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Serologic assessment for exposure to spotted fever group rickettsiae in dogs in the Arizona-Sonora border region

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Abstract

Rocky Mountain spotted fever (RMSF) is a severe tick-borne rickettsial illness. In the south-western United States and Mexico, RMSF displays unique epidemiologic and ecologic characteristics, including *Rhipicephalus sanguineus* sensu *lato* (brown dog tick) as the primary vector. Expansion and spread of the disease from hyperendemic regions of Arizona or Mexico to new areas is a key public health concern. Dogs are thought to play an important role in the emergence and circulation of *R. rickettsii* in these regions and are often one of earliest indicators of RMSF presence. A canine serosurvey was conducted in 2015 among owned and stray dogs at rabies clinic and animal shelters in three southern Arizona counties where RMSF had not previously been identified. Of the 217 dogs sampled, 11 (5.1%) tested positive for spotted fever group rickettsia (SFGR) IgG antibodies, with seropositivity ranging from 2.9% to 12.2% across the three counties. Large dogs were significantly more likely than small dogs to have positive titres reactive with *R. rickettsii;* no additional statistically significant relationships were observed between seropositivity of canine age, sex, neuter or ownership status. In addition, 17 (7.8%) dogs had ticks attached at the time of sampling, and stray dogs were significantly more likely to have ticks present than owned dogs ($p < 0.001$). All 57 ticks collected were identified as *Rh. sanguineus s.l.,* and four (7%) had DNA evidence of genera-wide Rickettsia species. The results of this project demonstrated canine seroprevalence levels lower than those previously reported from dogs in highly endemic areas, indicating a low risk of SFGR transmission to humans in the southern Arizona border region at this time. Continued surveillance is critical to identify SFGR emergence in new geographic regions and to inform prevention efforts for humans and dogs in those areas.

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CONFLICT OF INTEREST

The authors have no conflicts to disclose. The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.

Keywords

dog; *Rhipicephalus sanguineus*; Rickettsia rickettsii; Rocky Mountain spotted fever; serosurvey; spotted fever group; ticks

1. INTRODUCTION

Rocky Mountain spotted fever (RMSF), part of a family of diseases known as spotted fever group rickettsioses (SFGR), is recognized as one of the most prevalent and severe tick-borne rickettsial illnesses in the United States (U.S.). RMSF continues to be an emerging public health threat in the south-western United States since its recognition in the region in 2003 (Biggs et al., 2016; Demma et al., 2005). The disease is caused by the highly pathogenic intracellular bacterium *Rickettsia rickettsii,* with early nonspecific clinical manifestations including fever, headache and rash, which can progress to severe sepsis, widespread vasculitis and death. Doxycycline is the recommended antibiotic therapy and is most effective when administered early in the course of illness (Biggs et al., 2016; Demma et al., 2005; Openshaw et al., 2010).

The emergence of RMSF in Arizona in 2003 (McQuiston et al., 2014) led to the discovery of *Rhipicephalus sanguineus* sensu *lato,* the brown dog tick, as a newly identified vector for RMSF in the United States. (Demma et al., 2005). This finding was supported by a series of investigations in eastern Arizona that revealed a high level of *R. rickettsii* among *Rh. sanguineus s.l.* ticks (Demma et al., 2005; Eremeeva et al., 2006; Nicholson, Gordon, & Demma, 2006; Nicholson, Paddock, et al., 2006). The ecology of *Rh. sanguineus s.l.* is distinct from other tick vectors, and the epidemiology of RMSF in Arizona is unique compared to the rest of the United States, including infection in peridomestic settings, younger case age and higher case fatality rates (Demma et al., 2006, 2005; Openshaw et al., 2010). Since the discovery of RMSF in Arizona, over 385 human cases and 23 fatalities have been identified (ADHS et al., 2017). These cases have predominantly been identified on American Indian tribal lands, where large populations of free-roaming dogs support *Rh. sanguineus s.l.* populations (Demma et al., 2006; Diniz et al., 2009; Folkema, Holman, McQuiston, & Cheek, 2012; Holman, McQuiston, Haberling, & Cheek, 2009; Nicholson, Gordon, et al., 2006; Nicholson, Paddock, et al., 2006). In addition, binational cases have been identified among Arizona residents that become infected with RMSF while visiting Mexico, where the pathogen and vector are also present (Drexler et al., 2017).

