Ticks and Tick-borne Diseases xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Ticks and Tick-borne Diseases

journal homepage: www.elsevier.com/locate/ttbdis

Original article

Molecular detection of rickettsial tick-borne agents in white-tailed deer (*Odocoileus virginianus yucatanensis*), mazama deer (*Mazama temama*), and the ticks they host in Yucatan, Mexico

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ARTICLE INFO

Keywords:
Odocoileus virginianus yucatanensis
Mazama temama
Ehrlichia chaffeensis
Anaplasma phagocytophilum
Tick vectors
Molecular epidemiology

ABSTRACT

Twenty-five white-tailed deer (*Odocoileus virginianus yucatanensis*) (WTD), 4 mazama deer (*Mazama temama*) (MD), and the ticks they host in Yucatan, Mexico were sampled to run a molecular survey for tick-borne rickettsial agents. The prevalence of rickettsial agents was 20% in WTD (5/25) and 50% in MD (2/4). When sequencing the nested PCR products, *E. chaffeensis, A. phagocytophilum* and *A. odocoilei*, were identified as single infection or coinfecting cervids. None of the cervid samples were positive for *E. ewingii, E. canis,* nor *Rickettsia* spp. Overall, 355 individual ticks were collected. Species identified based on adult stages infesting cervids included *Amblyomma mixtum*, *A. parvum*, *A.* cf. *oblongoguttatum*, *Ixodes affinis, Rhipicephalus microplus*, *R. sanguineus* sensu lato, and *Haemaphysalis juxtakochi*. *Rhipicephalus microplus* was the tick species most commonly found infesting cervids with a frequency of 28.4%, and intensity of 25.2 ticks per animal. A pool of *Amblyomma* cf. *oblongoguttatum* adults and one of *Amblyomma* spp. nymphs were positive for *E. canis* and *E. chaffeensis,* respectively. None of the studied tick pools were positive for *E. ewingii, A. phagocytophilum*, nor *R. rickettsii*. To the best of our knowledge, this study is the first to report the prevalence of rickettsial agents in WTD and MD in Mexico. Our molecular study is the first to report the detection of *E. chaffeensis*, *A. phagocytophilum,* and *A. odocoilei* in MD in Mexico. The molecular detection of *E. chaffeensis, A. phagocytophilum,* and *A. odocoilei* in deer, and *E. chaffeensis* in *Amblyomma* spp. nymphs reported here raises the concern for the risk of human exposure to tick-borne rickettsial pathogens. Our findings highlight the need to apply the "One Health" approach to study ticks and tick-borne diseases. This science-based information could be used by state public-health programs to assess the risk for exposure to tick-borne Anaplasmataceae in Yucatan, Mexico.

1. Introduction

Several species in the Ixodidae tick family are biological vectors of bacteria classified in the order Rickettsiales (*Alphaproteobacteria*) (Kang et al., 2014). Among the families that compose the Rickettsiales, the Anaplasmataceae and Rickettsiaceae include tick-borne intracellular bacterial species that are pathogenic to humans and domestic animals (Dumler et al., 2001; Dahmani et al., 2017). *Anaplasma* and *Ehrlichia* are genera in the family Anaplasmataceae, which include intracellular Gram-negative organisms that multiply within membrane-bound vacuoles (Yabsley, 2010). Among the five described *Ehrlichia* species, *Ehrlichia canis, E. ewingii*, and *E. chaffeensis* cause infections in humans (Rar and Golovljova, 2011). Of the six confirmed *Anaplasma* species, *A. phagocytophilum*, *A. ovis* and *A. platys* cause infections in humans (Arraga-Alvarado et al., 2014; Breitschwerdt et al., 2014; Li et al., 2016).

The genus *Rickettsia* of the family Rickettsiaceae includes obligate intracellular Gram-negative coccobacillary forms that multiply within eukaryotic cells. This genus has been classically divided into the typhus, and the spotted fever groups. Infection with *R. rickettsii,* the causative agent of Rocky Mountain spotted fever (RMSF), induces an extremely severe, potentially fatal disease in people and animals (Paddock et al.,

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https://doi.org/10.1016/j.ttbdis.2018.11.018
Received 11 July 2018; Received in revised form 15 November 2018; Accepted 26 November 2018

2014).

