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Ticks infesting dogs in rural communities of Yucatan, Mexico and molecular diagnosis of rickettsial infection

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Abstract

Rickettsial infection in dog-associated ticks in three rural communities of Yucatan, Mexico was investigated using qPCR and nested PCR assays. A total of 319 dogs were studied and ticks samples were collected. A total of 170 dogs were infested with ticks (frequency of 53.4%). Overall, 1,380 ticks representing seven species were collected: *Amblyomma mixtum*, *A. ovale*, *A. parvum*, *A. cf. oblongoguttatum*, *Ixodes affinis*, *Rhipicephalus microplus*, and *R. sanguineus* sensu lato. The most abundant species was *R. sanguineus* s.l. with a mean intensity of 7.4 ticks/host. Dogs in the communities of Chan San Antonio and Yaxcheku were 2.84 and 2.41 times more likely to be infected with *R. sanguineus* compared with Sucopo ($p < 0.05$). Adult pools of *A. mixtum*, *A. parvum*, *I. affinis*, *R. microplus*, and *A. c.f. oblongoguttatum* were negative to *E. chaffeensis*, *E. ewingii*, *A. phagocytophilum*, and *R. rickettsii*. However, pools of *R. sanguineus* s.l. adults and *A. ovale* adults, as well as nymphs of *Amblyomma* spp. were positive to *E. canis*. Sequencing analysis of the nested PCR products amplifying the *16S rRNA* gene fragment of *E. canis* confirmed the results and revealed 100% identity with sequences of *E. canis*. This is the first report worldwide of *E. canis* infection in *A. ovale* by PCR. This finding does not necessarily indicate that *A. ovale* is a competent vector of *E. canis* because pathogen transmission of this specific tick to a naïve dog remains to be documented. This study documented that different tick species parasitize dogs in Yucatan, Mexico, where *R. sanguineus* s.l., *A. ovale*, and nymphs of *Amblyomma* spp. were shown to be infected with *E. canis*. These findings highlight the need for control strategies against tick infestations in dogs to prevent the risk of tick-borne disease transmission among companion animal and probably human populations.

KEYWORDS
dogs, Mexico, PCR, qPCR, tick-borne pathogens, ticks

1 | INTRODUCTION

Ticks represent a major threat to production, domestic, and wild animals worldwide due to their obligate blood feeding habit, irritation, pruritus, skin inflammation, self-wounding, stress, allergic responses, and pathogen transmission (Wall & Shearer, 2001). A range of viruses, protozoa, and bacteria causing tick-borne diseases (TBDs) induces economic losses in livestock production, and health of companion animals (Rodríguez-Vivas et al., 2016).

The increasing incidence of TBDs among companion animals and humans has been attributed to several factors, including climate change, habitat disruption, urbanization, socioeconomic situation,

changes in human behaviour, and increased number of wildlife hosts, which may together favour the spreading and establishment of selected tick species into some areas (Dantas-Torres, 2015).

In Mexico, ~100 tick species belonging to the Ixodidae and Argasidae families (Pérez, Guzmán-Cornejo, Montiel-Parra, Paredes-León, & Rivas, 2014) are estimated to infest animals and human and several species are capable of transmitting zoonotic pathogens (Solís-Hernández, Rodríguez-Vivas, Pérez-Barrera, Esteve-Gassent, & Apanaskevich, 2015). Many tick species are known or potential vectors of infectious agents that are pathogenic to human and dogs. In Yucatan, Mexico, the most common tick species infesting dogs reported to date are *Rhipicephalus sanguineus* sensu lato, *Amblyomma mixtum*, *A. sabanerae*, *A. parvum*, *A. ovale*, *A. auricularium*, *A. maculatum*, *Ixodes near affinis*, and *Dermacentor nitens* (Rodríguez-Vivas et al., 2016; Solís-Hernández et al., 2015).

Important endemic tick-borne pathogens infecting dogs in Yucatan, Mexico include *Babesia canis* (Rodríguez, Domínguez, & Cob, 2000), *Ehrlichia canis* (Pat-Nah, Rodriguez-Vivas, Bolio-Gonzalez, Villegas-Perez, & Reyes–Novelo, 2015; Rodriguez-Vivas, Albornoz-Rivero, & Bolio-González, 2005), *Borrelia burgdorferi s.l.* (Solís-Hernández, Rodríguez-Vivas, Esteve-Gassent, & Villegas-Pérez, 2018), *Rickettsia akari* (Zavala-Castro, Zavala-Velázquez, del Rosario García, León, & Dzul-Rosado, 2009), and *R. typhi* (Martinez–Ortiz et al., 2016). While most of the studies carried out on ticks infesting dogs in Yucatan have focused on *R. sanguineus* s.l. from urban areas (Rodríguez et al., 2000), there is limited reliable information on the diversity of ticks infesting dogs in rural communities and the pathogens they can transmit. To fill this knowledge gap, this study aimed to investigate the pathogens infecting ticks infesting dogs to evaluate the current risk of zoonotic infection in this group of companion animals living in rural communities of Yucatan, Mexico.

2 | MATERIALS AND METHODS

2.1 | Study area and sampling

The study was carried out in three rural communities of Yucatan state in Mexico. The studied rural communities were Chan San Antonio (21°21′66″N and 88°25′00), Sucopo (21°9′0″N and 88°4′0″O), and Yaxcheku (21°12′26″ N y 87°55′59″O) (Instituto Nacional de Estadística, Geografía e Informática, Censo General de Población y Vivienda, INEGI, 2010). These rural communities are located in the municipality of Tizimin, which is in region of Yucatan with a climate that is hot and subhumid with summer rains. The mean annual temperature is 25.4°C, and the area receives 1,167 mm of precipitation annually. In Chan San Antonio, there are 436 residents living in 98 houses. Seventy-five per cent of the households in this community have concrete floor. In Sucopo, there are 1,403 residents living in 309 houses (INEGI, 2010). Eighty-nine per cent of the households in this community have concrete floor. In Yaxcheku, there are 274 residents living in 63 houses (Instituto Nacional de Estadística, Geografía e Informática, Censo General de Población y Vivienda (INEGI), 2010). Eighty per cent of the households in this community have concrete floor (Instituto Nacional de Estadística, Geografía e Informática, Censo General de Población y Vivienda (INEGI), 2010).

In Chan San Antonio, Sucopo, and Yaxcheku, 69, 96, and 49 homes reporting ownership of at least one dog, respectively, were selected for convenience to enroll in this study. Each community was divided into four quadrants taken as reference the main streets crossing the towns south to north and from east to west. In each quadrant, 12–24 homes were selected for dog sampling. Each of the selected households was visited from June 2016 to April 2017. A total of 319 dogs were inspected and ticks samples were collected when infestation was detected.

