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Unbiased Assessment of Abundance of *Rhipicephalus sanguineus* sensu lato Ticks, Canine Exposure to Spotted Fever Group *Rickettsia*, and Risk Factors in Mexicali, México

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Abstract. An epidemic of Rocky Mountain spotted fever (RMSF) is ongoing in Mexicali, México. We visited 100 neighborhoods with diagnosed human cases and 100 control neighborhoods to evaluate knowledge of the epidemic; obtain data on the spatial distribution of dogs, canine seroprevalence and active infection, tick infestations, and presence of rickettsial DNA in ticks; and evaluate risk factors for human cases, seropositivity, and tick infestation within an unbiased study design. The majority (80%) of residents had heard of RMSF, but only 48% used acaricides in the home or on dogs. Case neighborhoods and those with high canine seroprevalence tended to be on the city periphery or in the agricultural valley. No dogs were polymerase chain reaction (PCR) positive for *Rickettsia rickettsii*, and the overall seroprevalence was 65% (titers from 64 to 1,024). PCR prevalence in ticks was 0.70%, confirmed by DNA sequencing as *R. rickettsii*; neighborhood prevalence ranged from 0.7% to 6.1%. Twelve percent of dogs had high tick burdens, and all ticks were *Rhipicephalus sanguineus*. Epidemiologically significant risk factors were ground covering for a neighborhood having a human case; dogs having poor body condition and weighing < 10 kg for canine seropositivity; dogs living at the home for the number of ticks in the environment; and being near canals, having trash on the patio, and a dog being thin for tick burdens on dogs. A One Health approach is crucial to understanding RMSF and brown dog ticks.

INTRODUCTION

Rocky Mountain spotted fever (RMSF), caused by the bacteria *Rickettsia rickettsii*, is responsible for more human fatalities than any other tick-borne disease in North America.[1] Most cases in the United States are associated with *Dermacentor variabilis*, which has a sylvatic feeding cycle on small mammals during immature stages. By contrast, the brown dog tick (*Rhipicephalus sanguineus* sensu lato)[2,3] vectors RMSF in eastern Arizona, México, and South America, and epidemics associated with this tick have incurred particularly high case fatality rates. The preferred host for all life stages of *R. sanguineus* s.l. is the domestic dog (*Canis lupus familiaris*), leading to a very different and inherently peridomestic ecology compared with *D. variabilis*. Since 2008, an ongoing RMSF epidemic in Mexicali, the capital of Baja California in México, has affected at least 1,000 people (although changes in case definition and confirmation make this a rough estimate).[4] After at least 13 deaths occurred in the impoverished neighborhood of Los Santorales, Mexicali, during the early stages of the epidemic, *R. rickettsii* was confirmed by PCR and DNA sequencing in human cases.[5,6]

Although RMSF has been described since 1910 in at least nine Mexican states, often from rural areas, this disease is now considered to be reemerging.[4,7] Recent epidemiological research has implicated poverty, stray dogs, and high burden of brown dog ticks on dogs as important risk factors.[4,8–10] Brown dog ticks can reach enormous densities in neighborhoods where free roaming or stray dogs are abundant,[2,11] and unlike many tick species, the brown dog tick thrives at high temperature with low humidity, as is characteristic during at least 7 months of the year in Mexicali.[12] Several reports even suggested that the tick could be more aggressive at elevated temperatures.[13,14] However, the true prevalence, spatial distribution, and risk factors for RMSF in northern México are not well understood.

Our goal was to better understand the exposure and pathogen prevalence in Mexicali. We aimed to evaluate people's knowledge of RMSF and the use of acaricides by questionnaires and to collect spatial coordinate data for canine and tick surveys to estimate the distribution of tick infestation, canine exposure, and canine and tick infection prevalence. We also aimed to identify risk factors for human RMSF cases, tick infestation, and tick and canine infection through comparison of high-risk sites (defined by recent human case detection) to putative low-risk control sites (no known human cases) using an unbiased study design.

MATERIALS AND METHODS

Data were collected from the city of Mexicali and the surrounding area, in an extensively modified desert habitat supporting rapid urban development and irrigated agriculture. There is limited rainfall, typically in winter, averaging 79 mm/year and annual temperature ranging from 15°C in winter to 50°C in summer. Questionnaire data were collected with informed consent, and the human subject research was in full compliance with the UC Davis Institutional Review Board. Animal sampling was subject to oversight of the UC Davis Institutional Animal Care and Use Committee (IACUC) and the Academic Group of Animal Health and the Academic Group for Diagnosis of Infectious Diseases of the Instituto de Investigaciones en Ciencias Veterinarias of Universidad Autónoma de Baja California (UABC).

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Before sampling, individuals conducting the study were trained on the protocol and tick bite prevention measures. Teams were composed of at least one student each from the UABC medical and veterinary schools and a supervisor who was either a UABC faculty member or a local health authority. Participants were instructed to wear white clothes, tuck their pant legs inside their socks, and have an identification badge. When sampling, appropriate personal protective equipment such as nitrile gloves were worn, and on completion of sampling, the teams examined each other for ticks and participants were instructed to take a shower and further examine themselves at the end of the day.

Sampling was organized into 20 Áreas Geoestadisticas Básicas (AGEBs), which are census-related geographical delimitations established by the Instituto Nacional de Estadística y Geografía (INEGI). Case AGEBs were defined as those from which a human case of RMSF confirmed by the Baja California Secretary of Health occurred in the 12 months preceding the study. A conservative case definition was used,[1] requiring clinical signs consistent with RMSF and laboratory confirmation by PCR. Fifteen urban (defined as contiguous with the city landscape) and five rural (in the southeastern agricultural valley outside Mexicali) case AGEBs were randomly chosen from among all available case AGEBs to provide coverage of the larger metropolitan area and the rural adjacent agricultural valley where reports of cases have been numerous despite low population density. Fifteen urban and five rural AGEBs were randomly chosen as controls from among AGEBs from which cases of RMSF had not been reported in the 12 months before the study. From each case and control AGEB, we randomly chose five "manzanas" (city blocks). The teams sampled one house in each manzana, initiated at the first house in the northwest corner by asking if the inhabitants consented to participate. If consent was not provided, the team continued clockwise around the manzana until obtaining consent. Participants were blinded to the case status of the AGEBs, and the study personnel did not enter homes.

