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Ixodes affinis (Acari: Ixodidae) in dogs from rural localities of Yucatán, Mexico: Prevalence, abundance and associated factors

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Abstract

The present study describes the prevalence and abundance of, as well as factors associated with, *Ixodes affinis* infestation of dogs from two environments in two rural localities (Tixméhuac with a medium sub-deciduous forest and Opichén with patches of low deciduous forest, cultivated lands, and grasslands) of Yucatán, Mexico. The associated factors were obtained by an X^2 analysis and variables where $P < 0.2$ were subjected to a logistic regression. A total of 33 adult ticks from the genus *Ixodes* were collected from 144 dogs. The infestation prevalence was found to be 11.11% (16/144) for *I. affinis*. When considering the prevalence of *I. affinis* infestation per environment, Tixméhuac had a prevalence of 19.6% (11/56) and Opichén had a prevalence of 5.6% (5/88). The abundance of *I. affinis* in both environments averaged 2.1 (1–4) ticks/dog. The factors associated with infestations of *I. affinis* in dogs were the locality of Tixméhuac (OR = 3.70, 95% C.I. = 1.24–11.08, P = 0.001), which is surrounded by a medium sub-deciduous forest, and the use of dogs for hunting in these forested areas (OR = 7.56, 95% C.I. = 1.63–35.04, P = 0.001). This study is the first report of the prevalence and abundance of infestation with *I. affinis* in dogs in Mexico, which is associated with the access of dogs to adjacent forested areas.

Keywords: *Ixodes affinis;* Dogs; Rural localities; Environments; Yucatán; Mexico.

Introduction

Ticks (Acari: Ixodoidea) are important vectors of a broad range of viral, rickettsial, bacterial and protozoan diseases that affect humans and animals (Estrada-Peña and Jongejan, 1999; Sonenshine, 1991). The genus *Ixodes* includes more than a quarter of the tick species worldwide with approximately 245 species described to date (Guglielmone *et al.*, 2014; Hornok *et al.*, 2015). Some *Ixodes* species have medical and veterinary relevance as they can transmit zoonotic pathogens (Sonenshine *et al.*, 2002; Goodman *et al.*, 2005). Despite the dominance of this genus in the northern hemisphere, few *Ixodes* species have been recorded in Mexico, with the primary species being *Ixodes boliviensis*, *I. luciae*, *I. rubidus*, *I. scapularis*, *I. spinipalpis*, *I. tancitarius*, *I. woodi* and *I. affinis*. The latter has been reported in only the state of Chiapas and was collected from a coati (*Nasua narica)* and a mazama deer (*Mazama americana*) (Hoffmann, 1962). In Yucatán, the sole *Ixode* specimen known to date was collected from cattle (*Bos taurus*) and was sent to the National Acari Collection from Universidad Nacional Autónoma de México as an archival voucher (Guzmán–Cornejo *et al.*, 2010). There was no morphological description of the specimen and the exact geographical location of the specimen when collected is unknown. In the United States (US), *I. affinis* has been reported as vector of pathogens, such as the bacterium *Borrelia burgdorferi* (Harrison *et al.*, 2010), that causes Lyme disease in many species of animals. In Mexico, this bacterium has been identified in white-tailed deer (*Odocoileus virginianus*), dogs (Salinas–Melendez *et al*., 1999) and humans (Gordillo *et al.,* 2003, 2007). However, there have been no studies on the role of *I. affinis* in the ecology of Lyme disease in Mexico.

Immature stages of *I. affinis* parasitize small mammals, reptiles and birds. The adults feed on large mammals such as ungulates (Mannelli *et al.*, 2011). Dogs can be parasitized by this species when they are in contact with forested and humid areas that contain tick populations (Harrison *et al.*, 2010). In Yucatán, dogs from rural localities have access to humid forested areas when they are taken out to hunt game (Segovia–Castillo *et al.,* 2010), which could increase the risk of their infestation by *Ixodes* ticks. Given the poor state of knowledge on *I. affinis* in the southeast of Mexico and its potential as a vector for zoonotic diseases, it is important to study its prevalence, abundance and other factors associated with infestation in rural localities of Yucatán. Consequently, the objective of the present study was to evaluate the prevalence of *I. affinis* in dogs from two environments in the Yucatán peninsula. This study provides the first evidence in Mexico of dog infestation by *I. affinis*.