2.2 | Tick collection and identification

All dogs from the selected households were inspected for ticks in the larval, nymphal, and adult stages. The inspection consisted of examining each dog for a period of 10–15 min to collect tick samples on the animal. Samples were collected with minimum stress to the dogs following current regulations for animal handling and sample collection in Mexico (NOM–062–ZOO–1999) and with the owners present. 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 & Salam, 2002). All ticks were transferred to 50 ml vials with ethanol 70%. 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 species level, accomplished with the aid of the taxonomic keys (Guzmán-Cornejo, Robbins, Guglielmone, Montiel-Parra, & Pérez, 2011), and morphological comparison with available images. Identified specimens were deposited at the Parasitology Laboratory, CCBA-UADY. To follow the taxonomic criteria proposed by Nava et al. (2014) for designed as *Amblyomma mixtum* within the *Amblyomma cajennense* species group, and the proposal of Lopes et al. (2016) to denominated *Amblyomma* cf. *oblongoguttatum*, as a tentative different species to *A. oblongoguttatum* from Brazil.

2.3 | Tick DNA extraction

For molecular diagnosis of rickettsial agents present in the ticks per infested dog, a pool was made with ticks of the same genus (2–8 nymphs) and the same species (1–4 adults), so that a total of 277 pools were made. DNA was extracted from ticks using a commercial kit (DNeasy Blood & Tissue Kit, QIAGEN, USA.). Prior to tick DNA extraction, ticks were transferred to prechilled 1.5-ml tubes. Liquid nitrogen-cooled disposable pellet pestles for 1.5-ml centrifuge tubes were used to grind each tick against the tube walls for 30 s. DNA samples were aliquoted into two groups for separate molecular analysis in two research centres.

2.4 | Polymerase Chain Reaction (PCR) procedures

The samples were initially screened for the Anaplasmataceae taxon by the proprietary TickPath Layerplex (qPCR) at Texas A&M

University (https://tvmdl.tamu.edu/tests/tickpath–layerplex–qpcr/) to amplify a fragment of the *16S rRNA* gene of *E. canis*, *E. chaffeensis*, and *E. ewingii*, a fragment of the *msp2* gene of *A. phagocytophilum*, and a fragment of *Rhhyp* gene of *R. rickettsii* (hypothetical protein A1G_04230).

Positive samples in the TickPath Layerplex were then analysed by nested PCR in the molecular biology laboratory at the Campus of Biological and Agricultural Sciences of the Autonomous University of Yucatan. Fragments of the *16S rRNA* and *ompB* genes were amplified in the nested PCR to detect *Ehrlichia/Anaplasma* (Massung et al., 1998; Murphy, Ewing, Whitworth, Fox, & Kocan, 1998) and *Rickettsia* spp. (Choi et al., 2005) respectively. External primers to distinguish genus, and internal ones to distinguish species were used. Primers, target gene, and conditions of the nested PCR used are mentioned in Table 1. *E. canis*, *E. chaffeensis*, *A. phagocytophilum*, and *R. coronii* plasmids were used as positive controls and nuclease–free water as a negative control.

All studies conducted at Texas A&M University were performed under biosafety level 2 conditions as permitted by the Institutional Biosafety Comittee (IBC–2013–039 and IBC–2016–051). Permits by the US Center for Disease Control and Prevention (CDC permit #2015–05–071) and US Department of Agriculture Animal and Plant Health Inspection Services (USDA APHIS VS permit #128538) allowed the importation of samples from Mexico for testing in the US.

2.5 | Sequencing and phylogenetic analysis

One adult pool of *A. ovale*, one adult pool of *R. sanguineus* s.l., and one nymph pool of *Amblyomma* spp. positives to a fragment of the *16S rRNA* of *E. canis* were sequenced. Products were purified using E.Z.N.A.® gel Extraction Kit (Omega Bio–tek, Inc, Norcross, Georgia, USA) and sequenced in the laboratory DIMYGEN® (Mérida, Yucatán, México; http://www.dimygen.com/). The resulting sequences were compared to sequences of *E. canis* deposited in GenBank® by using BLAST (http://www.ncbi.nlm.nih.gov/blast). Nucleotide sequences were edited and aligned using Mega 7.0 (Kumar, Stecher, & Tamura, 2015) software packages. The Kimura 2-parameter model was selected as the best-fit model using Model Test implemented in Mega 7.0 (Kumar et al., 2015). Maximum likelihood phylogenetic trees were constructed using Mega 7.0 (Kumar et al., 2015) with nodal support being assessed by 10,000 bootstrap. All sequences generated in this study were deposited in GenBank®.

2.6 | Questionnaire and statistical analysis

A questionnaire was submitted to each dog owner to obtain information about general ectoparasite control practices, communities, and season of the year. Infestation frequency was expressed as a percentage calculated with the formula: number of dogs infested with ticks/the number of examined dogs ×100%. Infestation intensity for each tick species was calculated with the following formula: total number of a tick species/number of infested dogs with the same species.

For tick associated factors, dogs infested with *R. sanguineus* s.l. were considered as dependent variable. General ectoparasite control practices (yes, no), communities (Chan San Antonio, Sucopo, and Yaxcheku), and season of the year (dry and rainy) were considered as independent variables. A univariate χ^2 analysis was used as a primary screening of exposure variables using the Statistix software, version 9. All variables with $p < 0.20$ were analysed by a multivariate analysis (logistic-binomial regression model of fixed effects) using the SPSS program version 18.0 for Windows, which provides exact regression estimates, 95% confidence intervals, odds ratio (OR-a measure of association that quantifies the relationship between the exposure variables and outcomes), and *p*-values (regression coefficient). We considered a *p*-value <0.05 as statistically significant.

3 | RESULTS

Three-hundred and nineteen dogs from three rural communities of Yucatan were inspected for tick collection. A total of 172 dogs were parasitized by ticks, which yielded an infestation frequency of 53.9%. The tick species collected from the dogs and their frequency are listed in Table 2. Overall, 1,380 individuals belonging to three genera and seven species were collected: *Amblyomma mixtum*, *A. ovale*, *A. parvum*, *A.* cf. *oblongoguttatum*, suggested by Lopes et al. (2016) to be different from *A. oblongoguttatum* from Brazil, *Ixodes affinis*, *Rhipicephalus microplus*, and *R. sanguineus* s.l. The most common tick species infesting dogs was *R. sanguineus* s.l. with frequencies of 48.4%, 27.1%, and 46.3%, and intensity of 9.8, 7.6, and 4.9 in the communities Chan San Antonio, Sucopo and Yaxcheku respectively (Tables 2 and 3).

Univariate analysis identified community and season ($p < 0.2$) as the variables that needed to be included in the multivariate analysis. Results of the logistic regression analysis are shown in Table 4. Dogs in Chan San Antonio and Yaxcheku were 2.84 and 2.41 (OR value; $p = 0.000$ and $p = 0.004$ respectively) times more likely to be infested with *R. sanguineus* s.l. than in Sucopo.

To determine the rickettsial agents present in the ticks per infested dog, a pool was made with ticks of the same genus (2–8 nymphs), and the same species (1–4 adults). This approach yielded a total of 277 pools. Using qPCR analysis, 6.1% tick pools were positive for *E. canis* (17/277). None of the tick pools were positive for *E. chaffeensis*, *E. ewingii*, *A. phagocytophilum*, or *R. rickettsii*.

