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Tick-associated diseases identified from hunting dogs during the COVID-19 pandemic in a Mayan community in Yucatan, Mexico

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Abstract

Background: Hunting activity in the Mayan communities has increased due to COVID-19 and domestic dogs have gained more importance. Due to their proximity to humans, domestic dogs are a bridge between tick-borne diseases (TBDs) and humans and their peri-domestic environment. In Mexico, and especially in rural regions, there were not adequate records of TBDs during the SARS-CoV-2 pandemic.

Aim: Identify TBD of ticks collected during the COVID-19 pandemic in a rural community.

Methods: Tick capture was carried out in March 2021, in Teabo, Yucatan. Ticks were removed using from domestic dogs and placed in ethanol. Collected ticks were morphologically identified and underwent DNA extraction and a partial segment of the mitochondrial 16S-rDNA gene was amplified to corroborate the tick species. The DNA was screened for the presence of *Anaplasma* spp., *Borrelia* spp., *Ehrlichia* spp., and *Rickettsia* spp. Purified amplification products were submitted for sequencing and the results were compared to those deposited in GenBank using BLAST.

Results: We collected 33 ectoparasites, *Ixodes affinis*, *Rhipicephalus sanguineus*, *Rhipicephalus microplus*, and *Amblyomma mixtum* on 11 hunting dogs. The most frequent ectoparasite was *R. sanguineus* (66%). We detected the presence of DNA of *Rickettsia endosymbiont* in *I. affinis* and *Anaplasma platys* in *R. sanguineus*. *Rickettsia endosymbiont* presented a similarity of 100% with the partial sequence of *R. endosymbiont* of *I. affinis* isolate IACACTM001 16S ribosomal RNA gene and the sequence of *A. platys* had a similarity of 100% with the partial sequence of the isolate 23-33TX 16S ribosomal RNA gene of *A. platys* from dogs from Texas, USA and with the partial sequence of the isolate L134 16S ribosomal RNA gene of *Ehrlichia canis* from dogs from Piura, Peru.

Conclusion: We confirmed for the first time the presence of *A. platys* in *R. sanguineus* and *R. endosymbiont* in *I. affinis* ticks from dogs in the state of Yucatan.

Keywords: Dogs, Hunting, Rural communities, Tick-borne diseases.

Introduction

Hunting in Mayan communities is an activity related to subsistence, natural resource management, and uses and customs. In these communities, hunting activity represents a protein contribution in their diet of at least 30%–50% (Montiel-Ortega and Arias-Reyes, 2008). Due to the conditions generated by the health emergency caused by SARS-CoV-2 virus, the inhabitants increased hunting activity in order to survive. In this context, domestic dogs play an important role as companion animals during this activity (Conexión Cinvestav, 2019).

Domestic dogs are sentinels and synanthropic animals and a bridge between tick-borne diseases (TBDs) and different hosts. Due to their proximity to humans, it is considered that dogs can be a risk factor for humans and their peri-domestic environment to be parasitized by ticks and potentially acquire the diseases they transmit (Solís-Hernández *et al.*, 2018).

Ticks are cosmopolitan ectoparasites and can occur in a wide variety of ecological niches. In Mexico, around 100 species of ticks have been described (Rodríguez-Vivas *et al.*, 2016). TBDs associated with

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causative agents such as *Rickettsia* spp., *Ehrlichia* spp., *Anaplasma* spp., *Borrelia* spp., *Babesia* spp., among others, represent a risk for humans and domestic and wildlife animals (Maggi and Krämer, 2019).

Worldwide during COVID-19 pandemic, animals came out to occupy the streets, looking for food or to enjoy the low pollution and little human activity (BBC News Mundo, 2020). Records of animals in urban areas located in Southeast Mexico include: deers, cows, foxes, pheasants, macaws, and toucans (Cerillo, 2020). Animals increased their nocturnal activity by an average factor of 1.36% and 92% of the world's forests were regenerated (FAO, 2020). The global mean temperature for 2020 (January to October) was $1.2^{\circ}\text{C} \pm 0.1^{\circ}\text{C}$ above the 1,850–1,900 baseline and is likely to be one of the three warmest years on record globally (World Meteorological Organization, 2021). Therefore, the geographical landscape and climate represent infection risk factors for humans due to the prevalence of ticks.