As tick-borne infectious agents, the prevalence of infections with *Anaplasma, Ehrlichia,* and *Rickettsia* species correlates with the ecology and distribution of their tick vectors (Ismail et al., 2010; Campos-Calderón et al., 2016). *Anaplasma* and *Ehrlichia* species are transmitted through the bite of an infected nymphal or adult tick vector that had been previously infected in the larval or nymphal stage, respectively, while feeding on a rickettsemic animal, usually a wildlife host that also serves as a reservoir. In the case of *R. rickettsii* and other spotted fever group rickettsiae, transovarial transmission enables the infection of larval ticks, and infection is maintained transstadially in subsequent life stages (Nicholson et al., 2010). However, in some parts of the world the involvement of wildlife, specifically cervids (Foley et al., 2016; Sayler et al., 2016), in the ecology of rickettsial tick-borne agents, some of which can be zoonotic, remains to be fully understood.

During the past three decades the population of white-tailed deer (*Odocoileus virginianus*) in Mexico has increased considerably (Ortega-Santos et al., 2011). Because they occupy sympatric ranges, white-tailed deer (WTD) and other cervids, and domestic animals, also may share pathogens. Pathogens may be transmitted from wildlife to domestic animals, domestic animals to wildlife, and occasionally to humans (Chomel et al., 1994). The ixodid ticks *Amblyomma mixtum, Haemaphysalis juxtakochi, Ixodes* near *affinis, A. parvum* and *Rhipicephalus microplus* are known to infest WTD in Yucatan, Mexico (Rodriguez-Vivas et al., 2016). This might indicates that WTD may serve as a reservoir of tick-borne rickettsial diseases. Earlier studies of cervids as hosts for tick-borne diseases in Yucatan focused on the bovine pathogen *Anaplasma marginale* (Rodriguez-Vivas et al., 2010, 2013). However, cervids play an important role as reservoirs of several species of *Ehrlichia* and *Anaplasma* causing human ehrlichiosis and anaplasmosis, respectively, in the USA, Europe and Latinoamerica (Kawahara et al., 2006; Murphy et al., 2017), including Mexico (Góngora-Biachi et al., 1999; Sosa-Gutierrez et al., 2016).

In the study presented here we sampled WTD, MD, and the ticks they host in Yucatan, Mexico to run a molecular survey for tick-borne rickettsial agents. Phylogenetic analyses were performed on the pathogenic *Anaplasma* and *Ehrlichia* detected. The apparent involvement of tick vectors and their cervid hosts in the epidemiology of tick-borne rickettsial pathogens in Yucatan, Mexico is discussed.

2. Materials and methods

2.1. Sample collection

Hunters participating in the study were trained to collect tissues and ticks from deer carcasses. Twenty-five WTD (*Odocoileus virginianus yucatanensis*) and 4 mazama deer (MD) were hunter-killed (permits: SGPA/DGVS/: 010533/17, 010534/17, 13892/16, 8165/17) during the 2016 and 2017 hunting seasons in Yucatan, Mexico. The deer were harvested in wildlife management and use units within the Sucila, Espita, Buctzotz, and Merida municipalities of Yucatan, Mexico.

Spleen and liver samples were obtained from the carcasses in the field by the hunters. Tissue samples (pieces of tissue ~1 cm^2) were deposited in tubes with 90% ethanol and identified by animal species and site. Samples were preserved and transported at 4 °C to the laboratory where they were kept under refrigeration until processed. Predilection sites for ticks, such as the ears, head, axillary, and inguinal regions, were examined rigorously by the hunters. Tick samples were removed manually, deposited in a vial with 10 ml of 90% methanol, and shipped to the laboratory. Tissue and tick samples were stored and processed at the Laboratory of Parasitology in the School of Veterinary Medicine at the Autonomous University of Yucatan.

