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Article

Brown Dog Tick (*Rhipicephalus sanguineus* sensu lato) Infection with Endosymbiont and Human Pathogenic *Rickettsia* spp., Northern Mexico

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Abstract: Of the documented tick-borne diseases infecting humans in Mexico, Rocky Mountain spotted fever (RMSF), caused by the gram-negative bacterium *Rickettsia rickettsia*, is responsible for most fatalities. Given recent evidence of brown dog tick, *Rhipicephalus sanguineus* sensu lato, as an emerging vector of human RMSF, we aimed to evaluate dogs and their ticks for rickettsiae infections as an initial step in assessing the establishment of this pathosystem in a poorly studied region of northeastern Mexico while evaluating the use of dogs as sentinels for transmission/human disease risk. We sampled owned dogs living in six disadvantaged neighborhoods of Reynosa, Northern Mexico to collect whole blood and ticks. Of 168 dogs assessed, tick infestation prevalence was 53%, comprised of exclusively *R. sanguineus* s. l. (n=2,170 ticks). Using PCR and sequencing, we identified an overall rickettsiae infection prevalence of 4.1% (n=12/292) in ticks, in which eight dogs harbored at least one infected tick. Rickettsiae infections included *R. amblyommatis* and *R. parkeri*, both of which are emerging human pathogens, as well as candidatus *R. andeanae*. This is the first documentation of pathogenic *Rickettsia* in *R. sanguineus* s.l. collected on dogs from northeastern Mexico. Domestic dog infestation with *Rickettsia*-infected ticks indicates ongoing transmission, thus humans are at risk for exposure and underscores the importance of public and veterinary health surveillance for these pathogens.

Keywords: *Rickettsia parkeri*; *Rhipicephalus sanguineus*; dogs; tick-borne disease; rickettsiosis

1. Introduction

Among the bacterial zoonoses, the gram-negative Rickettsiae are the most common vector-borne pathogens[1] and are responsible for most human fatalities in North America[2]. The most common tick-borne pathogenic *Rickettsia* species in the Americas include *Rickettsia rickettsii*, *R. parkeri*, and *R. africae* [3]. Tick vectors of these pathogens are distributed globally and vector species in the Americas include *Dermacentor variabilis*, *Amblyomma maculatum*, and *Rhipicephalus sanguineus* sensu lato [4]. Whereas *D. variabilis* and *A. maculatum* are generalist blood-feeders, *R. sanguineus* s. l. feed on dogs for all life stages, while occasionally feeding on humans [5,6].

Lyme disease, ehrlichiosis, spotted fever group rickettsiosis (SFGR) including and Rocky Mountain spotted fever (RMSF) have been reported in Mexico, [7–11] with RMSF as the most prevalent and fatal tick-borne disease in the country [12,13]. RMSF is especially devastating in Mexico as the majority of fatalities are children [14,15]. The tick-borne rickettsial zoonoses manifest similarly in human clinical diagnoses and symptoms, however, they are caused by genetically distinctly different bacteria species with different ecologies [16–18]. The distribution and prevalence of tick-borne pathogenic *Rickettsia* spp. in animals and ticks throughout Mexico is not well defined [19]. Furthermore, laboratory diagnostics of human rickettsiosis is challenging which complicates treatment of patients and the implementation of public health policy.

Across Mexico, rickettsiae pathogens have been described in vectors and hosts in Northern Baja California, Sonora, Chihuahua [20], Campeche [21,22], Yucatan [23], Tabasco [24], Veracruz [22,25], Tamaulipas [25–27], and Coahuila [4,12,28]. Ongoing outbreaks of RMSF since 2008, with high fatality rates, in Northwestern regions of Mexico have been much of the focused surveillance, with human disease also reported from adjacent indigenous communities of Arizona [29–33]. Studies have identified *R. rickettsia*, *R. amblyommatis* (formerly known as *R. amblyommii* or candidatus *R. amblyommii* [34]*),* and *R. rhipicephalus* from ticks removed from humans, dogs, deer, bobcats, and cattle, within the state of Tamaulipas [25–27], and the region is predicted highly suitable for *R. parkeri* to exist [35], yet there have been no reports of human tick-borne Rickettsial diseases in this region nor highly urbanized neighborhoods.

