Rickettsioses and other Tick-borne Diseases across the Border: Regional Focus in Chihuahua

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Abstract. Rocky Mountain spotted fever is of public importance from clinical and veterinary perspectives. During the last decade, cases have increased throughout Mexico, along with other tick-borne diseases. The number of cases reported has increased in northern states of the country that border the United States of America. Studies to understand the current state of affairs with the tick vector and identification of etiological agents also have increased. From the six border states, two in the northwest and central region are of special interest because of the close proximity of the populations from both regions -- Baja California (Tijuana-San Diego and Mexicali-Calexico) and Chihuahua (Ciudad Juárez-El Paso). However, not much information is available on the current situation of Rocky Mountain spotted fever and other rickettsiosis in the State of Chihuahua. Because of increased clinical cases reported, where Chihuahua was the State with the second most number of cases nationwide in 2020, the purpose of this study was to identify methods of detection and vector-collection strategies in the region.

Introduction

Tick-borne diseases are of clinical and veterinary importance in Mexico, especially Rocky Mountain spotted fever caused by *Rickettsia rickettsii*, a neglected disease important to public health. Despite Rocky Mountain spotted fever being a disease requiring immediate follow-up by the National Directorate of Epidemiology, with a total of 449 cases reported nationwide in 2019, the State of Chihuahua reported most cases, with 98 confirmed, of which 39 were caused by Rocky Mountain spotted fever and 59 by other rickettsiosis (Direccion General de Epidemiología 2019). In 2018, Chihuahua was second in the number of cases of rickettsiosis nationwide, behind the western neighboring State of Sonora with 77 cases, according to the state laboratory of Public Health. Despite multi-institutional efforts to prevent tick-borne diseases, emergence of Rocky Mountain spotted fever and other tick-borne diseases in different municipalities across the State has been a challenge since the first two

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confirmed cases were reported in 2013. Little is known of the current prevalence of ticks or identification of other pathogens related to vector-borne diseases such as *Anaplasma phagocytophilum* (Foggie), other *Rickettsia* spp., and *Borrelia burgdorferi* (Johnson). Literature on diagnostic tests approved by the World Health Organization (WHO) and Centers for Disease Control and Prevention (CDC) for tick-borne pathogens worldwide will not be reviewed here, but is available from Luce-Fedrow et al. (2015), Portillo et al. (2017), and Yazid et al. (2018).

The geographical location of the State that borders New Mexico and Texas in the US, and is the second largest binational metropolitan area at the US-Mexico border called el "Paso del Norte", emphasizes the importance of research on ticks and identification of etiological agents of clinical and veterinary importance in the region. Proper detection is highlighted by the overwhelming amount of work at the centralized National Institute of Epidemiological Reference (INDRE, in Spanish) and state-run laboratories. According to the National Center for Disease Prevention and Control (CENAPRECE, in Spanish) through the Program of Prevention and Control of Rickettsiosis, by November 2019 only 16% of probable cases of rickettsiosis were confirmed by a laboratory, of a total of 1,804 or 64% of 2,786 probable cases. Chihuahua was the State with most deaths, a total of 21. It is important to consider no official acknowledgment or updated knowledge of prevalence of other diseases similar to rickettsiosis with relevance in public health, such as Lyme disease, ehrlichiosis, and anaplasmosis. We reviewed current information on tick-borne disease in the State of Chihuahua in northern Mexico, specifically geographical distribution, and reported infections by ticks and diagnostic methods used.

Materials and Methods

For current information on Rocky Mountain spotted fever and other tick-borne diseases, we referred to PubMed and SciELO (for articles in Spanish) and searched "Chihuahua Rocky Mountain spotted fever", "Chihuahua ticks", "Chihuahua rickettsiosis", "Chihuahua tick-borne diseases", "Rocky mountain spotted fever in Chihuahua", "tick-borne disease(s) Mexico", "ticks in Chihuahua", and "rickettsiosis Mexico". The search of literature focused on tick-borne diseases in Mexico (Fig. 1), with a total of 373 articles from PubMed. We excluded 353 publications not related to the topic, leaving 20 full-text publications. Another publication from SciELO, two publications from bibliographies, three review papers from PubMed, one website, and seven historical papers not in PubMed also were included.