RMSF is re-emerging in Mexico, with multiple recent outbreaks in the states of Sonora, Sinaloa, and Baja California and an increasing number of cases in the border cities of Nogales and Agua Prieta along the Arizona-Sonora border region (Alvarez-Hernández, 2010; Alvarez-Hernández & Contreras Soto, 2013; Alvarez-Hernández et al., 2017; Alvarez-Hernández, Murillo-Benitez, Candia-Plata Mdel, & Moro, 2015). The epidemiologic and clinical features of cases in Mexico parallel what is seen on Arizona American Indian tribal lands, with primarily peridomestic transmission and higher incidence among children. Increased risk for RMSF exposure occurs predominantly in lower socio-economic populations; this is further influenced by increased tick harbourage areas, and an abundance

of free-roaming dogs that disperse infected *Rh. sanguineus s.l.* around human residences (Alvarez-Hernández, 2010; Alvarez-Hernández et al., 2017; Eremeeva et al., 2011; Openshaw et al., 2010).

Dogs are thought to play a major role in the emergence and continual circulation of *Rickettsia* spp. on Arizona American Indian tribal lands and in Northern Mexico. Dogs are the preferred host for the *Rh. sanguineus s.l.* tick in all tick life stages and are at high risk for exposure to RMSF in hyperendemic areas (Demma et al., 2006, 2005; Diniz et al., 2009; Elchos & Goddard, 2003; Gasser, Birkenheuer, & Breitschwerdt, 2001; Nicholson, Gordon, et al., 2006; Nicholson, Paddock, et al., 2006). Dogs are clinically susceptible to RMSF and develop similar signs as those exhibited by humans, including fever, myalgia, lymphadenopathy, oedema of the face or extremities and petechial rash (Gasser et al., 2001; Greene & Breitschwerdt, 2011; Nicholson, Allen, McQuiston, Breitschwerdt, & Little, 2010; Paddock et al., 2002). However, infection can also be asymptomatic in dogs. Asymptomatic infection can result in transmission of the bacterium to uninfected ticks that attach (Elchos & Goddard, 2003; Nicholson et al., 2010; Nicholson, Gordon, et al., 2006; Nicholson, Paddock, et al., 2006). While not well-documented, dogs with RMSF infection via a single tick bite mount an antibody response that can persist for up to a year; this particularly applies to dogs that have been exposed in areas of high tick activity (Nicholson et al., 2010). Knowledge of seropositivity rates in dogs and monitoring their trends over time can provide clues for human disease risk, as dogs are likely to be infected before human cases occur. Impacted communities have previously reported an increase in recent illnesses in or deaths of dogs around the home within the weeks prior to human illness (Diniz et al., 2009; Elchos & Goddard, 2003; Nicholson, Gordon, et al., 2006; Nicholson, Paddock, et al., 2006; Paddock et al., 2002). In this way, dogs can serve as an early warning system for RMSF emergence in new areas and help target prevention strategies.

Previous canine serologic surveys conducted in 2003–2004 revealed high levels of antibodies to Rickettsia in dogs from two neighbouring American Indian communities experiencing an outbreak of RMSF (Demma et al., 2006, 2005). Additional studies in 2005–2006 outside documented outbreak areas in Arizona evaluated dogs for exposure to SFGR and found an overall 5.7% (range: 0%−17.5%) seropositivity rate (McQuiston et al., 2011). At present, the burden of SFGR along the Arizona-Sonora border region is not well-studied (Diniz et al., 2009; Drexler et al., 2017; McQuiston et al., 2011; Openshaw et al., 2010). Infected ticks and rickettsemic dogs could be transported across the border and possibly be a source of RMSF introduction and expansion to nonendemic areas (Alvarez-Hernández et al., 2017; Demma et al., 2005; Eremeeva et al., 2011; Folkema et al., 2012; Fritz, 2009). The objective of this investigation was to assess seropositivity rates to SFGR in dogs along the Arizona-Sonora border region to determine human risk and improve early detection of human cases.

