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Seroprevalence of spotted fever group rickettsiae in canines along the United States–Mexico border

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Abstract

Portions of northern Mexico are experiencing a re-emergence of Rocky Mountain spotted fever (RMSF), a tickborne disease caused by *Rickettsia rickettsii*, a member of the spotted fever group of rickettsiae (SFGR). Infection with *R. rickettsii* can result in serious and life-threatening illness in people and dogs. Canine seroprevalence has been used as a sentinel for human RMSF in previous studies. This study aims to quantify SFGR seroprevalence in canines in three northern Mexican states and identify risk factors associated with seropositivity. A total of 1,136 serum samples and 942 ticks were obtained from dogs participating in government sterilization campaigns and from animal control facilities in 14 Mexican cities in three states. SFGR antibodies

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

DISCLOSURE

The findings and conclusions are those of the authors and do not necessarily reflect the views of the U.S. Centers for Disease Control and Prevention.

were detected using indirect immunofluorescence antibody assays at titre values ≥1/64. Six per cent (69 dogs) showed antibodies to SFGR, with the highest seroprevalence reported in Baja California (12%), Coahuila (4%) and Sonora (4%). Dogs from Baja California had three times higher odds of having SFGR antibodies compared to dogs from Sonora (OR = 3.38, 95% CI, 1.81–6.37). Roughly one quarter (25%) of surveyed dogs were parasitized by ticks (*Rhipicephalus sanguineus* sensu lato) at the time of sample collection. A portion of collected ticks were tested for rickettsial DNA using polymerase chain reaction. Positive samples were then sequenced, showing evidence of SFGR including *R. massiliae*, *R. parkeri* and *R. rickettsii*. Dogs that spent the majority of time on the street, such as free-roaming or community-owned dogs, showed a greater risk of tick infestation, seropositivity, bearing seropositive ticks, and may play a pivotal role in the spread of SFGR among communities. Estimating the seroprevalence of SFGR in the canine population can help public health campaigns target high-risk communities for interventions to reduce human RMSF cases.

Keywords

canine; canine SFGR seroprevalence; Mexico; RMSF; Rocky Mountain spotted fever

1 | INTRODUCTION

Spotted fever group rickettsiae (SFGR) are gram-negative, intracellular bacteria of the genus *Rickettsia* (Order Rickettsiales: Family Rickettsiaceae) that are usually transmitted via the bite of an infected arthropod. The most severe rickettsial disease in North America is Rocky Mountain spotted fever (RMSF), caused by infection with the bacteria *Rickettsia rickettsii* (Leighton, Artsob, Chu, & Olson, 2001; Paddock et al., 2008; Parola, Paddock, & Raoult, 2005; Piranda et al., 2008; Wachter et al., 2015). RMSF is a potentially life-threatening disease that can cause systemic vasculitis leading to organ failure and death if left untreated (Alvarez-Hernandez, Murillo-Benitez, Candia-Plata Mdel, & Moro, 2015; Hattwick et al., 1978; Warner & Marsh, 2002). RMSF can infect humans and wild and domestic animals, including dogs. Clinical disease in humans and dogs is similar (Alvarez-Hernandez et al., 2015; Keenan et al., 1977; Warner & Marsh, 2002; Yancey et al., 2014) including fever, abdominal pain, myalgia, petechial rash and renal failure. Over the last decade, RMSF has been responsible for hundreds of human deaths in Mexico and the United States (Alvarez Hernandez et al., 2017; Alvarez-Hernandez, 2010; Alvarez-Hernandez et al., 2015; Dahlgren, Holman, Paddock, Callinan, & McQuiston, 2012; Drexler et al., 2016; Field-Cortazares, Escarcega-Avila, Lopez-Valencia, Barreras-Serrano, & Tinoco-Gracia, 2015; Morano & Mendez, 2010; Regan et al., 2015; Tinoco-Gracia et al., 2009).

