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Vector and host factors driving the epidemiology of rickettsial pathogens across the western US-Mexico border

Author

Backus, Laura Harlan

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Vector and Host Factors Driving the Epidemiology of Rickettsial Pathogens Across the Western US-Mexico Border

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Janet Foley, Chair

Jonathan Dear

Heejung Bang

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Vector and host factors driving the epidemiology of rickettsial pathogens across the western US-Mexico border

Abstract

Tick-borne rickettsioses are an expanding threat to public health and veterinary health in western North America. The emergence of Rocky Mountain spotted fever (RMSF) in northern Mexico and Arizona in the last 20 years exemplifies the changing risk, with outbreaks carrying an unusually high human case fatality rate, ranging from 10% to over 30%. These outbreaks are associated two species of the brown dog tick, *Rhipicephalus sanguineus* s.l., which can spread *Rickettsia rickettsii* (the cause of RMSF) to dogs, humans, and potentially wildlife species. It is unknown whether risk factors for disease transmission differ between the two species, which are known as the tropical and temperate lineage. Known risk factors for RMSF in the region include a high density of domestic dogs, particularly free roaming dogs. However, this alone is not adequate to explain the sporadic but rapid emergence of RMSF, and the reasons why RMSF emerged in western North America when it did remain unknown. In addition, though *R. rickettsii* has been identified in wildlife species, a sylvatic or wildlife cycle has not been identified in this region. The focus of the three chapters of this dissertation were to investigate the factors influencing transmission of *R. rickettsii* and other tick-borne pathogens that may be associated with RMSF in southern California and northern Mexico. In Chapter 1, the effect of hot temperatures on the host preference of two lineages (tropical lineage and temperate lineage) of brown dog tick were assessed in the laboratory. In Chapter 2, to investigate the potential role of lagomorphs and their ectoparasites in the maintenance and transmission of rickettsial pathogens, a field study was studied in northern Baja California, testing rabbits, hares, and their ectoparasites for exposure to and infection with rickettsial organisms. Finally, a cross sectional study of tick-borne pathogen exposure and infection in sheltered dogs southern California and northern Baja California was conducted to determine the potential for pathogen transmission by brown dog ticks in this population.

Cases of rickettsial disease and rates of humans being bitten by brown dog ticks have been observed to increase during hot weather events in Europe. To investigate whether weather events may influence biting of humans in western North America, ticks of each lineage were tested to determine whether they chose to move towards a human or a dog at room temperature (23.5 °C) and hot temperature (38 °C). Adult tropical lineage ticks were 2.5 times more likely (95% CI: 1.71-3.65) to choose humans at 38 °C than at 23.5 °C, without a change in risk of choosing a dog, demonstrating a reversal in preference at hot temperatures. Temperate lineage adults were significantly less likely to choose the dog at 38 °C than 23.5 °C (RR 0.34, 95% CI 0.20-0.57) but the risk of ticks choosing humans was unchanged. Hot weather events may increase the risk of human exposure to bites by the brown dog tick, especially where the tropical lineage is present.

The role of lagomorphs in the transmission of RMSF has been hypothesized but not proven, and they are understudied for their role in rickettsial maintenance and amplification in western North America. Rabbits and hares were trapped in two locations and tested for infection with and exposure to rickettsial organisms, and ticks and fleas. Rabbits from Ensenada had significantly higher odds of being seropositive than rabbits from Mexicali ($p = 0.04$), and 88% of *Dermacentor parumapertus* ticks collected in that location (95% CI: 0.77-0.95) were infected with *Rickettsia bellii*, a rickettsial organism not known to be pathogenic. A single tissue sample from a hare in Ensenada was also positive for *R. bellii*. These findings indicate that rickettsial species that are not believed to cause disease may be affecting immunity and susceptibility in lagomorph populations.

Dogs may serve as sentinels for zoonotic pathogens and dogs in shelters may be at especially high risk of exposure. A cross sectional study of dogs from seven shelters in four locations (San Diego County,

Imperial County in southern California and Mexicali and Ensenada in northern Baja California) showed that dogs in all four locations had exposure to pathogens carried by brown ticks (*Anaplasma*, *Ehrlichia,* and *Rickettsia* species). In Tijuana, two dogs from different shelters were infected at the time of sampling with *R. rickettsii*. Infection with *E. canis* and *A. platys* ranged across shelters from 0 to 27% and 0 to 33%, respectively. Dogs in all four locations demonstrated exposure to all three pathogens, though *Rickettsia* and *Ehrlichia* seropositivity was highest in Mexicali (81% and 49%, respectively) and *Anaplasma* seropositivity was highest in Tijuana (45%). Findings from this research suggest that sheltered dogs may serve as valuable sentinels for RMSF in communities, and that while the risk of RMSF in southern California may be lower, the potential for transmission by brown dog ticks exists in both San Diego and Imperial counties.

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Introduction

An Introduction to Rickettsial Zoonoses at the Western US-Mexico Border

Laura Backus[1] and Janet Foley[1]

[1] Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California, Davis, CA 95616, USA

Background

Rickettsial zoonoses are an expanding health threat to humans and animals around the world, and represent the largest class of emerging vector-borne diseases (Jones et al., 2008). The worldwide burden of rickettsial disease in humans is underestimated, and will likely remain so on an international scale due to diagnostic challenges (Dye-Braumuller et al., 2022; Straily et al., 2020). Cases of tick-borne rickettsioses have markedly increased since the turn of the 21st century, even accounting for changes in reporting (Biggs, 2016; Eremeeva and Dasch, 2015). Similarly to other vector-borne diseases, potential drivers of increased rickettsial disease include climate change, land use change, and increased movement of humans and animals (Semenza and Menne, 2009). Rickettsial disease disproportionately affects communities and individuals in poverty, due to both reduced access to health care and increased exposure to vectors (Salje et al., 2021). The relationship of between rickettsial disease in domestic animals and wildlife is primarily studied due to their role in maintaining human pathogens, but morbidity and mortality occurs in animals as well (Beeler et al., 2011; Kidd et al., 2017). Understanding the drivers of rickettsial disease emergence is critical for predicting cases and protecting human and animal health, yet current knowledge is inadequate to provide specific and meaningful data to protect populations against disease before it occurs.

The epidemiology and human risk factors for rickettsial zoonoses are dependent on and intertwined with the ecology of the pathogens and their vectors. In western North America, along the US-Mexico border, outbreaks of Rocky Mountain spotted fever (RMSF) that began in the early 2000s exemplify the human health risk, ecological complexity, and difficulties in predicting emerging rickettsial disease. While RMSF in the United States has had a case fatality rate of 1-2% since antibiotic therapy became widely available, these outbreaks in the southwestern United States and northern Mexico carried extremely high case fatality rates, from 10% to over 30%, depending on location (Álvarez-López et al., 2021; Biggs, 2016).

The tendency for RMSF to appear in discrete geographic locations, then disappear, has been recognized at least since the time the diseases have been recorded in western literature. First described in scientific literature in the Bitterroot Mountains of Montana (the origin of the name), it caused severe disease in settlers in the late 19th and early 20th century, and subsequently vanished (Heyneman, 2001). The appearance and disappearance of the disease in Mexico was noted in the mid 20th century as well (Bustamante and Varela, 1943). From that time forward, however, RMSF essentially disappeared from western North America, and became a disease that in the United States was primarily associated with the American dog tick, *Dermacentor variabilis,* in the central and eastern portions of the country, causing individual or small sporadic clusters of cases (Schriefer and Azad, 1994). The outbreaks of RMSF in Arizona and northern Mexico were associated with the brown dog tick, *Rhipicephalus sanguineus* s.l., a tick that had, in the United States, been forgotten as a potential vector of the disease (Demma et al., 2005; Elchos and Goddard, 2003). It is unclear why RMSF erupted in this area when it did, but it is a region with marked rickettsial diversity occurring in domestic dogs, wildlife, and vectors (Eremeeva et al., 2006; López-Pérez et al., 2022). Disease emergence occurs when there is a change in the relationship between the pathogen, host, and vector. In this review, we discuss what is known about rickettsial pathogens in this region, and the interactions between them, their vectors, and hosts that influence spillover to humans.

Rickettsial classification

There are currently (as of August 1, 2022) 29 valid, unique species within the *Rickettsia* genus, though a significant number of new species are currently under description and characterization (https://www.bacterio.net/genus/rickettsia). All pathogenic members of the genus *Rickettsia* are obligate intracellular bacteria that depend on arthropod vectors (primarily fleas, ticks, lice, and mites) for maintenance and transmission (Fournier and Raoult, 2009; Socolovschi et al., 2009).