2. METHODS

2.1. Study location and population

An exploratory serosurvey of owned, stray and relinquished dogs was conducted in March and April 2015 in three southern Arizona counties: Cochise, Santa Cruz and Yuma (Figure

1). These counties share a border with Sonora, Mexico, and are noncontiguous to areas where RMSF is considered endemic. Pima County, which is also located in the border region, was not sampled due to known presence of RMSF in a defined region. Study sites included two rabies vaccination clinics, at which owned dogs were sampled, and seven animal shelters, at which stray or relinquished dogs were sampled. County public health and animal control partners assisted in the identification and recruitment of the study sites. In order for animal shelters to be included as a study site, the facility was required to maintain records for stray and relinquished dogs to allow for collection of demographic data and also had to be willing to have dogs enrolled in the project.

2.2. Data collection and canine serosurvey

A brief demographic questionnaire was developed to collect information about the dogs including age, sex, neuter status (intact or neutered) and size. For owned dogs, additional questions regarding travel to Mexico and potential risk factors for tick exposure were included. Location information was obtained for dogs as the place of owner residence for owned dogs, location of prior owner residence for relinquished dogs or address at which stray dogs were found. These data were geocoded by latitude and longitude and mapped using the ArcMap (ESRI, version 10.5) application. Dogs were included in the study if they met the following criteria: at least three months of age or older, nonaggressive disposition, and written consent obtained from owner or animal shelter management staff (for stray or relinquished dogs). To ensure safety of the dogs and study personnel, any dogs that exhibited signs of aggression, became overly stressed during sample collection, or were ill or under quarantine at the animal shelters were excluded from the study. All study personnel were provided with training on project protocols before data collection began.

Owners who presented with their dogs at rabies vaccination clinics were invited to have their dogs participate in the study. If the owner agreed, a summary of the project was explained by study personnel, written consent was obtained, and demographic information was collected about the dog. RMSF education was also provided, and owners were offered free topical tick prevention medication for their dog. Dogs at the animal shelters that met the inclusion criteria were sampled, and the demographic questionnaires were completed with the assistance of the facility staff. Blood was collected from dogs for serologic testing by cephalic or jugular venipuncture by trained project staff. A veterinarian was on site for supervision during all sampling. Dogs were also inspected for the presence of ticks; any ticks noted were collected and submitted for identification and testing.

2.3. Blood and tick analysis

Blood samples were kept refrigerated on ice packs and transferred to the Arizona State Public Health Laboratory for processing. Samples were centrifuged at 2,600 rpm and stored at −20°C after serum was aliquoted. Canine samples were sent to the CDC Rickettsial Zoonoses Branch, Disease Ecology Laboratory; serum samples were tested by indirect immunofluorescence antibody assay (IFA) for IgG reactivity to *R. rickettsii* (Demma et al., 2006; Kato et al., 2013; McQuiston et al., 2011; Nicholson, Gordon, et al., 2006; Nicholson, Paddock, et al., 2006). Standard assay format was followed using a 1:2 dilution serial dilution scale and FITC-labelled, goat anti-dog IgG (gamma chain specific) conjugate,

which does not react with other canine immunoglobulin classes. Slides were all examined under epifluorescence illumination at 400× magnification by one examiner for both the screening and titration runs. Serum samples were screened at 1/32 dilution and reactive samples were titred to endpoint. Antibody titres reactive at ≥1/64 were considered positive for this study.