A very sparse questionnaire was implemented based on our prior experiences analyzing data on ticks in northern California and northern México to use questions with high probability of revealing actionable or significant data without causing respondents to become impatient. The questionnaire was delivered orally in Spanish by a university student and asked whether participants had heard of RMSF or knew of someone in the home who had had the disease, whether they treated the house against ticks, whether they treated dogs against ticks, and whether the resident had seen ticks in the home or occupants been bitten by ticks. There were also questions needed for assessing risk factors in the case–control portion of the study, including number of people living in the home, number of dogs, whether dogs were allowed into the house, and whether the dog had been acquired as a stray. Questions were initially validated with our own project staff and piloted with a subset of local, Spanish-speaking residents to allow for ambiguous or problematic questions to be refined.

To obtain data on spatial distribution of canine infection and exposure, a maximum of five owned dogs present at each home were examined, subject to tick collection, and restrained, using a muzzle if necessary, to obtain a blood sample from any available vein. Dogs that could not be safely restrained were not sampled for blood, although, on occasion, the owner could perform restraint such that the researcher could observe ears, axillae, and groin for ticks. Approximately 1–3 mL of blood was collected in ethylenediaminetetraacetic acid (EDTA)-treated tubes and kept on ice in a cooler and then frozen at −20°C within 6 hours of collection. Blood samples were subjected to serology using indirect immunofluorescence antibody assays to detect antibodies to rickettsial pathogens. Plasma was serially diluted from 1/64 to end point. Dilutions and washes were performed in phosphate-buffered saline (PBS) and heat-inactivated goat serum, gamma globulin–free bovine serum albumin, and 0.01% thimerosal at pH 7.38. Diluted samples were spotted in 25-μL volumes on antigen slides for *R. rickettsii* (VMRD, Pullman, WA), incubated at 37°C in a humidity chamber for 30 minutes, and washed three times with the PBS mixture. A 1:100 dilution in the PBS mixture of fluorescein-conjugated anti-dog IgG heavy- and light-chain antibodies (KPL, Gaithersburg, MD) was applied to the wells and the slides incubated again for 30 minutes at 37°C in moisture. The slides were then washed again three times and Eriochrome Black was added as a counterstain in the last wash. Ten percent glycerol (pH 7.4) was added to each well, the slides were coverslipped, and then the wells were evaluated with a UV microscope. Samples reactive with the *R. rickettsii* antigen at a dilution of 1/64 or greater were considered positive. Confirmed positive canine patient sera and water negative controls were incorporated in each run.

The samples were also tested by PCR for *R. rickettsii* DNA. DNA was extracted using a blood and tissue kit (Qiagen, Valencia, CA) following the manufacturer's instructions. All extractions were eluted in 50 μL water. Real-time PCR for the detection of *Rickettsia* genus DNA was performed,[15] and the samples were considered presumptively positive if the threshold cycle was < 50 with a characteristic amplification curve. Three negative water controls and a sequence-confirmed *R. rickettsii*–positive control were included in each run.

The spatial distribution of tick infestations and tick infection was evaluated for ticks in the environment and on dogs. The tick burden on dogs was estimated as 0 ticks, 1–10, 11–30, and > 30 ticks, and a maximum of 20 ticks was collected from each infested dog using forceps. Ticks were also evaluated in the environment by direct observation and using a carbon dioxide (CO_2) trap.[16] Students spent 15 minutes per house observing and collecting ticks from the ground and exterior walls. The CO_2 trap was set in the first manzana per AGEB only. The trap slowly trickled lactic acid onto calcium carbonate, releasing CO_2 into the surrounding air. The apparatus was placed on top of a clean white 1-m^2 cotton cloth in a shaded location near the house where owners reported dogs might lie or near outside walls or doors. The trap was checked regularly for 2 hours, and any ticks observed on the white sheet were collected. After 2 hours, the cloth was carefully folded into a sealed plastic bag, and the bags were frozen at −70°C. All ticks observed in the environment were collected, kept cool, and then transferred to −70°C within 6 hours.

All ticks were confirmed as *R. sanguineus* s.l. under dissecting microscopy with the use of taxonomic keys.[14,17,18] The skew of adult tick sex ratio from one male:one female was evaluated using χ^2 tests. Using a random number generator, an initial selection of 50 adult ticks per AGEB (or all ticks in AGEBS where there were fewer than 50) and all immature stage ticks were removed from tubes, and then, the ticks were

surface-sterilized in 10% bleach. Following the first pass of PCR, all ticks from AGEBs containing a PCR-positive tick were then tested as well. DNA extraction from whole non-engorged ticks was performed using a modified method of boiling in ammonium hydroxide,[19] which provides a cost-effective rapid means to extract DNA in non-engorged ticks. Ticks were frozen in liquid nitrogen for 3 minutes and then crushed with a pestle. The ticks were then boiled for 15 minutes in 100 µL of 0.7 M NH_4OH, cooled quickly for 30 seconds on ice, and then boiled again for 15 minutes in open vials to evaporate ammonia. DNA was extracted from 5- to 10-mg fragments of engorged ticks (containing mouthparts, salivary glands, and portions of gastrointestinal tract) using the same kit as for canine blood to eliminate blood-borne PCR inhibitors. All extractions were eluted in 50 µL water. Comparability of the quality of DNA extracted from ticks by the two methods was verified by applying a commercial quantitative PCR (qPCR) control (Thermo Fisher Scientific, Waltham, MA) for eukaryotic 18S rRNA to randomly chosen tick samples and comparing the mean cycle threshold between the groups. Real-time PCR was performed as for dogs.

Presumptively positive PCR samples were subjected to conventional PCR and DNA sequencing. Amplification of the rickettsial outer membrane protein A (*ompA*) gene was performed using primers R190-70 and R190-602,[20] modified to use GoTaq Green Master Mix (Promega, Madison, WI) in 25-µL reactions containing 1.0 M of each primer and 3 µL of template DNA. Results of PCR were assessed by electrophoresis and UV transillumination of GelStar (Lonza, Rockland, ME)-stained 1% agarose gels. Bands of the expected size were excised and cleaned with a QIAquick gel extraction kit (Qiagen) as per the manufacturer's instructions. The products were sequenced in the forward direction in an ABI Prism 3730 Genetic Analyzer (UC DNA Sequencing Facility, Davis, CA). DNA sequences were manually trimmed and corrected if the nucleotide could be unambiguously determined and then compared with sequences in a large database (GenBank; NCBI, Bethesda, MD) by basic local alignment search tool (BLAST) search. The distribution of PCR positivity between adult and larval ticks was evaluated with a Fisher's exact test. Áreas Geoestadisticas Básica polygons provided by INEGI were plotted in ArcGIS (ESRI, Redlands, CA) for the production of maps of canine seroprevalence and tick abundance.