The 17 *E. canis* tick pools positive by qPCR were also positive in the nested PCR. Eleven pools of adult *R. sanguineus* s.l. (7.7%), four of *A. ovale* adult (12.9%), and two of *Amblyomma* spp. nymphs (4.4%) were positive for *E. canis*.

Sequencing the nested PCR products of the *16S rRNA* gene fragment of *E. canis* of the three positive pools confirmed the results and showed 100% identity with strain YZ-1 from China (CP025749.1), TWN1 (EU106856.1) from Taiwan and Jake from

TABLE 1 Tick-borne rickettsiae tested, primers, target gene, and conditions of nested PCR used in this study

Primer	Pathogen	Primer sequency	Name of primer (Reference)	PCR condition
External	*Ehrlichia* spp.	5′-agaacgaacgctggcggcaagcc-3′ 5′-cgtattaccgcggctgctggc-3′	ECC ECB Murphy et al. (1998)	Murphy et al. (1998)
Internal	*Ehrlichia canis*	5′-caattatttatagcctctggctataggaa-3′ 5′- tataggtaccgtcattatcttccctat-3′	ECAN5 HE-3 Murphy et al. (1998)	Murphy et al. (1998)
	Ehrlichia chaffeensis	5′-caattgcttataacctttttggttataaat-3′ 5′- tataggtaccgtcattatcttccctat-3′	HE1 HE3 Murphy et al. (1998)	Wen et al. (1997)
	Ehrlichia ewingii	5′-cgaacaattcctaaatagtctctgac-3′ 5′-tataggtaccgtcattatcttccctat-3′	EE52 HE3 Murphy et al. (1998)	Kocan et al. (2000)
External	*Anaplasma* spp.	5′-cacatgcaagtcgaacggattattc-3′ 5′-ttccgttaagaaggatctaatctcc-3′	Ge3a Ge10r Massung et al. (1998)	Massung et al. (1998)
Internal	*Anaplasma phagocytophilum*	5′-aacggattattctttatagcttgct-3′ 5′-ggcagtattaaaagcagctccagg-3′	Ge9f Ge2 Massung et al. (1998)	Massung et al. (1998)
External	*Rickettsia* spp.	5′-gtaaccggaagtaatcgtttcgtaa-3′ 5′-gctttataaccagctaaaccacc-3′	ompB-OF ompB-OR Choi et al. (2005)	Choi et al. (2005)
Internal	*Rickettsia* spp.	5′-gtttaatacgtgctgctaaccaa-3′ 5′- ggtttggcccatataccataag-3′	ompB SFG opmB SFG/TG Choi et al. (2005)	Choi et al. (2005)

USA (CP000107.1), and isolate 1576.2 from Mexico (MG029083). The phylogenetic tree of *E. canis* was inferred based on the *16S rRNA* sequences obtained in this study and compared with *Ehrlichia chaffeensis* strain Arkansas (NR_074500.2); *Cowdria* (*Ehrlichia*) *ruminantium* (X61659.1) and *Anaplasma phagocytophilum* str. JM (CP006617.1) (Figure 1).

Nucleotide sequences obtained in this study for the *E. canis* 16S rRNA gene fragment were deposited in the GenBank database. They can be retrieved using these acccesion numbers: MH374120, MH374121, and MH374122.

4 | DISCUSSION

Data from this study showed that dogs in rural communities of Tizimin municipality from Yucatan, Mexico are hosts of different tick species, which can be infected with zoonotic rickettsiae pathogenic to companion animals and humans. A relatively high frequency of tick infestation was observed in the dogs sampled (53.9%). Similar infestation rates have been reported in Brazil (58.5%, Dantas-Torres, Melo, Figueredo, & Brandão-Filho, 2009; 75.3%, Szabó, de Souza, Olegário, Ferreira, & de Albuquerque Pajuaba Neto, 2010), Nigeria (47.0%, Adamu, Adamu, & Salisu, 2012), and other areas of Mexico (59.5%, Tinoco-Gracia et al., 2009; 71;.5%, Galaviz-Silva, Pérez-Treviño, & Molina-Garza, 2013).

The seven tick species found infesting dogs in rural communities of Yucatan include *A. mixtum*, *A. ovale*, *A. parvum*, *I. affinis*, *R. microplus*, *R. sanguineus* s.l. and *A. c.f. oblongoguttatum*. With the exception of *A. oblongoguttatum*, these tick species have been reported to parasitize dogs in Mexico. Infestations with adults of *A. oblongoguttatum* have been reported on a variety of wild and domestic animals. Common vertebrate hosts parasitized by this tick species include Artiodactyla (Tayassuidae, Suidae, Cervidae), Perissodactyla (Tapiridae and Equidae), and Carnivora (mostly Canidae and Felidae) (Martins, Luz, Faccini, & Labruna, 2017).

In Mexico, *A. oblongoguttatum* infestations had been reported in cattle, deer (*Odocoileus virginianus* and *Mazama americana*), peccary (*Pecari tajacu* and *Tayassu pecari*), and tapir (*Tapirus bairdii*) (Guzmán-Cornejo et al., 2011). To our knowledge, this is the first report of *A. oblongoguttatum* infesting dogs from Mexico. This is a tick of public health importance. In its adult stage, *A. oblongoguttatum* is one of most frequent human-biting ticks in the Amazon biome of Brazil (Labruna et al., 2005; Martins et al., 2017).

Rhipicephalus sanguineus s.l. is the most common tick infesting dogs in Mexico (Cruz-Vazquez & Garcia-Vazquez, 1999; Rodríguez-Vivas et al., 2016). This tick species exhibits various disease agent vectoring capacity, hosts, environmental relationships and aggressiveness towards humans throughout the world (Dantas-Torres, 2010). Recently, Villarreal, Stephenson, & Foley, (2018) studied *R. sanguineus* near the Mexico–U.S. border and phylogenetic analysis showed that in the Mexican border (Mexicali) and western Mexico (Oaxaca) the tropical lineages was present, while the temperate lineage was dispersed in California and eastern Arizona, U.S. Moraes-Filho, Krawczak, Costa, Soares, and Labruna (2015) experimentally

TABLE 2 Number and frequency of tick species collected from dogs in rural communities of Tizimin municipality in Yucatan state, Mexico

	Communities							
	Chan San Antonio[a]		Sucopo[b]		Yaxcheku[c]		Total	
Specie ticks	Dogs with ticks	Frequency %	Dogs with ticks	Frequency %	Dogs with ticks	Frequency %	Total dogs with ticks	Frequency %
R. sanguineus s.l.	46	48.4a	35	27.1a	44	46.3a	125	39.1%
R. microplus	2	2.1c	1	0.7b	-	-	3	0.9c
A. mixtum	20	21.0b	7	5.7b	19	19.7b	46	14.4b
A. ovale	5	5.2c	9	7.0b	15	15.7b	29	9.11bc
A. parvum	-	-	-	-	1	1.0c	1	0.3c
A. cf. oblongoguttatum	1	1.0c	1	0.7b	7	7.3c	9	2.8c
Amblyomma spp.	17	17.8b	12	9.3b	14	14.7b	43	13.5b
I. affinis	-	-	-	-	6	6.3c	6	1.8c

[a]95 studied dogs.
[b]127 studied dogs.
[c]97 studied dogs.
R.: Rhipicephalus; A.: Amblyomma; I.: Ixodes; -: no cases; s.l.: sensu lato,
a, b, c = literals shown statistical differences.