The health emergency caused by SARS-CoV-2 caused an adaptation and prioritization of health services, neglecting other diseases, in addition to this, people in Mexico were insecure to attend health services, favored underdiagnosis of TBD. In Mexico, and especially in rural regions, there are no adequate records of the epidemiological behavior of TBD during the COVID-19 pandemic (Dzul-Rosado *et al.*, 2022).

The objective of this work was to identify TBD of ticks collected during the COVID-19 pandemic in a rural community. For this purpose, we selected the municipality of Teabo (20.4001°N , 89.2830°W) because there have been human cases of rickettsiosis and an increase in hunting activity with a closer approach of humans and hunting dogs to jungle areas.

Materials and Methods

Study site

Tick capture was carried out in the municipality of Teabo, Yucatan (Fig. 1A); it is located south of the city of Merida, and it means “your breath” in Maya. This municipality presents a high degree of marginalization (Secretaría de Bienestar, 2022). It consists mainly of conserved areas (medium forest, sub-deciduous), rural areas, and areas dedicated to agricultural activity (nomadic agriculture) (Secretaría de Fomento Económico y Trabajo, 2018).

Sampling

We selected houses belonged to families whose hunting was a priority activity for their economy during the COVID-19 pandemic. The sampling was a non-probabilistic type and was carried out on 15 and May 20, 2021 (dry season). This community has had previous intervention experiences on TBD which facilitated the acceptance of the activity. The participants were trained to remove ticks using tweezers from domestic dogs (*Canis lupus familiaris*), minimizing pain or

discomfort, immediately when they return from the hunt (Fig. 1D). Due to people of the community being Mayan speakers, researchers had the support of a bilingual community promoter (Spanish-Mayan). Ticks collected were placed in plastic tubes with 70% ethanol for their preservation. For the transportation of the samples, the plastic tubes were security closed and properly labeled and placed in a leak-proof secondary container and finally in a rigid outer container. The sampling, transportation, and management of the specimens were approved by the UADT's biosafety committee (Protocol Number: CIR-2020-0010).

Taxonomic identification

Ticks collected were morphologically identified following the taxonomic keys of (Keirans and Litwak, 1989; Delabra *et al.*, 1996; Guzmán-Cornejo and Robbins, 2010; Guzmán-Cornejo *et al.*, 2011) with a stereomicroscope Olympus SZX7 (Fig. 1C). 20 specimens were deposited at Colección de Artrópodos de Importancia Médica from the Laboratorio de Entomología Médica of the Instituto de Diagnóstico y Referencia Epidemiológicos (InDRE, Secretaría de Salud).

Molecular analysis

To confirm the morphological identification of the ticks, they underwent DNA extraction using the DNeasy Blood & Tissue Kit (Qiagen Hilden, Germany); a little incision was made below the fourth leg, and storing the empty body of the tick was in the Arthropod Collection of InDRE for further investigation, according with Lado *et al.* (2021).

Then, a partial segment of approximately 400 bp of the mitochondrial 16S-rDNA gene was amplified using primers and conditions reported elsewhere (Norris *et al.*, 1996) Thereafter, the extracted DNA was screened for the presence of bacterial pathogens (*Anaplasma* spp., *Borrelia* spp., *Ehrlichia* spp., and *Rickettsia* spp.). Conventional PCR was performed for the amplification of a fragment of 495 bp of the ribosomal 16S rDNA (16S) for *Anaplasma* spp., *Ehrlichia* spp., and *Rickettsia* spp. detection; a 300 bp of the flagellin gene (*flaB*) for *Borrelia* spp.; and fragments of 450, 532, and 800 bp of the 17 kDa and the outer membrane protein B for the identification of *Rickettsia* spp., using primers and thermal conditions reported (Sánchez-Montes *et al.*, 2021).

Purified amplification products were submitted for sequencing at the Pathogen Genome Laboratory of the Instituto de Diagnóstico y Referencia Epidemiológicos. The resulting sequences were compared to those deposited in GenBank using the BLAST tool (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). Bacterial nucleotide sequences of the valid species were retrieved and aligned using the Clustal W paired alignment in Mega 11.0. software packages.

Ethical approval

The study protocol was approved by the UADY's ethics committee (Protocol Number: CIR-2020-0010).



Fig. 1. (A) Satellite images show the municipality of Teabo, the red diamond shapes show the locations of the neighborhoods that were sampled (created by the authors using QGIS 3.28.2 with satellite images from Google maps). (B) White-tailed deer hunted by the inhabitant of Teabo. (C) Stereomicroscope view of the *I. affinis* tick collected from dogs of the community. (D) Collection of the ticks from domestic dogs.