Each species of the genus is classified into one of four groups: spotted fever, typhus, and transitional groups, with an "outlying" group of species, sometimes called the ancestral group (Fournier and Raoult, 2009; Gillespie et al., 2008). Pathogenicity of individual species is markedly variable, and genotype group is not itself associated with pathogenicity. Individual species range from non-pathogenic endosymbionts (that are part of a commensal or mutualistic relationship with their arthropod host, without causing illness in vertebrates) to highly pathogenic organisms that cause morbidity and mortality in mammalian hosts (Fournier and Raoult, 2009). Both the spotted fever group (SFG) and typhus group (TG) contain a number of well-established human pathogens, the best known of which are *R. rickettsii* and R. *conorii* (the cause of Mediterranean spotted fever) in the SFG and *R. typhi* in the TG; a few species that are believed to be non-pathogenic; and a large number of species whose pathogenicity is either considered to be low or is not well understood (Eremeeva and Dasch, 2015). Most SFG *Rickettsia* are tick-borne, while TG *Rickettsia* are primarily flea-borne (Eremeeva and Dasch, 2015). In the transitional group, *R. akari* is the cause of rickettsialpox and is transmitted by mites; *R. felis* is a widespread, emerging pathogen carried by fleas and potentially other vectors (Gillespie et al., 2007; Paddock et al., 2006). *Rickettsia bellii* and *R. canadensis* are the only described members of the ancestral group, are both tick-borne, and neither is considered pathogenic to humans or other mammals (El Karkouri et al., 2022). It should be noted that some authors have determined that based on phylogeny, the transitional group should be integrated with the SFG, and that *R. bellii* and *R. canadensis* are not closely enough related to be considered a single group; as new species are described, it is expected that interpretation of these relationships will continue to evolve (Parola et al., 2013).

Rickettsial Pathogens in Western North America

Rickettsia rickettsii

The diversity of rickettsial species found in the southwestern United States and northwestern Mexico is exceptionally broad, and spans all four rickettsial classifications. As discussed above, *R. rickettsii* is the pathogen with the greatest human disease concern. Two separate species in the *R. sanguineus* s.l. family—historically referred to as the temperate and tropical lineages—spread the pathogen, with the temperate lineage acting as a vector in the Arizona outbreaks and the tropical lineage in Mexicali in Baja California (Demma et al., 2005; Nava et al., 2018; Zemtsova et al., 2016). The two species have very similar life cycle characteristics, typically feeding on dogs at every life stage and thriving in peridomestic environments with high densities of dogs (Gray et al., 2013). Since they were first described as separate lineages and subsequently different species, they have been considered to have discrete geographic distributions controlled by environmental temperature (Jones et al., 2017; Zemtsova et al., 2016). However, in southern California, the tropical lineage appears to be moving northward, and geographic overlap has been identified in both southern California and Arizona, and it is unclear whether this overlap and expansion will affect the epidemiology of RMSF and other rickettsial diseases in the region (Brophy et al., 2022; Villarreal et al., 2018). It is well established that *R. rickettsii* isolates from different geographic locations and tick species have varying levels of pathogenicity when tested in the laboratory, and genetically, *R. rickettsii* isolated from brown dog ticks in Arizona falls into a separate clade from *R. rickettsii* from Mexicali (Clark et al., 2015; Eremeeva and Dasch, 2009; Paddock et al., 2014). It is not known whether this difference is associated with the different vector species, or whether it impacts virulence.

The association between RMSF in dogs and humans has been reported within households, where cases have occurred simultaneously in dogs and owners, and at higher rates in people reporting close contact with dogs in communities with outbreaks (Demma et al., 2006; Elchos and Goddard, 2003; Paddock et al., 2002). High dog density and free roaming dogs are associated with cases in Arizona and northern Mexico, risk factors that are also associated with poverty (Drexler et al., 2014; Reyes-Castro et al., 2021). Interventions resulting in decreased rates of tick infestation in dogs are associated with decreased human cases, and use of both long-lasting ectoparasite control on dogs and intensive chemical-based tick control in houses reduce risk to people (Alvarez-Hernandez et al., 2020). It is hypothesized that the population structure of dogs in the community plays a key role in driving tick populations and pathogen transmission, because younger dogs are susceptible to higher tick burdens and high birth rates mean a continual supply of puppies and young dogs susceptible to infection (Gray et al., 2013; López-Pérez et al., 2020). Because of this, spay and neuter for population control and restraint of dogs (i.e., not permitting dogs to roam freely) have been implemented where possible, but are resource intensive.

Dog and brown dog tick transmission appears to be adequate to sustain RMSF outbreaks, without the need for alternate vectors or pathogen hosts. This is likely enhanced by the ability of the brown dog tick to transmit *R. rickettsii* transovarially, so the pathogen can be maintained for multiple generations even if naïve hosts are not present (Piranda et al., 2010). However, elsewhere in North and South America, other species appear to play a key role in amplifying *R. rickettsii*. In South America, capybara—the largest extant rodent species in the world—are competent hosts for *R. rickettsii* and serve to infect *Amblyomma* species ticks (Polo et al., 2017; Ramírez-Hernández et al., 2020), and in the United States, rodents and lagomorphs have been proposed as rickettsial reservoirs or amplifiers (Adjemian et al., 2008; Burgdorfer et al., 1980; Gage et al., 1990; Kollars, 1996). It is possible, though unproven, that a sylvatic cycle for *R. rickettsii* is present in the western border region, or that wildlife and their tick vectors are contributing at least intermittently to pathogen transmission. An infected coyote was identified near an RMSF outbreak area in Mexicali, suggesting that other canid species could be responsible for maintenance and spread of the pathogen and infected vectors (López-Pérez et al., 2022). The relatively recent emergence of disease in this region and the potential ecologically complexity means that there is significant room for further investigation of alternative contributors to the eco-epidemiology of RMSF in the region.

Rickettsia parkeri

Rickettsia parkeri is an emerging tick-borne pathogen throughout the Americas that overlaps geographically and symptomatically with *R. rickettsii,* though disease (called "*Rickettsia parkeri* rickettsiosis") in humans tends to be a milder, self-limited febrile illness accompanied by an eschar at the site of tick bite (Paddock et al., 2004; Peniche-Lara and Lara-Perera, 2022; Silva-Ramos et al., 2021). Similarly to *R. rickettsii*, *R. parkeri* is vectored by multiple tick species in multiple host-tick cycles throughout the western hemisphere, but transmission, amplification, and disease burden are all incompletely described in the western border region. In the eastern United States, increases in *R. parkeri* rickettsiosis are correlated with the expansion of the Gulf Coast tick, *Amblyomma maculatum* (Sumner et al., 2007). Human cases of *R. parkeri* rickettsiosis have been diagnosed in Arizona associated with *Amblyomma triste*, a tick species closely related to the Gulf Coast tick, but the pathogen has also been detected in brown dog ticks in close proximity to reported RMSF cases (Herrick et al., 2016; López-Pérez et al., 2022; Yaglom et al., 2020). Dogs can become infected with *R. parkeri*, though whether they experience significant clinical signs with infection is unclear (Grasperge et al., 2012). Different strains of *R. parkeri* are associated with different vectors in different locations, and may have differential pathogenicity (Allerdice et al., 2021; Nieri-Bastos et al., 2018). The Arizona cases and infected brown dog ticks were associated with a strain associated primarily with *A. maculatum*, but the Black Gap strain has been found in *Dermacentor parumapertus* ticks feeding on hares (the black-tailed jackrabbit, *Lepus californicus*) in the border region and northwestern Mexico (Sánchez-Montes et al., 2018).

The overlap between vectors, clinical signs, and geographic distribution between *R. parkeri* and *R. rickettsii* in the southwestern border region highlights the necessity of delineating the transmission cycles. When serology is used to diagnose spotted fever rickettsiosis, it is not possible to differentiate between infection with the two pathogens using commercially available assays (Hechemy et al., 1989; Raoult and Paddock, 2005). Because it also occurs in dogs, and may be spread to both dogs and humans by the brown dog tick, the drivers of *R. parkeri* and *R. rickettsii* epidemiology could be similar in some locations. However, because *R. rickettsii* carries much greater clinical severity, at the epidemiological level, differentiating between them is vital.