Results

Taxonomic Identification. Since the early 20th Century, researchers like Hoffman (1925), Bustamante and Varela (1943), and Tovar (1944, 1945) began to report tick-borne diseases in Mexico where all effort was focused on rickettsiosis. One of most significant works for identification of ticks from North America was by Bishopp and Trembley (1945). Ticks identified in northern Mexico were *Amblyomma americanum* (Linnaeus), *A. cajennense* (Fabricius), *A. dissimile* (Koch), and *A. maculatum* (Koch), especially abundant in states bordering the Gulf of Mexico. *Dermacentor albipictus* (Packard) was found in northern Mexico and *D. andersoni* (Stiles) was identified in Arizona and New Mexico. *D. nitens* was abundant on the east coast of Mexico.

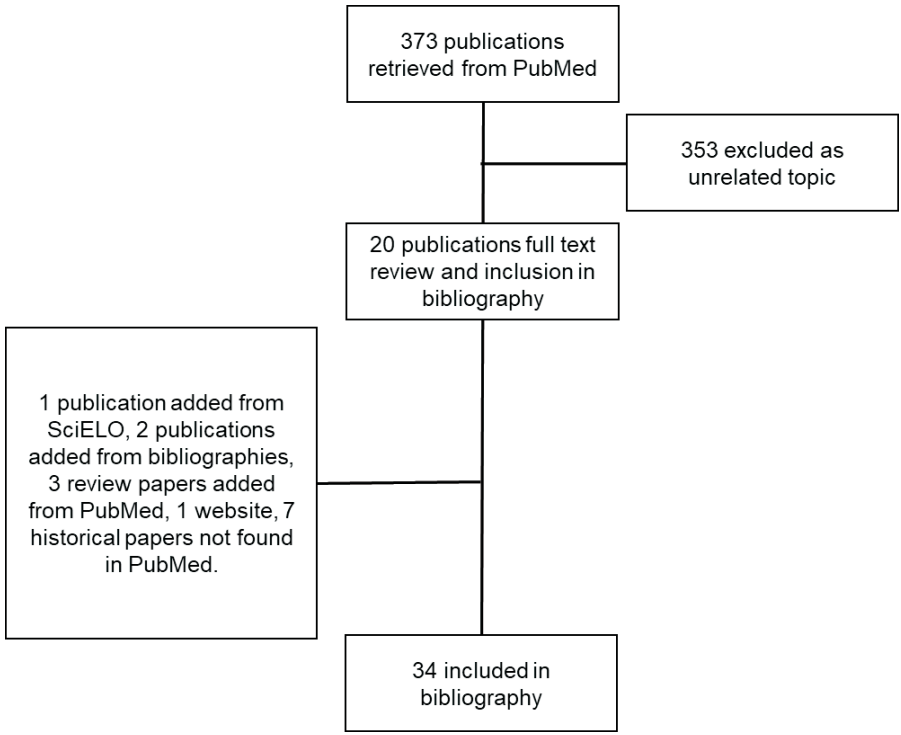

Fig. 1. Selection process for literature on "tick-borne disease(s) Mexico, Rocky Mountain spotted fever, rickettsiosis Mexico, Chihuahua Rocky Mountain spotted fever, Chihuahua ticks, Chihuahua rickettsiosis, Chihuahua tick-borne diseases" focused on Rocky Mountain spotted fever and other rickettsiosis in the State of Chihuahua, North Mexico.

The presence of *Rhipicephalus sanguineus* (Latreille) in South Texas also was highlighted. In the State of Chihuahua, reports by Chavarria (1941) identified *D. andersoni*, but the information still is not certain. Entomological surveys to collect ticks and report prevalence in Chihuahua are becoming more important. Recently, *D. parumapertus* (Neumann), *Ixodes hearlei* (Gregson), *I. kingi* (Bishopp), *Ornithodoros* sp., and *R. sanguineus* s.l. were reported (López-Pérez et al. 2019).

Rocky Mountain Spotted Fever in North Mexico. Refer to Table 1 and Figs. 2-3 for a summary. Rocky Mountain spotted fever is a potentially fatal disease caused by *Rickettsia rickettsii* (Brumpt), an intracellular obligate bacterium transmitted to humans by ticks (Harrell 1949). In the past decade, studies on the prevalence of *R. rickettsii* in dogs, *Canis lupus familiaris* (Linnaeus), and ticks were reported for Baja California (Tinoco-Gracia et al. 2009). In 2009, there was an outbreak of Rocky Mountain spotted fever among residents who reported *R. sanguineus* and tick bites at a community in Mexicali, Baja California, Mexico.

Table 1. Rocky Mountain Spotted Fever and Other Rickettsiosis Analysis by Sample and Diagnostic Test. Colors and numbers indicate location in Fig. 2.