Collected ticks were stored in 70% ethanol and sent to Northern Arizona University for identification and genetic analysis for *Rickettsia* spp. After identification of tick species using morphological keys (Furman & Loomis, 1984), DNA was extracted and assessed using qPCR assays that target the 50S ribosomal protein (Life Science Research, Bio-Rad, Hercules, CA) and a hybridization probe assay, one for genera-wide *Rickettsia* spp. (Panrickettsia, PanR8, FOR 5' - AGC TTG CTT TTG GAT CAT TTG G-3', REV 5'-TTC CTT GCC TTT TCA TAC ATC TAG T- 3') and a second assay that specifically detects *R. rickettsii* (RRi6, FOR 5' -AAA TCA ACG GAA GAG CAA AAC-3', REV 5' CCC TCC ACT ACC TGC ATC AT-3') (Kato et al, 2013). Positive- qPCR PanR8 samples were then subjected to a nested-PCR protocol using primers that detect the outer-membrane protein A (ompA) and differentiate members of the SFGR and then were sequenced using standard Sanger sequencing on an ABI 3,730 (Environmental Genetics and Genomics Laboratory, NAU) (Kato et al., 2013).

2.4. Data analysis

Relinquished dogs were combined into the same category as stray dogs for analysis. In addition, medium and large dogs were combined into one category for analysis, with small dogs defined as weighing less than 20 pounds and large dogs weighing 20 pounds or more based on similar studies (McQuiston et al., 2011). Epi Info™ and SAS (version 9.3) were used to conduct univariate and multivariate analyses of blood results, tick findings, demographic characteristics of dogs and county of sampling. We compared proportions to estimate relative risk of seropositive samples, as well as for the presence of ticks, using Fisher's exact test. Locations sampled and areas with positive results by canine serology or ticks with positive results for SFGR were mapped using ArcGIS (ESRI version 10.5).

This project was reviewed by the Arizona Department of Health Services Human Subjects Review Board and determined to be non-research and exempt from IRB-review.

3. RESULTS

Serum samples were collected from 217 dogs from Cochise (n = 41), Santa Cruz (n = 106) and Yuma (n = 70) counties (Table 1). The majority of dogs across all sites were adults (>1 year of age) (n = 185; 85%), male (n = 111; 51%) and reported to be stray rather than owned or relinquished (n = 123; 57%). Neuter status varied the most across sampling sites, with 41% spayed or neutered across all sites, and a range of 29%−44% at the individual sites (Table 1). Overall, 11 dogs (5.1%) had positive IgG titres reactive with *R. rickettsii;* with titres ranging from 64 to 1,024 and a geometric mean titre of 120 (CI=73–197). The reciprocal titres for all dogs sampled ranged from ≤32 to 1,024. Seropositivity across the three counties ranged from 2.9% to 12.2% (Table 2). A higher proportion of seropositive dogs were identified in Cochise County (Table 2), but this finding was not significantly

different from Santa Cruz and Yuma Counties. Figure 1 illustrates the locations of seropositive dogs by sampling region. Large dogs were significantly more likely to be seropositive (11/151; 7.3%) than small dogs, as no small dogs had positive serologic results. There were no statistically significant associations between seropositive status and dog's age, sex, neuter status or ownership status (Table 3).

Owned dogs ($n = 94$) comprised 43% of the total dogs sampled; 5 (5.3%) were seropositive for *R. rickettsii* antibodies. Among all owned dogs, 33 had been taken to Mexico as reported by the owner. Areas of frequent travel included Nogales and Algodones, Mexico. Dogs travelled with owners most commonly on a monthly basis (46%). Of the five owned dogs that were seropositive, only one was reported to have recent travel to Mexico, approximately 1–3 months prior to sampling. To assess whether owned dogs living in the border region had any increased risk for tick exposure, owners were asked whether their dogs mostly spent time inside the house and if their dog was contained by a leash or fence when outside. The majority of owned dogs lived inside the house (81%) and was contained by a leash or fence when allowed outside the house (84%). All of the owned dogs that were seropositive were reported to be kept mostly indoors.