TABLE 3 Intensity and range of tick infestation in dogs from three rural communities of Tizimin municipality in Yucatan, Mexico

	Communities							
	Chan San Antonio		Sucopo		Yaxcheku		Total	
Specie ticks	Intensity	Range	Intensity	Range	Intensity	Range	Intensity	Range
R. sanguineus s.l.	9.8	1–83	7.6	1–37	4.9	1–17	7.4	1–83
R. microplus	1	NA	1	-	-	-	1	1
A. mixtum	1.8	1–6	2.1	1–7	1.3	1–3	1.7	1–7
A. ovale	1.4	1–2	1.8	1–5	1.3	1–4	1.5	1–4
A. parvum	-	-	-	-	1	NA	1	NA
A. cf. oblongoguttatum	1	NA	1	NA	1.7	1–3	1.2	1–3
Amblyomma spp.	6.3	1–25	2.2	1–5	10.4	1–98	6.3	1–14
I. affinis	-	-	-	-	1.5	1–4	1.5	1–4

R.: Rhipicephalus; A.: Amblyomma; I.: Ixodes; -: no cases; NA: not applicable; s.l. sensu lato.

TABLE 4 Prevalence (%), odds ratio, and confidence interval at 95% of *Rhipicephalus sanguineus* s.l. infestation in dogs from three rural communities of Tizimin municipality in Yucatan, Mexico

Factor	Total tested dogs	No. Positive	Frequency	Odds ratio	CI 95%	*p-value*
Rural community						
Sucopo[a]	127	35	27.55	1		
Yaxcheku	97	45	46.36	2.41	1.32–4.41	**0.004**
Chan San Antonio	95	49	51.57	2.84	1.59–5.06	**0.000**
Season						
Dry[a]	148	54	36.48	1		
Rain	171	75	43.85	1.33	0.82–2.14	0.242

[a]Used as a reference, CI 95%: confidence interval at 95%.

demonstrated that tropical lineage of *R. sanguineus* are vectors of *E. canis*, while that temperate lineage were not. Further studies are required to know if the *R. sanguineus* tick population from Yucatan is similar to the one associated with Rocky Mountain Spotted Fever that recently re-emerged in the Mexico–U.S. (Álvarez-Hernández et al., 2017).

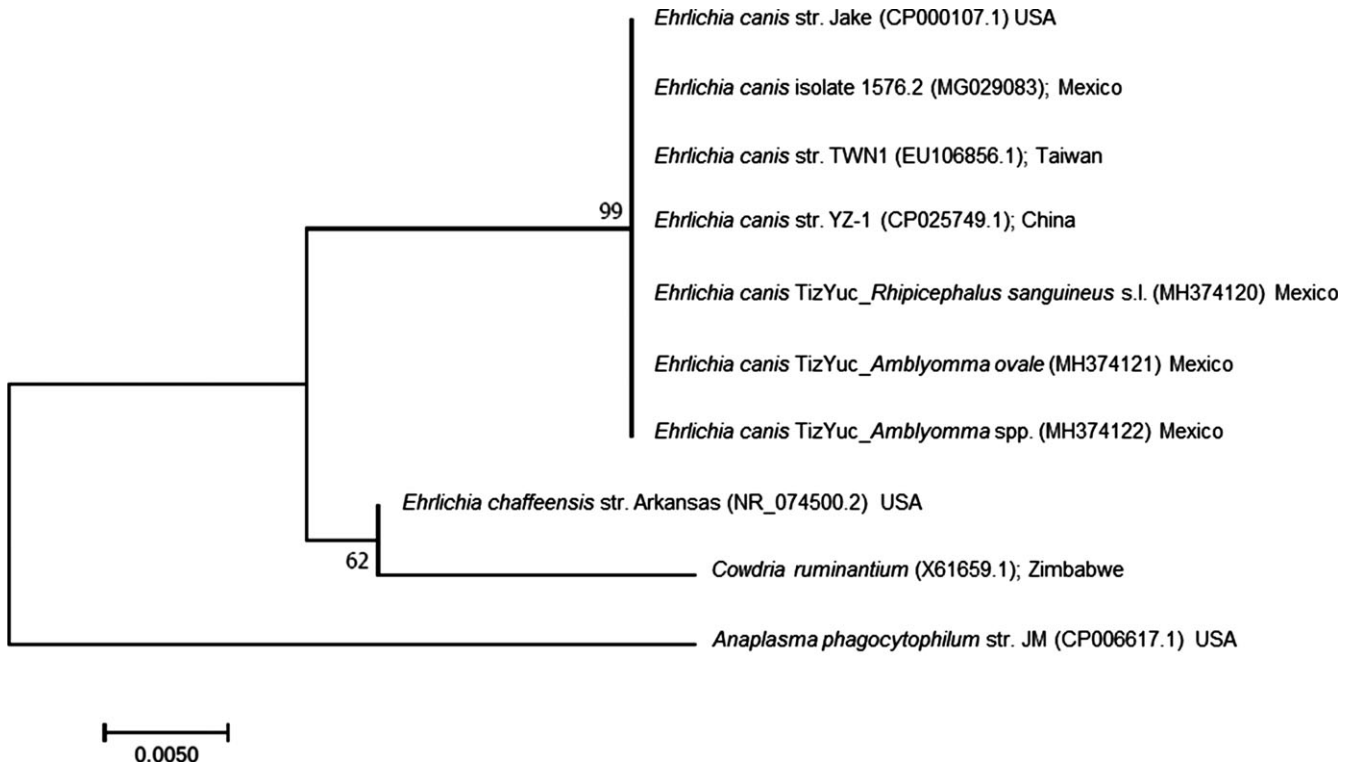

FIGURE 1 Phylogenetic trees based on the partial 16S rRNA sequences of *E. canis* isolates. Accession numbers for *E. canis* isolates and other sequences of *E. chaffeensis*, *Cowdria* (*Ehrlichia*) *ruminantium*, and *A. phagocytophilum* are given in parentheses. The scale bar indicates the number of substitutions per nucleotide position

Dogs can represent a model of tick-borne disease in humans. Several tick species that parasitize dogs in Mexico, including *R. sanguineus* s.l., can also bite humans, which represents a pathway for the probable transmission of zoonotic tick-borne pathogens from companion animals to humans (Rodríguez-Vivas et al., 2016). Data presented here indicate that the interaction of *R. sanguineus* s.l. with humans in rural communities studied of Yucatan warrants further investigations.