Sample	Geographic area	Diagnostic test	(Author), color/ number, Fig. 2
Tick	Janos Biosphere, Chihuahua, Mexico	Samples analyzed using PCR amplification gltA gene for *R. rickettsii* and flaB for *Borrelia* spp.	(López-Pérez et al. 2019) (Green, 1)
Dog	Cochise, Santa Cruz, Yuma, Arizona, USA	Serum samples tested by IFA.	(Yaglom et al. 2018) (Red, 1)
Dog	Imperial County, California, USA	Blood samples tested using IFA to detect *R. rickettsii*, *E. canis*, and *A. phagocytophilum*.	(Estrada et al. 2019) (Red, 2)
Dog and tick	Baja California, Coahuila, Sonora, Mexico	IFA in serum samples from dogs and RT-PCR targeting ompA in ticks.	(Pieracci et al. 2019) (Red, 3)
Human adult	Ensenada, Baja California, Mexico	Blood samples to detect antibodies against *R. rickettsii* measured with *R. rickettsii* ELISA in dogs, adapted to humans with anti-human IgG conjugate.	(Field-Cortazares et al. 2015) (Blue, 1)
Human adult (veterinary worker)	Ciudad Juarez, Chihuahua, Mexico	Blood samples evaluated using IFA and PCR targeting 16S rRNA to detect *R. rickettsii, Ehrlichia* spp., and *A. phagocytophilum*.	(Escárcega-Ávila et al. 2019) (Blue, 2)
Tick	South of Coahuila, Mexico	PCR using 23S-5S rRNA intergenic spacer and ompA *R. rickettsii.*	(Ortega-Morales et al. 2019) (Green, 2)
Pregnant woman	Hermosillo, Sonora	Confirmatory diagnoses made by PCR targeting gltA for *R. rickettsii* or serological reactivity by IFA.	(Licona-Enriquez et al. 2017) (Blue, 4)
Human adult	Hermosillo, Sonora	Blood samples analyzed by PCR targeting gltA gene or IFA to detect *R. rickettsia*	(Delgado-De la Mora et al. 2018) (Blue, 5)
Child	Hermosillo, Sonora, Mexico	Diagnosis of RMSF by IFA.	(Alvarez-Hernandez et al. 2015) (Blue, 3)
Tick	Calexico and El Centro, California, USA	RT-PCR targeting rOmpA gene from *R. rickettsii*.	(Fritz et al. 2012) (Red, 2)
Tick	Mexicali, Mexico	Sequencing 12S mitochondrial rRNA on DNA from ticks. *R. rickettsii* genetic typing of intergenic fragments RR0155-rpmB, cspA- ksgA, and RR1240-tlc5.	(Eremeeva et al. 2011) (Green, 3)
Tick and dog	Ciudad Juarez, Chihuahua, Mexico	Blood samples tested by PCR aimed at 16S rRNA gene for *Ehrlichia* spp. and *A. phagocytophilum*.	(Escárcega Ávila et al. 2018) (Red, 4)

Tick	Chihuahua City, Mexico	Samples tested using PCR.	(Prado et al. 2018) (Green, 6)
Horse and tick	Ciudad Juarez, Chihuahua, Mexico	Blood samples from horses and soft ticks analyzed by endpoint or nested PCR.	(Medrano-Bugarini et al. 2019) (Black, 1)
Human adult	Coahuila, Nuevo Leon, Tamaulipas, Mexico	Serum samples analyzed by ELISA to detect antibodies against *B. burgdorferi* and Western Blot to confirm.	(Gordillo-Pérez et al. 2003) (Blue, 6)
Tick	Texas, USA; Tamaulipas, Nuevo Leon, and Coahuila, Mexico	Samples tested using PCR amplification of 16SrRNA-23SrRNA to detect *B. burgdorferi.*	(Feria-Arroyo et al. 2014) (Green, 7)
White-tailed deer and coyote	South Texas	TickPath Layerplex qPCR targeting 23S/5S rRNA gene for *Borrelia*; 16SrRNA for *Rickettsia* and *Ehrlichia*; rrs gene for *Anaplasma*, 18SrRNA for *Babesia*, and 18SrRNA for *Theileria*.	(Yu et al. 2020) (Black, 2)
Human adult and tick	Allende and Linares, Nuevo Leon, and Mexico	Serum samples from humans tested using IFA for IgG antibodies against *Rickettsia prowazekii*, *R. typhi*, and *R. parkeri*. Ticks analyzed by PCR amplification of 17-kDa gene.	(Medina-Sanchez et al. 2005) (Blue, 7)

Fig. 2. Geographic representation of study areas with tick-borne pathogens at the Mexico-US border between 2003 and 2019. Legend: Color code by type of study. Green triangles and asterisk (*) indicate studies of ticks. Blue triangles, studies with blood human samples. Red triangles indicate studies with dog blood samples. Black triangles indicate studies of wild animals and horses. Maps were from Google Maps.