Among all dogs in the project, 17 (8%) had tick infestations at the time of sampling. None of these dogs had serologic evidence of exposure to SFGR. Stray dogs were 5.7 times) more likely to have ticks attached than owned dogs (95% CI 1.34–24.45; $p < 0.001$). Other factors possibly associated with the presence of ticks, including age, sex, size and neuter status, were not statistically significant between dogs with and without ticks (Table 4). The average tick count among dogs with ticks was 3.3 ticks per dog (range 1–15). A total of 57 ticks were collected during the investigation and all ticks were identified as *Rh. sanguineus s.l.* Four ticks (Figure 1) (7%) had DNA evidence of genera-wide *Rickettsia* species; however, none of the ticks yielded positive PCR results in the *R. rickettsii-specific* assay. Among stray dogs, six dogs sampled at one of the study sites were identified to be free-roaming dogs from American Indian tribal lands not known to be impacted by RMSF; all tested seronegative for SFGR antibodies.

4. DISCUSSION

This investigation aimed to address the prevalence and distribution of SFGR antibodies in canine populations in the ArizonaSonora border region to better understand the risk to human and animal health. The overall 5.1% seropositivity of dogs identified in this investigation demonstrates seroprevalence levels much lower than reports from highly endemic areas, which ranged from 12.5% to 100% in dogs from impacted American Indian tribes (Demma et al., 2006, 2005; McQuiston et al., 2014, 2011). The level of canine seroprevalence to SFGR found in this study is, however, similar to what was observed in nonendemic areas of Arizona (5.7%) (McQuiston et al., 2011), as well as on American Indian tribal lands in 1996 (5%) before RMSF was identified in humans (Demma et al., 2006, 2005; Folkema et al., 2012; Nicholson, Gordon, et al., 2006; Nicholson, Paddock, et al., 2006; Openshaw et al., 2010). Continued surveillance among dogs and humans for illness compatible with RMSF remains critical to identify any evidence of disease

introduction or spread. Serosurveys may specifically provide an effective method of active surveillance to monitor SFGR activity in a defined area.

Among the dogs that had evidence of exposure to SFGR, the only factor associated with seropositivity was size (Table 3), with a higher proportion of large dogs (7.3%) having antibodies compared with small dogs (0%). This finding is potentially an artefact of the overall low level of seropositivity, apparent low levels of tick exposure and small proportion of small dogs sampled in comparison with large dogs. Size might also represent the likelihood of the dogs being regularly housed indoors versus outdoors, and by association risk for tick exposure and seroprevalence. There was no difference in risk for SFGR exposure by ownership status, age, sex, neuter status, presence of ticks or sampling location (Table 3). In a surprising manner, dogs with reported history of travel to areas with evidence of RMSF, such as American Indian tribal lands and Mexico, did not show an increased risk for disease or presence of ticks compared with dogs coming from other areas. The only factor associated with the presence of ticks on the dog was ownership status (Table 4), with a higher proportion of stray dogs having ticks present (12%) compared with owned dogs (2%). None of the dogs with ticks observed at the time of sampling were seropositive. Ownership status likely contributes to dogs being regularly groomed and having tick prevention products applied, decreasing the risk for ticks. Moreover, pet ownership practices may differ in RMSF endemic areas, as dogs sampled during this project were more commonly kept leashed or in fenced yards which can be a protective measure for tick exposure. While ticks were collected from stray dogs, it is also likely that dogs sampled at animal shelters had lower tick burdens than expected due to veterinary care (i.e. tick removal, treatments or baths) received upon intake.

Although this serosurvey indicates limited exposure to SFGR among dog populations in southern Arizona, it does not quantify the risk for disease from ticks transported by dogs directly from Mexico or tribal lands. For example, a fatal case of RMSF reported in California occurred in a 53-year-old female who was exposed to infected ticks that were on a dog brought over from an endemic area in Mexico (Drexler et al., 2017). While trans-national exposure may be rare, this case exemplifies the need for increased education about the risk of RMSF on both sides of the border and the importance of treating dogs with tick preventive products before travel.