Materials and methods

Study area

The study was carried out in the localities of Opichén and Tixméhuac, in the state of Yucatán, Mexico (Fig. 1). Tixméhuac is 251.6 km^2 in area, with a climate that is hot and sub-humid with summer rains. The mean annual temperature is 26 ºC, and the area receives 1,050 mm of precipitation annually. The predominant winds come from the southeast and northeast. The surrounding areas are composed of medium sub-deciduous forest (MSDF) inhabited by white-tailed deer, jaguars (*Panthera onca*), jaguarundis (*Herpailurus yagouaroundi*), coatis (*Nasua narica*), squirrels (*Sciurus yucatanensis*), gray foxes (*Urocyon cinereoargenteus*), rabbits (*Sivilagus floridanus*), raccoons (*Procyon lotor*), mazama deer (*Mazama americana*) and small rodents such as cotton mice (*Sigmodon hispidus*) and deer mice (*Peromyscus yucatanicus*). The primary economic activities of the Tixméhuac's 4,746 residents (INEGI, 2010) are agriculture, hunting, and construction (rural migrant workers).

Opichén is 268.2 km^2 in area, with a climate that is hot and sub-humid with summer rains. The mean annual temperature is 28 ºC. The predominant winds come from the southeast, and the area receives 1,100 mm of precipitation annually. In Opichén the surrounded areas of medium sub-deciduous forest was replaced by patches of low deciduous forest, cultivated lands ("milpas"), and grasslands (PLDFCG). Species that inhabit these forest patches include rabbits (*Sylvilagus* sp.), white-tailed deer (*Odocoileus virginianus*), opossums (*Didelphis virginiana)*, pigeons (*Zenaida asiatica*), and quails (*Dactylortyx thoracicus*). There are 6,285 residents living in Opichén (INEGI, 2010), and the main source of family income is agriculture and hunting as well construction (rural migrant workers), supplemented by such activities as raising backyard animals.

Household and dog selection

In each of the two localities, 50 households that contained at least one dog were selected. The dog owners were asked for their consent to participate in the study; in instances when an owner declined, another household was selected. Sampling was undertaken from August to November 2013. A structured interview was conducted to obtain the following information about each of the dogs included in the study: age (by owner's reference), physical examination, physical activity outside the locality, body condition according to Laflamme (1997), and frequency of acaricide treatments.

Tick collection and identification

All dogs from the chosen households were inspected for ticks of different instars. The inspection consisted of examining each dog for a period of 10 to 15 min to collect all ticks found on the animal. Samples were collected with minimum stress to the dogs and with the owners present, and all regulations for animal handling and sampling in Mexico were followed (NOM-062-ZOO-1999). Ticks were manually removed with the aid of fine-point forceps as close to the dog's skin as possible without compromising the ticks' mouthparts (Gammon and Salam, 2002). All ticks were placed in 50 mL vials containing a 70% ethanol solution. Specimens were taken to the Parasitology Laboratory at the Campus of Ciencias Biológicas y Agropecuarias of Universidad Autónoma of Yucatán (CCBA-UADY) for taxonomic classification to the genus level, which was accomplished with the aid of the taxonomic keys described by Keirans and Litwat (1989) and Guerrero (1996), and morphological comparison with available images.

All specimens belonging to the genus *Ixodes* were sent to the United States National Tick Collection (USNTC) of Georgia Southern University for species determination. Ticks were examined with a stereomicroscope (Olympus SZX16, Olympus Corporation). Identified specimens were deposited at USNTC (The James H. Oliver, Jr. Institute of Coastal Plain Sciences, Georgia Southern University, Statesboro, GA) with accession numbers of USNMENT 00860890-00860896, and at the Parasitology Laboratory, CCBA-UADY.

Figure 1. Map of the state of Yucatán and the sites where ticks were collected. A) Map of Mexico showing the location of the state of Yucatán. B) Location of Opichén and Tixméhuac, the two localities where ticks were collected.

Data analysis

The prevalence, abundance, and intensity of *I. affinis* infestation on dogs were calculated in both localities. The prevalence and abundance were calculated as follows:

Prevalence:
(Number of dogs infested with *I. affinis*/Total number of studied dogs) x 100

Abundance:
Total number of *I. affinis* collected/Number of dogs with *I. affinis* ticks

To identify the associated factors, infested dogs from both localities were considered. The variables different environments (Tixméhuac with MSDF, Opichén with PLDFCG), age (≤ 1 year, > 1 year), physical activity (hunting dogs with access to forested areas, household dogs with no access to forested areas), body condition (good, regular, poor), and acaricide treatment (no treatment, every 1–3 months, > 3 months) were analyzed by the X^2 univariate test. Variables for which $P \leq 0.20$ were analyzed afterward with a logistical binomial multivariate test with fixed effects, using SPSS 15 software (SPSS, 2006). The odds ratio (OR), 95% confidence intervals (CI) and probability values (P) were calculated. Values with $P < 0.05$ were considered to be statistically significant.

