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Molecular detection of *Rickettsia* species in ticks collected in the Mexico-USA transboundary region

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Abstract

Zoonotic tick-borne diseases, including those caused by *Rickettsia* species, continue to have serious consequences for public health worldwide. One such disease that has emerged as a major problem in several countries of the American continent is the Rocky Mountain Spotted Fever (RMSF) caused by the bacterium *Rickettsia rickettsii*. Several tick species are capable of transmitting *R. rickettsia,* including *Amblyomma cajennense, A. aureolatum, A. imitator, Rhipicephalus sanguineus, Dermacentor andersoni, D. variabilis* and possibly *A. americanum.* Despite previous reports in Mexico linking new outbreaks of RMSF to the presence of these tick species, no robust measures have tackled transmission. In the present study, we amplified *R. rickettsii* from 109 test DNA samples extracted from ticks collected from several animals and humans of Tamaulipas, Mexico, between November 2015 and December 2017. Our analysis revealed the presence of *R. rickettsii* in six samples and these findings contribute to a spatial distribution map that is intended to minimize the risk of transmission to humans.

Keywords Rickettsia · Diagnostic · Ticks · Tamaulipas · Transboundary

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Introduction

Tick-borne diseases (TBDs) are increasing and have become a worldwide public health issue due to the many zoonotic pathogens that ticks can transmit (Parola and Raoult 2001). From these, rickettsiosis or diseases caused by *Rickettsia* species, represent a very important group due to the emergent character of the illness (Jones et al. 2008).

New outbreaks of rickettsiosis have been of major concern in several countries on the American continent (Treadwell et al. 2000; Zavala-Castro et al. 2006; Guedes et al. 2005). In humans the initial and classic symptoms of a rickettsial infection are headache, fever, vomit, muscle pain, and rash that commonly begins on the wrists, ankles and forearms and then spreads to the rest of the body. The diagnostics for the disease have been based on the presence of all of these symptoms; however, the appearance of new symptoms and the difficulty of early identification of these pathogens makes treatment difficult and thus threatens the life of the patient. Therefore, a rapid differential diagnostic for rickettsial infection based on molecular techniques is needed (Chaudhry and Scofield 2013).

Rickettsial pathogens are transmitted via the bite of several tick species like *Amblyomma cajennense, A. aureolatum A. imitator, Rhipicephalus sanguineus, Dermacentor andersoni, D. variabilis* and possibly *A. americanum,* (Saraiva et al. 2014; Soares et al. 2012; Breitschwerdt et al. 2011; Peniche-Lara et al. 2015). Most of these tick species have been reported in Mexico (Cruz-Vazquez and Garcia-Vazquez 1999; Galaviz-Silva et al. 2013), and may pose a high risk for human and animal health (Nava et al. 2014; Szabó et al., 2013; Woods 2013; Breitschwerdt et al. 2011; Guzmán-Cornejo et al. 2011). In fact, recent outbreaks of rickettsiosis have been published in Mexico and have been linked to the presence of the aforementioned tick species (Eremeeva et al. 2011; Zavala-Castro et al. 2006; 2008; Alvarez and Contreras 2013). Despite the presence of these known vectors and the reported cases of rickettsiosis, there have been no steps taken to establish new prevention and control programs. The present research genotypes a randomly collected pool of tick samples from the state of Tamaulipas in order to identify the presence of rickettsial agents and characterize which species represent a threat for human and animal health.

Material and methods

Study area

The state of Tamaulipas (24° 17′ 14″ N, 98° 33′ 48″ W) is located in northern Mexico. Tamaulipas has 43 municipalities and is bordered on the north by the Rio Grande Valley of southeast Texas, USA, on the east by the Gulf of Mexico, on the west by the state of Nuevo Leon, and by the states of Veracruz and San Luis Potosi on the south.

Tick sampling

Tick samples were collected from 16 municipalities of Tamaulipas. Samples were obtained from bovines, horses, wild boars, dogs and humans present at the sampling sites. Primary attention was given to ticks obtained from humans and dogs, because they are considered to be reservoirs and are susceptible to infection by *R. rickettsii.* All samples were identified, labeled with relevant host and collection data and stored in 70% ethanol. Morphological classification

and identification of ticks was carried out using 'Ticks (Acari: Ixodidae y Argasidae) Invertebrate Collection of the Provincial Museum of Natural Sciences Florentino Ameghino' (Faccioli 2011), the 'Pictorial Key to the Identification of ticks in Colombia and Northern and South America' (Benavides-Ortiz and López-Valencia 2005), the tick identification guide of the Tick Encounter Resource Center (https://tickencounter.org/tick_identification 2019), and '*Amblyomma* (Acari: Ixodida: Ixodidae) of Mexico: Identification Keys, Distribution and Hosts' (Guzmán-Cornejo et al. 2011).