Dogs are commonly involved in human *Rickettsia* outbreaks, as they are integrated into human communities and support tick populations [29–31,33,36,37]. The widespread nature of *R. sanguineus* s. l. on dogs, and ubiquity of dogs within human domiciles, suggest that routine surveillance of these ticks on dogs can provide useful information for both veterinary and human health risk assessments [15,31,36,38]. Here, we sampled *R. sanguineus* s. l. from dogs in predominantly low-income neighborhoods of northeastern Mexico. Our objectives were to (i) describe the infestation prevalence of ticks on privately-owned dogs across six neighborhoods in relation to dog demographic data; and (ii) characterize tick infection prevalence with *Rickettsia* species.

2. Methods

2.1. Sample collection

We sampled dogs in six different neighborhoods within Reynosa, Tamaulipas of Northern Mexico (Fig, 1) between April 4 through August 31 2019, as the summer season is representative of historical RMSF case reports [12]. These neighborhoods included: Aquiles Serdán, Pedro J. Méndez, Margarita Maza de Juárez, 15 de Enero, Villa Florida, and La Cima, as previously described [39]. Each neighborhood was selected based on either their low or low-medium socioeconomic status (S-Table 1), and the available support of the local health neighborhood committees as the homes within these neighborhoods are built with weak infrastructure to minimize cost and development time. These neighborhoods typically have little veterinary care for their owned dogs, as well as large populations of free roaming dogs. All neighborhoods were sampled once, except for the neighborhood 15 de Enero, which was sampled once in May and once in June. Dogs were enrolled during neighborhood visits usually in a centralized home provided by a health neighborhood member, in empty nearby lots, and in door-to-door visits. As an incentive for participation free rabies vaccination were provided as a public health protective measure. Each dog was inspected for ticks which were removed with forceps into 70% ethanol. Blood was collected into EDTA tubes. Animal use protocols were approved by Texas A&M University Institutional Animal Care and Use Committee (IACUC 2018-0460 CA) with written informed consent obtained by dog owners. Ticks and blood were exported to Texas A&M University for processing. From each dog, basic demographics were obtained including sex, age, and an estimation of breed and age was provided by the owner.

2.2. Tick identification

All ticks were identified to species, life stage, and sex, under a dissecting microscope (Furman and Loomis 1998). Representative ticks of each life stage and sex were submitted as voucher specimens to the Texas A&M Insect Collection of the Department of Entomology (Accession No. X1689674), with collection information of these voucher specimens also submitted to the open-access Global Biodiversity Information Facility data source. We scored the engorgement status of ticks on a scale of 0-5, in which a 0 was used for flat ticks with no appreciable bloodmeal whereas a 5 was extremely engorged and presumed to be near repletion (Fig. 2a). Adult male ticks all appeared flat and were not given an engorgement status.

2.3. DNA extractions

A stratified random subset (292 of 2,170) of ticks was selected for molecular analysis. After tallying the number of ticks collected per dog (burden), a minimum 20% of each dog's tick burden was selected for DNA extraction, up to a maximum of ten ticks on a dog with 200 or more ticks present. The first selection stratum was tick life stage; due to the rarity of immature ticks in the sample set, larvae and nymphs were always selected for processing when present. The second selection stratum was engorgement score, and those with higher engorgement scores for processing over flat ticks to better represent any pathogens circulating in the dog's blood. Each individual tick was sliced repeatedly using a sterile number 11 scalpel blade and then subjected to DNA extraction using a commercially available kit (E.Z.N.A Tissue DNA Kit; Omega Bio-Tek, Norcross, GA, USA) and overnight incubation for lysis, with a two-step final elution bringing the final volume to 50 µL. For any dog that had one or more *Rickettsia*-positive tick, we subsequently extracted DNA from 50 µL of whole dog blood using this same extraction kit, in which the incubation time for lysis was 10 minutes. In the case of a tick found to be positive for a pathogenic SFGR, all remaining ticks from that dog that did not meet the initial selection criteria were then processed in full.