Rickettsia massiliae

After first being formally described in 1993, *R. massiliae* has been identified as one of the most cosmopolitan spotted fever group pathogens (Beati and Raoult, 1993). It is an emerging human pathogen with an increasing number of cases being reported, although the incidence is unknown (García-García et al., 2010; Vitale et al., 2006). Clinical signs reported in humans thus far include fever, spotted-fever like petechiation, and a clinical syndrome called Scalp Eschar and Neck Lymphadenopathy After Tick Bite (SENLAT), a condition more commonly associated in Europe and Asia with *R. raoultii* and *R. slovaca* (Cascio et al., 2013; García-García et al., 2010; Zaharia et al., 2016). Human cases have not been diagnosed in the United States or Mexico, but dogs with presumptive infection and signs similar to RMSF were diagnosed in Southern California (Beeler et al., 2011), and infected brown dog ticks have been identified across California, Arizona, and northern Mexico (Beeler et al., 2011; Eremeeva et al., 2006; López-Pérez et al., 2019). Because human infections with *R. massiliae* have not been reported in the United States, it is unknown at this time whether the strains found in North America are pathogenic to humans, but diagnostic challenges, and overlap with other spotted fever rickettsias in the same vector species, currently limit the understanding the impact of this pathogen in the region (Eremeeva et al., 2006). No wildlife or other domestic species have been found infected with *R. massiliae*, and thus far the pathogen has only been found in brown dog ticks in North America. Because *R. massiliae* is transmitted transovarially, the infection can be efficiently maintained in a tick population without requiring feeding on an infected host, so it is possible that a continual enzootic transmission cycle is not present (Beeler et al., 2011; Matsumoto et al., 2005). However, it has been shown that in dogs, inoculation with one strain of *R. massiliae* from Arizona (AZ80) induces spotted fever group antibodies as well as cross protection from pathogenic *R. conorii* (Levin et al., 2014). Therefore, while it remains unclear whether dogs are amplifying *R. massiliae*, exposure to *R. massiliae* may induce SFG antibodies that prevent infection with other, more pathogenic species. Further research is indicated to determine whether this effect is seen with natural exposure via feeding by infected ticks, as it could significantly impact rickettsial transmission dynamics in areas of high brown dog tick density.

Rickettsia 364-D (Candidatus Rickettsia philippi)

Of the SFG pathogens discussed here, *Rickettsia* 364-D is the only rickettsial agent not known to be transmitted by the brown dog tick. First recognized as a human pathogen in 2008, the pathogen is the cause of Pacific Coast tick fever and is spread by *Dermacentor* species ticks, most notably by the Pacific Coast tick, *D. occidentalis* (Paddock et al., 2018; Shapiro et al., 2010). Pacific Coast tick fever is characterized by a low grade febrile illness and eschar at the site of tick bite, similar to other SFG pathogens (Johnston et al., 2013). While the Pacific Coast tick has a very narrow distribution range compared to the brown dog tick, it occurs from Baja California northward and infected ticks have been identified in San Diego County in southern California (Furman and Loomis, 1984; Guzmán-Cornejo et al., 2016; Wikswo et al., 2008). Risk factors for exposure to infected Pacific Coast ticks are very different from those associated with exposure to brown dog ticks—they are typically acquired during work or recreation in natural landscapes, and do not occur indoors—but there may be general geographic overlap, and infection with 364-D may not be distinguishable from other eschar-causing SFG rickettsial illnesses based on clinical signs or serology (Johnston et al., 2013; Shapiro et al., 2010).

Rickettsia typhi

As the cause of flea-borne typhus or murine typhus, *R. typhi* occurs in markedly different cycles than the tick-borne, SFG pathogens described here, yet is present in rodent and their fleas throughout the same region. Unlike SFG carried by brown dog ticks, *R. typhi* cases are not associated with contact with dogs, and instead occur in western North America when there is spillover from one of two transmission cycles: either from rats (*Rattus rattus* or *Rattus norvegicus*) and the Oriental rat flea *Xenopsylla cheopsis*, or the opossum (*Didelphus virginianus*) and the cat flea, *Ctenocephalides felis* (Blanton et al., 2015; Wiggers and Stewart, 2002). While infection with *R. typhi* does not cause the classical "spotted fever" signs of the spotted fever group, non-specific febrile illness with a rash is a common presentation (Civen and Ngo, 2008; Green et al., 2011). *R. typhi* is considered endemic in southern California, where most of the cases in this region are diagnosed annually, but is found in fleas of wildlife in Baja California, suggesting the disease may be underrecognized in these regions (California Department of Public Health, 2022; López-Pérez et al., 2022).

Rickettsia felis

Rickettsia felis is an emerging pathogen that was named after it was first described from the cat flea, *C. felis* (Adams et al., 1990; Higgins et al., 1996). Since that time, it has been found on six continents in a markedly diverse variety of arthropods, including brown dog ticks, multiple species of *Haemaphysalis* and *Amblyomma* ticks, a non-parasitic booklouse, mites, and additional flea species (Angelakis et al., 2016; Reif and Macaluso, 2009). It is also increasingly recognized as a human pathogen, having been isolated from patients worldwide (Reif and Macaluso, 2009). It has been detected in asymptomatic individuals; in those that are symptomatic, a non-specific febrile illness very similar to flea-borne typhus occurs (Pérez-Osorio et al., 2008). Cats infested with fleas carrying *R. felis* will produce anti-rickettsial antibodies, but dogs appear more likely to be competent reservoirs, achieving rickettsemia capable of infecting feeding fleas (Hii et al., 2011; Wedincamp and Foil, 2000). Though not a member of the typhus group, some cross reaction between antibodies to *R. typhi* and *R. felis* may occur, meaning that serologic testing against only one pathogen or the other may be misleading (Teoh et al., 2016). Fleas from the same host may also carry both *R. felis* and *R. typhi*, further complicating identification of etiologic agents (Abramowicz et al., 2010). Fleas from a variety of wildlife, ranging from Norwegian rats to opossums to bobcats, and domestic pets, including dogs and cats, have been found to positive for *R. felis* in southern California and northwestern Mexico (Abramowicz et al., 2012, 2010; López-Pérez et al., 2022). In southern California, *R.felis* has been associated with cases of rickettsial disease in urban areas, although given the bacteria's ubiquitous nature in the face of relatively few rickettsiosis cases, the role of *R. felis* relative to *R. typhi* in causing disease in the region has been questioned (Abramowicz et al., 2010; Billeter and Metzger, 2017).

Conclusion

Tick-borne rickettsioses are a transboundary, emerging One Health issue. The markedly diverse rickettisal pathogens present in the border region of the southwestern United States and northern Mexico have similar geographic, symptomatic, and vector characteristics, yet profoundly different impacts on human health. Their similarities make detection through case-based, syndromic, or serologic based surveillance very difficult, yet detecting highly pathogenic species like *R. rickettsii* is paramount for intervening and preventing illness, especially in the face of a geographically expanding epidemic. In addition, understanding the ecological characteristics that drive the epidemiology of disease emergence is essential for targeting surveillance. As is evident from the above discussion, a large number of critical knowledge gaps exist in our understanding of the forces behind rickettsial disease transmission at the vector, host, and pathogen levels. Interdisciplinary collaboration is critical to filling those gaps for the prevention of tick-borne rickettsioses. While the role of the brown dog tick is a common thread for many of the SFG pathogens in this region, drivers of human biting behavior and differences in vectorial competence between the lineages remain underexplored. The role of wildlife in serving as a source for pathogens and hosts for ticks is incompletely described, but this is key information for identifying communities and locations at risk. Finally, identification of effective sentinels and implementation of a sentinel surveillance program that allows for differentiation between rickettsial agents is needed to actively protect human health. Filling these gaps in knowledge will allow surveillance, prevention, and mitigation strategies to be employed effectively and, with adequate continuity of resources, sustainably, protecting the health of humans and animals.

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Chapter 1

Effect of temperature on host preference in two lineages of the brown dog tick, *Rhipicephalus sanguineus*

L.H. Backus[1], A.M. López Pérez[1], and J.E. Foley[1]

[1] Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California, Davis, CA 95616, USA

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Abstract.

Rhipicephalus sanguineus is a species complex of ticks that vector disease worldwide. Feeding primarily on dogs, members of the complex also feed incidentally on humans, potentially transmitting disease agents such as *Rickettsia rickettsii*, *R. conorii*, and *Ehrlichia* species. There are two genetic *Rh. sanguineus* lineages in North America, designated as the temperate and tropical lineages, which had occurred in discrete locations, although there is now range overlap in parts of California and Arizona. *Rh. sanguineus* in Europe are reportedly more aggressive towards humans during hot weather, increasing the risk of pathogen transmission to humans. The aim of this study was to assess the impact of hot weather on choice between humans and dog hosts among tropical and temperate lineage *Rh. sanguineus* individuals. Ticks in a two-choice olfactometer migrated towards a dog or human in trials at room (23.5°C) or high temperature (38°C). At 38°C, 2.5 times more tropical lineage adults chose humans compared with room temperature, while temperate lineage adults demonstrated a 66% reduction in preference for dogs and a slight increase in preference for humans. Fewer nymphs chose either host at 38°C than at room temperature in both lineages. These results demonstrate that risk of disease transmission to humans may be increased during periods of hot weather, where either lineage is present, and that hot weather events associated with climate change may result in more frequent rickettsial disease outbreaks.