Results and discussion

A total of 144 dogs (88 in Opichén and 56 in Tixméhuac) were sampled, and 846 ticks (672 in Opichén and 174 in Tixméhuac) were collected. Of those, 27 were subsequently identified as *Amblyomma* spp. (*A. maculatum*, *A. mixtum*, *A. ovale* and *A. auricularium*), 786 were identified as *Rhipicephalus sanguineus*, and 33 were identified as *Ixodes* ssp. The *Ixodes* specimens were compared to other *Ixodes* species (i.e., *I. scapularis*, *I. aragaoi*), and all *Ixodes* specimens were classified as *I. affinis*. All key morphological features (pattern of punctations, dentition and shape of hypostome, and size of coxal spurs) used in the identification of *I. affinis* throughout its geographic distribution were present in the Yucatán specimens. However, the number of large punctations along both the posterior margin of the female's scutum and in the center of the male's conscutum was lower than in specimens of this species from other parts of its range (Fig. 2).

Considering both environments, the total infestation prevalence of dogs with *I. affinis* was 11.11% (16/144) with an abundance of 2.1 (1–4) ticks/dog. Information related to dog infestation (prevalence and abundance) with *I. affinis* has not been published elsewhere; however, in Panama, Bermudez and Miranda (2011) found that *I. affinis* often co-existed with *R. sanguineus*, *Amblyomma cajennense*, *A. oblongoguttatum*, *A. ovale*, and *Haemaphysalis juxtakochi* on dogs, horses and cattle. This co-existence occurred in rural populations, indigenous towns and in suburban areas near forests. Immature stages of these species parasitize mostly small mammals and birds, whereas adults parasitize medium to large-sized mammals, including dogs (Guglielmone *et al.*, 2004), which have been described as accidental hosts (Mannelli *et al.*, 2011). In the US (Georgia and South Carolina), the abundance of *I. affinis* has been shown to be associated with the distributions

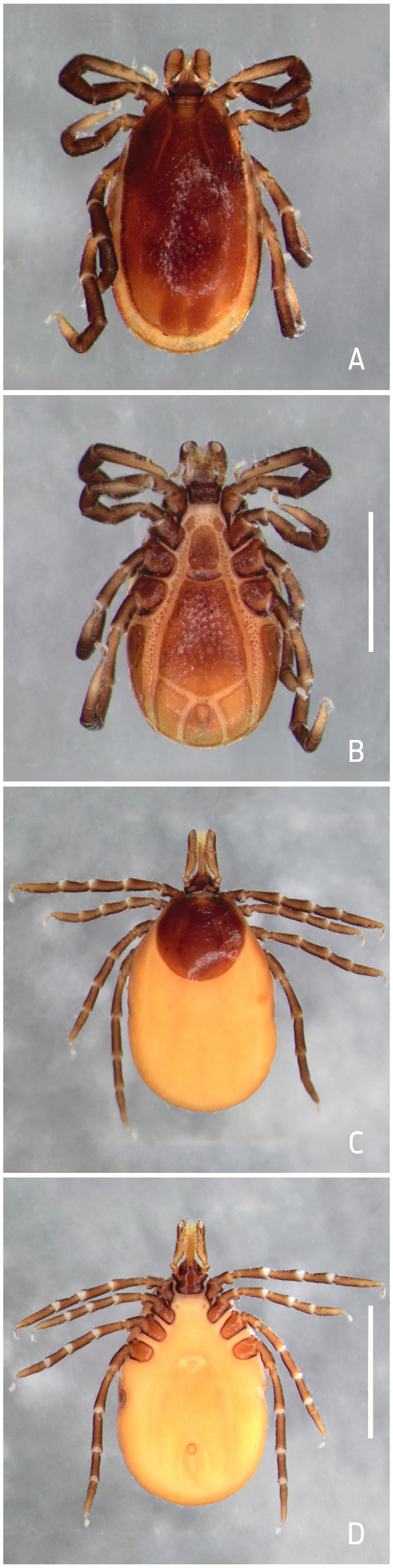

Figure 2. *Ixodes affinis* from Opichén, Yucatán, Mexico. A and B - male, A - dorsally, B - ventrally, bar = 1 mm; C and D - female, C - dorsally, D – ventrally; bar = 2 mm.

of three rodent species: cotton mouse (*Peromyscus gossypinus*), hispid cotton rat (*Sigmodon hispidus*), and eastern wood rat (*Neotoma floridana*) (Oliver *et al.*, 2003). These rodents serve as the primary hosts for the immature stages of *I. affinis* (Clark *et al.*, 1998). *I. affinis* occurs in Central and South America and is found less often in North America. Adults have been frequently reported feeding on white-tailed deer (*Odocoileus virginianus*), bobcat (*Lynx rufus*), raccoon (*Procyon lotor*), and domestic dogs (*Canis familiaris*) (Oliver *et al.*, 1987).