Evaluation of risk factors for human RMSF cases, canine seroprevalence, tick infestation, and tick PCR prevalence was performed with a case–control study design. All data were maintained in Excel (Microsoft Corp, Redmond, WA) and analyzed using the program R (R Core Team, Vienna, Austria). Candidate risk factors for AGEBs with reported human RMSF cases were mean number of residents per home; whether the location was a single-family home, apartment, or other type of building; presence of trash in front of the home (yes/no); presence of an irrigation canal within a 5-minute walk (yes/no); ground surface in vegetation (lawn or weeds), paved, gravel, shrubs, and scrubs; whether or not the respondent was familiar with RMSF (from the questionnaire); and whether or not the house was fumigated at least twice a year. Although instructed to record proportion of each type of ground cover at the home, students sometimes only indicated presence or absence, requiring analysis to be adjusted to use only two predictors: any of each cover and majority cover class. The mean number of residents was compared using Student's *t*-test, whereas univariate logistic regression was used for other risk factors to yield odds ratios (ORs) and a test of significant differences by the χ^2 test.

The overall serostatus in dogs between case and control AGEBs was compared with Fisher's exact test. Multiple candidate risk factors of the canine serostatus were explored. Students stood within the front area of each house and observed visible yards, houses, and street areas to quantify dogs during a standardized 15 minutes. This time period was chosen after pilot observations, which indicated that almost all dogs were actually seen within 5 minutes. We also obtained data directly from the owners as to how many dogs resided at the address. Student's *t*-tests compared mean numbers of dogs seen and the resident in the homes between case and control AGEBs. Physical examinations yielded the body condition score (BCS) using a scale of 1–5 (with 5 representing obesity; Hill's Pet Nutrition, Topeka, KS), and a Wilcoxon test was used to compare BCS's between seropositive and seronegative dogs. Remaining candidate risk factors were assessed using mixed-model logistic regression using GLMER in lme4 in R[21] with the house included as a random effect. Approximate body mass (small was < 10 kg, medium 10–20, and large > 20), sex, and number of ticks on the dogs (none, few = 1–10, moderate = 11–30, and many > 30) were obtained during physical examination. During these home visits, the owners provided data on dogs' ages (adult/juvenile), whether they were allowed into the house (yes/no), whether the dogs had initially been stray, and whether acaricides were used on the dog at least two times per year (yes/no). Variable collinearity was ruled out using variable inflation factors; then, predictors were assessed individually and then included in a multivariable model if individually significant at $P < 0.1$ or considered likely to modify effects. The most parsimonious model was chosen with backward removal to minimize Akaike Information Criterion (AIC).

Before analyses of ticks, data were initially checked to ensure comparable ambient temperature, cloud cover, and precipitation among sampling days. Then, three analyses were conducted to assess associations of variables with ticks: 1) correlation of AGEB PCR prevalence in ticks and canine seroprevalence (excluding AGEBs where there were no PCR-positive ticks); 2) assessment of risk factors for abundances of ticks in environments; and 3) assessment of risk factors for tick burdens on dogs. Possible risk factors for the number of ticks in an environment were assessed with univariate Poisson regression using the R sandwich package.[22] Candidate factors were the home being in a case AGEB (yes/no), near a canal (yes/no), treated with acaricides at least twice per year (yes/no), type of house and the presence of grass, earth, or pavement (as described for the human case analysis), patio with debris (yes/no), dogs at the home (yes/no), and dogs allowed into the home (yes/no).

Possible predictors for tick burden on dogs (graded as none, few, moderate, or many as described previously) were evaluated using ordinal logistic regression using the function *polr* in the MASS library.[23] Risk factors were the home being in a case AGEB (yes/no), near a canal (yes/no), type of house, most landscape type (where the referent category consisted of data with ties between two different landscape types and landscaping with shrubs), patio with debris (yes/no), on-dog acaricide use at least two times/year (yes/no), dog serostatus (positive/negative), dog size as described for the serostatus, dog age (adult/juvenile), and dog body condition. For this

analysis, the dog body condition was considered thin if the BCS was 1 or 2, normal if the BCS was 3, and overweight if the BCS was 4 or 5.

RESULTS

Our team of 68 veterinary and 20 human medical students and supervisors evaluated 20 case and 20 control AGEBs across Mexicali and Mexicali Valley in late August and September 2017. They deployed questionnaires and assessed for ticks and environmental risk factors in 200 homes; a total of 284 dogs were sampled. All sampling was conducted during morning hours, with no rain or clouds present in the sky and ambient temperatures ranging from 17°C to 42°C (typically in the high part of the temperature range). Questionnaire results indicated that, although 80% of people had heard of RMSF, only half reported that they used pesticides or fumigated their premises against ticks at least twice a year. Questionnaire results are summarized in Table 1.

Spatial assessment documented the presence of dogs overall and local canine rickettsial seroprevalence. All AGEBs had dogs at homes except Nos. 27 and 31, and 142/200 (71.0%) total homes had dogs. Serology and PCR were performed on 213 dogs. The overall seroprevalence was 86.4%. Titers ranged from 64 to 1,024 as follows: 141 at 64 (76.6% of all seropositive dogs), 31 at 128 (16.8%), eight at 256 (4.3%), and two each at 512 and 1,024 (1.1%). The overall geometric mean titer (excluding 0s) was 79.1. No dogs were PCR positive. Seroprevalence in dogs was notably higher in outlying neighborhoods of Mexicali, whereas some of the inner city areas appeared to have lower rates of seropositivity (Figure 1). There were no obvious exposure patterns in the valley, with seroprevalence ranging from zero to the highest category (> 80%).

Focal areas of high tick infestation and PCR-positive ticks were detected as well. Overall, a total of 2,970 ticks were collected from CO_2 traps, floors and walls, and dogs (Table 1). Among the 200 houses, 107 (53.5%) had at least one tick collected from the environment or dogs. Twenty-eight ticks were excluded from statistical analysis because they were poorly labeled and laboratory staff could not determine an AGEB or manzana. The few ticks recovered from CO_2 traps were three from AGEB 19 and four from AGEB 25. The vast majority of ticks were adult, and the sex ratio in adult ticks was significantly (P = 0.0002) skewed toward males overall, skewed toward females (39 M:49 F) in the environment, and skewed ($P = 1.20 \times 10^{-7}$) toward males on dogs (1,481 M: 1,148 F). Very few larvae were found questing, compared with 45% of the nymphs, but only 3.5% of adults. On average, 1.8 ticks (SD: 5.9) were seen per home, with numbers ranging from 0 to 33. Visual inspection of spatial patterns in tick numbers did not correspond well with serology: tick numbers were typically lower in areas on the city margin and higher in the core (Figure 1).