Results and Discussion

Due to the risk of contagion during the COVID-19 pandemic, most indigenous communities decided to close their borders to prevent the spread of the virus (Cohen and Mata-Sánchez, 2021). It was during this period, in which Mexico was in lockdown when we carried out the sampling of the present study. To cope with the decrease in income and food shortages caused by the isolation, the inhabitants of the rural indigenous communities turned to the consumption of traditional foods, favoring the increase in activities such as agriculture (e.g. *solar*, *milpa*) and

hunting (Briceño-Méndez *et al.*, 2021; Cohen and Mata-Sánchez, 2021).

Mayan peasant-hunters who own dogs, in addition to hunting wild animals, are engaged in other complementary practices such as agriculture (*milpa*), the creation of kitchen gardens (*solares*), and the raising of backyard animals (Plata and Montiel, 2020). The animals that they mainly hunt are Great Curassow (*Crax rubra*), Ocellated Turkey (*Meleagris ocellata*), paca (*Cuniculus paca*), white-tailed deer (*Odocoileus virginianus*) (Fig. 1B) and the collared peccary (*Pecari tajacu*) (Santos-Fita *et al.*, 2012). Hunters have always

used domestic dogs as hunting animals to support themselves in this activity; Domestic dogs are mainly indoor animals and go out freely touring rural areas that are dedicated to agriculture, and conserved areas (Plata and Montiel, 2020), this behavior represents a risk factor for animals tick infestation (Ojeda-Chi et al., 2019).

We recovered 33 ectoparasites belonging to 4 species, *Ixodes affinis* [7 (6F, 1M)], *Rhipicephalus sanguineus* [22 (12F, 6M, 4N)], *Rhipicephalus (Boophilus) microplus* (1), *Amblyomma mixtum* (3F), on 11 hunting dogs of Teabo, Yucatan, Mexico (Table 1). The infestation interval ranged between 1 and 8 specimens. Tick species found in the present study have been previously identified in Yucatan, Mexico (Solís Hernández et al., 2015; Rodríguez-Vivas et al., 2016; Martínez-Ortiz et al., 2019).

The most frequently recovered ectoparasite species from dogs was *R. sanguineus* found in 66% of the dogs. In similar studies, in dogs from rural areas of Yucatan, the most frequently identified species were

also *R. sanguineus* (Ojeda-Chi et al., 2019). The tick *R. sanguineus* has been described as a vector for different species of *Rickettsia* spp. (Sánchez-Montes et al., 2021). In Dzul-Rosado et al. (2017), *R. sanguineus* tick obtained from domestic dogs in rural areas were found to be positive for *Rickettsia typhi*, the causative agent of murine typhus, suggesting that the tick and the dog could be actively participating in the transmission of murine typhus. In another study, carried out in marginalized areas of Yucatan, the presence of *Rickettsia rickettsii* was identified in *R. sanguineus* obtained from dogs in the area (Peniche-Lara et al., 2018).

In the present study, we detected the presence *Anaplasma platys* DNA in *R. sanguineus* (Table 1). The sequence of *A. platys* had a similarity of 100% with the partial sequence of the isolate 23-33TX 16S ribosomal RNA gene of *A. platys* from dogs from Texas, USA (GenBank Accession number MH620179.1) and with the partial sequence of the isolate L134 16S ribosomal RNA gene of *Ehrlichia*

Table 1. Ecological parameters of ectoparasites selected for bacterial detection in dogs at Teabo, Yucatan, Mexico.

Host	Activity	Ectoparasite species	Development stage			PCR	Sequence
			F	M	N		
1	Hunting	<i>I. affinis</i>	X			+	<i>R. endosymbiont</i>
		<i>I. affinis</i>	X			+	
2	Hunting	<i>R. sanguineus</i>	X				<i>R. endosymbiont</i>
		<i>R. sanguineus</i>		X			
3	Agriculture	<i>R. sanguineus</i>	X			+	<i>A. platys</i>
		<i>R. sanguineus</i>			X		
		<i>R. sanguineus</i>			X		
		<i>R. sanguineus</i>			X		
		<i>R. sanguineus</i>	X				
4	Agriculture	<i>R. sanguineus</i>	X				
		<i>R. sanguineus</i>	X				
		<i>R. sanguineus</i>	X				
		<i>R. sanguineus</i>	X				
		<i>R. sanguineus</i>		X			
		<i>R. sanguineus</i>		X			
		<i>R. sanguineus</i>			X		
5	Agriculture	<i>I. affinis</i>	X			+	<i>R. endosymbiont</i>
		<i>Rhipicephalus (Boophilus) microplus</i>					
6	Hunting	<i>Amblyomma mixtum</i>	X				Insufficient
		<i>I. affinis</i>	X			+	<i>R. endosymbiont</i>
6	Hunting	<i>I. affinis</i>	X			+	<i>R. endosymbiont</i>
		<i>I. affinis</i>		X			