2.2. Tick identification

Ticks were taxonomically classified to species level using taxonomic keys described by Guzmán-Cornejo et al. (2011), and by morphological comparison with available images. The taxonomic criteria proposed by Nava et al. (2014) were applied to recognize *Amblyomma mixtum* within the *Amblyomma cajennense* species group. The criteria by Lopes et al. (2016) was followed to separate *Amblyomma* cf. *oblongoguttatum* from *A. oblongoguttatum* present in Brazil.

2.3. DNA extraction of liver, spleen and ticks

The liver and spleen samples from each animal were washed in bidistilled water until the excess alcohol was removed. A piece of 0.02 g each tissue was obtained and deposited in 1.5-ml vials and frozen at −20 °C for subsequent DNA extraction. A commercial blood and tissue DNeasy kit from Qiagen was used for DNA extraction of spleen and liver samples.

Specific adult and nymphal ticks were handled in "pools" for each infested animal. Tick DNA was extracted using the DNeasy tissue kit (DNeasy Blood & Tissue Kit, QIAGEN, USA). Ticks were transferred to prechilled in 1.5-ml tubes and introduced to liquid nitrogen for 1 min. Twenty microliters of sample buffer extraction were added to the tube and a disposable pellet pestle was used to grind the ticks against the tube wall for 20 s until close visual inspection revealed that each tick was broken into several fragments. The tube was briefly microcentrifuged and placed in a boiling water bath for 1 h. The DNA was stored at −20 °C. The quality of the extracted DNA was determined with a spectrophotometer (NanoDrop). This DNA solution was used in each PCR reaction.

2.4. Polymerase chain reaction (PCR) procedures

Samples were initially screened for pathogens within the Anaplasmataceae and Rickettsiaceae families using the TickPath Layerplex qPCR at Texas A&M University. This patent pending quantitative real-time PCR platform (patent application Serial No. 16/130,177) was used 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 the *R. rickettsia Rhhyp* gene (hypothetical protein A1G_04230).

Positive samples obtained with the TickPath Layerplex qPCR were then analyzed by nested PCR in the molecular biology laboratory at the Campus of Biological and Agricultural Sciences of the Autonomous University of Yucatan. In the nested PCR, the fragments of the *16S rRNA* and *ompB* genes were amplified to detect *Ehrlichia/Anaplasma* and *Rickettsia*, respectively. External primers to distinguish genus, and internal to distinguish species, were used (primers, target gene and conditions of the nested PCR used are described 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 according to Institutional Biosafety Permit (IBC-2013-039 and IBC-2016-051). Additionally, samples from Mexico were imported into the US with permits approved 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).

2.5. Sequencing

Two individual samples of WTD, one of MD, as well as one pool of *Amblyomma* spp. nymphs that were positive for a fragment of the *16S rRNA* of *E. chaffeensis* were sequenced. One pool of *A.* cf. *oblongoguttatum* positive for a fragment of the *16S rRNA* of *E. canis* was also sequenced. Additionally, two individual samples of WTD, and one MD that were positive for a *16S rRNA* fragment of *A. phagocytophilum* were sequenced. Products were purified using E.Z.N.A.® gel extraction kit (Omega Bio-tek, Inc, Norcross, Georgia, USA) and sequenced by the

Table 1
Pathogens tested, primers, target gene and conditions of nested PCR used in this study.

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

laboratory DIMYGEN® (Mérida, Yucatán, México; http:// http://www.dimygen.com/). The resulting sequences were compared to sequences of *E. chaffeensis, E. canis, A. phagocytophilum*, and *A. odocoilei* deposited in GenBank® by using BLAST (http://www.ncbi.nlm.nih.gov/blast). All the sequences generated by this study were deposited in GenBank®.

2.6. Statistical analysis

Prevalence of tick-borne Rickettsiales was calculated (number of deer with rickettsial agent or pathogen infection/number of evaluated deer × 100). The χ^2 analysis was used to know the statistical differences between sample (spleen and liver) sources to detect rickettsial pathogens. Infestation intensity for each tick species was calculated using this formula: total number of tick species/number of infested deer with the same species.