TABLE 1

Sex and stage of *Rhipicephalus sanguineus* sensu lato ticks collected from different sources in homes in Mexicali, México, in summer 2017

Source	CO_2 trap	Dog	Walls and floors	NA	Total
Larvae	0	20	2	0	22
Nymphs	0	32	26	0	58
Female adult	3	1,148	49	91	1,291
Male adult	4	1,481	39	72	1,596
NA	0	3	0	0	3
Total	7	2,684	116	163	2,970

NA = not available.

Cycle thresholds on 18S PCR of 1,719 ticks ranged from 22 to 23; values did not differ among ticks extracted with Qiagen and ammonium hydroxide (data not shown). Twelve ticks (0.42%) were presumptively PCR positive for a *Rickettsia* sp. by the real-time assay, although three of these with cycle thresholds (CTs) > 40 were not confirmed because the conventional PCR had inadequate sensitivity to allow for DNA sequencing. Of the 12 PCR-positive ticks, two were from CO_2 traps, one from the environment, eight from eight different dogs, and the origin of one was not recorded. Nine were adult and three were larvae. When summarized by AGEB, case AGEB 8 had one positive of 110 tested for a local prevalence of 0.91%, with values for other PCR-positive AGEBs as follows: case AGEBs 19 (3/49, 6.1%), 20 (1/29, 3.4%), and 23 (2/139, 1.4%), and control AGEBs 12 (3/273, 1.1%), 38 (1/143, 0.7%), and 39 (1/94, 2.0%). These AGEBs are urban (AGEB 8), within city limits but approaching agricultural land to the southwest (19) or southeast (12), and in the Mexicali Valley to the east and southeast of the city (23, 38, and 39). After excluding AGEBs where there were no PCR-positive ticks, canine seroprevalence and tick PCR prevalence were not significantly correlated (P = 0.9). With 22 total larvae collected and three that were PCR positive, the prevalence among larvae was 13.6%, which is significantly higher than that for adults ($P = 6 \times 10^{-5}$). The positive larvae came from case AGEB 23 (two from the same dog) and a dog in control AGEB 38. The other larvae included two PCR-negative specimens from the same dog from which the positive larvae came in AGEB 23, one from the environment, and others from diverse AGEBs. DNA sequencing of a 245-bp segment of the *ompA* gene from PCR-positive ticks revealed 100% identity with the homologous *ompA* segment of multiple strains of *R. rickettsii* and with sequences amplified previously from humans in Mexicali.[6] DNA of no other *Rickettsia* sp. was detected in the sample set.

Analysis of candidate risk factors sought to determine attributes that differentiated AGEBs where people had been diagnosed with RMSF from AGEBs lacking such diagnosis. Most houses had approximately four residents, and the majority (approximately 93%) of locations sampled were houses (Table 2), approximately half of which had refuse in front. About a quarter of sampled homes in case AGEBs had an irrigation canal within a 5-minute walk, compared with about one-fifth in control AGEBs. Only substrate at the home was significantly associated with the case status, with elevated risk in homes where the majority landscape type was earth (OR: 3.8), or any pavement (OR: 1.85) or grass (OR: 2.68). Case and control homes did not differ in awareness of RMSF nor in the use of fumigation to control ticks.

A similar analysis was undertaken to detect factors associated with canine seropositivity in case AGEBs. Typically, approximately three dogs were seen by the team during the 15-minute observation period regardless of whether it was a case or control; the mean number of owned dogs per home (from the questionnaire) was 2.4 in case AGEBs and 3.0 in controls (Table 3). Seroprevalence was 63.1% (95% CI: 53.0–72.2) in case AGEBs and 67.3% in controls (95% CI: 57.7–75.6), and these differences were not statistically

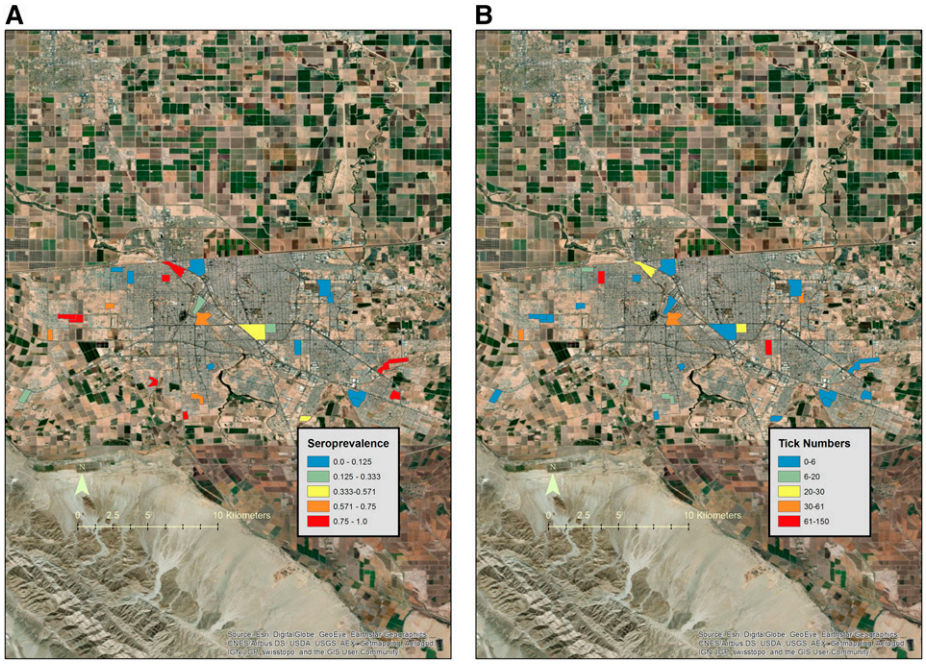

FIGURE 1. Map of Mexicali, Baja California, México, sampled for ticks and canine risk factors for Rocky Mountain spotted fever (RMSF) in August and September 2017. Sample Áreas Geoestadisticas Básicas (AGEBs) are color-coded by local RMSF seroprevalence in dogs (**A**) and by the mean number of *Rhipicephalus sanguineus* ticks observed in a standardized 15-minute observation period and on dogs at each house in an AGEB (**B**). Áreas Geoestadisticas Básicas in the Mexicali Valley are not displayed.