Fig. 3. Most common diagnostic tests used on blood samples from dogs/wild animals (orange circle), humans (blue circle), and ticks (green circle). All genes are used to detect *R. rickettsia* except flab and 16SrRNA-23SrRNA used for *Borrelia burgdorferi.*

A later study determined the prevalence of vector ticks as a possible cause of outbreak on 45 dogs at the Calexico shelter and 71 dogs at El Centro, CA. In total, 200 ticks were collected among the dogs. All the ticks were morphologically identified as *R. sanguineus*. All were tested by real-time PCR targeting the 154-bp fragment of the ompA gene from *R. rickettsii*, but none was detected (Fritz et al. 2012).

In contrast, a previous study at Mexicali collected 96 adult ticks on 11 stray dogs in a neighborhood where one patient was diagnosed with Rocky Mountain spotted fever. The 12S mitochondrial rRNA was sequenced from DNA from ticks, and *R. rickettsii* was detected by genetic typing of intergenic fragments RR0155-rpmB, cspA-ksgA, and RR1240-tlc5. Thirty of 96 ticks (31%) were positive for *R. rickettsii* (Eremeeva et al. 2011). This was one of the first studies to show *R. rickettsii* in ticks where Rocky Mountain spotted fever had been reported. Between 2016 and 2017, seroprevalence was surveyed in 752 dogs from Imperial County, a US county

that borders Mexicali, Mexico. Blood samples were collected and tested using immunofluorescence antibody assay (IFA) to detect *R. rickettsii*, *Ehrlichia canis* (Moshkovski), and *A. phagocytophilum*. Total prevalence was 12% for *R. rickettsia* in dogs (Estrada et al. 2019). To the west during the same period, animals in shelters and rabies clinics in three southern Arizona counties (Cochise, Santa Cruz, and Yuma) were studied near the Mexican border with the State of Sonora. In total, serum from 217 owned and stray dogs was tested by IFA and showed total prevalence of 5% for spotted fever group rickettsia (SFGR) IgG antibodies, with seropositivity ranging from 3 to 12% across the counties (Yaglom et al. 2018). Later studies found similar prevalence among dogs from Arizona and Baja California. In Mexico, one study of ticks from five municipalities in the State of Coahuila confirmed *R. rickettsii* in engorged female ticks from 253 free-roaming dogs. In total, 1,238 ticks were morphologically identified as *R. sanguineus*, of which 86% were engorged females and 14% engorged nymphs. The ticks were put into 30 groups, their DNA was purified, and conventional PCR assays were done using 23S-5S rRNA intergenic spacer and the outer membrane protein (ompA) gene of *R. rickettsii*. The six groups (each with six females) were positive for *R. rickettsii* DNA, with minimum infection of 3% (Ortega-Morales et al. 2019). In another study at Baja California, Coahuila, and Sonora, seroprevalence of *R. rickettsii* was evaluated in 1,136 serum samples from dogs and 942 ticks (413 adult females, 313 adult males, 215 nymphs, and one larva morphologically identified as *R. sanguineus* s.l.*)* using IFA antibody assays in dogs and real-time PCR assays targeting the first ompA gene in ticks. In total, 6% (69 dogs) had antibodies to *R. rickettsii*, with most in Baja California (12%), followed by Coahuila (4%), and then by Sonora (4%). Positive samples on ticks were sequenced using assays for genus-specific *Rickettsia*, showing evidence of *R. massiliae* (Beati and Raoult), *R. parkeri* (Lackman), and *R. rickettsii* by ompA PCR with further sequencing (Pieracci et al. 2019).