2.4. PCR for ompA gene of the genus Rickettsia and DNA sequencing

To test for the presence of *Rickettsia* species in hard ticks [17,18,40,41] within each extracted tick, we adapted the semi-nested protocol from Wikswo *et al.* 2008 [42] to amplify ompA gene of *Rickettsia*, a protein important in pathogenesis and common target for detecting several species of SFG *Rickettsia* [43]. To reduce the potential for PCR inhibitory effects of hemoglobin [44], we added 1µL of 1 mg/mL bovine serum albumin (final PCR concentration of 0.04 µM) for every reaction [42]. Further alterations of the established PCR protocol included using touchdown thermocycling to minimize non-target amplification[45] and FailSafe™ 2X P CR Premix E and PCR enzyme (Lucigen, Middleton, WI). PCR conditions were 10 cycles of amplification, each of 30 s at 95°C. Touchdown annealing step between 56.5°C and 57.5°C for 30 s followed by 20 cycles of elongation, each of 72°C for 1 min. An initial and final step of 95°C for 1 and 5 min were conducted to ensure complete denaturation and elongation of the template DNA, respectively. Semi-nested conditions were 10 cycles, each of 30 s at 95°C. Touchdown annealing step between 59°C and 60°C for 30 s followed by 30 cycles of elongation, each of 72°C for 1 min. An initial and final step of 95°C for 1 and 5 min were conducted to ensure complete denaturation and elongation, respectively. Every PCR reaction used a SFGR positive control [46,47] and a negative control of PCR water. Prior to reconstructing this PCR protocol, we used up to five published PCR protocols targeting different genes (Table 1)[42,48,49]. However, these protocols would produce multiple bands of variable fragment sizes per reaction, which sequenced to dog DNA or tick DNA.

Table 1. Primers used to test for Rickettsiae in this study.

Gene	Primers	Nucleotide sequence (5′-3′)	Amplicon size	Reference
Citrate synthase	RrCS.372 RrCS.989	TTTGTAGCTCTTCTCATCCTATGGC CCCAAGTTC CTTTAATACTTCTTTGC	617 bp	[49]
Citrate synthase	RpCs.877p RpCs.1258n	GGGGGCCTGCTCACGGCGG ATTGCAAAAAGTACAGTGAACA	381 bp	[84]
rOmpB	120-M59 120-807	CCGCAGGGTTGGTAACTGC CCTTTTAGATTACCGCCTAA	862 bp	[85]
OmpA	Rr190-70 Rr190-701	ATGGCGAATATTTCTCCAAAA GTTCCGTTAATGGCAGCATCT	632 bp	[86]
OmpA	Rr190-70 Rr190-701 Rr190-602	ATGGCGAATATTTCTCCAAAA GTTCCGTTAATGGCAGCATCT AGTGCAGCATTCGCTCCCCCT	550 bp	[42] (modified for touchdown PCR in this study)

All PCR products were visualized via gel electrophoresis and resulting amplicons were purified with ExoSAP-IT (Affymetrix, Santa Clara, CA, USA). Bidirectional Sanger sequencing was performed (Eton Bioscience Inc, San Diego, CA, USA). In Geneious (v 9.1.8), the forward and reverse sequences were trimmed, edited, and aligned to determine a consensus sequence which was compared to published sequences in NCBI GenBank [50]. Our criteria for concluding a sample as positive and identifying the rickettsial spp. included a distinct band of approximately 550 bp with a sequence at least 97% identical to a published sequence (Table 2). Sequences were submitted to NCBI GenBank (accession numbers of OM743005-OM743016).

Table 2. A collection summary of each neighborhood sampled in Reynosa Northern México. The Table indicates all dogs enrolled in the study, their total ticks removed, the average tick burden, the dogs tick infestation prevalence, the Rickettsiae prevalence, and the *Rickettsia* spp. amplified from ticks removed from each of the six neighborhoods. Overall metrics are also given.

Neighborhood	Dogs	Total Ticks	Mean Tick Burden	Dog Infestation Prevalence	Rickettsiae Prevalence of Ticks	Rickettsiae Species
15 de Enero	21	497	23.67	67% (14/21)	9.38% (6/64)	*R. amblyommii, R. andeanae*
Aquiles Serdán	22	84	3.82	36% (8/22)	5.88% (1/17)	*R. amblyommii*
La Cima	45	939	20.87	60% (27/45)	0	NA
Col. Margarita Maza de Juárez	9	18	2.00	67% (6/9)	16.67% (1/6)	*R. andeanae*
Pedro J. Méndez	19	347	18.26	63% (12/19)	3.45% (2/58)	*R. parkeri, R. andeanae*
Villa Florida	52	285	5.48	37% (19/52)	1.69% (1/59)	*R. andeanae*
Overall	168	2,170	12.92	51% (86/168)	4.11 % (12/292)	