Continued

Host	Activity	Ectoparasite species	Development stage			PCR	Sequence
			F	M	N		
7	Agriculture	<i>R. sanguineus</i>	X				insufficient
		<i>R. sanguineus</i>		X			
		<i>R. sanguineus</i>		X			
8	Hunting	<i>Amblyomma mixtum</i>	X				
9	Agriculture	<i>R. sanguineus</i>	X				Insufficient
		<i>R. sanguineus</i>	X				
		<i>R. sanguineus</i>		X			
		<i>R. sanguineus</i>		X			
		<i>R. sanguineus</i>	X				
10	Agriculture	<i>R. sanguineus</i>	X				Insufficient
		<i>R. sanguineus</i>	X				
11	Agriculture	<i>I. affinis</i>	X				
		<i>Amblyomma</i>	X				

F = Female, M = Male, N = Nymphs.

canis from dogs from Piura, Peru (GenBank Accession number MF153971.1). The bacteria *A. platys* is the causative agent of canine infectious cyclic thrombocytopenia and was first reported in Mexico in *R. sanguineus* s.l. ticks obtained from dogs from the states of Coahuila and Durango (Almazán *et al.*, 2016). The organisms *A. platys* and *E. canis* are frequently found in the same geographic region and usually infest the same host (Soares *et al.*, 2017). In Yucatan, the prevalence of *E. canis* infection in dogs has been reported formerly (Rodríguez-Vivas *et al.*, 2005), but not for *A. platys*, so in this study, we confirmed, for the first time, the presence of *A. platys* in *R. sanguineus* of dogs in the state of Yucatan.

We also detected the presence of DNA of *Rickettsia endosymbiont* in *I. affinis* (Table 1). The *I. affinis* tick has been previously reported in the state of Yucatán in dogs from rural communities (Solís Hernández *et al.*, 2015). This species has been associated with the transmission cycle of *Borrelia burgdorferi* (the causative agent of Lyme disease) in dogs, which highlights the high risk of transmission of *B. burgdorferi* from animals to humans (Solís-Hernández *et al.*, 2018). In addition, in the study by Solís Hernández *et al.* (2015) it was observed that hunting dogs were more likely to be infested with *I. affinis* compared to dogs that remained inside the houses. As mentioned above, this increases the likelihood that hunting dogs are a risk factor for the transmission of TBDs.

The sequences of *R. endosymbiont* obtain in this study presented a similarity of 100% with the partial sequence of *R. endosymbiont* of *I. affinis* isolate IACACTM001

16S ribosomal RNA gene (GenBank Accession number MT535907.1). The *Rickettsia endosymbiont* of *Ixodes scapularis* (REIS), is a symbiont of the deer tick vector of Lyme disease in North America. Robust phylogeny estimation suggests REIS is ancestral to the virulent spotted fever group of rickettsia (Gillespie *et al.*, 2012). Sánchez-Montes *et al.* (2021), also found *Rickettsia* DNA in *I. affinis* positive for *Rickettsia monacensis* and *R. endosymbiont* of *Ixodes pacificus* from the USA. However, the aspects of pathogenesis, transmission life cycles, and vector competence were not investigated in the study. The results of the present study exhibit for the first time the presence of *R. endosymbiont* in *I. affinis* ticks from dogs in Yucatan, However, it is important to elucidate the relationship between pathogen and host to know how people could be at risk of exposure.

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Conflict of interest

The authors declare no conflict of interest.

Author contributions

KRDR: Conceptualization, project administration, writing, investigation. KAAS: Conceptualization, writing, investigation. AJTM: data curation, investigation, methodology. JJAL: visualization, investigation. GFP: visualization, investigation. FIPM: ormal analysis, investigation. MGLF: visualization,

investigation, methodology. JCML: visualization, investigation, methodology. BSS: Conceptualization, visualization, writing, investigation, methodology.

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