Most studies (three of six) of rickettsiosis in humans were from the State of Sonora (Table 1). Analysis of 104 children diagnosed with Rocky Mountain spotted fever by IFA at a hospital at Sonora, indicated 20% died (Alvarez-Hernandez et al. 2015). During 2015 and 2016, four pregnant women were infected by Rocky Mountain spotted fever. Diagnoses were confirmed by PCR targeting a conserved fragment of 805 bp of the citrate synthase (gltA) gene for *R. rickettsii* or by serological reactivity to *R. rickettsii* antigens by IFA (Licona-Enriquez et al. 2017). Blood samples analyzed by PCR targeting the gltA gene or IFA in 47 human deaths from 2013 to 2016 showed all were by *R. rickettsii* (Delgado-De la Mora et al. 2018). At Ensenada, Baja California, a cross-sectional study between 2009 and 2011 collected 384 human blood samples from patients older than 1 year. Antibodies against *R. rickettsii* were measured in samples using *R. rickettsia* ELISA in dogs but adapted to humans with an anti-human IgG conjugate. Sensitivity and specificity were determined using IFA in 32 samples. Results showed adjusted seroprevalence of 4% of rickettsiosis for *R. rickettsii* not associated with the sex, age, or occupation of humans or mobility of dogs between the exterior and interior of houses according to seropositivity (Field-Cortazares et al. 2015). Four years later, two more studies were published: one at Ciudad Juarez, Chihuahua, that evaluated blood samples from 106 veterinarians, 36 veterinary assistants, 19 pet groomers, and six veterinary administrative workers by IFA and PCR targeting 16S rRNA to detect *R. rickettsii, Ehrlichia* spp., and *A. phagocytophilum*. Results showed 54% of the participants were seropositive for at least one of the pathogens. The percentage of coinfection with two or three pathogens was remarkable. In total, 3% had co-exposure to *R. rickettsii* and *A.*

phagocytophilum, 9% to *Ehrlichia* spp. and *A. phagocytophilum*, 2% to *Ehrlichia* spp. and *R. rickettsii*, and 2% were positive for all three pathogens. Veterinarians and pet groomers were at great risk of being infected with *R. rickettsii* (Escárcega-Ávila et al. 2019).

Other Tick-borne Diseases. Other tick-borne diseases were reported recently in the region. Etiological agents were reported for Lyme disease, anaplasmosis, ehrlichiosis, and other rickettsiosis detected in humans, ticks, dogs, or wild animals. Most findings were reported from northeastern states, in contrast to the few publications from the northwestern region.

Researchers of a cross-sectional descriptive study at Mexico City, Coahuila, Nuevo León, and Tamaulipas between 1987 and 1988 used ELISA to test 2,346 human serum samples to detect antibodies against *B. burgdorferi*, and Western Blot to confirm positive samples. Twelve percent of the samples were positive by ELISA, and 122 of the samples were confirmed by Western Blot. Seroprevalence by region was 3% for Mexico City and 6% for the northeastern area of the country (Gordillo-Pérez et al. 2003). A decade later, analysis focused on Texas and partly the states of Tamaulipas, Nuevo León, and Coahuila where 1,235 samples of ticks were collected from vertebrate hosts. In total, 109 (9%) of ticks were identified as *Ixodes scapularis* (Say). *B. burgdorferi* was detected by PCR targeting 16SrRNA-23SrRNA and was in 45% of the ticks (Feria-Arroyo et al. 2014). In counties from South Texas near the Mexican border where the purpose was to know molecular prevalence of tick-borne pathogens, 245 white-tailed deer, *Odocoileus virginianus* (Zimmermann), and 122 coyotes, *Canis latrans* (Say), were analyzed. Whole-blood samples were evaluated by TickPath Layerplex qPCR test, targeting multiple genes for *Borrelia*, *Rickettsia*, *Ehrlichia*, *Anaplasma*, *Babesia*, and *Theileria* genera. Coyotes had total prevalence of 9% for *Babesia vogeli* (Reichenow) and 0.8% for *Borrelia turicatae* (Brumpt), while white-tailed deer had total prevalence of 0.4% for *Anaplasma platys* (Dumler), 1.6% for *Ehrlichia chaffeensis* (Anderson), and 7% for *Theileria cervi* (Denier). The study indicated wild animals could be sentinels for a number of zoonotic tick-borne pathogens (Yu et al. 2020).