Previous findings bear relevance to the relatively high frequency of *R. sanguineus* s.l. in dogs from Yucatan (Solís-Hernández et al., 2018). Dogs apparently lack the ability to acquire protective immunity against that ectoparasite (Szabó, Mukai, Rosa, & Bechara, 1995). *R. sanguineus* s.l. prefers the domestic dog as a host for all stages of development and has a high reproductive potential. Under warm subhumid climatic conditions in Mexico, Cruz-Vazquez and Garcia-Vazquez (1999) reported that *R. sanguineus* s.l. has the potential to complete at least 2.5 generations per year.

Here, we report a mean intensity of 7.4 (range 1–83) for *R. sanguineus* s.l. infestations in dogs. Similar results of 3.8, 5.4, and 7.8 *R. sanguineus* s.l. per dog have been reported in north-western Georgia (United States) (Goldberg & Recha, 2002), north-eastern Brazil (Dantas-Torres et al., 2009), and south-eastern Brazil (Soares et al., 2006) respectively. The prevalence and intensity of *R. sanguineus* s.l. in dogs varies according to diverse factors such as host living conditions, season of the year, dog population density, and the proportion of dogs treated with ectoparasiticides or tick repellents within a population, age, and breed (Dantas-Torres, 2010). However, in some rural areas, *R. sanguineus* s.l. may be absent and dogs can be infested by other tick species including *Amblyomma mixtum*, *A. sabanerae*, *A. parvum*, *A. ovale*, *A. auricularium*, *A. maculatum*, *Ixodes near affinis*, and *Dermacentor nitens* (Rodríguez-Vivas et al., 2016; Solís-Hernández et al., 2015).

In this study, *A. mixtum* and *A. ovale* presented intensities of 1.7 and 1.5 respectively. Jones, Clifford, Keirans, and Kohls (1972) stated that rodents appear to be the preferential hosts for immature stages of *A. ovale*. However, Guglielmone et al. (2003) found that the great majority of adult specimens (84.3%) were found on Carnivora. Males and females of this tick species occasionally infest domestic animals, such as dogs, and man. In a previous study conducted in Nuevo Leon, Mexico, Galaviz-Silva et al. (2013) reported a *A. mixtum* intensity of 0.18 in dogs, which is lower than what we detected in Yucatán. *Amblyomma mixtum* is an eclectic feeder and is recognized as a serious pest of wildlife, livestock and humans. The adults usually feed on equids, cattle and dogs but the immature stages have also been found on several rodent species (Almazan, Tipacamu, Rodriguez, Mosqueda, & Perez de Leon, 2018; Estrada-Peña, Guglielmone, & Mangold, 2004; Piña et al., 2017).

Dogs in Chan San Antonio and Yaxcheku were 2.84 and 2.41 times more likely to become infected with *R. sanguineus* s.l. than dogs in Sucopo. The poor environmental sanitation prevailing in rural communities of Yucatan likely contributes to the high survival and propagation of this tick species among dogs. Chan San Antonio and Yaxcheku are rural communities with reduced incomes, decreased number of household dogs, as well as reduced number of houses with cemented floors, which are factors that could also favour the proliferation of this tick species in both communities. Demma et al. (2005) and Parola et al.

(2008) mentioned that *R. sanguineus* s.l. exhibits behaviour that can be classified as nidicolous, e.g., inhabiting burrows, artificial shelters, between rocks and crevices, etc., and seeking harbourage, i.e., sequestered in the immediate vicinity of host dwellings.

None of the studied pools of adult ticks were positive for *E. chaffeensis*, *E. ewingii*, *A. phagocytophilum*, or *R. rickettsii*. These tick-borne pathogens can infect a wide range of vertebrate hosts including humans (Atif, 2015; Yabsley, 2010). Their ecology involves hard ticks (Ixodidae) as vectors, wildlife and domestic animals, and human as hosts (Atif, 2015). In Mexico, cases of human ehrlichiosis and infection of *E. chaffeensis* in *R. sanguineus* s.l. and *Amblyomma mixtum* have been documented (Gongóra-Biachi, Zavala-Velázquez, Castro-Sansores, & González–Martínez, 1999; Sosa-Gutiérrez, Vargas, Torres, & Gordillo-Perez, 2014; Sosa-Gutierrez et al., 2016), as well as *A. phagocytophilum* in opossums and dogs (Rojero-Vázquez, Gordillo-Pérez, & Weber, 2017). Further studies are needed to ascertain the presence of these pathogens in dogs, wildlife, and their tick vectors in Yucatan, Mexico.

Pools of *R. sanguineus* s.l. adults, *A. ovale* adults, and *Amblyomma* spp. nymphs were positive for *E. canis*. Canine monocitic ehrlichiosis is endemic to Yucatan, Mexico, largely because regional climate conditions in this tropical zone provide an adequate environment for its main tick vector, *R. sanguineus* s.l. (Rodríguez-Vivas et al., 2016). The presence of *E. canis* in dogs by blood smear visualization, molecular detection of the *16s rRNA* gene, as well as serology have all been reported previously in Yucatan (Pat-Nah et al., 2015; Rodriguez-Vivas et al., 2005). Reports of the prevalence of *E. canis* in *R. sanguineus* s.l. can range from 18.5% in Mexico (Pat-Nah et al., 2015), to ~21% in Cameroon and Brazil (Ndip et al., 2007; Souza et al., 2010), to 27% in Ivory Coast (Socolovschi et al., 2012). These findings highlight the importance *R. sanguineus* s.l. as a vector of *E. canis* in dogs worldwide, including Yucatan in Mexico.

Human infection by *E. canis* or novel genotype of *E. canis* has been reported in Venezuela (Perez, Rikihisa, & Wen, 1996), Costa Rica (Bouza-Mora et al., 2017), and Mexico (Silva et al., 2014). The presence of *E. canis* in human blood samples in Latinoamerica may be a result of the high prevalence of the agent in canines and the ticks they host (Bouza-Mora et al., 2017), which increases the likelihood of human exposure to infected ticks. In this context, tick control on dogs is critical to prevent human infection (Barrantes-González, Jiménez-Rocha, Romero-Zuñiga, & Dolz, 2016). Further field surveillance for infected ticks and reservoir hosts is required to shed light on human and animal risk to tick-borne disease exposure.

Tick species in the genus *Amblyomma* (i.e., *A. americanum*) have been incriminated as vectors of *E. ewingii* in dogs (Murphy et al., 1998). In our study, two pools of *Amblyomma* spp. nymphs (4.4%), and four of *A. ovale* (12.9%) were positive for *E. canis*. To our knowledge, this is the first report worldwide of *A. ovale* infection with *E. canis*. *Amblyomma ovale* is the vector of *R. belli* and *R. parkeri* strain in the Atlantic rain forest of Brazil (Sabatini, Pinter, Nieri-Bastos, Marcili, & Labruna, 2010). In Belize, Lopes et al. (2016) collected ticks from wild animals and reported *R. parkeri* strain Atlantic rain forest in *A. ovale*. Since *A. ovale* ticks were collected directly from dogs, our results for *E. canis* could have originated from infected blood ingested by these ticks. Further studies are required to confirm the role of *Amblyomma* species, and especially *A. ovale*, as vectors of *E. canis* able to transmit this pathogen to dogs and humans in Yucatan, Mexico.