5. LIMITATIONS

The authors recognize several limitations to this investigation. Cross-reactions are possible between antibodies for *R. rickettsii* and other SFGR family members (Greene & Breitschwerdt, 2011). Serologic evaluation of dogs for other SFGR was not conducted as part of this study, and it is possible that the antibody titres detected were caused by exposure to other closely related SFGR (e.g. *R. rhipi- cephali, R. massiliae,* or *R. parkeri)* (Eremeeva et al., 2006; Nicholson et al., 2010; Herrick, et al, 2016; Allerdice, et al, 2017) that have been identified in the state. Also, the convenience sampling design through rabies clinics and animal care facilities might not be representative of all dogs in the border region. Owned dogs which presented at rabies vaccine clinics likely have a different risk for exposure than unowned dogs, or owned dogs not regularly vaccinated for rabies; however, we did not

observe any difference in risk for SFGR infection between owned and stray dogs. In addition, because ill dogs were excluded from the study, it is possible that either tick-infested dogs or those with possible infection were not included in the sampling. At last, antibodies represent a cumulative response to single exposures and multiple exposures to both heterologous and homologous rickettsial antigens. IgG antibodies against *Rickettsia spp.* can persist for lengthy time periods after initial infection, and a positive result does not indicate when infection occurred; conversely, antibodies levels could wane after initial exposure and some dogs could have negative results that were previously infected. In hyperendemic areas, repeated exposures result in high titre values and increased prevalence of antibodies, while other areas have sporadic elevated titres (Demma et al., 2006; McQuiston et al., 2011; Nicholson et al., 2010).

6. CONCLUSIONS

This study provides additional information about the potential geographic distribution of SFGR in the southern Arizona border region. While canine seroprevalence in this investigation was low, these findings indicate that dogs in the region have been exposed to SFGR, as seen in all three counties sampled. Although these data indicate a relatively low risk of RMSF infection to humans, other tick vectors infected with *Rickettsia* spp. could pose a risk to human health (Nicholson et al., 2010). Because of the growing importance of *R. sanguineus s. l.* as a vector of *Rickettsia* spp., continued surveillance for tick populations and symptomatic cases of disease among canine or human populations is needed.

Previous studies (Demma et al, 2006; Diniz et al., 2009; McQuiston et al., 2011) describe associations between canine se- roprevalence and human cases, and provide evidence that dogs can be effective sentinels for RMSF. Antibody seroprevalence investigations of SFGR among dogs in high-risk regions for RMSF introduction can provide valuable evidence about the potential human risk and geographic distribution where risk levels are unknown. Given the nonspecific clinical signs and lack of rapid diagnostic tests for RMSF for canine and human populations, there is a need for empiric antibiotic treatment for dogs or humans with clinically compatible illness and a high degree of suspicion in known endemic areas.

The current study helped to better define the risk of SFGR in the border region, and additional investigations in future years may be beneficial to identify early emergence or spread of SFGR into new geographic boundaries. This project provided an opportunity to educate animal control staff and pet owners and highlighted the importance of education, particularly for owners that travel with their dogs to hyperendemic areas. Continued outreach about the risk of disease and prevention methods is critical to detect and prevent further spread of SFGR in Arizona. Perhaps most importantly, testing and education will lead to greater knowledge and awareness of the disease throughout the border region, tools for prevention and guidance for more effective disease control strategies.

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Impacts

- About 5.1% of owned and stray dogs sampled along the Arizona, USA, and Sonora, MX, border region were seropositive for SFGR antibodies.
- There were no statistically significant associations between seropositive status and age, sex, neuter status or ownership status of sampled dogs, although stray dogs were significantly more likely to have ticks attached than owned dogs.
- Rocky Mountain spotted fever continues to be a public health threat in the south-western United States and Mexico, and surveys of dogs may serve as an early warning system for disease emergence in new areas and help to target prevention strategies.