2.5. Statistical analysis

We tested for differences in the mean tick burdens (mean number of ticks attached per dog) among dogs from the different neighborhoods and between dog sexes using Kruskal-Wallis rank sum test, followed by a Dunn's post-hoc test. These calculations were run with R Version 1.2.5042 using the *'dunn.test'* and *'stats'* packages [51,52]. Generalized linear mixed models (GLMM) with negative binomial error distribution and neighborhood as a random variable were used to determine the effect of dog sex and estimated age (continuous data ranging from 1 month to 10 years) on the outcome of tick burden. Similarly, GLMMs with a binomial error distribution and neighborhood as a random effect were used to determine the effects of the ticks life stage, ticks level of engorgement, tick burden of a host, host age, and host sex had any interaction on the outcome of a tick harboring rickettsiae. Lastly, a GLM with binomial error distribution was used to test the effect neighborhood has on the probability of identifying a tick positive for a Rickettsia. These models were calculated with the *'lme4'* and *'MASS'* packages[53,54]. Models with multiple predictor variables were checked for multicollinearity using the *'vif'* function within the *'car'* package[55], predictor variables with variance of inflation factors 5 or greater were either excluded from the models or set as a random variable.

3. Results

3.1. Sample collection

Overall, 168 dogs were enrolled in this study across 6 neighborhoods (collection sites) in Reynosa, Northern Mexico. Dog enrollment varied by neighborhood, where the most enrolled was 45 dogs from La Cima, and the least enrolled was 9 dogs from Margarita Maza de Juárez (Table 3). The sex ratio was nearly equal (females, n=83; males, n=81; unknown, n=4). Throughout the six neighborhoods the average age of the dogs sampled was three years, with an age range of one month to fourteen years. Eighteen different dog breeds or mixes were recorded, with 50% (n=84) of them described as mixed and 24% (n=40) were Chihuahuas.

Table 3. Host and tick attributes for ticks infected with *Rickettsia* species from Reynosa, Northern Mexico.

Dog Identification	Dogs Sex	Dogs Age (years)	Dog Breed	Dog Tick Burden	No. Ticks Processed	Tick Infection Prevalence	Life Stage	Sex	Engorgement	Rickettsiae
19PJMD1	F	2	Mix	22	4	25% (1/4)	N	NA	5	*R. parkeri*
19PJMD6	M	1	Mix	88	10	20% (2/10)	A	F	3	*R. andeanae*
							N	NA	4	*R. andeanae*
19MMJD01	F	2	Mix	2	1	100% (1/1)	A	F	1	*R. andeanae*
19VFD30	F	4	Chihuahua	7	1	100% (1/1)	L	NA	4	*R. andeanae*
190615DED1	M	5	Mix	75	10	10% (1/10)	A	M	NA	*R. andeanae*
1915DED10	F	1	Mix		10	40% (4/10)	A	F	3	*R. andeanae*
							A	F	2	*R. andeanae*
							N	NA	3	*R. andeanae*
							A	F	5	*R. amblyommii*
190615DED4	F	0.33	Mix	13	3	33% (1/3)	A	F	0	*R. amblyommii*
19ASD11	F	0.83	Mix	74	10	10% (1/10)	N	NA	4	*R. amblyommii*

Eighty-nine of 168 dogs harbored at least 1 tick for an overall infestation prevalence of 53% (Table 3). A total of 2,170 ticks were collected from the 89 infested dogs. Across all neighborhoods, the average tick burden was 13 ticks per dog (n=168; ± 47 SD), with the largest tick burden of 546 ticks attached to a Chihuahua dog (Fig, 2b). Mean tick burdens were significantly different across neighborhoods (Kruskal-Wallis chi-square test = 17.02, df = 5, p-value = 0.005). Dogs living in 15 de Enero (23.7 ± 8.4 SE) had significantly greater mean tick burdens than that of Aquiles Serdán (3.8± 0.97 SE, p-value=0.02) and Villa Florida (Fig. 4; 5.5± 1.9 SE, p-value=0.02). Dog age (p-value = 0.64) and sex (p-value= 0.16) were not predictive of tick burden (S-Table 2).

All ticks identified from dogs were *R. sanguineus* (n=2,149, Table 4). There was a total of 21 ticks (<1%) that were unidentifiable due to poor condition or missing anatomic parts.