5 | CONCLUSIONS

We documented that ticks infesting dogs in rural communities of Yucatan, Mexico are infected with tick-borne rickettsia. Molecular assays showed that *R. sanguineus* s.l., *A. ovale*, and nymphs of *Amblyomma* spp. were positive for *E. canis*, which is known to cause disease in dogs and probably infection in humans. The infection of *A. ovale* with *E. canis* is reported here for the first time. Our findings highlight the need to control tick infestations in dogs that can also mitigate the risk of tick-borne disease transmission among companion animal and humans living in rural communities of Yucatan, Mexico.

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CONFLICT OF INTEREST

The authors declare no conflict of interests.

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REFERENCES

Adamu, N. B., Adamu, J. Y., & Salisu, L. (2012). Prevalence of ecto-, endo- and haemoparasites in slaughtered dogs in Maiduguri, Nigeria. *Revue de Médecine Vétérinaire*, *163*(4), 178–182.

Almazan, C., Tipacamu, G. A., Rodriguez, S., Mosqueda, J., & Perez de Leon, A. (2018). Immunological control of ticks and tick-borne

diseases that impact cattle health and production. *Frontier in Bioscience (Landmark Ed)*, *23*, 1535–1551. https://doi.org/10.2741/4659

Álvarez-Hernández, G., González-Roldán, J. F., Hernández-Milan, N. S., Lash, R. R., Barton- Behravesh, C., & Paddock, C. D. (2017). Rocky Mountain spotted fever in Mexico: Past, present, and future. *The Lancet*, *17*, e189–e196. https://doi.org/10.1016/S1473-3099(17)30173-1

Atif, F. A. (2015). *Anaplasma marginale* and *Anaplasma phagocytophilum*: Rickettsiales pathogens of veterinary and public health significance. *Parasitology Research*, *114*, 3941–3957. https://doi.org/10.1007/s00436-015-4698-2

Barrantes-González, A. V., Jiménez-Rocha, A. E., Romero-Zuñiga, J. J., & Dolz, G. (2016). Serology, molecular detection and risk factors of *Ehrlichia canis* infection in dogs in Costa Rica. *Ticks and Tick Borne Diseases*, https://doi.org/10.1016/j.ttbdis.2016.07.006

Bouza-Mora, L., Dolz, G., Solórzano-Morales, A., Romero-Zuñiga, J. J., Salazar-Sánchez, L., Labruna, M. B., & Aguiar, D. M. (2017). Novel genotype of *Ehrlichia canis* detected in samples of human blood bank donors in Costa Rica. *Ticks and Tick Borne Diseases*, *8*(1), 36–40. https://doi.org/10.1016/j.ttbdis.2016.09.012

Choi, Y. J., Jang, W. J., Ryu, J. S., Lee, S. H., Park, K. H., Paik, H. S., … Kim, I. S. (2005). Spotted fever group and typhus group rickettsioses in humans, South Korea. *Emerging Infectious Diseases*, *11*, 237–244. https://doi.org/10.3201/eid1102.040603

Cruz-Vazquez, C., & Garcia-Vazquez, Z. (1999). Seasonal distribution of *Rhipicephalus sanguineus* ticks (Acari: Ixodidae) on dogs in an urban area of Morelos, Mexico. *Experimental and Applied Acarology*, *23*, 277–280. https://doi.org/10.1023/A:1006075232455

Dantas-Torres, F. (2010). Biology and ecology of the brown dog tick *Rhipicephalus sanguineus*. *Parasites and Vectors*, *3*(26), 1–11. https://doi.org/DOI.org/10.1186/1756-3305-3-26

Dantas-Torres, F. (2015). Climate change, biodiversity, ticks and tick–borne diseases: The butterfly effect. *International Journal for Parasitology: Parasites and Wildlife*, *4*(3), 452–461. https://doi.org/10.1016/j.ijppaw.2015.07.001

Dantas-Torres, F., Melo, M. F., Figueredo, L. A., & Brandão-Filho, S. P. (2009). Ectoparasite infestation on rural dogs in the municipality of São Vicente Férrer, Pernambuco, Northeastern Brazil. *Revista Brasileira de Parasitologia Veterinaria*, *18*, 75–77. https://doi.org/10.4322/rbpv.01803014

Demma, L. J., Traeger, M. S., Nicholson, W. L., Paddock, C. D., Blau, D. M., Eremeeva, M. E., … Mcquiston, J. H. (2005). Rocky Mountain spotted fever from an unexpected tick vector in Arizona. *New England Journal of Medicine*, *353*, 551–553. https://doi.org/10.1056/NEJMoa050043

Estrada-Peña, A., Guglielmone, A. A., & Mangold, A. J. (2004). The distribution and ecological 'preferences' of the tick *Amblyomma cajennense* (Acari: Ixodidae), an ectoparasite of humans and other mammals in the Americas. *Annals of Tropical Medicine & Parasitology*, *98*(3), 283–292. https://doi.org/10.1179/000349804225003316

Galaviz-Silva, L., Pérez-Treviño, K. C., & Molina-Garza, Z. J. (2013). Distribution of ixodid ticks on dogs in Nuevo León, Mexico, and their association with *Borrelia burgdorferi* sensu lato. *Experimental and Applied Acarology*, *61*, 491–501. https://doi.org/10.1007/s10493-013-9707-5

Gammon, M., & Salam, G. (2002). Tick removal. *American Family Physician*, *66*, 643–645.

Goldberg, M., & Recha, Y. (2002). Durden LA: Ticks parasitizing dogs in northwestern Georgia. *Journal of Medical Entomology*, *39*, 12–114. https://doi.org/10.1603/0022-2585-39.1.112

Gongóra-Biachi, R. A., Zavala-Velázquez, J., Castro-Sansores, C. J., & González–Martínez, P. (1999). First case of human ehrlichiosis in Mexico. *Emerging Infectious Disease*, *5*(3), 481. https://doi.org/10.3201/eid0503.990327

Guglielmone, A. A., Estrada-Peña, A., Mangold, A. J., Barros-Battesti, D. M., Labruna, M. B., Martins, J. R., … Keirans, J. E. (2003). *Amblyomma aureolatum* (Pallas, 1772) and *Amblyomma ovale* Koch, 1844 (Acari: Ixodidae): Hosts, distribution and 16S rDNA sequences. *Veterinary Parasitology*, *113*, 273–288. https://doi.org/10.1016/S0304-4017(03), 00083-9

Guzmán-Cornejo, C., Robbins, R., Guglielmone, A. A., Montiel-Parra, G., & Pérez, T. M. (2011). The *Amblyomma* (Acari: Ixodida: Ixodidae) of Mexico: Identification Keys, Distribution and Hosts. *Zootaxa*, *2998*, 16–38. https://doi.org/10.1016/j.vetpar.2004.08.022

Instituto Nacional de Estadística, Geografía e Informática, Censo General de Población y Vivienda (INEGI) (2010). http://inegi.org.mx. Accessed 03 March 2018

Jones, E. K., Clifford, C. M., Keirans, J. E., & Kohls, G. M. (1972). The ticks of Venezuela (Acarina: Ixodoidea) with a key to the species of *Amblyomma* in the Western Hemisphere. *Brigham Young University Science Bulletin, Biological Series*, *17*, 1–40.