DNA extraction

All collected ticks were used to obtain DNA. Samples from bloodless internal tissues (intestine, salivary glands, ovaries, and rectal pouch synganglion) were dissected out, frozen with liquid nitrogen and then macerated using a mortar and pestle. Homogenates were transferred to 1.5-ml Eppendorf tube (SSI, Lodi, CA, USA) for DNA extraction using TRI Reagent Kit (MCR Molecular Research Center, Cincinnati, OH, USA) following the manufacturer´s instructions. DNA was quantified using a Jenway 6405UV/VIS spectrophotometer (Keison Products, Oldham, UK) and stored at − 20 ºC until use.

PCR protocol

DNA samples were screened by PCR methodology using GoTaq Green Master Mix, 2x (Promega, Madison, WI, USA) following the manufacturer's instructions. To detect the presence of *Rickettsia* spp. the primers R. rick-F: 5′-TGT CTA TCA ATT CAC AAC TTG CC-3′ and R. rick-R: 5′-GCT TAC AAA ATT CTA AAA ACC ATA TA-3′, which amplify a total of 547 bp of the gene encoding for the 17-kDA protein of *R. rickettsii* were used. Reactions were run in a thermocycler (AB 2720) with the following PCR cycling conditions 1 cycle: 94 °C for 5 min; 35 cycles 94 °C for 30 s, 54 °C for 30 s, 72 °C for 30 s; with a final extension at 72 °C for 7 min. Reactions were on hold at 4 °C until further analysis was done. PCR products were separated by electrophoresis in 1.5% agarose gels. Subsequently, gels were stained using Diamon Nucleic Acid Dye (Promega), visualized, and photographed under ultraviolet light using an ENDURO GDS gel documentation system (LABNET).

Nucleotide sequencing

PCR products of the expected size were purified and sequenced using primers R. rick-F: 5′-TGT CTA TCA ATT CAC AAC TTG CC-3′ and R. rick-R: 5′-GCT TAC AAA ATT CTA AAA ACC ATA TA-3′. Sequences were clean at the 5′ and 3′ ends and chromatogram picks were checked for any potential sequencing error. Assembled sequences were submitted to BLAST analysis to determine similarities with any of the *Rickettsia* species. All final sequences were aligned to representative sequences published in GenBank using the program T-COFFEE v. 11.00.

Results

In total 110 tick samples were obtained from five different host species including canines (64.5%), bovines (26.4%), equines (3.6%), wild porcine (0.90%) and humans (4.54%) from 16 municipalities of Tamaulipas, Mexico (Table 1). After collection, morphological identification was carried out.

Of the 110 collected samples, 82 belonged to the genus *Rhipicephalus*, representing 74.6% of specimens collected (Table 2). Eighteen *Amblyomma* specimens were collected (16.4%) and 10 specimens belonged to the genus *Dermacentor* (9.1%). After identification, DNA was extracted from all samples and subsequently used to detect rickettsial DNA through PCR; six samples had an amplified band with the expected size for rickettsia. Of the six positive specimens, five were identified as *Amblyomma* and the sixth was a *Rhipicephalus* sp. (Table 3).

The ticks infected with *Rickettsia* spp. were collected from two different sites in the state. It is important to note that sample numbers 8 and 9 were collected from the same host animal, but the species of ticks collected were *A. mixtum* and *R. sanguineus.* Both samples amplified a fragment of the expected size (547 bp) for rickettsia (Fig. 1).

Samples that amplified a band of the expected size were sent for sequencing and according to the results obtained from the sequencing service, three of them obtained a higher homology to *Candidatus* Rickettsia amblyommi. These ticks were previously identified as A. *mixtum* and obtained from two animals (a dog and a bovine) and one more from a human. Another sample also identified as *A. mixtum* and obtained from a dog resulted in a higher homology to *R. rhipicephali.* Two additional samples were 100% identical to *R. amblyommatis,* these samples were identified as *A. cajennense* and *R. sanguineus,* respectively (Table 3). PCR-positive products that were sent for sequencing showed at least 95%

Table 1 Number of tick samples obtained from different hosts and municipalities from Tamaulipas, Mexico