Of those for which life stage was assigned, 50% were adults (n=1,074); 40% were nymphs (n=866) and 10% were larvae (n=217). Adults were 58% male and 42.0% female. The average engorgement score of adult females was 2.1, nymphs were 3.0, and larvae were 3.0 (Table 5).

Table 4. Summary of attached ticks by life stage and engorgement status. Engorgement not scored is representative of most male ticks but also includes some ticks that had ruptured during the removal process.

Engorgement Score	Adult Females	Adult Males	Nymphs	Larvae	Total Ticks (%)
0	119	na	226	42	387 (18%)
1	94	na	68	11	173 (8%)
2	107	na	122	46	275 (13%)
3	64	na	180	71	315 (15%)
4	32	na	128	18	178 (8%)
5	22	na	115	20	157 (7.3%)
Engorgement not scored	13	621	32	9	675 (31%)
Total ticks	451	621	871	217	2160

3.2. Molecular testing for Rickettsiae

Two-hundred-ninety-two individual ticks met the selection criteria and were tested for rickettsiae. Overall, there was a 4.1% infection prevalence for rickettsiae (n=12/292) through amplification of the ompA gene via the touchdown PCR protocol followed by sequencing to identify the genospecies. Candidatus *R. andeanae* was the most common (N=8/12), followed by *R. amblyommii* (N=3/12) and a single tick with *R. parkeri* (Table 3). Of the nine dogs which had *Rickettsia*-positive ticks, all dog blood samples tested negative for rickettsiae. GLMM analyses of the individual ticks life stage (p-values=0.32 and 0.78), level of engorgement (p-value=0.39), and the dog tick burden (p-value= 0.47), had no significant effects on the outcome of tick infection (S-Table 3). Younger dogs (p-value= 0.03) and male dogs (p-value= 0.02), were found to be less likely associated with harboring a *Rickettsia* positive tick. There was no significant association between neighborhood of collection and the outcome of tick infection (S-Table 4).

The *R. parkeri*-positive tick was attached to a two-year old female, mixed breed dog from the neighborhood Pedro J. Mendez. This was the only infected tick among the total of 22 ticks on the dog (four ticks processed in the initial stratified random screening; the remaining 18 were processed following the finding of a pathogen-infected tick on the dog). The *R. parkeri*-positive tick was scored to have an engorgement of 5, while the other ticks on this dog had engorgement scores of 0-3 (Table 3).

The majority of dogs that harbored rickettsiae positive ticks (n= 6/8) had only a single positive tick. Two dogs had multiple ticks test positive for endosymbiotic rickettsiae in the subset of ticks that were tested. One dog from the neighborhood Pedro J. Mendez harbored two candidatus *R. andeanae*-positive ticks among the 10 that were tested; there were a total of 88 ticks present on this dog. One 1-year-old female mixed breed dog from the neighborhood 15 de Enero harbored four infected ticks (three with candidatus *R. andeanae* and one with *R. amblyommatis*) among the 10 that were tested (118 ticks present on this dog).

4. Discussion

We document three species of *Rickettsia* in brown dog ticks removed from owned dogs in low-income neighborhoods of Reynosa in Northern Mexico. In particular, we found *R. parkeri*, a pathogenic SFGR in *R. sanguineus*; this pathogen has not previously been detected in Northern Mexico [4,56]. This bacteria causes *Rickettsia parkeri* rickettsiosis and is most commonly transmitted by *A. maculatum* (gulf coast ticks), with similar symptomatic manifestation in humans as RMSF, but slightly less severe [57]. Human clinical diagnostic tests often cross-react between *R. rickettsia* and *R. parkeri* [57], leading to misdiagnosis.

The *R. parkeri*-infected tick was a fully engorged nymph, and direct testing of the host dog blood as well as the other 21 ticks attached to this dog yielded a negative result. Typically, rickettsiae circulate in the blood and then establish in endothelial cells of tissues such as skin and other organs [43]. The lack of *R. parkeri* found in the whole blood of the dog could represent either that this nymphal tick had acquired *R. parkeri* i) from the previous larval blood meal; ii) from transovarial transmission [58], iii) that the dog had an established infection in the skin rather than circulating *R. parkeri* in the bloodstream, or iv) the level of rickettsemia was below the limit of detection of the assay; as such, a negative blood test does not rule out canine rickettsiae infection [59] . Skin biopsy of this dog to test for *Rickettsia* spp. could further illuminate the infection status of the dog [60–63]. *Rickettsia* spp. are in the salivary glands of infected ticks and can transmit to the host as fast as 10 – 30 minutes from the onset of blood feeding [64,65] .