Kocan, A. A., Levesque, G. C., Whitworth, L. C., Murphy, G. L., Ewing, S. A., & Barker, R. W. (2000). Naturally occurring *Ehrlichia chaffeensis* infection in coyotes from Oklahoma. *Emerging Infectious Diseases*, *6*(5), 477–480. https://doi.org/10.3201/eid0605.000505

Kumar, S., Stecher, G., & Tamura, K. (2015). MEGA7: Molecular evolutionary genetics analysis version 7.0. *Molecular Biology and Evolution.*, *33*(7), 1870–1874. https://doi.org/10.1093/molbev/msw054

Labruna, M. B., Camargo, L. M. A., Terrassini, F. A., Ferreira, F., Schumaker, T. T., & Camargo, E. P. (2005). Ticks (Acari: Ixodidae) from the state of Rondoˆnia, western Amazon, Brazil. *Systematic and Applied Acarology*, *10*, 17–32. https://doi.org/10.1603/0022-2585-41.6.1073

Lopes, M. G., Junior, J. M., Foster, R. J., Harmsen, B. J., Sanchez, E., Martins, T. F., … Labruna, M. B. (2016). Ticks and rickettsiae from wildlife in Belize, Central America. *Parasites & Vectors*, *9*, 62. https://doi.org/10.1186/s13071-016-1348-1

Martinez–Ortiz, D., Torres–Castro, M., Koyoc–Cardena, E., Lopez, K., Panti–May, A., Rodriguez Vivas, I., … Manrique–Saide, P. (2016). Molecular evidence of *Rickettsia typhi* infection in dogs from a rural community in Yucatan, Mexico. *Biomedica*, *36*, 45–50.

Martins, T. F., Luz, H. R., Faccini, J. L. H., & Labruna, M. B. (2017). Life–cycle of *Amblyomma oblongoguttatum* (Acari: Ixodidae) under laboratory conditions. *Experimental and Applied Acarology*, *71*(4), 415–424. https://doi.org/10.1007/s10493-017-0135-9

Massung, R. F., Slater, K., Owens, J. H., Nicholson, W. L., Mather, T. N., Solberg, V. B., … Olson, J. E. (1998). Nested PCR assay for detection of granulocytic ehrlichiae. *Journal of Clinical Microbiology*, *36*, 1090–1095.

Moraes-Filho, J., Krawczak, F. S., Costa, F. B., Soares, J. F., & Labruna, M. B. (2015). Comparative evaluation of the vector competence of four South American populations of the *Rhipicephalus sanguineus* group for the bacterium *Ehrlichia canis*, the agent of canine monocytic ehrlichiosis. *PLoS ONE*, *10*, https://doi.org/10.1371/journal.pone.0139386.

Murphy, G. L., Ewing, S. A., Whitworth, L. C., Fox, J. C., & Kocan, A. A. (1998). A molecular and serologic survey of *Ehrlichia canis*, *E. chaffeensis*, and *E. ewingii* in dogs and ticks from Oklahoma. *Veterinary Parasitolology*, *79*, 325–339. https://doi.org/10.1016/S0304-4017(98), 00179-4

Nava, S., Beati, L., Labruna, M. B., Cáceres, A. G., Mangold, A. J., & Guglielmone, A. A. (2014). Reassessment of the taxonomic status of *Amblyomma cajennense* (Fabricius, 1787) with the description of three new species, *Amblyomma tonelliae* n. sp., *Amblyomma interandinum* n. sp. and *Amblyomma patinoi* n. sp., and reinstatement of *Amblyomma mixtum* Koch, 1844; and *Amblyomma sculptum* Berlese, 1888 (Ixodida: Ixodidae). *Ticks and Tick Borne Disiases*, *5*, 252–276. https://doi.org/10.1016/j.ttbdis.2013.11.004

Ndip, L. M., Ndip, R. N., Ndive, V. E., Awuh, J. A., Walker, D. H., & McBride, J. W. (2007). Ehrlichia species in *Rhipicephalus sanguineus* ticks in Cameroon. *Vector-Borne and Zoonotic Diseases*, *7*(2), 221–227. https://doi.org/10.1089/vbz.2006.0608

Parola, P., Socolovschi, C., Jeanjean, L., Bitam, I., Fournier, P. E., Sotto, A., … Raoult, D. (2008). Warmer weather linked to tick attack and emergence of severe rickettsioses. *PLoS Neglected Tropical Diseases.*, *2*, e338. https://doi.org/10.1371/journal.pntd.0000338

Pat-Nah, H., Rodriguez-Vivas, R. I., Bolio-Gonzalez, M. E., Villegas-Perez, S. L., & Reyes–Novelo, E. (2015). Molecular diagnosis of *Ehrlichia*

canis in dogs and ticks *Rhipicephalus sanguineus* (Acari: Ixodidae) in Yucatan, Mexico. *Journal of Medical Entomology*, *52*(1), 101–104. https://doi.org/10.1093/jme/tju010

Pérez, T. M., Guzmán-Cornejo, C., Montiel-Parra, G., Paredes-León, R., & Rivas, G. (2014). Biodiversidad de ácaros en México. *Revista Mexicana de Biodiversidad*, *85*, S399–S407. https://doi.org/10.7550/rmb.36160

Perez, M., Rikihisa, Y., & Wen, Y. (1996). *Ehrlichia canis*–like agent isolated from a man in Venezuela: antigenic and genetic characterization. *Journal of Clinical Microbiology*, *34*, 2133–2139.

Piña, F. T. B., da Silva Rodrigues, V., de Oliveira Souza Higa, L., Garcia, M. V., Barros, J. C., Pérez de León, A. A. P., & Andreotti, R. (2017). Life cycle of *Amblyomma mixtum* (Acari: Ixodidae) parasitizing different hosts under laboratory conditions. *Experimental and Applied Acarology*, *73*, 257–267. https://doi.org/10.1007/s10493–017–0178–y

Rodríguez, V. R. I., Domínguez, A. J. L., & Cob, G. L. (2000). Hemoparásitos en bovinos, caninos y equinos diagnosticados en el laboratorio de Parasitología de la FMVZ–UADY (1984–1999). *Revista Biomédica*, *11*, 277–282.