KEY WORDS: *Rhipicephalus sanguineus*, lineage, host preference, dog, human

INTRODUCTION

The *Rhipicephalus sanguineus* complex comprises *Rh. sanguineus* sensu stricto (s.s.) as well as a group of morphologically and biologically similar ticks designated *Rh. sanguineus* sensu lato (s.l.).[1] The two lineages of *Rh. sanguineus* in North America have been designated as "temperate" and "tropical" lineages.[2] While the taxonomic status of the complex is not fully resolved, the temperate lineage is well-defined molecularly and corresponds to the *Rh. sanguineus* sensu stricto (s.s.) species, whereas a separate phylogenetic branch contains ticks in the tropical lineage and several other *Rhipicephalus* entities.[1] Both tropical and temperate lineage ticks are competent vectors for zoonotic pathogens including *Ehrlichia canis, E. chaffeensis*, *E. ewingii*, *Coxiella burnetti* and numerous spotted fever group rickettsias—notably *Rickettsia rickettsii*, *R. conorii*, and *R. massiliae.*[3–7] In North America, the temperate lineage was associated with an outbreak of Rocky Mountain spotted fever (RMSF) caused by *R. rickettsii* in Arizona in 2004,[6] while the tropical lineage has been implicated in ongoing outbreaks in Baja California Norte and Sonora, Mexico.[8–10]

All members of the *Rh. sanguineus* complex are endophilic and monotropic (all stages tend to feed on dogs), with incidental feeding on humans and other mammalian hosts.[11–15] Historically, however, the temperate and tropical lineages have occupied separate ranges in North America with the distribution likely influenced by local climate.[16] Globally, the tropical lineage is found where annual mean temperature exceeds 20 °C while the temperate lineage is restricted to mean temperatures below that.[2] Specific lineage adaptations likely drive the disparate distributions; for example, the temperate lineage has greater molt success at lower temperatures than the tropical lineage.[17] In North America, the tropical lineage is present across the far southern portions of the United States.[16] However, areas of overlap between the temperate and tropical lineages occur in both Southern California and Arizona, with evidence that the tropical lineage is moving progressively northward at the margins of its range in these areas[10,18].

Beyond ecological characteristics associated with presence and survival, comparisons of behavior and vector competence between the tropical and temperate lineages are limited. While both lineages are competent vectors, changes in the distribution of lineages, with differential survival and behavior under different climatic conditions, may result in changing patterns of rickettsial disease. Host attraction and feeding preferences are critical components of the eco-epidemiology of tick-borne disease. A network of preferred and pathogen-competent hosts is required to maintain an arthropod-borne pathogen within a system, and transmission of a pathogen is determined by the arthropod's host preferences as well as host availability.[19,20] Host preference and tick attachment are influenced by host factors (e.g. presence/absence of hosts, host behavior and physical and chemical signals from hosts) and environmental off-host factors (e.g. climate conditions and presence of microhabitat).[21–23] Shifts in host preference could affect feeding frequency and pathogen maintenance and disease spillover into alternate hosts.[19]

There is evidence that *Rh. sanguineus* may be more aggressive towards human hosts at increased ambient temperatures.[7] Attachment and initiation of feeding also occurs more rapidly at high temperatures.[24] Higher temperatures and lower humidity are associated with greater aggression (increased host seeking behavior or biting rate) in *Ixodes persulcatus*, and *I. scapularis* host choice is influenced by humidity.[25,26] Rickettsial disease emergence and reports of increased *Rh. sanguineus* aggression during heat waves in Europe may be due to an increased proportion of ticks feeding on humans compared to dogs, an absolute increase in aggression, or a combination.[7,27] The annual number of days over 38 °C (100 °F) is expected to increase markedly across most of the continental United States in next decade,[28] increasing concern for heat-driven emergence of tickborne disease. The aim of this study was to quantify differences in host preference and the effect of high ambient temperatures on host choice in two North American *Rh. sanguineus* lineages to determine whether climate and changes in lineage distribution may contribute to changing disease patterns.

MATERIALS AND METHODS

Tick Sources and Colony Maintenance

Engorged adult female tropical lineage *Rh. sanguineus* s.l. were collected from dogs who were infested on arrival at an animal shelter facility near Maricopa, Arizona, USA, and temperate lineage larvae were obtained from the colony maintained at Oklahoma State University. Partial sequencing of the 16s ribosomal subunit was performed on a subset of females after oviposition[29] and GenBank Blast search performed to confirm conspecificity with the tropical lineage. Both lineages were reared concurrently and maintained at 85-95% humidity with a 12h light/dark cycle. Approximately 80 days post-eclosure, larvae of both lineages were simultaneously fed on New Zealand white rabbits (Charles River Laboratories, Wilmington, MA) and permitted to molt. Behavior trials using nymphs were performed between 40 and 50 days post-molt. Nymphs were fed on naïve New Zealand white rabbits approximately 60 days post-molt. Adults resulting from fed nymphs were used in trials 40-50 days post-molt.

Olfactometer Design

A two-choice olfactometer was designed and constructed to conduct behavior trials, with design adapted from published olfactometer studies. [30,31] Two wooden boxes (91cm x 76cm x 122cm) with rear hinge-attached access doors were connected with a 122 cm long, 6.35cm diameter polyethylene terephthalate glycol plastic heavy wall tubing (see Figure 1.1). A small fan was placed in each box to facilitate air flow. The center tube was marked on both sides 30.5cm and 45cm from the midpoint, and a capped tube was inserted at the midpoint to allow tick placement. Fabric mesh was used to confine ticks to the center tube and prevent access to the human or dog. Ambient temperature was controlled by placing the center tube in a large Styrofoam cooler and heating the surrounding air with two household electric fan-powered space heaters for high temperature trials. Temperatures were recorded at the start of each trial and monitored continuously using a digital thermometer adjacent to the tubing.

Behavior Trials

For nymphs, five trials for each lineage were performed at each temperature (23.5 ± 1 °C and 38 ± 2.5 °C) for 20 trial iterations and 800 total ticks tested. Ambient humidity ranged from 10 to 42% in the 23.5°C trials and 10-35% in the 38 °C trials. For adults, 10 trials (5 of each sex) were performed at each temperature for a total of 40 trial iterations and 800 ticks tested. Ticks used in the high temperature trials were held for at least 6 hours at 40 °C and 80-90% relative humidity prior to testing.

For each trial, a dog occupied one box and a human the other box, with 10 minutes elapsed prior to starting the trial to allow accumulation of scent and carbon dioxide. All dogs were neutered or spayed and mid-size (15-30kg) owned pets with owner consent. For nymphs, five trials were conducted for each temperature/lineage combination, with 10 combinations of dogs and humans used (8 humans and 5 dogs). For adults, 10 trials (5 each for male and female ticks) were conducted for each temperature/lineage combination, with 6 combinations of dogs and humans used (4 humans and 3 dogs).

Ticks were deposited at the midpoint at the tube. For nymphs, 40 ticks of a single lineage were used for each trial. Trials of adult ticks were performed in groups of 20 of a single sex. Trials were performed for 20 minutes past the time ticks were placed in the tube. Every two minutes, the number of ticks in each section were counted. Ticks moving beyond 30.5cm (nymphs) or 45cm (adults) from the midpoint were considered to have demonstrated preference at that time point, but were free to move backwards and forwards.

Data Analysis

Data were managed and analyzed in R (R Foundation for Statistical Computing, Vienna, Austria). Separate log-binomial mixed effect generalized linear models were used for each stage to compare the effects of temperature on host choice using the "lme4" package in R.[32] Separate models were used for overall host choice, human host choice, and dog host choice. Tick choice for human, dog, or both were used as the dependent variable, temperature and lineage used as independent variables, and tick sex (for adult ticks) and interaction between temperature and lineage considered as additional predictors. The dog-human combination used for the trial was included as a random intercept. Temperature and lineage were both included as binary variables, with room temperature and tropical lineage used as reference. Individual model effect estimates were considered significant if $p<0.05$ when compared to the reference value, and relative risks calculated from the model were considered significant when the 95% Wald confidence interval did not include 1.

RESULTS

The two lineages varied markedly in their preference for humans versus dogs at room temperature. At room temperature, the temperate lineage showed greater preference for humans over dogs than the tropical lineage, with nearly twice as many temperate nymphs and 2.2 times more temperate adults choosing the human compared with the tropical lineage (Table 1.1).

High temperatures suppressed host choice in nymphs in both lineages, with fewer nymphs choosing any host at high temperatures, although the effect was non-significant in the temperate lineage (Figure 1.2). In tropical lineage nymphs, high temperature reduced the probability of ticks choosing either host by 43% (RR 0.57, 95% CI 0.35 to 0.92), a reduction primarily due to fewer ticks choosing dogs (RR 0.27, 95% CI 0.09-0.82) while the probability of a nymph choosing the human was unchanged (RR 1.02, 95% CI 0.54-1.89).