significant (Table 3). Geometric mean titers were 78.2 in case-positive AGEBs and 79.4 in case-negative AGEBs. Although the median BCS for both seropositive and seronegative dogs was 3, the BCS of seropositive dogs overall was significantly lower than that of seronegative dogs ($P = 0.04$). There were no significant differences between seronegative and seropositive dogs based on the univariate assessment of whether dogs were allowed into the home, whether owners used on-dog

TABLE 2

Characteristics of locations sampled in AGEBs in and near Mexicali, Baja California, México, in summer 2017 for ticks and other risk factors for a house being within an AGEB classified as positive for a human case of RMSF

Physical characteristic or risk factor	Case	Control	*P*-value	Estimate	Standard error	OR	95% CI
Number of residents in the house (mean, SD)	3.95, 1.68	3.86, 1.75	0.51	–	–	–	–
Housing type							
House	153 (93.9)	168 (93.3)	0.9	0.094	0.82	1.1	0.21–5.71
Farm	4 (2.5)	0	0.99	−17.7	1,200	NA	NA
Commercial	0	6 (3.3)	0.99	16.6	979.6	NA	NA
Apartment	3 (1.8)	3 (1.7)	Referent	–	–	–	–
Patio with trash							
Yes	74 (45.4)	96 (53.3)	0.13	0.33	0.22	1.39	0.90–2.16
No	88 (54.0)	80 (44.4)	Referent	–	–	–	–
Canal within a 5-minute walk							
Yes	36 (22.1)	31 (17.2)	0.16	−0.38	0.27	0.68	0.40–1.18
No	127 (77.9)	149 (82.8)	Referent	–	–	–	–
House with most outside space in landscape type							
Earth	78 (83.0)	63 (54.8)	0.0001	1.34	0.35	3.82	1.90–7.69
Grass	0	8 (7.0)	0.94	−15.44	848.37	NA	NA
Scrub	1 (1.1)	1 (0.9)	0.44	1.12	1.45	3.06	0.17–55.70
Paved	15 (16.0)	43 (37.4)	Referent	–	–	–	–
House with any of landscape types							
Any paved (referent = no paved)	99 (61.1)	131 (74.4)	0.009	–	–	1.85	1.16–2.97
Any earth (referent = no earth)	141 (87.0)	143 (81.3)	0.08	–	–	1.65	0.94–2.01
Any grass (referent = no grass)	32 (19.8)	70 (39.8)	<< 0.0001	–	–	2.68	1.63–4.43
Familiarity with RMSF							
Yes	130 (79.8)	152 (86.9)	0.08	0.52	0.30	1.68	0.93–3.04
No	33 (20.2)	23 (13.1)	Referent	–	–	–	–
House fumigated at least 2× per year							
Yes	75 (48.1)	87 (48.3)	0.96	0.01	0.22	1.01	0.65–1.57
No	81 (51.9)	93 (51.7)	Referent	–	–	–	–

AGEB = Áreas Geoestadisticas Básicas; OR = odds ratio; RMSF = Rocky Mountain spotted fever. Unless otherwise indicated, values in the cells are the number of positive responses for that feature (and percentage of responses in that feature that were positive). NA responses were not included in the percentage calculations. Exact *P*-values are given for statistical tests as described in the text. If relevant, univariate logistic regression estimates, standard errors, ORs, and 95% CIs are provided.

TABLE 3
Summary of canine risk factors, activity, and seroprevalence for rickettsiosis in and near Mexicali, Baja California, México, in summer 2017

	Case	Control	*P*-value	Estimate	Standard error	OR	95% CI
Mean number of dogs seen	3.13	3.17	0.86	–	–	–	–
SD of dogs seen	3.65	2.9		–	–	–	–
Mean number of dogs in the house	2.43	2.97	0.34	–	–	–	–
SD of dogs in the house	1.82	2.55		–	–	–	–
Overall seroprevalence	63.1	67.3	0.45	–	–	–	–
Body condition score (median)	3	3	0.04	–	–	–	–
Dogs enter the house							
Yes	58 (36.0)	63 (36.8)	0.77	0.14	0.49	1.15	0.43–3.06
No	103 (64.0)	108 (63.2)	Referent	–	–	–	–
Dogs treated with acaricides							
Yes	82 (63.6)	73 (48.3)	0.11	−0.85	0.52	0.43	0.15–1.21
No	47 (36.4)	78 (51.7)	Referent	–	–	–	–
Dog stray status							
Originally stray	19 (18.1)	14 (13.0)	0.77	−0.19	0.62	1.21	0.35–4.18
Not originally stray	86 (81.9)	94 (87.0)	Referent	–	–	–	–
Dog size							
Small	56 (53.3)	51 (0.47)	0.07	1.06	0.59	2.89	0.89–9.39
Medium	29 (27.6)	36 (0.33)	0.23	0.73	0.61	2.08	0.61–7.03
Large	20 (19.1)	21 (0.19)	Referent	–	–	–	–
Dog sex							
Male	48 (46.1)	59 (54.6)	0.95	0.03	0.40	1.03	0.46–2.29
Female	56 (53.9)	49 (45.4)	Referent	–	–	–	–
Dog age							
Juvenile	39 (37.9)	32 (30.2)	0.78	0.12	0.43	1.13	0.48–2.66
Adult	64 (62.1)	74 (69.8)	Referent				
Number of ticks on the dog							
Many	10 (9.6)	18 (16.8)	0.59	0.41	0.76	1.51	0.33–6.89
Moderate	16 (15.4)	19 (17.8)	0.33	−0.65	0.67	0.52	0.14–1.99
Few	35 (33.7)	27 (25.2)	0.44	−0.41	0.53	0.66	0.23–1.92
None	43 (41.3)	43 (40.2)	Referent	–	–	–	–

OR = odds ratio. Unless otherwise indicated, values in the cells are the number of positive responses for that feature (and percentage of responses in that feature that were positive). NA responses were not included in the percentage calculations. Exact *P*-values are given for statistical tests as described in the text. If relevant, univariate logistic regression estimates, standard errors, ORs, and 95% CIs are provided.

acaricides at least twice a year, whether the dogs had been acquired as a stray, dog size, sex, age, and number of ticks on the dog. However, based on a *P*-value of 0.07 for significance of dog body size ($P = 0.07$), size and age (as a possible confounder) were included in a multivariable mixed generalized linear model to predict the serostatus. Inclusion of age changed the effect of dog size by > 10%, and the final model that minimized AIC was serostatus ~ dog size + dog age + 1|house. With an adjusted OR of 3.7 (estimate = 1.31, 95% CI: 1.02–13.5), being a dog < 10 kg was significantly ($P = 0.04$) associated with being seropositive.