In Mexico, RMSF is considered a re-emerging disease (Alvarez Hernandez et al., 2017; Alvarez-Hernandez, 2010; Drexler et al., 2016; Eremeeva et al., 2011). Outbreaks were recognized in northern Mexico during the 1940s and were associated with the brown dog tick, *Rhipicephalus sanguineus* sensu lato (Alvarez Hernandez et al., 2017). Baja California, Coahuila and Sonora are states located in northern Mexico along the United States–Mexico border. All of these states have reported an increasing number of RMSF cases in humans within the past 10 years likely due to both increasing surveillance and re-emergence of the

disease (Alvarez Hernandez & Contreras Soto, 2013; Alvarez Hernandez et al., 2017; Alvarez-Hernandez, 2010; Alvarez-Hernandez et al., 2015; Field-Cortazares et al., 2015; Morano & Mendez, 2010; Tinoco-Gracia et al., 2009; Zavala-Castro, Dzul-Rosado, Leon, Walker, & Zavala-Velazquez, 2008). In Mexico, children are the most impacted age group, with up to 67% of cases occurring in patients under 15 years of age (Alvarez Hernandez & Contreras Soto, 2013). This may be in part to their high rates of exposure to dogs who transmit the disease to humans (Alvarez Hernandez et al., 2017). The case fatality rate (CFR) in children from Sonora is as high as 20%, compared to the U.S. CFR for RMSF, which ranges from 5% to 10% (Alvarez-Hernandez et al., 2015). *Rickettsia rickettsii* is transmitted by several species of ticks. In Mexico and the south western U.S. states, the brown dog tick is understood to be one of the primary vectors for RMSF (Demma et al., 2005; Eremeeva et al., 2011; Parola et al., 2005). *Rickettsia rickettsii* has been documented in dogs, *Rh. sanguineus* sensu lato ticks and humans throughout the border region (Alvarez-Hernandez et al., 2015; Demma et al., 2005; Diniz et al., 2010; Eremeeva et al., 2011; Field-Cortazares et al., 2015; McQuiston et al., 2011; Yancey et al., 2014; Zavala-Castro et al., 2008). *Rhipicephalus sanguineus* sensu lato preferentially feeds on dogs in all life stages (Warner & Marsh, 2002), increasing dogs' exposure to ticks and to *R. rickettsii*. Dogs serve as valuable sentinels of risk for *R. rickettsii* infection in people for several reasons: dogs are susceptible to *R. rickettsii*, have higher rates of tick exposure thus increasing risk for disease, develop measurable serologic response to *R. rickettsii* infection (Demma et al., 2006) and can be systematically sampled more easily than humans (McQuiston et al., 2011). Dogs characteristically live in close proximity to people, making them valuable sentinels for human disease risk given their frequent contact with humans. Rates of previous SFGR exposure in these dog populations may be more reflective of the probability of human exposure. Clusters of disease have been reported in defined geographic areas, and temporally associated infections can be seen in dogs and humans (Paddock et al., 2002; Rozental et al., 2014). This interconnected relationship between ticks, dogs and humans ultimately increases human risk of exposure to *R. rickettsii* (Drexler et al., 2014).

The primary objectives of the evaluation were to identify the prevalence of antibodies reactive with SFGR antigens in canines in three northern Mexican states and to identify potential risk factors associated with canine seropositivity.

2 | METHODS

2.1 | Study population

Researchers in 14 cities from the northern Mexican border states of Baja California, Coahuila and Sonora recruited domestic dogs through government sponsored sterilization clinics and animal control facilities (Figure 1). The 14 cities were selected by the State Health Departments' Zoonosis Program in each of the three Mexican states, the Centro Nacional de Servicios de Constatación en Salud Animal, the Centro Nacional de Programas Preventivos y Control de Enfermedades, Secretaria de Salud and the U.S. Centers for Disease Control and Prevention due to their proximity to the U.S. border, interest from states and availability of sterilization clinics within the cities. At sterilization clinics, owners were asked to volunteer their dogs for the study. For inclusion in the study, dogs had to weigh a

minimum of 3 kilograms and be at least 6 months of age. The general health status of the dogs, such as mentation, lameness, body condition score, coat appearance, skin lesions and external parasites were assessed during the intake examination by local veterinary staff prior to sterilization and enrolment in the study.