In this study, only 4.8% (7/144) of the dogs received acaricide treatment, which may explain the high prevalence of infestation with adult *I. affinis*, without a significant impact of acaricide treatment on the prevalence of infection. The two variables from the X^2 univariate analysis that presented values of $P < 0.2$ were different environments and physical activity. Table 1 shows the results of the logistic regression on these two variables. Dogs in Tixméhuac were 3.7 times more likely to become infested with *I. affinis* than were dogs in Opichén (Table 1), which may be due to the medium sub-deciduous forest surrounding Tixméhuac sustaining a larger population reservoir of *I. affinis* (Bermudez and Miranda, 2011). Lindström and Jaenson (2003), in a study of tick abundance in Sweden, found that the abundance of *I. ricinus* nymphs was significantly higher in forested areas than in open fields, supporting the view that differing vegetation types influences tick abundance. Furthermore, a study on *I. scapularis* in Wisconsin, USA, showed that the presence and abundance of this tick varied among different habitats: tick presence correlated positively with deciduous forests and negatively with grasslands (Guerra *et al.*, 2002). The lower probability of finding *I. affinis* ticks in Opichén might be explained by the existence of a transition ecotone caused by the conversion of forest to agriculture, thereby diminishing the risk of exposure to the tick (Segovia–Castillo *et al.*, 2010). Vanwambeke *et al.* (2010) observed that the incidence of tick-borne disease was lower not only where there were relatively large areas of unfavorable land cover, such as arable land, but also where forests were surrounded by more agricultural land.

Hunting dogs were 7.56 times more likely to become infested by *I. affinis* than household dogs (Table 1). A similar result was reported by Bermudez and Miranda (2011), who found that hunting dogs were more likely to become infested with three-host ticks, including *I. affinis*, than household dogs. The hunting dogs usually carry infected ticks from the forest to the human environment, where the female ticks lay eggs and the larvae preferentially feed on small mammals and rodents (Shimada *et al.*, 2003; Bhide *et al.*, 2004). The presence of rodents in the peridomestic area facilitates feeding of larvae and nymphs, and consequently helps establish the tick population (Bhide *et al.*, 2004). These ixodid nymphs have a wide range of hosts, including dogs and humans.

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Table 1. Results of the logistical regression to identify factors associated with infestation by *Ixodes affinis* in dogs from two environments in Yucatán, Mexico.

Variable	Total	Positives	Prevalence of infestation (%)	OR	95% CI	P
Environments						
PLDFCG (Opichén)	88	5	5.6	1		
MSDF (Tixméhuac)	56	11	19.6	3.70	1.24 - 11.08	0.01
Physical activity						
Household dogs	58	2	3.4	1		
Hunting dogs	86	15	17.4	7.56	1.63 - 35.04	0.001

OR: Odds ratio; CI: Confidence interval; P: Probability value; PLDFCG: patches of low deciduous forest, cultivated lands, and grasslands; MSDF: medium sub-deciduous forest.

These nymphs metamorphosize into adults, which are the primary source of infection for dogs (Smith *et al.*, 1993). The hunting dogs thus likely play a key role in tick dispersion and subsequent pathogen transmission, and consequently may represent a potential health risk to these localities.

Taken together, these observations suggest that hunting dogs may play an important role in the spread of ticks in rural localities of the state of Yucatán as a result of their exposure to ticks in adjacent forested areas. Thus, both dogs and humans might be accidental hosts of pathogens. *Ixodes affinis* usually do not bite people (Rudenko *et al.*, 2012), but Allan (2001) observed this species feeding on a human. Additional studies are therefore needed to confirm the role of humans as incidental hosts of *I. affinis*.

The importance of *I. affinis* in Yucatán, Mexico, is related to the role this tick species may have in the maintenance of certain pathogens, such as *B. burgdorferi*. In particular, this could be of great importance in regard to the reservoir hosts on which *I. affinis* feeds, acting as bridge vectors that under certain environmental/ecological conditions could parasitize humans (Oliver, 1996). Although *I. affinis* has been previously reported in Mexico, this study is the first record of this tick species parasitizing dogs in rural localities.

Conclusion

This is the first report in Mexico of the prevalence and abundance of infestation of domestic dogs by *I. affinis*. This is likely a result of the access that these dogs have to adjacent forested areas. The role of hunting dogs as possible spreaders of *I. affinis* and reservoirs of tick-borne diseases transmissible to humans in rural areas of Mexico should be considered and deserves further investigation.

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Conflicts of interest

The authors declare that they have no competing interests.

Author contributions

Analilia Solís Hernández, Roger Iván Rodríguez Vivas, and María Dolores Esteve Gassent: Conducted the study, and critically reviewed and approved the manuscript for publication.

Mario Antonio Pérez Barrera: Reviewed the statistical analysis and drafted the manuscript for publication.

Dmitry A. Apanaskevich: Performed the taxonomic classification for tick species determination, and critically reviewed and approved the manuscript for publication.

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