3. Results

Results obtained with the TickPath Layerplex qPCR revealed that 5 out of 25 WTD (prevalence: 20%), and 2 out of 4 MD (prevalence: 50%) were positive for tick-borne rickettsial agents. These 7 positive deer were also positive in the nested PCR. *E. chaffeensis, A. odocoilei*, and *A. phagocytophilum* were identified by sequencing the nested PCR products (Figs. 1 and 2). None of the deer samples were positive for *E. ewingii, E. canis*, nor *Rickettsia* spp. Single infections and coinfections of WTD and MD with *E. chaffeensis, A. odocoilei*, or *A. phagocytophilum* are listed in Table 2. Five positive cases of Anaplasmataceae infection (*A. odocoilei, A. phagocytophilum* and *E. chaffeensis*) were detected in spleen (17.2%, 5/29) and six in liver (20.6%, 6/29) ($P > 0.05$).

Overall, 355 individual ticks (340 adults and 15 nymphs) belonging to four genera were collected. The seven species identified were *A. mixtum*, *A. parvum*, *A.* cf. *oblongoguttatum*, *I. affinis, R. microplus*, and *R. sanguineus* sensu lato, and *H. juxtakochi*. The most common tick species infesting cervids in this study was *R. microplus*, with a frequency of 28.4%, and an intensity of 25.2 (Table 3).

A pool was made with ticks of the same genus (2–8 nymphs), and the same species (1–4 adults) to characterize the rickettsial agents present in the ticks infesting each deer. Twenty pools were obtained. Twenty % (5/20) of the tick pools were positive for rickettsial agents by qPCR. None of the tick pools were positive for *E. ewingii, A. phagocytophilum*, nor *R. rickettsii*. One pool of adult *A.* cf. *oblongoguttatum* and one of *Amblyomma* spp. nymphs were positive for *E. canis* and *E. chaffeensis,* respectively.

The nested PCR products of the *16S rRNA* gene fragment of *E. chaffeensis,* and *A. phagocytophilum* for seven positive deer, and two pools of ticks positive for *E. canis* and *E. chaffeensis,* were sequenced. Four nested PCR products of the *16S rRNA* gene fragment of *E. chaffeensis* revealed 99–100% homology with isolate D11 (KY644145.1) of *Blastocerus dichotomus* deer from Argentina, and str. West Paces (CP007480.1), str. Arkansas (CP000236.1), and str. Jax (CP007475.1)

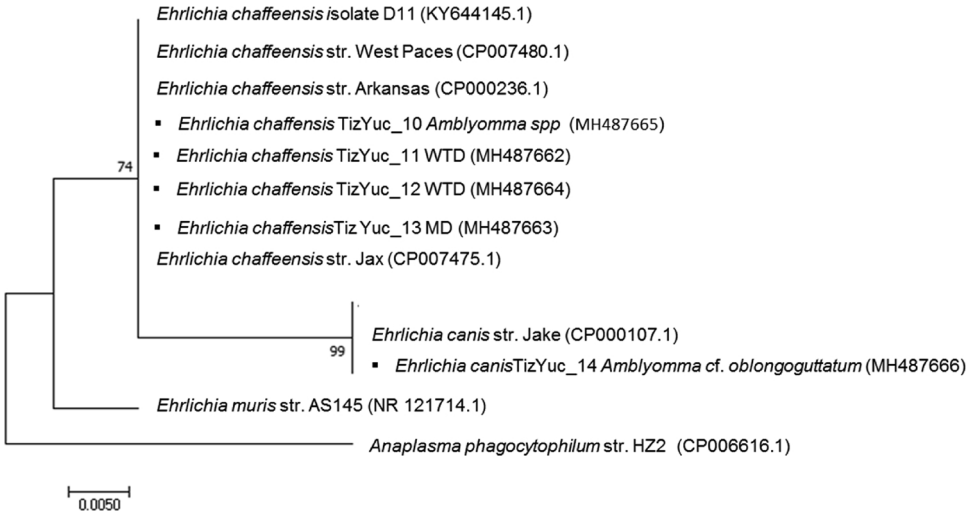

Fig. 1. Phylogenetic trees based on the partial *16S rRNA* sequences of *Ehrlichia* spp. Accession numbers for *E. chaffeensis* and *E. canis* and other sequences of *E. muris*, and *A. phagocytophilum* are given in parentheses. The scale bar indicates the number of substitutions per nucleotide position.