The effect of heat on host choice in adults differed significantly by lineage for human, dog, and overall host choice, with a greater effect observed in the tropical lineage as shown by the significant interaction between lineage and temperature (Table 1.2). Tick sex was not significant in any of the host choice models so was dropped from further analysis. In temperate lineage adults, overall host choice was not significantly different between room and high temperature, but there was a relative shift in preference for the human over dog, with a 66% decrease in the risk of a tick choosing a dog (RR 0.34, 95% CI 0.19-0.57) and a small but not significant increase in the risk of choosing a human (RR 1.22, 95% CI from 0.93 to 1.60). In the tropical lineage, high temperature increased the mean number of ticks choosing a host, which occurred primarily due to an increased preference for humans, with the risk of a tick choosing the human being 2.5 times greater at high temperature than at room temperature (95% CI 1.71-3.65); the probability of a tick choosing the dog did not change significantly.

DISCUSSION

In the first experiment to quantify the effect of temperature on host preference in *Rh. sanguineus*, we show that there is an increased risk of humans being bitten by adult ticks as temperatures increase. In temperate lineage *Rh. sanguineus*, high temperatures increased the relative preference of humans over dogs (a similar number of ticks made a host choice at each temperature, but a greater proportion selected the human), while in the tropical lineage, high temperatures increased the total number of ticks choosing humans. While it has been noted in multiple tick species that host aggression in general is higher under hot weather conditions,[7,26] these findings demonstrate that relative preference may also increase at high temperatures, so preference is increased more for one host than another. These findings suggest that changing climate may increase human bites and subsequent pathogen transmission, particularly in areas occupied by the tropical lineage. Expansion of the tropical lineage may result in climatic or weather events being stronger drivers of disease emergence in these areas, or overall increased risk of human disease, especially if the temperate lineage is not displaced.

In nymphs, there appears to be a smaller shift during increasing temperature towards relative preference for humans than was observed in adults, although the host drive decreased overall at higher temperatures. This is in contrast to the observation that attachment to humans occurs in greater numbers and more rapidly in nymphal *Rh. sanguineus* that are held at higher temperatures than at room temperature.[7] High temperatures may therefore suppress nymphal hunting or ambush behaviors while simultaneously increasing how rapidly they attach to a host as mechanism to allow nymphs to survive in environments where temperatures exceed our experimental conditions.[2,6] Unfed *Rh. sanguineus* nymphs are much more sensitive to heat and desiccation than adults,[33] so it is possible that host seeking was impaired under hot conditions. Drop-off of engorged nymphs and adult females tends to occur at night,[34] but the hunting behavior of nymphs in the environment has not been well described.

Experimental and surveillance studies suggest that typically a very small percentage of *Rh. sanguineus* attach to human hosts,[35,36] and olfactory preference rather than attachment was measured here. However, surveys of ticks feeding on humans have reported instances of human parasitism by both the temperate and tropical lineages around the world, suggesting that feeding on humans by *Rh. sanguineus* may be underrecognized.[37–40] Temporal and spatial clustering of human parasitism events have also been described, again in both lineages and with a broad geographic distribution in the Americas and Europe.[7,12,41,42] Clusters of human parasitism are less likely to be detected if not associated with cases of disease, but their occurrence suggests that there are forces driving *Rh. sanguineus* in these instances to feed on humans with increasing intensity that may be related to either tick behavior or focus of high density.

Genetic shifts in populations have been postulated to play a role in increased human parasitism.[12] Because groups within the *sensu lato* complex are morphologically similar or indistinguishable, invasion or expansion of other *Rh. sanguineus* lineages may occur undetected.[10,43,44] If the invading genotype differs in its behavior or vectoral capacity, a subsequent shift in human parasitism or disease transmission could be expected. The results of this experiment, however, demonstrate that behavioral changes due to environmental factors may be at least partially responsible for clusters of human parasitism and that epidemic transmission could be driven by weather events and climatic patterns.

The finding that high temperatures drive human parasitism is further supported by the relationship between rickettsial disease cases caused by *Rh. sanguineus* and weather events. Mediterranean spotted fever, caused by *R. conorii*, is also vectored by *Rh. sanguineus* and cases in Europe have been correlated repeatedly with periods of higher temperature.[45,46] In France in 2007, spring temperatures 3-4 °C above normal (over 25 °C) were associated with both observed intense human parasitism and rickettsial disease cases.[7] All life stages of *Rh. sanguineus* feed most frequently in the summer and fall[47], which is also when RMSF cases in the United States are most frequently reported,[6,48] though correlation between unusually hot weather events and specific cases or outbreaks has not been described. In Arizona, cases including the eastern Arizona epidemic display an extended seasonality, with cases reported into December, indicating a shift in tick activity.[49,50] Across the United States, temperatures greater than 35 °C are associated with cases of spotted fever rickettsiosis, although many of these are vectored by *Dermacentor variabilis*.[51] Taken together, these findings warrant further investigation into both transient weather events and climate trends as predictors of RMSF cases. In light of the experimental results here, the magnitude of effect on changes in human biting rates may depend on the particular population and lineage of *Rh. sanguineus*, although shifts away from dogs and toward humans may occur in areas where either lineage is present.

The finding that the temperate lineage had markedly higher preference for humans at room temperature was unexpected. However, some of the variation in baseline preferences between the tropical and temperate lineages may be attributable to the tick origins. The temperate lineage was obtained from colony-maintained ticks, which may have affected host seeking beavior through selection for feeding on rabbits over multiple generations, while the tropical lineage originated from wild-caught ticks feeding on dogs. This highlights a concern about the use of colony raised ticks for behavioral and life history studies and suggests that caution is warranted in extrapolating results from colony raised to wild ticks. In addition, variation may not be strictly due to genetic lineage, as local populations of the same species of tick may show different behavioral characteristics, as is the case for *I. scapularis*.[52]

This study was performed comparing only two temperatures, and further experiments are warranted to test lower temperatures (below 23 °C), at gradations between the temperatures used here, and higher temperatures to determine whether host preference varies linearly with temperature or whether there is a threshold effect. While this study shows that there is a shift in preference at hot temperatures, it does not quantify the change in number of bites that is expected to occur, and further research is needed to provide this data across the spectrum of environmental conditions expected to occur. The chemical or physiologic basis for host preference is driven by a number of mechanisms, not all of which are well understood in *Rh. sanguineus* ticks. The design of the two-direction olfactometer using actual hosts was intended to capture the cumulative effects of each of these factors and better replicate the conditions produced when ticks are host-seeking in the environment, where perception of two hosts may happen simultaneously, with ticks able to change direction as they get closer to a host. Ticks are attracted to hosts by chemical signals (host pheromones or kairomones), heat or infrared light,[53] and, in some cases, visual and auditory cues.[54,55] Carbon dioxide produced by host respiration is attractive to ticks across species, with the gradient likely used for host localization [56], and *Rh. sanguineus* specifically are responsive to ammonia.[22] These factors may vary between demographic groups of both dogs and humans.[57,58] Comparing a wider variety of dog breeds and sizes with more demographic variation of the humans, including children, would allow more refinement of the risk to humans at high temperatures.

From these results, we conclude that short term weather patterns and climatic change may both impact the transmission of pathogens transmitted by *Rh. sanguineus* to humans. It is difficult to directly correlate large-scale climate effects on the expansion of ticks and emergence of tick-borne diseases,[59] but these results suggest that even short term weather events may significantly affect disease transmission to humans and provides evidence that increased caution and tick protection is warranted under hot weather conditions. Hot weather events may be predictive of increased cases of *Rh. sanguineus*-borne zoonotic disease, particularly in areas where the sensu lato group may be present; range expansion of the tropical lineage with concurrent climatic changes may significantly alter pathogen transmission patterns in those areas. These results also highlight the importance of further and ongoing work to describe the changing distribution of the different lineages within the *Rh. sanguineus* complex in North America.

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Table 1.1. Mean and standard deviation of number of *Rh. sanguineus* ticks selecting dog or human per trial at room (23.5°C) and high (38°C) temperatures.

Nymphs

	Room Temperature	**High Temperature**	**T**	***p*-value**
Temperate lineage				
Mean # choosing dog ± SD (%)	1.4 ± 1.7 (3.7%)	1.0 ± 2.2 (2.6%)	-0.32	0.758
Mean # choosing human ± SD (%)	9.2 ± 3.3 (23.7%)	7.8 ± 5.9 (20.2%)	-0.46	0.658
Tropical lineage				
Mean # choosing dog ± SD (%)	8.4 ± 3.6 (21.9%)	2.8 ± 2.7 (7.1%)	-2.77	0.027
Mean # choosing human ± SD (%)	5.2 ± 3.7 (13.8%)	5.6 ± 2.4 (14.4%)	0.20	0.845

Adults

	Room Temperature	**High Temperature**	**T**	***p*-value**
Temperate lineage				
Mean # choosing dog ± SD (%)	4.9 ± 3.3 (25.8%)	1.6 ± 1.4 (8.2%)	- 2.92	0.013
Mean # choosing human ± SD (%)	6.5 ± 3.8 (33.7%)	7.9 ± 2.8 (41.2%)	0.94	0.361
Tropical lineage				
Mean # choosing dog ± SD (%)	5.2 ± 2.5 (22.5%)	4.4 ± 2.3 (25.3%)	0.74	0.471
Mean # choosing human ± SD (%)	2.9 ± 2.5 (14.8%)	7.5 ± 4.1 (37.4%)	3.03	0.009

Table 1.2. Beta coefficient estimates and associated relative risks (RR) with confidence intervals for log binomial models predicting the risk of *Rh. sanguineus* ticks choosing either host, human, or dog subject by temperature and lineage. Room temperature (23.5°C) and tropical lineage are used as reference categories.