Our survey is the first to document candidatus *R. andeanae* from a *R. sanguineus* s. l. in Northern, Mexico [66]. This uncultured rickettsiae is regarded as an endosymbiont [67–70]. Candidatus *R. andeanae* has been isolated from *A. maculatum* in both Perú, México, and the United States [26,27,66,67,69,71], but only documented in *R. sanguineus* sensu lato in Perú [66]. Most studies found candidatus *R. andeanae* to be sympatric with *R. parkeri*, as we have found in our samples (Table 3), or co-infecting *A. maculatum*.

We detected *R. amblyommatis*, also known as '*R. amblyommii*'. The pathogenicity of this species is undeclared medically but current investigations suggest that it can be opportunistically pathogenic [72–76]. Studies have reported *R. amblyommatis* to cause fever in guinea pigs [75], has been isolated from a rash of a human [77], has been associated with some pathology in humans [73,76], and recently has shown load dependency to cause morbidity or mortality in mice [72]. This species is geographically widespread and usually detected in tick species that encounter humans quite frequently and *A. americanum* serving as vector [71,78,79].

Rhipicephalus sanguineus s. l. has been implicated in recent human epidemics of RMSF, in which high tick burdens on dogs were associated with human disease cases [15,28–33,37]. Our analyses did not find an interaction between rickettsia within attached ticks and the tick burden of dogs (S-Table 4). In fact, the *R. parkeri* infected tick was from a neighborhood that had one of the lowest average tick burdens on dogs of the enrolled neighborhoods (Fig. 2). Furthermore, contrary to some studies, we did not find any life history data of the dogs correlated with tick burdens [31,33,80]. Although, we did find that mean *R. sanguineus* burdens did vary significantly among neighborhoods (Fig. 4). Further, we found that tick burdens among dogs were aggregated, as expected based on the parasite burden literature [81]. The highest tick burden was from a neighborhood that is relatively more exposed to the forest edge (Fig. 1) than some other neighborhoods. Prior studies have found that areas with dogs that roam more and unique landscape risk factors often associated with poverty (e.g. presence of trash) increased risk of RMSF [31,33]. The *R. parkeri*-positive *R. sanguineus* was from a dog in a neighborhood adjacent to a lagoon and having the third highest average tick burden, suggesting that infection may not be able to be predicted alone by tick burdens or the exposure to the rural areas [29–31]. A prior study found that dogs living at homes near an agricultural canal had higher *R. sanguineus* tick burdens [31].

No active surveillance of rickettsiosis is currently underway across Mexico by the Ministry of Health although 1,113 human cases of RMSF and 559 of other *Rickettsia* etiology was reported between 2017-2021. Signs and symptoms of these rickettsioses might be misdiagnosed of other endemic diseases in the region, such as dengue fever. Serology testing of humans have also indicated high exposure rate to these pathogens; only in 2012, the Ministry of Health had identified 465 cases of rickettsiosis in 27 States of México (highest numbers in Southern Baja California, Coahuila, Michoacán, Nuevo Leon, and Sinaloa), including *R. rickettsii*, (68.4% - 318 cases), and other (e.g. *R. prowazekii*, *R. typhi*, and

Ehrlichia) [82] . We believe that monitoring of dogs for rickettsiosis can supplement the detection of tick-borne pathogen surveillance by Mexican health authorities.

Limitations of the study include that all enrolled dogs were owned dogs and therefore may not represent the feral/stray dogs that occur in the same neighborhoods. Furthermore, not all collected ticks or dog blood was tested to conserve resources. Nonetheless, our criteria for prioritizing ticks for testing based on individual dogs burdens and the engorgement score for ticks may be useful for other investigations that wish to establish similar protocols for representative testing of a subset of collected ectoparasites. Further, the sequence data come from a single Rickettsia gene. Although we attempted up to five PCR protocols [42,48,49], results included multiple bands of variable fragment sizes per reaction, which sequenced to dog DNA or tick DNA, suggesting that those protocols were not suited for use on engorged ticks where the host DNA is abundance.

