4	0	4	C, P
Ae. (*Ochlerotatus*) *taeniorhynchus*	14	107	13	120	C, Ch, P, S.
Ae. (*Stegomyia*) *aegypti*	22	48	35	83	C, Ch, P, R
Anopheles (*Nyssorhynchus*) *albimanus*	6	6	4	10	C, Ch, P
An. (*Anopheles*) *pseudopunctipennis*	4	4	3	7	C, Ch, R
An. (*Anopheles*) *quadrimaculatus*	16	44	186	230	C, Ch, R
Coquilletidia (*Coquilletidia*) *perturbans*	4	4	1	5	C, P
Culex (*Culex*) *quinquefasciatus*	335	1394	1611	3005	C, Ch, P, R, S
Cx. (*Melanoconion*) *erraticus*	7	17	16	33	C, Ch
Cx. (*Melanoconion*) *nigripalpus*	105	734	119	853	C, Ch, P
Cx. (*Melanoconion*) *pilosus*	2	2	0	2	C, R
Mansonia (*Mansonia*) *titillans*	68	240	11	251	C, Ch, P, R
Ma. (*Mansonia*) *dyari*	2	2	0	2	P
Uranotaenia (*Uranotaenia*) *lowii*	8	12	1	13	C, Ch
Total	595	2618	2004	4622	

*Males were not assayed by PCR. C—Centro; Ch—Chontalpa; P—Pantanos; R—Rios; S—Sierra.

DISCUSSION

This study describes one of the first PCR-based surveys designed to assay dogs and mosquitoes in Mexico for evidence of *D. immitis* infection. The overall prevalence of *D. immitis* in dogs was 8%, which is not dissimilar to the national prevalence (5.3–7.5%); although, caution is required when comparing these findings because the nationwide data were collected using non-molecular assays[9–10]. PCRs are more sensitive than traditional assays used for *D. immitis* detection[21–22]. The prevalence of *D. immitis* in dogs has been reported to vary from 8.3 to 59.8% in regional studies performed in Mexico[8, 11–12]. For example, 17 of 86 (19.8%) dogs in Villahermosa (the largest city in Tabasco) were positive for *D. immitis* by TBS and modified Knott's test[11]. Dogs ≤3 yr-old were excluded from the study cohort and this could be one reason why the prevalence was two-fold higher in the Villahermosa study compared to this study. In another investigation, *D. immitis* DNA was detected by PCR in 167 of 279 (59.8%) dogs in Celestun (a coastal town in Yucatan) in 2007 and 2008[12]. Nationwide surveys have demonstrated that the prevalence of *D. immitis* in dogs along the Gulf of Mexico coastline is often higher than dogs in non-coastal areas[1, 10] potentially because competent vectors are more abundant along the coast[12].

Several demographic variables have been shown to be significantly associated with *D. immiti*s prevalence. Higher age was identified as a risk factor, consistent with other studies[9, 23–25]. Regular use of anthelmintic treatment reduced the likelihood of infection and this too is consistent with the findings of others[26–27]. However, routine anthelmintic treatment was not completely effective because a small percentage of dogs in this category were infected. Explanations for this finding include parasite resistance or product failure due to incorrect storage, dosage or application. Purebred dogs were less likely to test positive for evidence of *D. immitis* infection than their non-purebred counterparts. This could be coincidental because it was also observed that purebred dogs were more likely to receive regular anthelmintic treatment than non-purebred dogs (275/339, 81% *vs* 310/708, 44%, respectively). Others studies have also demonstrated that purebred dogs are significantly less likely to be infected than non-purebred dogs[28–29]. For example, antibodies to *D. immitis* were detected in 8 of 167 (4.8%) purebred dogs and 17 of 147 (11.6%) non-purebred dogs in Costa Rica[28]. Others have reported no significant differences between purebred and non-purebred dogs[22, 27, 30– 31].

Four pools of *Cx. quinquefasciatus* were positive for *D. immitis* DNA and therefore, it is speculated that this species could be an important vector of *D. immitis* in Tabasco. In this regard, dogs living in houses where *Cx. quinquefasciatus* were collected, were significantly more likely to be infected than dogs living in other places. Likewise, dogs living within 100 m of standing open water were more likely to be infected. This could be because standing water, particularly stagnant standing water, provides ideal breeding habitats for various species of mosquitoes including *Cx. quinquefasciatus* which was implicated as a potential vector of *D. immitis* in this study and in other studies[32–34].

In Celestun, distance from a water source was not positively associated with prevalence[12]. However, in the Celestun study, a water source was defined as a wetland and distance was classified as ≤300 m or 301–1000 m[12]. These differences could explain the contrasting findings between the two studies. Another explanation is that wetlands do not provide suitable breeding habitats for the principal vectors of *D. immitis* in Celestun. However, *Ae.* (*Ochlerotatus*) *taeniorhynchus* has been implicated as a principal vector of *D. immitis* in Celestun[13–14] and this species is common in wetland areas[35]. Few *Ae.* (*Ochlerotatus*) *taeniorhynchus* were collected in this study (the species comprised 2.6% of the total sample population) and all were negative. In other parts of the world, many other species of mosquitoes have been implicated as principal vectors of *D. immitis.* For example, in a study performed in the western United States, *Cx. pipiens* and *Cx. tarsalis* were found to be major vectors, and *Ae. vexans*, *Ae. melanimon*, *Cs. incidens*, *Cs. inornata* and *Cx. erythrothorax* were observed as secondary vectors of *D. immitis*[36]. In another study, *Ae. scapularis*, *Ae. taeniorhynchus* and *Cx. quinquefasciatus* were implicated as principal vectors in Brazil[37]. In Italy, *Cx. pipiens* is considered to be an important vector[38]. One limitation of the present entomologic investigation is that the PCRs were not L3-specific. To address this issue, additional *Cx. quinquefasciatus* need to be collected from diverse habitats throughout Tabasco and assayed by RT-PCR using primers that detect L3-specific transcripts.

The detection of *A. reconditum* in several dogs in the sample population was not unexpected because the parasite has a worldwide distribution[39–43]. *Acanthocheilonema reconditum* usually causes inapparent infections in dogs but sometimes there are clinical consequences[39, 44]. The parasite is not typically considered to be a zoonotic agent, although one case of human subconjunctival infestation has been documented[45]. Nevertheless, this investigation was not limited to *D. immitis*. It is important to assay for other filarial nematodes, including inconsequential pathogens, to minimalize the likelihood of misdiagnosis. In this regard, misdiagnosis commonly occurs when using the Knott's test if species differentiation is not considered[46].

CONCLUSION

This study identified several factors positively associated with an increased risk of *D. immitis* infection in domestic dogs in Tabasco. This information can be used by local veterinarians and dog owners to reduce the incidence of *D. immitis* infections in dogs in the region. Additionally, this study provides evidence that *Cx. quinquefasciatus* could be an important vector of *D. immitis* in the region. Further studies focusing specifically on L3 larvae are needed to increase our understanding of the transmission dynamics of *D. immitis* in Tabasco.

Conflict of interest

The authors declare no conflicts of interest.

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