FIGURE 1.

Address of residence or collection for seropositive dogs *(n* = 11) and ticks (n = 4) by county sampled, March-April 2015. Dog serum tested for IgG antibodies by IFA specific for R. rickettsii. Ticks tested by PCR for genera wide Rickettsia species

Table 1

Characteristics of dogs sampled in Arizona, March-April 2015 ($n = 217$)

County (number of sites)	Cochise (4)	Santa Cruz (3)	Yuma (2)	Total (9)
Number of dogs	41	106	70	217
Adult dogs >1 year (%)	34 (82.9)	91 (85.8)	60 (85.7)	185 (85)
Male dogs (%)	22 (53.7)	54 (50.1)	35 (50.0)	111 (51)
Neutered dogs (%)	12 (29.3)	46 (43.4)	31 (44.3)	89 (41)
Large dogs >20 pounds(%)	39 (95.1)	59 (55.6)	53 (75.7)	151 (70)
Stray dogs (%)	41 (100.0)	32 (30.1)	50 (71.4)	123 (57)
Dogs with ticks present (%)	5 (12.2)	4(3.8)	8 (11.4)	17 (8)

Table 2

Antibody (IgG) titres by IFA[a] to *Rickettsia rickettsii* among dogs sampled in Arizona, 2015 (n = 217)

County	Cochise	Santa Cruz	Yuma
IgG titre			
≤32	36	102	68
64	3	2	1
128	1	1	0
256	1	0	1
1,024	0	1	0
Number positive/ total tested (%)	5/41 (12.2)	4/106 (3.8)	2/70 (2.9)
Chi-square test p-value	0.07		

[a]Indirect immunofluorescence antibody assay.

Table 3

Associated factors for *R. rickettsii* antibody-positive status among stray and owned dogs sampled in Arizona, 2015 *(n* = 217). The ‘denotes statistical significance

Variable	Number of seropositive dogs/total	Percent	Relative risk (95% confidence interval); p-value (Fisher exact)
Adult			
>1 year	10/185	5.4	RR 1.02 (0.95–1.09)
<1 year	1/32	3.1	
Male	6/111	5.4	RR 1.01 (0.95–1.07)
Female	5/106	4.7	
Neutered	5/89	5.6	RR 1.01 (0.94–1.08)
Not neutered	6/128	4.7	
Large dog	11/151	7.3	RR 1.08 (1.03–1.12); p=0.02*
Small dog	0/66	0	
No ticks present	11/200	5.5	RR 1.06 (1.02–1.09); p = 0.19
Ticks present	0/17	0	
Stray	6/123	4.8	RR 1.05 (0.94–1.08)
Owned	5/94	5.3	
n = 94			
No travel	4/61	6.6	RR 1.04 (0.95–1.14)
Travel to Mexico	1/33	3.0	

Table 4

Associated factors for presence of ticks among stray and owned dogs sampled in Arizona, 2015 (n = 217). The *denotes statistical significance

Variables	Number with ticks present/total	Percent	Relative risk (95% confidence interval); p-value (Fisher exact)
Adult			
>1 year	13/185	7.0	RR 1.78 (0.62–5.11)
<1 year	4/32	12.5	
Female	11/106	10.4	RR 1.91 (0.74–5.00)
Male	6/111	5.4	
Neutered	7/89	7.9	RR 1.01 (0.39–2.54)
Not neutered	10/128	7.8	
Small dog	6/66	9.1	RR 1.25 (0.48–3.23)
Large dog	11/151	7.3	
Stray dog	15/123	12.2	RR 5.7 (1.34–24.45); $p < 0.001$*
Owned dog	2/94	2.1	
n = 94			
No travel	2/61	3.3	RR undefined; p=0.54
Travel to Mexico	0/33	0	