2.2 | Sample collection

Prior to sterilization surgery, or during the time of stay at the animal control facility, five millilitres (5 ml) of blood was drawn from the cephalic or lateral saphenous vein of the dogs, allowed to clot and serum separated by centrifugation. Owners completed a questionnaire with demographic information (age, sex) for their dog and additional questions on where the dog spent time (location) and from where the dog originated (origin). Upon arrival, dogs were visually inspected for tick infestation. Engorged and non-engorged ticks were removed from infested dogs using fine-tipped forceps and stored in vials containing 70% ethanol until assayed. All ticks found by the researchers were removed from dogs. Ticks removed from an individual dog were labelled with the dog's unique identifier. Up to 10 ticks were tested from at least five dogs at each site by convenience sampling. A total of fifty ticks from each state were selected for PCR analysis (convenience sample).

Serum samples were tested by indirect immunofluorescence antibody (IFA) assays using *R. rickettsii* whole-cell antigen as described previously (Kato et al., 2013; McQuiston et al., 2011). The assay detects IgG-specific antibodies reactive with *R. rickettsii* and other spotted fever group rickettsiae. Samples were screened at dilutions of 1/16 and 1/32 (McQuiston et al., 2011). Collected blood samples were centrifuged and serum aliquoted in tubes for storage. The antigen of *Rickettsia rickettsii* (yolk sac antigen supplied by the CDC Biologics, Division of Scientific Resources, Atlanta, GA) was resuspended in 0.5 ml of water free of nucleases containing 0.01% thimerosal. The antigen was dotted onto the wells of teflon-templated slides and fixed with the use of cold acetone at room temperature for 15 min. Slides were dried at room temperature for the removal of traces of acetone. Dilution of samples was conducted on U-bottomed plates with dilutions of 1/16 and 1/32 used for screening. The dilutions were added to the antigen slides in a volume of 10 μl per well. The slides were incubated at 37°C for 30 min in a humidified chamber. After incubation, the slides were washed three times for 5 min each with PBS. Fluorescein isothiocyanate (FITC) labelled, goat anti-canine IgG, gamma chain-specific (Kirkegaard & Perry Laboratories) conjugate was applied at 1/150 dilution. The slides were incubated at 37°C for 30 min in the humidified chamber and then washed twice with PBS and once with PBS containing counterstain solution (Eriochrome black T). Slides were dried and coverslips mounted with mounting medium. Any samples showing specific rickettsial fluorescence at the screening dilutions were then titrated to endpoint using 2-fold serial dilutions. The slides were observed at 250× and endpoints determined at 400×.

Ticks were taxonomically identified based on morphologic characteristics by species, life stage and sex (adults), and results were recorded by entomologists at Centro Nacional de Servicios de Constatación en Salud Animal (CENAPA), Mexico.

PCR analysis and nucleotide sequencing were also performed at CENAPA. The pooled ticks were homogenized, and DNA was extracted using blackPREP Tick DNA Kit (Analytik jena,

No Cat: 845-BP-3100050 kit). Individual adult or tick pools (5 larvae and/or nymph tick samples all relating to the same dog) were placed in lysing reagent tubes with steel beads and 50 μl of nuclease-free water. Using the MagNA Lyser (Roche Diagnostics), ticks were homogenized at 7,000 rpm for 15 s. After disruption, 300 μl of lysis buffer and 25 μl of Proteinase K were added to the sample, stirred for 5 s and incubated at 95°C for 10 min. The sample was then transferred to a tube with a pre-filter and centrifuged at 10,000 g for 1 min. The supernatant was mixed with 300 μl of binding solution, and the solution was passed to a tube with filter and centrifuged at 10,000 g for 2 min; and the resulting supernatant was discarded. A total of two washes with 500 μl of wash buffer were completed. Finally, the filter was centrifuged at 12,000 g for 2 min to remove remains of ethanol. The sample was eluted in 70 μl of elution buffer. The tick DNA samples were tested for the presence of *Rickettsia* species and *R. rickettsii* by two real-time PCR assays using PanR8 and RRi8 assays, respectively, as described previously (Kato et al., 2013). The samples testing positive for the genus and negative for *R. rickettsii* were amplified using a PCR assay targeting the *ompA* gene and sequenced to identify *R. parkeri*, and *R. massiliae* as referenced in Eremeeva et al. (2006). PCR sequencing amplified oligonucleotides R17-122 and R17-500 using generated fragment lengths of 378 base pairs. Due to pooling techniques, PCR results for adult males, nymphs and larva can only be tied to an individual dog and not to a specific tick.