In characterizing the infestation of *R. sanguineus* s. l. on dogs, and tick infection with rickettsial species in a disadvantaged region of Mexico, we provide evidence that noninvasive monitoring of dogs can be utilized for efficient detection of tick-borne pathogen surveillance. These results illustrate the value of using dogs as sentinels and highlight the potential to use dogs as key targets for vector control techniques to prevent human tick-borne disease emergence [29,30]. Recent trials suggest that warmer temperatures induce *R. sanguineus* s. l. to bite humans more often [83]; accordingly, canine surveillance has increasing potential to provide information critical for assessing transmission/human risk especially in a warming climate. Long term monitoring programs of dogs should be emphasized for early detection of changing tick abundance and infection prevalence on dogs in Northern Mexico, which may be predictive of human disease risk.

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Figure legends

Figure 1 Map of dog sampling locations in Reynosa, Northern Mexico. Figure is an original map, created by the authors using QGIS 3.18.2 with public domain map data from INEGI, and satellite images from Google maps. (https://qgis.org/en/site/) with public domain map data from INEGI (https://www.inegi.org.mx/app/mapas/), and satellite images from Google maps (https://www.google.com.mx/maps).

Figure 2 a. Ticks were scored for engorgement on a scale from 0 to 5. This image is an example of the scoring scale for *R. sanguineus* s. l. adult female. Each life stage was scaled appropriately, except males were grossly indistinguishable and were therefore not scored for engorgement. Ticks that were damaged during the removal process were also not scored for engorgement. Figure 2 b. Dogs were checked for ticks and all found were removed with forceps and stored in 70% ethanol. This individual Chihuahua had 526 *R. sanguineus* s. l. attached.

Figure 3 Tick burdens of these dogs from Reynosa, Northern Mexico is highly skewed, where most dogs have no ticks but one has over 500 ticks.

Figure 4 Mean attached *R. sanguineus* of all our collection sites. Error bars represent the standard error of the mean. The white number inside the bars represent how many dogs were enrolled in the study at each neighborhood. Significant differences are indicated as "*", where p<0.05.