The last two sets of analyses were to attempt to predict tick abundances. Notably elevated numbers of ticks were found in case AGEBs and on farms, although these risk factors were not statistically significant (Table 4). Borderline significance was seen when dogs were present (*P*-value = 0.06), but, importantly, there were also 0.45 ticks on average in homes that had no dogs. The mean number of ticks observed in each AGEB was not significantly associated with seroprevalence in dogs ($R^2 = 0.03$, $P = 0.14$). Other nonsignificant predictors included house being near a canal, whether or not the house was treated with acaricides, landscape type, whether the front had debris, and whether or not dogs were allowed into the house.

With respect to the canine tick burden, almost half of the dogs (44.8%) had no ticks; percentages of dogs with few, moderate, and high tick burdens were 27.4%, 15.7%, and 12.1%, respectively (Table 5). Tick burden on dogs was not significantly associated with case AGEBs, treating the dog at least twice a year with acaricides, dog age, or dog size (Table 5). Dogs that lived near canals had significantly ($P = 0.04$) different tick burdens: those near canals tended to have disproportionately higher percentages of dogs with high (13%) and moderate (25%) tick burdens, whereas high and moderate tick burdens in dogs not near canals were 12% and 13%, respectively. Homes where most of landscaping was paved had lower tick burdens on dogs ($P = 0.03$), whereas homes with trash on the patio tended to have dogs with higher tick burdens ($P = 0.005$). Seropositive dogs were marginally ($P = 0.06$) more likely to have higher tick burdens, and tick burdens were significantly higher on thin dogs ($P = 0.04$).

Individuals in eight homes (4%) in case AGEBs Nos. 3, 9, and 29, and control AGEBs 14, 22 (two cases), 26, and 27 reported that someone in the home had been diagnosed with RMSF, although they did not specify when. We examined reports from these homes carefully for commonalities. These locations were either on the edges of the city (to the west, southwest, and northeast) or outside the city in agricultural or rural villages. Areas tended to be impoverished or in one case characteristic of a slum and near agricultural fields. Substrates near the houses were mixed in most cases, dirt in two, concrete in one, and scrub in one. Three families (1.5% of all families queried) did not have dogs, whereas the other five did; of those with dogs, two did not allow them into the house. Tick infestation was detected in two of the homes. In one home in AGEB 3, residents reported that they did not have a tick infestation, even though students directly observed ticks in the dirt. The family also owned two dogs (which were tick free at the time of examination), permitted them into the home, and neither fumigated nor applied acaricides to the dogs. In AGEB

TABLE 4

Summary of attributes and risk factors associated with differing numbers of *Rhipicephalus sanguineus* seen in the environment during a 15-minute survey per house in and near Mexicali, Baja California, México, in summer 2017

	No. of ticks seen/house (mean, SD)	*P*-value	Estimate	Standard error	OR	95% CI
Case AGEBs	2.06 (6.31)	0.54	0.29	0.48	1.34	0.51–3.49
Control AGEBs	1.54 (5.42)	Referent	–	–	–	–
House near canal						
Yes	5.49 (1.69)	0.9	–0.07	0.58	0.93	0.29–2.97
No	5.98 (1.81)	Referent	–	–	–	–
House treated with acaricides at least 2×/year						
Yes	2.46 (6.96)	0.21	0.61	0.48	1.84	0.70–4.81
No	1.34 (4.94)	Referent	–	–	–	–
Housing type						
House	1.82 (5.99)	0.42	–0.72	0.9	0.49	0.08–2.94
Farm	5.00 (NA)	0.74	0.29	0.87	1.34	0.23–1.92
Commercial	0 (0)	1	NA	–	–	–
Apartment	3.75 (7.5)	Referent	–	–	–	–
House with any of landscape types						
Any paved: yes/no	2.13 (6.53)/1.25 (4.50)	0.32	0.53	0.54	1.7	0.58–5.02
Any earth: yes/no	1.94 (6.24)/1.33 (4.25)	0.54	0.37	0.61	1.45	0.43–4.90
Any grass: yes/no	1.87 (5.80)/1.81 (5.98)	0.95	0.03	0.53	1.03	0.36–2.97
Patio with trash						
Yes	1.81 (5.60)	0.97	–0.02	0.48	0.98	0.37–2.56
No	1.85 (6.25)	Referent	–	–	–	–
Dogs present						
Yes	2.24 (6.57)	0.06	1.6	0.86	4.95	0.89–27.66
No	0.45 (2.37)	Referent	–	–	–	–
Dogs enter the house						
Yes	1.93 (4.78)	0.96	0.02	0.46	1.02	0.41–2.56
No	1.89 (6.56)	Referent	–	–	–	–

AGEBs = Áreas Geoestadisticas Básicas; OR = odds ratio. Exact *P*-values are given for statistical tests as described in text. If relevant, Poisson regression coefficients, standard errors, ORs, and 95% CIs are provided. NA responses were not included in the percentage calculations.

27, residents reported a tick infestation (which we verified), even though they did not own dogs and no dogs were observed. Three families reported that they fumigated the house every 6 months, one of which treated the dogs every month and another once a year; no ticks were observed in these homes. Among the eight homes, four had dogs for a total of seven dogs of which two were seronegative, four had titers of 1:64, and one had a titer of 1:256.

DISCUSSION

Since 2008, a large RMSF epidemic in northern México has affected more than a 1,000 people and likely many 1,000 dogs with high case fatality rates.[4] Here, based on a door-to-door survey of 200 residences in 40 AGEBs in Mexicali and Mexicali Valley, we show that there was a high level of awareness of RMSF and the ongoing epidemic, yet inadequate preventive management. There was a heavy burden of brown dog ticks in many neighborhoods, with ticks in the environments of 54% of homes (even in homes without dogs), and 65% of dogs were seropositive. Although the overall PCR prevalence in ticks was low, it was as high as 6% in particular AGEBs. People tended to be well aware of RMSF, but still often did not use acaricides.

We performed our study at the height of summer to target times of high tick activity and also because historical epidemiologic data implicate March to October as the time of most case reports.[4] Our large group of native Spanish-speaking human and veterinary medical student volunteers allowed for large amounts of data collection in a short time window and also served to perpetuate interest and the need to expand awareness of the epidemic within the medical community and the next generation of providers.