Municipality	Dogs	Bovines	Horses	Wild boars	Humans	Total
Victoria	25	10	–	–	1	36
San Fernando	3	11	2	–	1	17
Bustamante	1	–	–	–	–	1
Tula	1	–	–	–	–	1
Rio Bravo	6	–	–	–	–	6
Güémez	14	–	–	–	2	16
Soto la Marina	2	1	–	–	–	3
Aldama	3	3	2	–	1	9
Mante	1	–	–	–	–	1
Villagrán	–	1	–	–	–	1
Mainero	–	2	–	1	–	3
Llera	1	–	–	–	–	1
San Carlos	5	–	–	–	–	5
Ocampo	–	1	–	–	–	1
Reynosa	8	–	–	–	–	8
Matamoros	1	–	–	–	–	1
Total	71	29	4	1	5	110

Table 2 Tick genera collected by municipalities of Tamaulipas, Mexico

Municipality	*Rhipicephalus*	*Amblyomma*	*Dermacentor*	Total
Victoria	32	2	2	36
San Fernando	14	3	–	17
Bustamante	1	–	–	1
Tula	1	–	–	1
Rio Bravo	6	–	–	6
Güémez	11	–	5	16
Soto la Marina	1	2	–	3
Aldama	1	8	–	9
Mante	1	–	–	1
Villagrán	1	–	–	1
Mainero	2	1	–	3
Llera	–	1	–	1
San Carlos	2	–	3	5
Ocampo	–	1	–	1
Reynosa	8	–	–	8
Matamoros	1	–	–	1
Total	82	18	10	110

of similarity in the sequence. Two samples showed 100% similarity with sequences from GenBank.

The phylogenetic inference based on the six rickettsia sequences (Fig. 2), placed samples 1 and 2 in the same clade, also samples 3 and 5. Sample 4, the only sample that came from a *Rhipicephalus* tick, represented a higher distance between clades (Fig. 2).

Discussion

Since the discovery of TBDs, more than 35 have been partially characterized with 16 diseases affecting humans and 19 affecting livestock and companion animals. The discovery of new TBDs will likely continue (de la Fuente et al. 2015). Due to the severity and increase in the number of cases reported, the tick-borne rickettsial diseases (TBRD) are getting more attention by veterinarians and physicians (Dantas-Torres et al. 2012; Gleim et al. 2016; Zavala-Castro et al. 2008). For over a century the tick-borne pathogen *R. rickettsii* was considered the only pathogenic agent for humans on the American continent, nowadays several other agents have been identified (Parola et al. 2009). Results obtained in this research demonstrate that the brown dog tick *R. sanguineus* was the most prevalent tick capable of transmitting TBRD in Tamaulipas. These results are similar to other results from Mexico (Sosa-Gutierrez et al. 2016; Cruz-Vazquez and Garcia-Vazquez 1999; Galaviz-Silva et al.2013); however, another study performed in the southern Yucatan peninsula found that the most prevalent tick genus for that region of Mexico was *Amblyomma* (Arana-Guardia et al. 2015). The two most prevalent tick genera from Tamaulipas found in this study were *Rhipicephalus and Amblyomma.* Both genera are important vectors of TBRD (Dantas-Torres 2008; Nunes et al. 2015).

Results obtained from the sequenced amplicons suggest that *Rickettsia amblyommatis* (formerly *Candidatus* R. amblyommii) is infecting *A. mixtum.* The pathogenicity of this

Table 3 Tick samples positive for *Rickettsia* spp.

PCR product number	Tick species	Host	Municipality	Identity %	GenBank	*Rickettsia* species
1	*Amblyomma mixtum*	Dog	Soto la Marina	97	DQ517291	*Ca.* Rickettsia amblyommii
2	*A. mixtum*	Human	Aldama	99	AY375162	*Ca.* Rickettsia amblyommii
3	*A. mixtum*	Bovine	Aldama	99	DQ517291	*Ca.* Rickettsia amblyommii
4	*A. mixtum*	Dog	Aldama	95	LAOC01000001	*R. rhipicephali*
5	*A. mixtum*	Dog	Aldama	100	LANR01000001	*R. amblyommatis*
6	*Rhipicephalus sanguineus*	Dog	Aldama	100	LANR1000001	*R. amblyommatis*