2.3 | Statistical analysis

Questionnaires and laboratory results were entered into an Epi Info database, version 7.1.5.0 (Centers for Disease Control and Prevention). Analyses were done using STATA, version 13.1 (STATACorp) statistical software. Descriptive statistics are presented as proportions and ranges. Pearson's chi-square test was used to compare categorical data; Fisher's exact test was used when any cell contained fewer than five observations. For analysis, dogs were grouped by age into two categories: <2 years of age and ≥2 years of age. Variables found to be significantly associated on initial univariate analysis were considered for further multivariate analysis. Data were further analyzed by state for associations when sample size was adequate. Sonora was arbitrarily selected as the reference category for all regional comparisons, and differences between states were evaluated when sample sizes were sufficiently large. Geometric mean titres were calculated using log (base 2) transformed titres for samples with endpoint titres 64. Odds ratios (OR) and 95% confidence intervals were calculated when appropriate. All *p*-values are two-sided and were evaluated for statistical significance at the .05 level. The map was produced using Arc GIS.

2.4 | Ethics statement

This protocol was approved by the ethics committee of the Secretary of Agriculture, Cattle Breeding, Rural Development, Fishing, and Food (SAGARPA), Mexico, and the U.S. Centers for Disease Control and Prevention's Institutional Animal Care and Use Committee.

3 | RESULTS

During May–August 2015, 1,136 dogs were enrolled in the evaluation. The majority of dogs enrolled were female (56%) and 2 years of age or greater (53%). Sixty-eight per cent (*n* =

778) of dogs used in this survey were owned, and samples were obtained at sterilization clinics. Nearly half of the owners reported their dogs spent the majority of their time on the patio (the outdoor space around the home) (51%). One or more ticks were present on 278 (25%) dogs. Dogs had a mean tick count of 6.1 ticks (*SD*, ±4.8; range, 0–28). Demographic and epidemiologic information for the dogs is listed in Table 1.

Sixty-nine (6%) dogs had IgG antibodies reactive with SFGR at reciprocal titers of at least 64 (Table 1). The geometric mean titre for SFGR-positive dogs was 130.7 (*SD*). A range of endpoint titres was seen in the dogs that screen positive ($n = 69$), with reciprocal titres of 32 ($n = 29$, 30%), 64 ($n = 13$, 13%), 128 ($n = 23$, 23%), 256 ($n = 1$, 1%), 512 ($n = 20$, 20%) and 1,024 ($n = 12$, 12%) recorded. State of residence was significantly associated with the presence of SFGR antibodies (Table 2). Dogs from Baja California had three times higher odds of having SFGR antibodies compared to dogs from Sonora (OR = 3.38; 95% CI, 1.81–6.38).

Dogs were further analysed using the molecular results of ticks as the dependent variable (Table 3). These results showed that state of residence was found to be associated with positive PCR results; dogs from Baja California had over seven times the odds of having a SFGR-positive tick compared to Sonora (OR = 7.19; 95% CI, 2.58–20.03). Free-roaming dogs were more likely to be infested with SFGR-positive ticks than those who remained near the residence, as dogs that spent the majority of time on the street had over three times the odds of having a SFGR-positive tick compared to dogs that spent the majority of time on the patio (OR = 3.86; 95% CI, 1.22–12.25). A total of 278 dogs had at least one tick: 138 from Coahuila, 99 from Baja California and 41 from Sonora. Out of the 1,136 dogs evaluated, 514 had no ticks. Two hundred twenty-five (36%) dogs had 1–10 ticks, 49 (8%) dogs had 11–20 ticks, and four (0.6%) dogs had over 20 ticks. Three hundred forty-four dogs had no tick information provided on the survey form.