Approximately half of the dogs were infested with ticks and some dogs carried thousands of ticks. Before detection of the epidemic (in 2006), 65% of dogs were reported infested.[9] In 2017, ticks were observed walking across open dirt or paved yards and up walls, sometimes initially with few ticks observed and then increasing in number during the standardized 15-minute observation periods. In light of high numbers of ticks, the results from the CO_2 traps were disappointing in an assay that has been shown effective in the past (e.g., ref. 24). The use of dry ice was impractical, necessitating the use of validated traps with chemical reactions to release CO_2. Contributory factors to the poor success of these traps in Mexicali could be the very hot and dry environment or the holes that allow the acid to drip on to the calcium carbonate were too small. If such traps were to be used in future work at the same site, it would be helpful to quantify the CO_2 released to ensure that the traps are functioning optimally.

Seroprevalence among dogs was 65% and humans were not tested, but a recent study showed a seroprevalence in people in Ensenada, also in northern Baja California, of 4%.[25] Several studies have followed canine seroprevalence in Mexicali, including a pilot of 21 symptomatic dogs in the known high-risk Los Santorales neighborhood in 2008–2009 in which 85% of dogs were seropositive,[26] a survey before the epidemic of 384 owned dogs from veterinary clinics across the city with a seroprevalence of 64%,[27] a survey of stray dogs from the same time period yielding 60% seroprevalence,[28] and a survey of rural dogs from 2009 with a 74% seroprevalence.[29] The assay we used is sensitive for antibodies but has poor specificity because of serological cross-reactivity among rickettsial species.[30,31] Thus, documenting seroprevalence among dogs allows detection of suspect at-risk areas only. In the present study, it was not surprising that none of the dogs were PCR positive because *R. rickettsii* has a tropism for

TABLE 5

Summary of attributes and risk factors associated with tick burden of *Rhipicephalus sanguineus* on dogs in and near Mexicali, Baja California, México, in summer 2017

	High tick load (> 30 ticks)	Moderate tick load (11–30 ticks)	Low tick load (1–10 ticks)	No ticks	*P*-value	Estimate (standard error)	OR (97.5% CI)
Case AGEBs	15 (11.4%)	19 (14.4%)	38 (28.8%)	60 (45.5%)	0.66	–0.09 (0.22)	0.91 (0.59–1.40)
Control AGEBs	19 (12.6%)	25 (16.6%)	39 (25.8%)	68 (45.0%)	Referent	–	–
House near canal							
Yes	7 (12.7%)	14 (25.5%)	15 (27.3%)	19 (34.6%)	0.04	0.54 (0.27)	1.72 (1.0–2.94)
No	27 (11.8%)	30 (13.2%)	62 (27.2%)	109 (47.8%)	Referent	–	–
House with most outside space in landscape type							
Earth	12 (10.7%)	19 (17.0%)	33 (29.5%)	48 (42.9%)	0.40	–0.17 (0.24)	0.84 (0.52–1.36)
Grass	3 (37.5%)	0 (0%)	1 (12.5%)	4 (50.0%)	0.86	0.14 (0.77)	1.14 (0.24–5.28)
Paved	4 (8.9%)	3 (6.7%)	12 (26.7%)	26 (57.8%)	0.03	–0.76 (0.34)	0.47 (0.24–0.90)
No majority	15 (13.3%)	22 (19.5%)	30 (26.5%)	45 (39.8%)	Referent	–	–
Patio with trash							
Yes	25 (16.3%)	26 (17.0%)	42 (27.5%)	60 (39.2%)	0.005	0.63 (0.22)	1.87 (1.2–2.9)
No	9 (6.9%)	18 (13.8%)	35 (26.9%)	68 (52.3%)	Referent	–	–
Dog treated with acaricides at least 2×/year							
Yes	15 (9.9%)	27 (17.8%)	41 (27.0)	69 (45.4%)	0.89	–0.03 (0.22)	0.97 (0.62–1.5)
No	18 (14.6%)	15 (12.2%)	34 (27.6%)	56 (45.5%)	Referent	–	–
Dog serostatus							
Positive	20 (16.4%)	19 (15.6%)	34 (27.6%)	49 (40.2%)	0.06	0.14 (0.26)	1.15 (0.7–1.9)
Negative	8 (9.2%)	16 (18.4%)	28 (32.2%)	35 (40.2%)	Referent	–	–
Dog age							
Juvenile	12 (12.4%)	18 (18.6%)	30 (30.0%)	37 (38.1%)	0.14	0.34 (0.23)	1.4 (90.9–2.2)
Adult	22 (12.0%)	25 (13.7%)	47 (25.7%)	89 (48.6%)	Referent	–	–
Dog size							
Small	16 (10.5%)	26 (17.1%)	43 (28.3%)	67 (44.1%)	0.62	0.15 (0.31)	1.16 (0.64–2.15)
Medium	10 (12.4%)	16 (19.8%)	19 (23.5%)	36 (44.4%)	0.53	0.21 (0.34)	1.24 (0.64–2.43)
Large	8 (16.3%)	2 (4.1%)	15 (30.6%)	24 (49.0%)	Referent	–	–
Dog body condition							
Thin	11 (32.4%)	11 (26.2%)	22 (28.6%)	20 (16.4%)	0.04	0.52 (0.26)	1.7 (1.01–2.8)
Overweight	1 (2.9%)	4 (9.5%)	9 (11.7%)	17 (13.9%)	0.24	–0.43 (0.37)	0.65 (0.31–1.3)
Normal	22 (64.7%)	27 (64.3%)	46 (59.7%)	85 (69.7%)	Referent	–	–

AGEBs = Áreas Geoestadisticas Básicas; OR = odds ratio. Unless otherwise indicated, values in the cells are the number of dogs for that feature (and percentage of responses in that feature that had a given tick load). NA responses were not included in the percentage calculations.

endothelial cells, and negative PCR does not rule out canine RMSF.[32] It is unlikely that the cause of zero PCR prevalence in dogs was low-titer bacteremia as the PCR assay reportedly has analytical sensitivity as low as one molecule and is specific to rickettsial species per se excluding *Rhipicephalus bellii*.[19] The high seroprevalence suggests that many dogs may have some level of acquired immunity to infection, contributing to herd immunity; such recovered dogs are unlikely to be actively infected. However, chances of detecting rickettsial DNA in circulating blood could be improved with better surveillance for acutely ill dogs with clinical signs of disease.