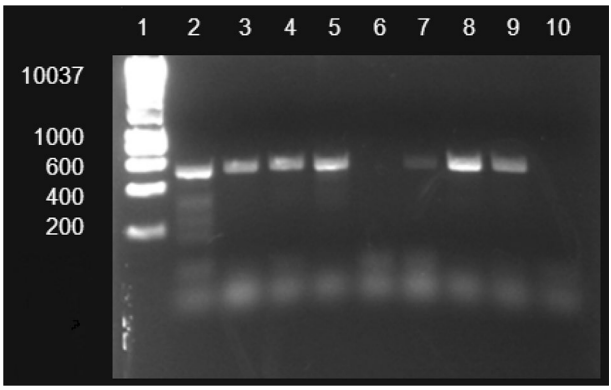

Fig. 1 Purified positive-PCR products on a 1% agarose gel stained with Diamond Nucleic Acid Dye. Lane 1, molecular weight marker; lane 2, positive control; lanes 3–5 and 7–9, positive samples; lanes 6 and 10, negative control (double distilled water)

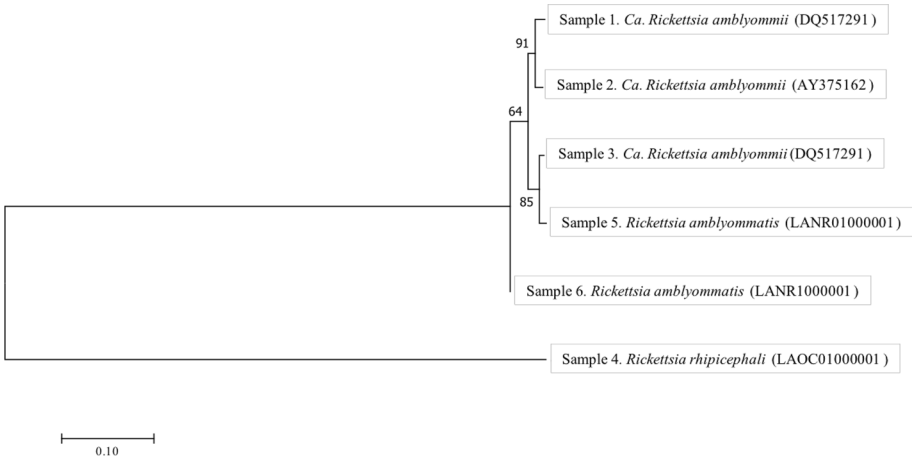

Fig. 2 Phylogenetic relationship of the six rickettsia sequences found. The evolutionary history was inferred using a nucleotide substitution Tamura and the evolutionary distances were computed using the maximun-composite-likelihood method. Bootstrap values for 1000 replicates are displayed next to the branches

organism is still unknown and represents a risk for human health (Apperson et al. 2008; Costa et al. 2017). These results are similar to those found in other research (Sánchez-Montes et al. 2016); nevertheless, the location of these findings is of high importance because Tamaulipas is a transboundary region and the epidemiology of this pathogen indicates that *A. mixtum* may play an important role in the cycle of *R. amblyommatis* (Esteve-Gassent et al. 2014; Costa et al. 2017). Moreover, in the USA the geographical range of some species is constantly expanding. This expansion coincides with a rise in human cases of spotted fever group (SFG) rickettsiosis, making it important to anticipate the appearance of new cases in this area (Harris et al. 2017).

Another interesting result of the sequencing was that an amplified segment of a tick obtained from a dog coming from Aldama had a 95% of homology with *R. rhipicephali.* Described for the first time in 1975 in the brown dog tick, *R. sanguineus,* this pathogen has been subsequently found in other tick species like *Dermacentor occidentalis*, *D. andersoni* and *D. variabilis* across the USA, in particular the western part of the country (Wikswo et al. 2008). The findings of this research suggest that *R. rhipicephali* could generate a cross-reactive immune response with *R.rickettsii* antigens and thus serve as a natural protective antigen (Zeringóta et al. 2016; Cage and Jerrells 1992) or it may act by interfering with the acquisition of another *Rickettsia* as was proposed for by Burgdorfer et al. (1981) and Niebylski et al. (1997) for *R. peacockii* in the tick *D. andersoni*.

Conclusions

Our study provides the first molecular data on the presence and species identification of SFG rickettsiae in the Mexico-USA transboundary region. This information represents a great opportunity to understand the epidemiology of rickettsial pathogens in this region and it is necessary for the adequate diagnostic and prevention of rickettsial diseases. The tick *A. mixtum* resulted as the species with most rickettsial pathogens. As this tick may infest several hosts, it represents a high risk for domestic and wild animals and also for humans. Although results obtained in this research represent an important step in the characterization of tick-borne pathogens in the transboundary region it is necessary to increase the information of the vector-host–pathogen interaction in order to improve the control of tick-borne diseases.

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