A total of 942 ticks were collected from all three states: 413 adult females, 313 adult males, 215 nymphs and 1 larva. These were all identified as *Rhipicephlaus sanguineus* sensu lato. There were 360 ticks collected from 50 dogs in Baja California, 190 ticks collected from 55 dogs in Sonora and 392 ticks collected from 173 dogs in Coahuila. Many owners reported removing ticks from their dogs prior to attending the clinic which directly impacted our observed tick numbers and the density of ticks per dog. Thus, the calculated infestation rates are conservative estimates. A total of 150 ticks were selected for PCR analysis (50 ticks from each state) by convenience sampling. Tick samples were pooled for testing, and results are not available based on sex and life stage for adult males, nymphs and larva. Thirty-eight (25%) ticks were found to be infected by *Rickettsia* spp. by using the genus-wide real-time PCR assay. One (0.67%) tick was determined to harbour *R. rickettsii* based on results from the species-specific real-time PCR assay.

Sequence analysis of 7 ticks confirmed specific identity: four (3%) were positive for *R. massiliae*, two (1%) were positive for *R. parkeri*, and one (0.66%) was positive for *R. rickettsii*. Six ticks were adult females (stage of engorgement unknown), and one tick was from a pooled sample containing two adult males and three nymphs. All *Rickettsia* spp.-positive ticks were collected from dogs in Baja California, four dogs from Mexicali, 2 from

Ensenada and 1 from Rosarito. The remaining 31 ticks (3 from Sonora, 12 from Coahuila, 16 from Baja California) did not have suitable DNA content to successfully conduct sequencing.

3.1 | State-level analysis

In Baja California, 35 (12%) dogs sampled had antibodies reactive to SFGR. There was variation between the cities in Baja California, 12 of 70 (17%) dogs sampled in Ensenada had titres ≥64 as did 8 (9%) dogs from Tijuana, 6 (21%) from Rosarito, 5 (8%) from Mexicali, 2 (10%) from Ejido Oaxaca and 2 (8%) from Tecate. There were no statistically significant differences in SFGR seroprevalence in dogs or proportion of dogs with SFGR-positive ticks among cities within the state of Baja California.

In Coahuila, 17 (4%) dogs sampled had antibodies reactive to SFGR. Fifteen out of 214 (7%) dogs sampled in Ciudad Acuna had titres ≥64 as did 2 (1%) dogs from Piedras Negras. There were no statistically significant differences in SFGR seroprevalence in dogs or proportion of dogs with SFGR-positive ticks among cities within the state of Coahuila.

In the state of Sonora, 17 (4%) dogs sampled had antibodies reactive to SFGR. Five out of 166 (3%) dogs sampled in Puerto Penasco had titres ≥64 as did 6 (4%) dogs from San Luis Rio Colorado and 6 (11%) from Agua Prieta. There were no statistically significant differences in SFGR seroprevalence in dogs or proportion of dogs with SFGR-positive ticks among cities within the state of Sonora.

In Baja California, 23 (6%) dogs sampled carried ticks that were PCR positive for *Rickettsia* spp.: 13 (57%) ticks were collected from dogs in Ensenada, 5 (22%) from Mexicali, 4 (17%) from Rosarito and 1 (4%) from Ejido Oaxaca. Twelve (3%) dogs in Coahuila carried ticks that were PCR positive for *Rickettsia* spp.: 10 (83%) ticks were from Ciudad Acuna and 2 (17%) from Piedras Negras. Three (2%) dogs in Sonora carried ticks that were PCR positive for *Rickettsia* spp. and all 3 (100%) ticks were from Puerto Penasco. The one tick positive for *R. rickettsii* was collected from a dog in Mexicali, Baja California. The seven *Rickettsia* spp.-positive ticks were collected from six dogs in Baja California.