Figures

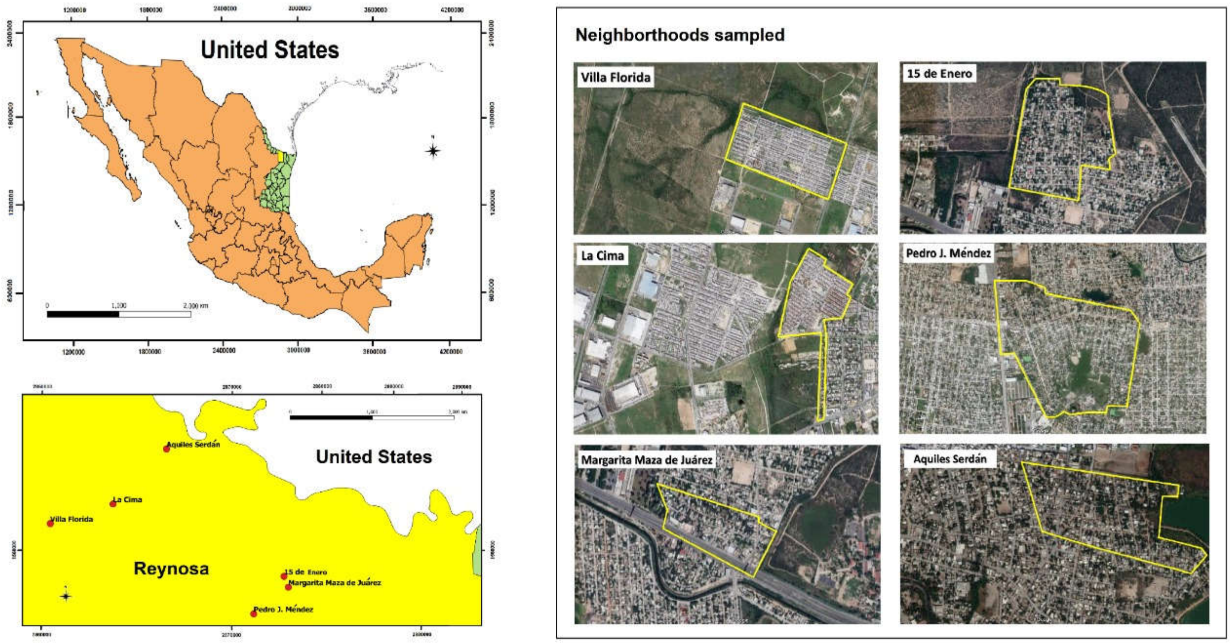

Figure 1

Figure 2

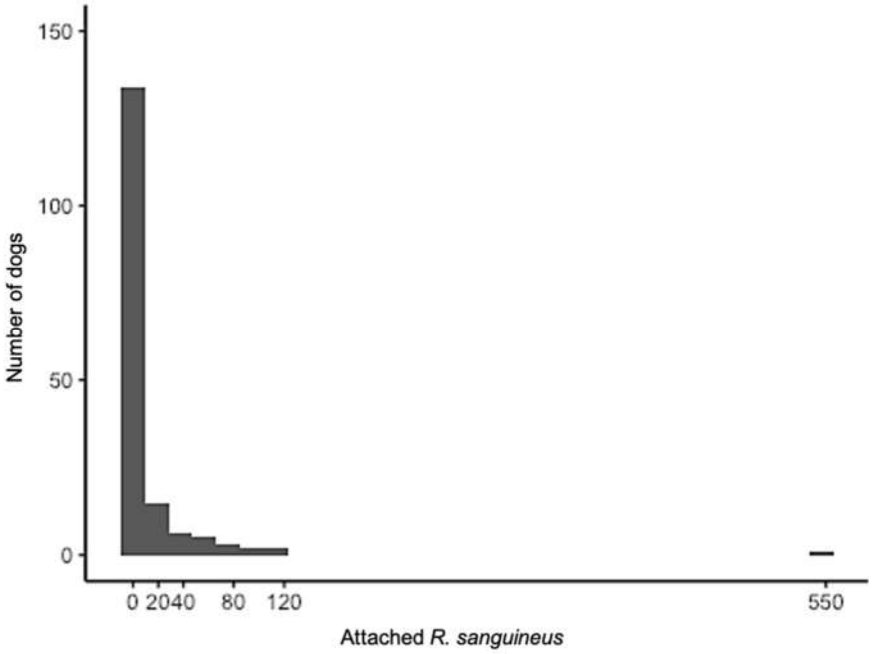

Figure 3

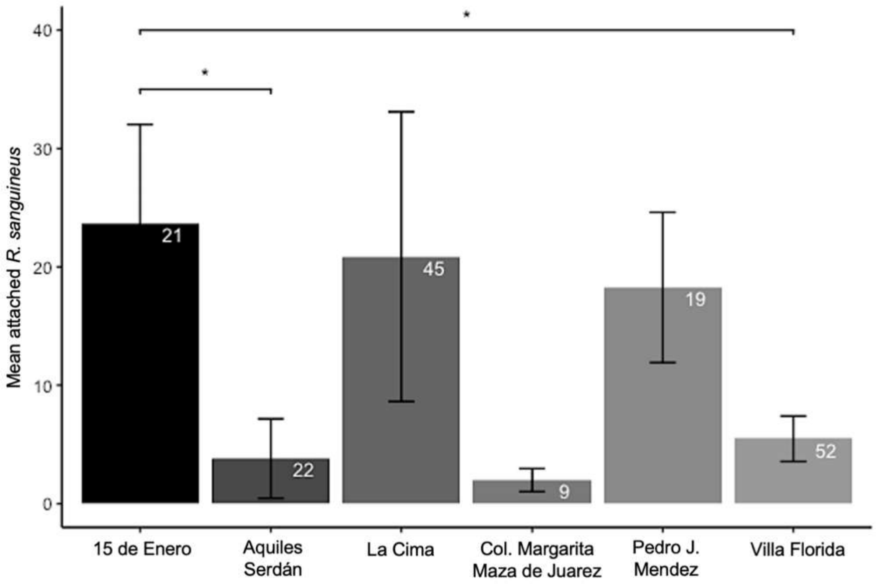
Mean attached *R. sanguineus*
0
10
20
30
40
*
*
21
22
45
9
19
52
15 de Enero
Aquiles Serdán
La Cima
Col. Margarita Maza de Juarez
Pedro J. Mendez
Villa Florida

Figure 4