The number of PCR-positive ticks overall was relatively low (0.7%), but as high as 6.1% in AGEB 19. This percentage is consistent with other studies, for example, 0.75% prevalence by fluorescent antibodies among 1,168 *D. variabilis* in an endemic focus in Ohio[33] and 3% of brown dog ticks by PCR in an eastern Arizona outbreak.[34] In this light, a prior report from Mexicali in 2009 of 31% PCR prevalence among 188 brown dog ticks from seropositive dogs is anomalous.[35] Tick PCR was confirmed reliable and not impacted by inhibitors in blood in ticks or the use of bleach to sterilize, on the basis of the strong signal on 18S control PCR. It is possible that our PCR primers could fail to initiate a reaction for a strain of *R. rickettsii* with mutated primer sites, although there is no known published precedent for that with this universal protocol used here, rather these primers initiate PCR of all known spotted fever rickettsial species. The positive results do not appear to be due to contamination with PCR-positive dog blood because none of the dogs from which positive ticks were removed were PCR positive, although we cannot rule out that the dogs were carriers of rickettsial infection, possibly at very low levels or in endothelial cells but not circulating blood.

The findings of disproportionately high prevalence of infection (13.6%) in the larval ticks and in ticks on CO_2 traps (16.7%) are fascinating and deserve further evaluation. High prevalence in larvae is consistent with maturing ticks losing infection because of negative fitness costs of the bacterium on the tick,[36] although small sample sizes of PCR-positive ticks preclude being able to confidently evaluate the numerous possible contributing factors. Still, further work to discover the true reservoir for *R. rickettsii* in the city—whether in patchy high-risk areas in mature ticks, among larvae, or dogs—is important for next steps in epidemic management. All of the tick PCR data help highlight focal areas in the city where risk is elevated. Such data allow people who are bitten by a tick to work with their physicians to monitor for disease and are necessary for the implementation of possible guidelines such as the use of prophylactic antibiotic treatment after every tick bite.

Risk factors identified previously for the Mexicali epidemic were typically those of rural residences or poverty (nearby canals, lack of home security, dirt floors, presence of broken-down cars, and dogs having ticks),[37] whereas prior epidemics, for example, in Arizona, have been associated primarily with stray dogs.[2] In the present study, risk factors for neighborhoods having human cases were related to landscaping; risk factors for seropositive dogs were being small and having poor BCS; risk factors for high tick numbers in the environment were having dogs; and risk factors for high tick burdens

on dogs were proximity to agricultural canals, landscaping, the patio having trash, the dog being seropositive, and the dog being thin. Some of these risk factors may reflect risk for undetected and unmanaged human disease (drug use from prior studies and trash from this and prior studies), some are likely associated with the dog population maintaining ticks (poor canine BCS and small dogs), and some reflect conditions for tick persistence (ground covering). Dog numbers per se including stray dogs were not exceptional in this study, with the average only three dogs seen per 15-minute observation, and human cases and environmental tick infestations both occurring in homes that did not have any dogs. Given the very minimal ability of these ticks to move over a distance (not directly studied for *R. sanguineus*, but reported as from 2.8 to 4.8 m for *Rhipicephalus appendiculatus* and *Rhipicephalus evertsi*[38]), the occurrence of such infestations and case risk could either occur because the properties are so small that ticks can move across property lines (e.g., if a dog sleeps next to a fence line) or because unrestrained dogs enter neighbors' yards. The significance of proximity to canals and high numbers of human cases in the valley is not known but could suggest a role of wildlife (including possibly coyotes, foxes, and mesocarnivores) transporting infected ticks to new areas. Possible roles of wildlife and infestations in homes without dogs together suggest that entire communities must be managed for RMSF control rather than individual homes.

Most interesting were the findings of similarity between case and non-case AGEBs in canine seropositivity, tick numbers, and risk factors for the presence of a human disease, probably reflecting misclassification, particularly of control AGEBs. Human cases likely represent only a small portion of the circulating bacteria in the environment and mild cases are almost certainly overlooked, suggesting that there may have been unreported recent cases in some control AGEBs (as some of our participants reported). There may have been a questionnaire bias, particularly in identification of human cases. People may have both under- and overestimated RMSF cases because of the lack of confirmatory testing and nonspecificity of symptoms in many cases. For other questions, such as whether dogs were allowed into the home, there would be little concern about bias. Bias in case confirmation and questionnaire responses further underscores the value of looking to tick sampling, detection of clinical signs of RMSF in dogs, and RMSF diagnostic testing to further understand the disease risk. It is also possible that a sample of five randomly chosen manzanas and one house within each is insufficient to characterize local attributes and risk factors of a given AGEB. As implemented, these five houses constitute only a sample, and it is not known how many houses are truly needed to accurately characterize each predictor we explored. Satellite imagery or citizen science-based data could be more efficient means to capture features of AGEBs, such as dog abundance and vegetation types. Other data such as awareness of RMSF, whether dogs are allowed inside, etc., require the direct sampling of the sort we performed.

Participants were generally aware of RMSF and knowledgeable about the role of the brown dog tick as the vector. Nevertheless, the use of acaricides in homes and on dogs was highly inconsistent. Our past data in other areas suggest that people use a diversity of products against ticks, including products perceived as nontoxic or natural but with minimal anti-tick efficacy. Tick-borne disease and RMSF in particular are expanding as serious veterinary and public health problems. Over the last 15 years, there have been RMSF epidemics in Arizona, Sonora, México, and Baja California.[2,39,40] In the United States, the incidence of RMSF has increased over 4-fold since 2000.[41] In Arizona, aggressive multidimensional intervention campaigns achieved partial resolution of the epidemic, although in areas that were smaller than Mexicali. Key aspects of those programs included dog spay and neuter programs, treatment of houses with pesticides, and the use of a long-acting tick collar.[11] However, control of the much larger RMSF epidemic in Mexicali will require a multifaceted approach. Spay and neuter campaigns may not be as urgently needed to control dog numbers in Mexicali as they were in eastern Arizona, but reducing the proportion of the canine population that are puppies could help boost herd immunity if the older dogs have been exposed and become immune. Importantly, officials must continue to educate residents as to the magnitude of the tick burden and best practices for use of effective acaricides.

Mexicali is a large, rapidly developing city, and this RMSF epidemic may be subject to poorly understood impacts of climate change, land conversion, and other anthropogenic influences. Moreover, there continue to be outbreaks in the Sonora state and other towns in Baja California.[4] Emphasis on the diagnosis of RMSF in dogs has previously been proposed as a means to improve public health.[42] Our data strongly support a One Health approach to understanding RMSF transmitted by brown dog ticks, incorporating the extensive use of dogs as sentinels, with the added benefit that management of tick and pathogen burdens on dogs will serve to improve the lives of dogs and the people living near them.

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