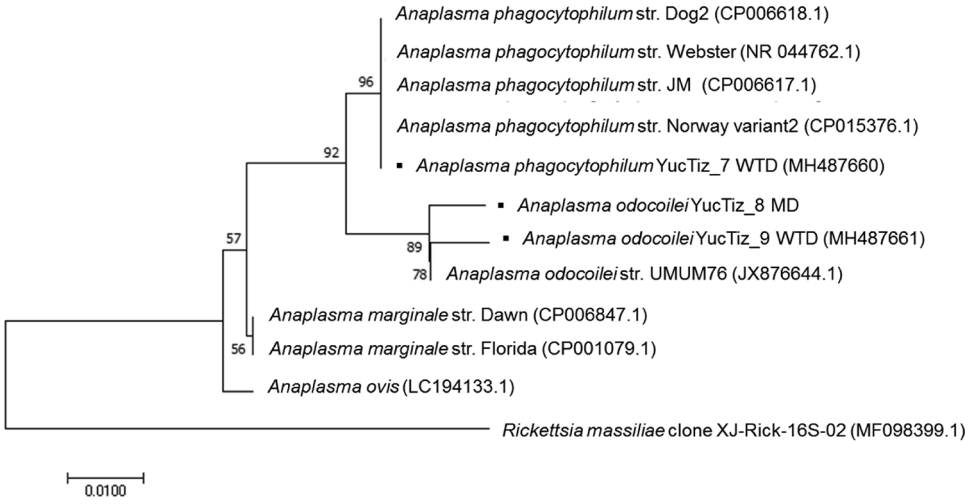

Fig. 2. Phylogenetic trees based on the partial *16S rRNA* sequences of *Anaplasma* spp. Accession numbers for *Anaplasma odocoilei* and *A. phagocytophilum* and other sequences of *Anaplasma marginale*, *A. ovis* and *Rickettsia massiliae* are given in parentheses. The scale bar indicates the number of substitutions per nucleotide position.

Table 2
White-tailed deer (n = 25) and Mazama deer (n = 4) infected with *E. chaffeensis, A. odocoilei*, or *A. phagocytophilum* in Yucatan, Mexico.

Tick-borne Anaplasmataceae	WTD	MD	Positive	Prevalence (%)
Ehrlichia chaffeensis	1	–	1	3.44
Anaplasma odocoilei	2	–	2	6.89
E. chaffeensis + *A. phagocytophilum*	2	1	3	10.34
E. chaffeensis + *A. odocoilei*	0	1	1	3.44
Total	5	2	7	24.13%

- : no infection detected; WTD: white-tailed deer (*Odocoileus virginianus yucatanensis*), MD: mazama deer (*Mazama temama*).

Table 3
Intensity, range, and frequency of tick infestation in white-tailed deer and mazama deer from Yucatan, Mexico.

	Intensity	Range	Frequency (%)
Amblyomma mixtum	2.6	1-7	7.3
Amblyomma parvum	3.8	1-8	18.6
Amblyomma cf. *oblongoguttatum*	5.6	1-31	11
Haemaphysalis juxtakochi	3.9	1-45	26.1
Ixodes affinis	3	1-3	3.4
Rhipicephalus sanguineus s.l.	1	1	0.28
Rhipicephalus microplus	25.2	1-92	28.4
Amblyomma spp.*	2.8	1-5	4.9

* nymphs.

from humans in the USA. The same gene fragment of one *E. canis*-positive pool obtained from *A.* cf. *oblongoguttatum* adults revealed 100% homology with the *E. canis* strain Jake (CP000107.1) from USA. The phylogenetic tree of *E. canis* was inferred based on the *16S rRNA* sequences obtained in this study and compared with *E. muris* str. AS145 (NR 121714.1), and *A. phagocytophilum* str. HZ2 (CP006616.1) (Fig. 1).