		Nymphs					**Adults**				
		Estimate ± SE	**Z**	***p*-value**	**RR**	**95% CI**	**Estimate ± SE**	**Z**	***p*-value**	**RR**	**95% CI**
Either Host											
Intercept		-1.05 ± 0.15	-6.99	<0.01	0.35	0.25-0.48	-1.01 ± 0.09	-10.67	<0.01	0.36	0.30-0.43
Temperature	High (38 deg)	-0.56 ± 0.24	-2.28	0.02	0.57	0.33-0.94	0.55 ± 0.11	5.00	<0.01	1.74	1.42-2.17
Lineage	Temperate	-0.25 ± 0.15	-1.65	0.10	0.78	0.58-1.05	0.45 ± 0.11	3.95	<0.01	1.57	1.26-1.97
Interaction	High temperature * Temperate lineage	0.35 ± 0.24	1.44	0.15	1.42	0.88-2.29	-0.73 ± 0.15	-5.00	<0.01	0.48	0.36-0.64
Human											
Intercept		-2.02 ± 0.22	-9.13	<0.01	0.13	-0.08-0.20	-1.91 ± 0.17	-11.12	<0.01	0.15	0.10-0.20
Temperature	High (38 deg)	0.02 ± 0.32	0.06	0.96	1.02	0.53-1.96	0.91 ± 0.19	4.71	<0.01	2.50	1.73-3.72
Lineage	Temperate	0.55 ± 0.22	2.45	0.01	1.72	1.13-2.71	0.77 ± 0.20	3.84	<0.01	2.16	1.48-3.26
Interaction	High temperature * Temperate lineage	-0.19 ± 0.32	-0.60	0.55	0.83	0.44-1.54	-0.71 ± 0.24	-3.00	<0.01	0.49	0.30-0.77
Dog											
Intercept		-1.63 ± 0.33	-4.99	<0.01	0.20	0.09-0.41	-1.49 ± 0.14	-11.11	<0.01	0.23	0.17-0.29
Temperature	High (38 deg)	-1.30 ± 0.56	-2.31	0.02	0.27	0.07-0.83	0.15 ± 0.18	-3.84	0.40	1.16	0.82-1.66
Lineage	Temperate	-1.77 ± 0.39	-4.50	<0.01	0.17	0.07-0.34	0.11 ± 0.18	-4.53	0.59	1.10	0.77-1.58
Interaction	High temperature * Temperate lineage	0.77 ± 0.64	1.21	0.23	2.17	0.59-7.64	-1.24 ± 0.32	1.17	<0.01	0.29	0.15-0.54

Figure 1.1. Diagram of olfactometer for assessment of host preference between a dog and human in ticks. Ticks are inserted at center point of middle tube (solid arrow) and prevented from accessing human or dog by fabric mesh (hollow arrows). A fan in each box facilitates air flow into the tubing. The center tube (between hollow arrows) measures 122cm in length and each box measures 91cm x 76cm x 122cm.

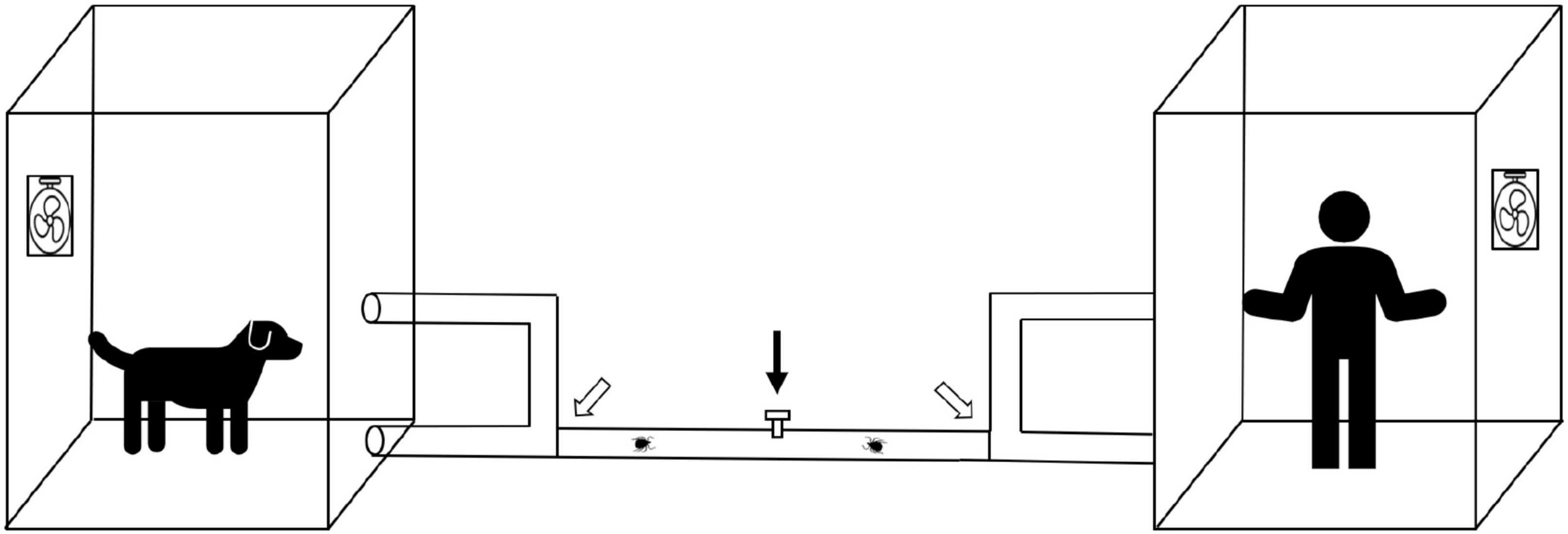
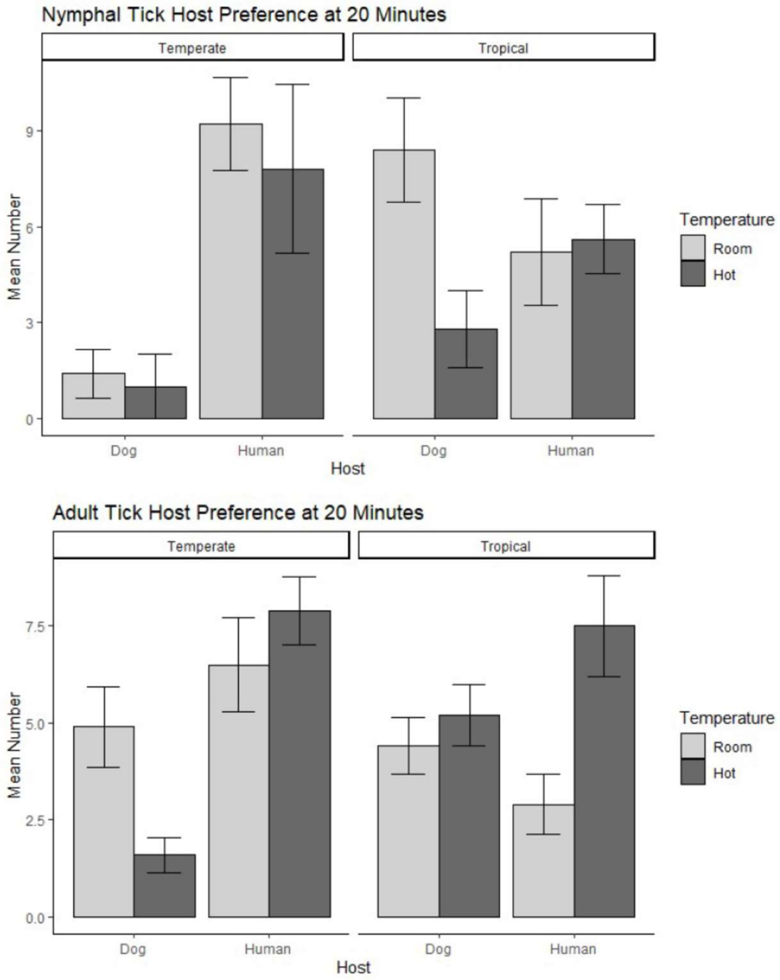


Figure 1.2. Mean number of ticks (± standard deviation) of temperate and tropical lineage choosing dog or human at room temperature (23°C) or high temperature (38°C). Nymph trials were performed with 40 ticks and adult trials were performed with 20 ticks.