4 | DISCUSSION

The brown dog tick, *Rhipicephalus sanguineus* sensu lato, was shown to be a vector of *R. rickettsii* in at least four Mexican states in the 1940s, yet the role of this tick in the natural ecology has been largely discounted over the years. Resurgence of infection in the last 10 years has shown that dogs and brown dog ticks contribute to a growing problem in Mexico and the south western United States. SFGR antibodies were present in 6% of dogs in this study. Previous studies have reported antibodies to SFGR in 5.7% of canines in a non-outbreak setting, and rates as high as 77% during a RMSF outbreak in Arizona (Demma et al., 2006; McQuiston et al., 2011). Endpoint titres seen in this study ranged from 32 to 1,024, but previous studies have shown much higher titres (up to 262,144) in outbreak areas (Demma et al., 2006). The geometric mean titre in this evaluation was low, while it may reach higher values in outbreak areas. Studying the seroprevalence of SFGR in dogs can help establish levels of human risk in communities, as SFGR antibodies in canines precede

the first human case reports of RMSF in communities (Demma et al., 2006; Nicholson, Gordon, & Demma, 2006). A recent study by Foley et al. (2019) found seroprevalence rates in dogs as high as 65%; however, the paper by Dr. Foley focuses on Mexicali, a location of hyperendemicity for RMSF in recent years, and they focused on blocks where human cases of RMSF had been found (Foley et al., 2019). Whereas our evaluation sampled from multiple cities in three states and mostly involved owned dogs in good health, we expect lower seropositivity, as seen in other recent, similar studies (Yaglom, Nicholson, Casal, Nieto, & Adams, 2018).

Twelve per cent of dogs sampled from Baja California had antibodies reactive to SFGR compared to 4% of dogs from Sonora and Coahuila. This 3-fold difference highlights a potentially significant difference between the states. Dogs from Baja California also had over seven times higher odds of harbouring *Rickettsia* spp.-positive ticks. This difference may be due to differences present between dog populations in the states, such as total numbers, differences in dog owners who participated in sterilization clinics or factors that impact tick proliferation, such as climate. Although the authors noted interstate variability in this evaluation, intrastate variability was not seen as there were no significant differences between cities within each state.

It is important to note that all three *Rickettsia* species identified in this report (*R. rickettsii*, *R. parkeri* and *R. massiliae*) are known to cause human disease. Their distribution and maintenance in North America are not fully understood and dogs may play a role. To our knowledge, this is one of the first reports of *R. parkeri* and *R. massiliae* in Mexico detected in *Rh. sanguineus* sensu lato; *R. massiliae* had previously been identified across the border in Arizona (Eremeeva et al., 2006). The *R. rickettsii*-positive tick in this study was collected in Mexicali; infected ticks had previously been found from that city (Eremeeva et al., 2011). Due to its proximity to the border, animals in California have been monitored for tick infestation and rickettsial infection (Fritz et al., 2012).

Ticks that live in climates suitable for reproduction, development and infestation, such as that found in northern Mexico, play a critical role in pathogen transmission in both dogs and humans (Nicholson, Allen, McQuiston, Breitschwerdt, & Little, 2010; Nicholson et al., 2006). In experimental studies, *Rh. sanguineus* sensu lato was readily infected by *R. rickettsii* (89%–100% infected) and mortality due to rickettsial infection was not significantly different between infected and uninfected ticks (8%–21% died over the experimental period) (Labruna, Ogrzewalska, Martins, Pinter, & Horta, 2008). Brown dog ticks move among canine hosts during high tick activity, and the interrupted feedings may shorten transmission times to the second host (Little, Hostetler, & Kocan, 2007). Nocturnal detachment of nymphal and adult ticks can concentrate ticks in an area and facilitate contact with new canine hosts and with humans (Dantas-Torres, 2008). It has been shown that the human biting rate by *Rh. sanguineus* sensu lato may be increased with elevated temperature (Parola et al., 2008), and the brown dog tick can survive temperatures and humidities in which other ticks cannot (Yoder, Benoit, Rellinger, & Tank, 2006; Yoder, Bozic, Butch, Rellinger, & Tank, 2006).