The approach has been used in other regions of Mexico, in the Yucatan Peninsula where overall prevalence of tick-borne rickettsial agents was 20% in white-tailed deer and 50% in *Mazama* sp. (Ojeda-Chi et al. 2019). Some tick-borne diseases can be confused with each other. In the State of Nuevo Leon in 2001, the Public Health Department collected sera from 345 human febrile patients at Allende and Linares that were suspected to have dengue fever. IFAs were used for IgG antibodies against *Rickettsia prowazekii* (da Rocha-Lima), *R. typhi* (Wolbach and Todd), and *R. parkeri* (Lackman). In total, 25% had antibodies reactive with typhus group rickettsiae and 16% against *R. parkeri*. Also, 190 ticks (*A. cajennense* and *A. imitator* (Kohls)) from livestock and tested in groups showed 50% were positive for *R. prowazekii* (Medina-Sanchez et al. 2005). One of the most ambitious analyses of tick-borne pathogens in Mexico to date was of samples collected between 1997 and 2013. About 1,107 ticks were collected from a range of mammalian hosts in forests and ecotourism parks in 22 of 32 states of the Republic of Mexico. *R. sanguineus* was collected most frequently (43%). PCR was used on the 16S rRNA gene for *A. phagocytophilum*, *E. chaffeensis*, and *E. canis*; gltA and 17 kDa protein-encoding genes for *Rickettsia* spp. were used to assay 477 groups of ticks. *A. phagocytophilum* was the most common pathogen followed by *E. canis* in 45 and 42 groups evaluated. *R. rickettsii*, *E. chaffeensis*, and *Ca. R. amblyommii* also were found in 4.2% of the total evaluated (Sosa-Gutierrez et al. 2016).

Tick-borne Diseases and Ticks at Chihuahua. At Chihuahua, in the "Paso del Norte" region, *Ehrlichia* spp. and *A. phagocytophilum* were reported in 30 dogs from Ciudad Juarez, Chihuahua infested by at least five female engorged *R. sanguineus*. In the study, blood samples from dogs and ticks were tested using PCR by amplification of the 16S rRNA gene for *Ehrlichia* spp. and *A. phagocytophilum*. *Ehrlichia* spp. was found in 40% of dogs and 66% of ticks. *Anaplasma* spp. was detected in 27% of dogs and 66% of ticks (Escárcega Ávila et al. 2018). Results might suggest more prevalence of bacteria in the ticks collected. However, follow-up studies need to clarify this contrast with reports from other regions.

At the city of Chihuahua (capital of the State) between August and November 2015, 664 ticks were extracted from 99 dogs in neighborhoods with reported clinical cases of Rocky Mountain spotted fever. Results showed 99.5% of the ticks were *R. sanguineus*. Ticks were grouped and evaluated by PCR. All were negative for *R. rickettsii* ompA, but two were positive for *A. phagocytophilum*, for total prevalence of 7% of the grouped samples tested (Prado et al. 2018). To date, this has been the only report of another tick-borne disease bacteria related to Rocky Mountain spotted fever from endemic neighborhoods in the State of Chihuahua. Recently, a study at Janos Biosphere Reserve in northwest Chihuahua collected 45 hard and soft ticks from wild animals. All were tested using PCR amplification of the gltA gene for *R. rickettsii* and a fragment of 470 bp of the flagellin (flaB) gene for *Borrelia* spp. Results showed *B. burgdorferi s.s*. in an *I. kingi* collected from a kit fox, *Vulpes macrotis* (Merriam), and *R. massiliae* was in 6.5% of 31 *R. sanguineus* from free-roaming dogs (López-Pérez et al. 2019). At Ciudad Juarez, a study with 144 blood samples from horses and soft ticks on them were analyzed by endpoint or nested PCR. Total prevalence of *Babesia caballi* was 2.8% in horse blood and 6% in soft ticks (Nuttall and Strickland). One sample tested positive for *A. phagocytophilum*, indicating prevalence of 0.8% in horse blood (Medrano-Bugarini et al. 2019).

In conclusion, despite efforts focused on molecular diagnosis of tick-borne disease in the State of Chihuahua, it is fundamental to develop strategies according to tools available by human and financial resources that could impact the prevalence and mortality of the exposed population in endemic counties. For example, in the neighboring State of Sonora, it was possible to decrease the prevalence of tick-infested dogs from 32.5 to 8.8% by using long-acting acaricidal collars, applying acaricides, and educating people about Rocky Mountain spotted fever (Alvarez-Hernandez et al. 2019). It is important to maintain ongoing collection and taxonomic identification of ticks throughout the State, which will aid in understanding current cases of Rocky Mountain spotted fever. To date (47th week of epidemiological surveillance in 2020), 34 cases of FMMR and other rickettsiosis have been reported, and Chihuahua is the second state with the most cases nationwide. Intersectoral participation of Public Health Departments at national and state levels and participation of the two public Autonomous Universities in the State are encouraged to address the diseases.

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