A nested PCR product of the *16S rRNA* gene fragment of *A. phagocytophilum* shared 98.5% homology with strain Webster (NR_044762.1), strain MJ (CP006617.1), strain Norway (CP015376.1), and strain Dog2 (CP006618). Likewise, two sequences of the *16S rRNA* gene fragment of *A. odocoilei* showed 98.4–99.2% homology with strain UMUM76 (NR_118489.1). The phylogenetic tree of *A. phagocytophilum* and *A. odocoilei* was inferred based on the *16S rRNA* sequences obtained in this study and compared with *A. marginale* strain Dawn (CP006847.1), strain Florida (CP001079.1), *A. ovis* (LC194133.1), and *R. massiliae* clone XJ-Rick-16S-02 (MF098399.1) (Fig. 2).

4. Discussion

Cervids are an important wildlife reservoir of several species of pathogenic *Ehrlichia* and *Anaplasma* known to cause human ehrlichiosis and anaplasmosis (Nair et al., 2014). While some studies have demonstrated that various *Ehrlichia* and *Anaplasma* species circulate among dog and ruminants and tick vectors worldwide (Said et al., 2015; Aktas and Ozubek, 2018) and specially in Mexico (Rodriguez-Vivas et al., 2005; Pat-Nah et al., 2015); to the best of our knowledge, this study is the first to report the prevalence of rickettsial agents in WTD and MD in Mexico.

According to Waner and Harrus (2013), the spleen is shown to be a major reservoir of ehrlichial organisms due to the abundance of hosting macrophages in this organ. The spleen apparently is the last organ to contain ehrlichial agents before they are eliminated (Harrus et al., 2004). This study found no significant difference in positive cases to ehrlichia infections when the spleen and liver were used (5 vs. 6). However, the spleen has proven to be a rich source of ehrlichial organisms for diagnostic purposes and is considered therefore as the organ of choice for molecular diagnosis during the different phases of the disease (Waner and Harrus, 2013).

In this study, *A. mixtum, A. parvum, A.* cf. *oblongoguttatum, I. affinis, R. microplus*, *R. sanguineus* s.l. and *H. juxtakochi* were found infesting deer. All these tick species have been reported to infest cervids in Mexico. However, the ability of ixodid ticks to vector rickettsial agents to cervids in Mexico remains to be fully ascertained. *R. microplus* was the tick species found to be infesting WTD and MD most frequently. Although cervids may not be the preferred host for *R. microplus*, their proximity to cattle herds may facilitate their infestation (Szabó et al., 2003). In Latino America, *R. microplus* is a well-known vector of *A. marginale*, *Babesia bovis* and *B. bigemina* in ruminants (Scoles et al., 2007; Rojas Ramírez et al., 2011).

WTD are suspected to be a reservoir host of *E. chaffeensis* by studies that included experimental infection and detection of natural infection (Yabsley et al., 2003; Nair et al., 2014). Anti-*E. chaffeensis* antibodies are common in WTD in the USA with decreasing numbers of seropositive deer found near the periphery of the range of the lone star tick, *A. americanum* (Mueller-Anneling et al., 2000). No differences in prevalence were noted among age classes, suggesting that the majority of deer in endemic areas are exposed to *E. chaffeensis* while they are fawns (Yabsley et al., 2003). In our study 20% (5/25) of WTD, 50% of MD (2/4), and one pool of *Amblyomma* spp. nymphs were positive to Anaplasmataceae infection. Despite these findings, further studies are required to confirm the possible role of deer as reservoirs of

Anaplasmataceae in Yucatan.

Increasing deer populations in Southern Mexico (Ortega-Santos et al., 2011), and the expanding distribution of ticks infesting them (Rodriguez-Vivas et al., 2016) may be factors driving the increased risk for human monocytic ehrlichiosis, as it has been previously reported in Yucatan (Góngora-Biachi et al., 1999). Besides being affected by hunting and habitat destruction, the fact that WTD and MD may live in areas that are close to populations of domestic animals may be a threat to these animals due to the risk of disease transmission. *E. chaffeensis* is transmitted to humans and animals by infected *A. americanum* in the USA (Wright et al., 2014). However, this tick species is not present in Yucatan. Detailed studies are needed to evaluate the species of *Amblyomma* involved in the transmission of *E. chaffeensis* to cervids and possibly to humans. Our molecular study is the first to report the detection of *E. chaffeensis* in MD in Mexico.