Chapter 2

Rickettsial species in two lagomorph populations and their ectoparasites in northern Baja California, Mexico

Laura H. Backus[1]*, Andrés M. López-Pérez[1], Jacob Marcek[1], Laura Shultz[1], Oscar Efrén Zazueta[2], Janet Foley[1]

[1]Department of Veterinary Medicine and Epidemiology, School of Veterinary Medicine, University of California, Davis, CA, USA

[2]Dirección de Enseñanza y Vinculación, Instituto de Servicios de Salud Pública del Estado de Baja California, Mexicali, México

*Corresponding author

Abstract

Lagomorphs—principally rabbits and hares—have been implicated as hosts for vectors and reservoirs for pathogens of multiple rickettsial diseases. Western North America is home to a diverse range of rickettsial pathogens which circulate between multiple wildlife and domestic hosts and tick and flea vectors. It is also a site of multiple outbreaks of fatal Rocky Mountain spotted fever in humans. The desert cottontail, *Sylvilagus audubonii*, and black tailed jackrabbit, *Lepus californicus*, are ubiquitous in the region but understudied for their potential role as hosts and reservoirs. The purpose of this study was to live-trap and sample lagomorphs in two locations in northern Baja California, Mexico, to assess exposure to and infection with rickettsial organisms. In total, 55 *S. audubonii* and two *L. californicus* were captured. Hosts in Ensenada had significantly higher prevalence of rickettsial antibodies than hosts in Mexicali (52.3% vs 21.4%), and the identified rickettsial agents based on polymerase chain reaction (PCR) and DNA sequencing were *Rickettsia bellii* in *Dermacentor parumapertus* and *Haemaphysalis leporispalustris* ticks from Ensenada. In addition, a single tissue sample from a hare was positive for *R. bellii* by real-time PCR. These findings suggest that rickettsial organisms not regarded as pathogenic may contribute to host immunity. In addition, the distribution of ectoparasites was markedly different between the two locations, suggesting that potential for vector-borne disease transmission in this species may vary widely even within a region.

Key Words: Hares, *Dermacentor parumapertus*, *Haemaphysalis leporispalustris*, rabbits, spotted fever group, *Rickettsia bellii,* Rocky Mountain spotted fever

Introduction

Understanding sylvatic epidemiological cycles and identifying factors underlying disease spillover from animals to humans have become increasingly urgent with recent outbreaks of rickettsial diseases. Rickettsial bacteria are transmitted by tick, flea, and louse vectors, and are classified into spotted fever, typhus, transitional, and ancestral groups (Fournier and Raoult, 2009). The spotted fever group includes three tick-borne threats to human health in North America: *Rickettsia rickettsii*, the cause of Rocky Mountain spotted fever, *Rickettsia parkeri*, the agent of *R. parkeri* rickettsiosis, and *Rickettsia* 364D (Biggs et al., 2016). Of these, *R. rickettsii* has the highest morbidity and mortality in humans, with Rocky Mountain spotted fever outbreaks in Arizona, USA having case fatality rates of up to 10% and exceeding 30% in Baja California and Sonora, Mexico (Álvarez-Hernández et al., 2017; Biggs et al., 2016; Demma et al., 2005). Flea-borne typhus has emerged in western North America in the last two decades, associated with both *R. typhi* and *R. felis* (Blanton et al., 2015; Nelson et al., 2018). For all of these pathogens, knowledge of full host and vector range is incomplete, limiting the capacity to use ecological information for surveillance and prevention (Eremeeva and Dasch, 2015).

Lagomorphs—primarily the black-tailed jackrabbit (*Lepus californicus*) and desert cottontail (*Sylvilagus audubonii*)—are common species in both rural and peri-urban environments across the southwestern United States and northwestern Mexico (Reid, 2006). They serve as hosts and reservoirs for vector-borne zoonotic pathogens, including *Francisella tularensis*, *Bartonella* spp., and *Babesia* sp. MO1 (Sato et al., 2020; Yabsley et al., 2006). In Europe, rabbits and hares may be reservoirs for *R. conorii* (the cause of Mediterranean spotted fever) and are primary hosts of *Rhipicephalus pusillus*, a vector of *R. massiliae*, and the European hare *(Lepus europeaus*) can be infected with *R. slovaca* (Eremeeva and Dasch, 2015; Fernández de Mera et al., 2009; Rehácek et al., 1978). Lagomorph species were also implicated as potential hosts for *R. rickettsii* in several studies during the 20th century. Through experimental inoculation, both rabbits and hares were shown to maintain at least transient rickettsemia, and *R. rickettsii* was isolated from wild-caught eastern cottontails (Burgdorfer et al., 1980; Lundgren et al., 1968; Schriefer and Azad, 1994; Shirai et al., 1961). Data on non-spotted fever group rickettsias (SFGR) in lagomorphs is sparse, although during *R. typhi* vector transmission studies, rabbits seroconverted but it is unclear whether they became rickettsemic (Houhamdi et al., 2003). In addition, significant portions of past work relied primarily on immunological assays which do not differentiate among members of the spotted fever group or potentially even between spotted fever and typhus groups (Hechemy et al., 1989; Henke et al., 1990; Raoult and Paddock, 2005). Advances in molecular diagnostic techniques (i.e. polymerase chain reaction or PCR) can help clarify which specific pathogens infect lagomorphs.

Regardless of lagomorphs' reservoir capacity for rickettsiae, they may have an important role as hosts for arthropod vectors of pathogens. For example, tick species parasitizing lagomorphs are able to maintain and transmit a multitude of both pathogenic and nonpathogenic SFGR agents. These include *Haemaphysalis leporispalustris*, the rabbit tick, which is highly lagomorph-specific and a competent vector for *R. rickettsii* (Freitas et al., 2009). *Haemaphysalis leporispalustris* was implicated in *R. rickettsii*-caused human disease in Costa Rica (Fuentes et al., 1985; Hun et al., 2008). *Dermacentor parumapertus*, or the hare tick, also tends to specialize on rabbits and hares, and *D. parumapertus* removed from black-tailed jackrabbits carried the Black Gap strain of *R. parkeri* (Paddock et al., 2017). In the eastern United States, the primary vector for *R. rickettsii*, *Dermacentor variabilis*, feeds readily on *Sylvilagus* sp. rabbits (Cooney et al., 2005). While the vector-competence of the newly-differentiated *Dermacentor similis* in western North America is unclear, this tick also feeds on lagomorphs (Furman and Loomis, 1984; Lado et al., 2021). Finally, the brown dog tick, *Rhipicephalus sanguineus*, which is the main vector of *R. rickettsii* in the western United States and Mexico, feeds incidentally on lagomorphs (Gray et al., 2013).

This study was intended to address several questions about the ecology and epidemiology of rickettsias in the western US and the role of lagomorphs in this ecology. Human epidemics of Rocky Mountain spotted fever in western North America are closely tied to dogs, because *Rh. sanguineus* populations are dependent on dogs, particularly free-roaming dogs, and dogs are also competent hosts for *R. rickettsii*, (Alvarez-Hernandez et al., 2020). However, infected dogs and ticks are identified infrequently, even during outbreaks, which suggests that there may be an alternate reservoir or vector species that maintains the pathogen (Demma et al., 2005). In addition, there are a number of circulating rickettsial species within the epidemic region, which may complicate both the transmission and detection of *R. rickettsii* (López-Pérez et al., 2022). The goal of this study, therefore, was to identify the role that lagomorphs and their ectoparasites may play in the maintenance and transmission of rickettsial species. Two locations were selected in northern Baja California, Mexico due to their proximity to ongoing Rocky Mountain spotted fever outbreaks.

Methods

Study Site

Two study sites were utilized in northern Baja California, one in the Mexicali Valley (MV; (−115.1966°N, 32.5024°W) and the other approximately 40km east of Ensenada (ENS, −116.1189°N, 31.9076°W) (Figure 2.1). Trapping occurred between May and July of 2021.

Lagomorph Trapping and Sample Collection

At each study site, Tomahawk traps (66cm x 22.9cm x 22.9cm, Tomahawk Live Trap Co., Hazelhurst, WI) were set in 8 transects of 6 traps each, placed 8-10m apart, for 4-5 trap nights each. Traps were baited with alfalfa hay, timothy hay rabbit pellets (Volkman Pet Products, Ceres, CA, USA), rolled oats, and apple juice. Animals were weighed and sexed. Blood was collected through venipuncture of the lateral saphenous vein or auricular artery and divided between collection tubes containing ethylene di-amine tetra-acetic acid (EDTA) anticoagulant or serum separator. Tissue samples were collected from the lateral aspect of the ear. Systematic examination for ectoparasites was performed, examining the internal and external pinnae, orbital region, cheeks, and trunk from head to tail for five minutes. All visualized ectoparasites were collected and placed in 70% ethanol. This research was approved by the UC Davis Institutional Animal Care and Use Committee (Protocol #21705) and the Mexican Secretary of Environment and Natural Resources (Permit FAUT-0250).