In this study, ticks were present on 25% of dogs, and dogs were infested with a mean tick count of 6.1 ticks per dog. McQuiston et al. (2011) found 6.6% of stray dogs in Arizona animal shelters were infested with a mean tick count of 8.5 ticks per dog. Previously, free-roaming dogs have been documented to play a major role in the dissemination of infected ticks and subsequent RMSF infections in humans (McQuiston et al., 2011; Nicholson et al., 2010). The dogs in our study were primarily owned dogs which may account for the lower tick per dog average; however, we discovered that a high proportion of the dogs in our study were cleaned of ticks prior to participating. A greater percentage of dogs in our study were noted to have at least one tick compared to findings in the southern United States (24% vs. 6.6%) which may suggest a higher tick burden among dogs in northern Mexico. A previous study in Mexicali reported the presence of at least one tick on 60% of owned and stray dogs surveyed (Tinoco-Gracia et al., 2009). Through comprehensive canine health programmes that include spay/neuter services and sustained control of free-roaming dog populations (Fritz et al., 2012), it may be possible to decrease the prevalence of highly infested dogs, thus interfering at key points to reduce human exposure to RMSF. Prevention strategies, such as tick control in owned dogs and the environment, have been shown to reduce tick burden in and around homes and decrease cases of RMSF in humans (Drexler et al., 2014; Straily, Drexler, Cruz-Loustaunau, Paddock, & Alvarez-Hernandez, 2016). Due to the high proportion of free-roaming dogs in highly impacted communities, increasing tick prevention in family-owned dogs only will be less impactful than a combined approach. A One Health strategy that emphasizes responsible and sustained control of free-roaming dog populations while simultaneously engaging in tick prevention campaigns for owned and free-roaming dogs and the environment may be the best approach to reduce *Rhipicephalus*-associated RMSF in humans.

There were several limitations in this study. First, the interpretation of canine serologic assays can be difficult because there is cross-reactivity between different SFGR and the aggregate immune response measured by such assays are cumulative over time. Rickettsial agents, such as *R. rhipicephali*, *R. parkeri*, *R. rickettsii* and *R. massiliae*, have been identified in the border region (Eremeeva et al., 2006; Herrick et al., 2016; Sanchez-Montes et al., 2018) and may account for an undetermined portion of the SFGR antibodies present in dogs. Second, the dogs sampled in this study were a convenience sample and may not be representative of the canine population in Mexico. The majority of dogs sampled for this study were generally owned dogs in good health, with good body condition scores and healthy coat appearance. Rates of infection between owned and unowned dogs were not compared; however, any free-roaming dog, regardless of ownership status, is at risk of tick infestation and potential infection. Third, the completeness and accuracy of information available on the questionnaires varied. Some questionnaires lacked significant amounts of data and could affect the statistical strength of the results. Finally, the state governments' request that dogs presented for spay/neuter services be cleared of ticks prior to sterilization likely reduced the number of ticks available for counting and collection; therefore, we believe this study underestimates the true tick burden of dog populations in northern Mexico. In addition, only a limited number of ticks were tested per state due to limited resources; therefore, the proportion of rickettsia-positive ticks may not be representative of the burden of rickettsia-positive ticks per locality.

High-risk communities should approach RMSF elimination using a comprehensive, One Health approach that includes enhanced surveillance, increased laboratory diagnostic capacity, improved clinician access to oral and intravenous doxycycline (the preferred treatment for RMSF), public health campaigns targeting population control of free-roaming dogs, as well as tick control in owned and free-roaming dogs and the environment.

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Impacts

- Six per cent of dogs tested had antibodies to SFGR, with the highest seroprevalence reported in Baja California.
- Dogs that spent the majority of time on the street, such as free-roaming or community-owned dogs, showed a greater risk of tick infestation, SFGR seropositivity, and may play a pivotal role in the spread of SFGR among communities.
- Estimating the seroprevalence of SFGR in the canine population can help public health campaigns target high-risk communities for interventions to reduce human RMSF cases.

FIGURE 1.