The single infection of WTD with *E. chaffeensis*, and coinfection with *A. phagocytophilum* and *A. odocoilei* indicates high levels of exposure to Anaplasmataceae in this wildlife species in Yucatan. This also raises the possibility that individual WTD could be simultaneously coinfected with the three tick-borne rickettsial agents. In Europe and America, *A. phagocytophilum* has been reported infesting ungulates such as roe deer (*Capreolus capreolus*), red deer (*Cervus elaphus*), and elk (*Alces alces*) (De la Fuente et al., 2005; Woldehiwet, 2006).

Earlier studies in Mexico demonstrated that *A. marginale* is present in WTD (Rodriguez-Vivas et al., 2010, 2013). In this study, two WTD and one MD were positive to *A. odocoilei* showing 99.9% identity with isolate UMUM76 T. *Anaplasma* sp., also called *Ehrlichia*-like sp., is common among WTD populations in the southeastern United States (Arens et al., 2003). However, experimental infection, morphologic, serologic, and molecular studies of this pathogen led to the reclassification of this *Anaplasma* under the name *Anaplasma odocoilei* sp. nov. (Tate et al., 2013). This species infects platelets of acutely infected deer; but, clinical signs of illness were not apparent in experimentally infected WTD (Tate et al., 2013). Further studies are required to determine the route of transmission of the agent in both WTD and MD. Our finding represents the initial report of *A. odocoilei* in MD from Mexico.

None of the 29 cervids tested in the present study were positive for *E. canis*. However, a pool of *A.* cf. *oblongoguttatum* was *E. canis*-positive. This *Ehrlichia* species has been detected in cervids in China (Li et al., 2016). Nonetheless, the lack of detection by PCR in WTD and MD is not unexpected because previous studies reported that *E. canis* does not establish infection or cause seroconversion in WTD (Dawson et al., 1994; Arens et al., 2003).

We did not detect *Rickettsia* by PCR in the cervids and ticks tested. These findings are in contrast with reports of sika deer infection with *R. helvetica* (Inokuma et al., 2008), and *R. asiatica* (Jilintai et al., 2008), as well as *A. maculatum* with *R. parkeri* (Parola et al., 2013), and *A. triste* with *R. parkeri* (Nava et al., 2008). Additional studies are needed to clarify if cervids and the ticks they host play a role in the epidemiology of *Rickettsia* spp. in Yucatan, Mexico.

Deer are used as natural sentinels to determine the geographic distribution of *E. chaffeensis*, and risk of human infection in the USA (Yabsley et al., 2005). Yucatan only has one confirmed case of *E. chaffeensis* in humans (Góngora-Biachi et al., 1999). The molecular detection of *E. chaffeensis* and *A. phagocytophilum* in deer, and *E. chaffeensis* in *Amblyomma* spp. nymphs reported here raises the concern for the risk of human exposure to tick-borne rickettsial pathogens.

5. Conclusions

In conclusion, our molecular survey revealed that WTD and MD are infected with *E. chaffeensis* and *A. phagocytophilum*. *Amblyomma* spp. infesting deer were infected with *E. chaffeensis*, which provides a potential pathway for the transmission of this zoonotic agent to humans. Our findings highlight the need to apply the One Health approach to study ticks and tick-borne diseases. This science-based information could be used by state public-health programs to assess the risk for exposure to tick-borne Anaplasmataceae in Yucatan, Mexico.

Funding sources

This work was supported by the FMVZ-2016-0007, and Texas AgriLife and TVMDL's seed grant, Molecular Diagnosis of Zoonotic Tick-Borne Diseases. Also by USDA-ARS (3094-32000-039-00D, 3094-32000-03). USDA is an equal opportunity provider and employer.

Ethics statement

The protocol was approved by the Biomedic Committee of CCBA-UADY (CB-CCBA-2016-003).

Conflicts of interest

None.

Acknowledgements

We thank farmers and hunters of Yucatan for providing us deer and tick samples. M.M. Ojeda-Chi was funded by CONACYT-Mexico for the scholarship provided to pursuing a PhD degree from CCBA-UADY, Yucatán, Mexico.

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