Rodriguez-Vivas, R. I., Albornoz-Rivero, E. F., & Bolio-González, M. E. (2005). *Ehrlichia canis* in dogs in Yucatan, Mexico: Seroprevalence, prevalence of infection and associated factors. *Veterinary Parasitology*, *127*, 75–79. https://doi.org/10.1016/j.vetpar.2004.08.022

Rodríguez-Vivas, R. I., Apanaskevich, D. A., Ojeda-Chi, M. M., Trinidad-Martínez, I., Reyes–Novelo, E., Esteve–Gassent, M. D., & Pérez de León, A. A. (2016). Ticks collected from humans, domestic animals, and wildlife in Yucatan, Mexico. *Veterinary Parasitology*, *215*, 106–113. https://doi.org/10.1016/j.vetpar.2015.11.010

Rojero-Vázquez, R., Gordillo-Pérez, G., & Weber, M. (2017). Infection of *Anaplasma phagocytophilum* and *Ehrlichia* spp. in opossums and dogs in Campeche, Mexico: The role of tick infestation. *Frontiers in Ecology and Evolution*, *5*, 1–19. https://doi.org/10.3389/fevo.2017.00161

Sabatini, G. S., Pinter, A., Nieri-Bastos, F. A., Marcili, A., & Labruna, M. B. (2010). A survey of ticks (Acari: Ixodidae) and their rickettsia in an atlantic rain forest reserve in the state of São Paulo, Brazil. *Journal of Medical Entomology*, *47*(5), 913–916. https://doi.org/10.1603/ME10073

Silva, A. B., Piña, S., Gabriel de la Torre, M., Mayoral, A., Mayoral, M. A., Pérez-Campos, L., … Pérez-Campos, E. (2014). Infección humana asintomótica por contacto con perros. Un caso de ehrlichiosis humana. *Gaceta Médica de México*, *150*, 171–174.

Soares, A. O., Souza, A. D., Feliciano, E. A., Rodrigues, A. F., D'Agosto, M., & Daemon, E. (2006). Avaliação ectoparasitológica e hemoparasitológica em cães criados em apartamentos e casas com quintal na cidade de Juiz de Fora, MG. *Revista Brasileira de Parasitologia Veterinaria*, *15*, 13–16.

Socolovschi, C., Gomez, J., Marié, J. L., Davousta, B., Guigal, P. M., Raoult, D., & Parola, P. (2012). *Ehrlichia canis* in *Rhipicephalus sanguineus* ticks in the Ivory Coast. *Ticks and Tick–borne Diseases*, *3*(5–6), 411–413. https://doi.org/10.1016/j.ttbdis.2012.10.005

Solís-Hernández, A., Rodríguez-Vivas, R. I., Esteve-Gassent, M. D., & Villegas-Pérez, S. L. (2018). Detección de *Borrelia burgdorferi* sensu lato en perros y sus garrapatas en comunidades rurales de Yucatán, México. *Revista de Biologia Tropical*, *66*(1), 428–437. https://doi.org/10.15517/rbt.v66i1.27776

Solís-Hernández, A., Rodríguez-Vivas, R. I., Pérez-Barrera, M. A., Esteve-Gassent, M. D., & Apanaskevich, D. A. (2015). *Ixodes affinis* (Acari: Ixodidae) in dogs from rural localities of Yucatán, Mexico: Prevalence, abundance and associated factors. *Veterinaria México*, *2*(3), 1–9. https://doi.org/10.21753/vmoa.2.3.351

Sosa-Gutierrez, C. G., Solorzano-Santos, F., Walker, D., Torres, J., Serrano, C. A., & Gordillo-Perez, G. (2016). Fatal Monocytic Ehrlichiosis in woman, Mexico. *Emerging Infectious Disease*, *22*(5), 871–874. https://doi.org/10.3201/eid2205.151217

Sosa-Gutiérrez, C. G., Vargas, S. M., Torres, J., & Gordillo-Perez, M. G. (2014). Tick-borne rickettsial pathogens in rodents from Mexico. *Biomedical Science and Engineering*, *7*, 884–889. https://doi.org/10.4236/jbise.2014.711087

Souza, B. M. P. S., Leal, D. C., Barboza, D. C., Uzeda, R. S., De Alcantara, A. C., Ferreira, F., Labruna, M. B., … Franke, C. R. (2010). Prevalence of ehrlichial infection among dogs and ticks in Northeastern Brazil. *Revista. Brasileira de Parasitologia Vetinaria*, *19*, 89–93. https://doi.org/10.4322/rbpv.01902004

Szabó, M. P., de Souza, L. G., Olegário, M. M., Ferreira, F. A., & de Albuquerque Pajuaba Neto, A. (2010). Ticks (Acari: Ixodidae) on dogs from Uberlândia, Minas Gerais, Brazil. *Transboundig Emerging Diseases*, *57*(1–2), 72–74. https://doi.org/10.1111/j.1865-1682.2010.01111.x

Szabó, M. P. J., Mukai, L. S., Rosa, P. C. S., & Bechara, G. H. (1995). Differences in the acquired resistance of dogs, hamsters, and guinea pigs to repeated infestations with adult ticks *Rhipicephalus sanguineus* (Acari: Ixodidae). *Brazilian Journal of Veterinary Research and Animal Science*, *32*(1), 43–50. https://doi.org/10.11606/issn.1678-4456.bjvras.1994.52089

Tinoco-Gracia, L., Quiroz-Romero, H., Quintero-Martínez, M. T., Rentería-Evangelista, T. B., González-Medina, Y., & Vinasco, J. (2009). Prevalence of *Rhipicephalus sanguineus* ticks on dogs in a region on the Mexico–USA border. *Veterinary Record*, *164*, 59–61. https://doi.org/10.1136/vr.164.2.59

Villarreal, Z., Stephenson, N., & Foley, J. (2018). Possible northward introgression of a tropical lineage of *Rhipicephalus sanguineus* ticks at a site of emerging Rocky Mountain Spotted Fever. *Journal of Parasitology*, *104*(3), 240–245. https://doi.org/10.1645/18-10

Wall, R., & Shearer, D. (2001). *Veterinary Ectoparasites, Biology, Pathology andControl*, 2nd ed.. London: Blackwell Science. https://doi.org/10.1002/9780470690505

Wen, B., Rikihisa, Y., Mott, J. M., Greene, R., Kim, H. Y., Zhi, N., … Bartsch, R. (1997). Comparison of nested PCR with immunofluorescent-antibody assay for detection of *Ehrlichia canis* infection in dogs treated with doxycycline. *Journal of Clinical Microbiology*, *35*(7), 1852–1855.

Yabsley, M. J. (2010). Natural history of *Ehrlichia chaffeensis*: Vertebrate hosts and tick vectors from the United States and evidence for endemic transmission in other countries. *Veterinary Parasitology*, *167*, 136–148. https://doi.org/10.1016/j.vetpar.2009.09.015

Zavala-Castro, J. E., Zavala-Velázquez, J. E., del Rosario García, M., León, J. J., & Dzul-Rosado, K. R. (2009). A dog naturally infected with *Rickettsia akari* in Yucatan, México. *Vector-Borne and Zoonosis Diseases*, *9*(3), 345–347. https://doi.org/10.1089/vbz.2008.0189

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