Cities that participated in the seroprevalence evaluation of spotted fever group rickettsiae in canines, 2015 [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 1

Demographic information for canines in northern Mexico, 2015

	Dogs included in study (*N* = 1,136)	Dogs with SFGR antibodies (≥64) by IFA (*N* = 69)	Dogs with ≥1 tick present (*N* = 278)	Dogs with ≥1 *Rickettsia* spp.-positive tick by PCR (*N* = 38)
Age				
<2 years	404 (35%)	20 (29%)	85 (31%)	11 (29%)
≥2 years	605 (53%)	40 (58%)	175 (63%)	18 (47%)
Missing data	127 (12%)	9 (13%)	18 (6%)	9 (24%)
Sex				
Male	377 (33%)	25 (36%)	133 (48%)	17 (44%)
Female	635 (56%)	36 (52%)	129 (46%)	14 (37%)
Missing data	124 (11%)	8 (12%)	16 (6%)	7 (18%)
Location				
Patio	561 (51%)	37 (54%)	115 (41%)	20 (52%)
Street	189 (16%)	13 (19%)	115 (41%)	7 (18%)
Inside House	142 (12%)	5 (7%)	3 (1%)	0 (0%)
Missing data	244 (21%)	14 (21%)	45 (17%)	11 (29%)
State of residence				
Baja California	289 (25%)	35 (51%)	99 (35%)	23 (65%)
Coahuila	399 (35%)	17 (27%)	138 (50%)	12 (21%)
Sonora	448 (40%)	17 (27%)	41 (15%)	3 (14%)
Ticks present				
No ticks observed	514 (45%)	28 (41%)	–	–
Dogs with ≥1 tick	278 (25%)	20 (29%)	278 (100%)	33 (87%)
Missing data	344 (30%)	21 (30%)	–	5 (6%)
Total	1,136	69	278	38

TABLE 2

Results of univariate and multivariable logistic regression for risk of SFGR antibodies ≥64 by IFA among dogs in northern Mexico, 2015

Variable	*N*(%)	Univariate OR (95% CI)	AOR* (95% CI)
Age (years)			
<2	20 (1.7%)	Reference	Reference
≥2	40 (3.5%)	1.35 (0.78–2.36)	1.43 (0.80–2.55)
Sex			
Male	25 (2.2%)	Reference	Reference
Female	36 (3.2%)	0.85 (0.50–1.43)	0.91 (0.53–1.60)
Where dog spends time			
Patio	37 (3.3%)	Reference	Reference
Street	13 (1.1%)	1.04 (0.54–2.01)	**2.37 (1.01–5.55)**
Inside house	5 (0.4%)	0.51 (0.20–1.33)	0.74 (0.28–1.99)
State of residence			
Sonora	17 (1.5%)	Reference	Reference
Baja California	35 (3.1%)	**3.49 (1.91–6.36)**	**3.38 (1.81–6.38)**
Coahuila	17 (1.5%)	1.12 (0.56–2.24)	0.62 (0.27–1.42)

Note: For purposes of this analysis, dogs were considered seropositive if they had *Rickettsia spp.* Antibodies ≥64 in serum by IFA. Values of $p < .05$ were considered significant.

*AOR, adjusted odds ratio.

TABLE 3

Results of univariate and multivariable logistic regression for risk of *Rickettsia* spp.-PCR positive tick infestation among dogs in northern Mexico, 2015

Variable	*N* (%)	Univariate OR (95% CI)	AOR* (95% CI)
Age (years)			
<2	11 (1.0%)	Reference	Reference
≥2	18 (1.6%)	1.09 (0.51–2.34)	1.04 (0.46–2.34)
Sex			
Male	17 (1.5%)	Reference	Reference
Female	14 (1.2%)	**0.47 (0.23–0.97)**	0.51 (0.23–1.10)
Where dog spends time			
Patio	20 (1.7%)	Reference	Reference
Street	7 (0.6%)	1.04 (0.43–2.50)	**3.86 (1.22–12.25)**
Inside house	0 (0%)	1 (0)	1 (0)
State			
Sonora	5 (0.4%)	Reference	Reference
Baja California	25 (2.2%)	**8.39 (3.17–22.18)**	**7.19 (2.58–20.03)**
Coahuila	8 (0.7%)	1.81 (0.59–5.58)	0.57 (0.16–2.08)

Note: For purposes of this analysis, dogs were considered tick infested if they had one or more ticks attached to their body during sample collection. Values of $p < .05$ were considered significant.

*AOR, adjusted odds ratio.