Identification of Ectoparasites

Ticks were identified morphologically prior to extraction using published dichotomous keys (Brinton et al., 1965; Furman and Loomis, 1984). Fleas were subject to DNA extraction as described below and then prepared for identification. Exoskeletons were placed in a saturated potassium hydroxide solution for 24 hours, then dehydrated through a series of increasingly concentrated ethanol solutions (75, 85, 95, and 100%, for 30 minutes each), and then identified using dichotomous keys (Stark, 1958).

DNA Extraction and PCR

The Qiagen DNEasy Blood and Tissue extraction kit (Valencia, CA, USA) was used for DNA extraction of blood samples, engorged ticks, tissue, and fleas. Engorged ticks were sliced in half and incubated overnight at 56°C in ATL buffer and proteinase K. Five fleas per host were incised perpendicular to the long axes at the tergum and incubated overnight in kit-provided proteinase K and Buffer ATL before proceeding with extraction. Remaining extraction steps were according to kit manufacturer directions. Unfed ticks and tick larvae were extracted using a modified ammonium hydroxide protocol as published previously (Foley et al., 2019). Larvae were grouped into pools of up to 5 ticks from a single host for extraction and analysis.

All DNA samples from ticks, fleas, blood, and tissue were screened for rickettsial organisms using a real-time PCR protocol that detects most organisms in the spotted fever, typhus, and transitional groups (Stenos et al., 2005). All tick and host samples were also tested using a real-time PCR protocol that detects *R. bellii* (Hecht et al., 2016).

Serology

Indirect immunofluorescent assay (IFA) slides for Rocky Mountain spotted fever were used to assess rickettsial antibodies in serum (VMRD, Pullman, WA, USA). Serum was diluted using phosphate buffered saline (PBS) and spotted on 25µL volumes on slides, incubated at 37ºC for 35 minutes, then washed in PBS, spotted with 25µL volumes of 1:100 dilution fluorescent conjugated anti-rabbit immunoglobulin (KPL, Gaithersburg, MD, USA) and incubated again for 35 minutes at 37ºC. Slides were then washed with PBS and counterstained with Eriochrome Black, dotted with 10% glycerol to fill wells, and covered with a glass coverslip. Wells were evaluated using a fluorescent microscope. A positive control (RMSF FA Positive Control from VMRD) and negative control (known negative sample) were included in each run. Samples were considered positive at 1:64 dilution.

Data Analysis

Data were stored in Excel (Microsoft Corp., Seattle, WA, USA) and analyzed using R Studio (RStudio Team, 2019). Generalized linear models were utilized to identify relationships between host and location characteristics and rickettsial seropositivity (logistic regression) and ectoparasite burden (Poisson regression). Univariate analysis was used to identify predictors with a significant association, then stepwise addition made and predictors retained in model if significant ($p < 0.05$). Due to small sample size, hare data was not included in models. Ectoparasites collected from individuals that were recaptured were identified and screened for pathogens, but were not included in models.

Results

A total of 55 individual rabbits (*S. audubonii*) and 2 hares (*L. californicus*) were captured and sampled, out of 85 capture events. More rabbits were captured in Mexicali than Ensenada (Table 2.1). Ectoparasite burden varied markedly between locations. In Mexicali, ticks were collected from 44% (14/32) of individuals, and were exclusively *H. leporispalustris,* the majority of which were larvae; in Ensenada, ticks were collected from 70% (16/23) individuals, and 95% were *D. parumapertus* adults. *Euhoplopsyllus glacialis* fleas were collected from rabbits and the one hare from Mexicali, while the few fleas found on hosts in Ensenada were *Echidnophaga gallinacea* (3 from 3 hosts) and *Cediopsylla inaequalis* (1 from 1 host) (Table 2.2).

All host blood samples were negative for rickettsial pathogens on PCR (Table 2.3), while two fleas (both *E. glacialis* from Mexicali) were positive on the panrickettsial PCR (Table 2.4). However, DNA sequencing of the 17kDa gene failed, precluding identification of the rickettsiae. One tissue sample, from the single hare captured in Ensenada, was positive for *R. bellii*. Of the 63 ticks collected in Ensenada, 55

(87%) were also PCR positive for *R. bellii*; positive ticks included both *D. parumapertus* and *H. leporispalustris* (Table 2.3). No ticks from Mexicali were positive for rickettsiae.

Rabbits from Ensenada had significantly higher seropositivity for SFGR (52.3% positive) than rabbits from Mexicali (21.4% positive, p= 0.035). When controlling for location, there was no relationship between the presence or burden of either ticks or fleas and rickettsial seropositivity. Host body mass was significantly, negatively associated with tick burden of both *D. parumapertus* and *H. leporispalustris* in rabbits (Table 2.5). Results from the Poisson model showed that for *D. parumapertus* in Ensenada, every 100g increase in body weight was associated with a 28% decrease in tick burden (0.72, 95% CI 0.54-0.95), while for *H. leporispalustris* in Mexicali, every 100g increase was associated with a 27% decrease in tick burden (0.73, 95% CI 0.64-0.83). Male sex was negatively associated with *H. leporispalustris* burden but there was no significant relationship between sex and burden of *D. parumapertus* (Table 2.5). In contrast, increasing size and male sex were significantly associated with a higher *E. glacialis* burden in Mexicali. In addition, in Mexicali, where there were high numbers of both fleas and *H. leporispalustris*, no relationship between flea and tick burden was found. Interactions between sex and body mass were tested and not significant.

Discussion

In this study, we identified a high prevalence of rickettsial antibodies in lagomorphs associated with infestation with *R. bellii*-infected ticks. Along with the finding of PCR-confirmed *R. bellii* infection in lagomorph tissue, our results suggest that lagomorphs are extensively exposed to rickettsial organisms including those not regarded as pathogenic but which may lead to infection and immune response. In addition, these exposures to ectoparasites and pathogens may be very different between locations within the same region.

There was a marked difference in seroprevalence between our two study locations. Given the finding of an *R. bellii* PCR-positive hare in Ensenada, this suggests that lagomorphs in Ensenada at least might have seroconverted to *R. bellii.* It is also possible that animals had been exposed to other rickettsial pathogens but which were not detected by PCR and are known to be present in the region (López-Pérez et al., 2022) which may not have been detected due to the cross-sectional nature of the study. Cross-reaction among SFG rickettsiae is widely recognized, with implications for serologic diagnosis (Hechemy et al., 1989; Raoult and Paddock, 2005), and there is evidence in humans that cross-reactivity between SFG and typhus group rickettsia may occur as well (Ormsbee et al., 1978; Valbuena et al., 2004). While *R. bellii,* a member of the ancestral group, is not known to be pathogenic in any species, capybaras exposed via tick bite developed a serologic response specifically to *R. bellii* (Fortes et al., 2011; Pacheco et al., 2007).

Whether immune response to a nonpathogenic or ancestral rickettsial species confers immunological protection against infection with a pathogenic species is unclear. Inoculation experiments in guinea pigs using *Rickettsia amblyommatis* resulted in immunity to pathogenic *R. rickettsii* strains (Blanton et al., 2014); while *R. amblyommatis* is classified within the spotted fever group, it is apparently nonpathogenic in guinea pigs and has limited pathogenicity in other species (Karpathy et al., 2016). Any similar effect of *R. bellii* not been experimentally tested. In addition to host level immunity, at the tick level, circulation of one rickettsial species may also impact acquisition and transmission of another. Infection with low-pathogenicity or endosymbiont species in ticks has been reported to reduce successful maintenance and transmission of pathogens (Macaluso et al., 2002) but for other rickettsial and tick species, the effects have been equivocal (Levin et al., 2018). The effect of *R. bellii* on transmission of pathogenic rickettsias has not been investigated in the laboratory, although reported co-infection of *D. parumapertus* with *R. parkeri* and *R. bellii* suggests that at the tick population level, the two species do not completely exclude one another (Paddock et al., 2017).

The burden and distribution of ectoparasites were highly disparate between study locations, with a high infestation prevalence and overall burden of *H. leporispalustris* larvae and *E. glacialis* fleas in Mexicali, while at the Ensenada site, adult *D. parumapertus* were the predominant ectoparasite. While both study sites were sampled in similar seasons (Mexicali in May/June and Ensenada in July), they are climatically somewhat different, with contrasting landscape and anthropogenic impact, so ectoparasite life cycles and phenology may differ between them. The Mexicali Valley site is located at sea level, with desert conditions and exceptional heat (with daily high temperatures averaging over 37 ºC) during the summer, while the Ensenada site is approximately 500m above sea level and slightly cooler, with summer high temperatures ranging between 32 and 36ºC (National Oceanic and Atmospheric Administration, 2022). The Mexicali site is surrounded by irrigated crop agriculture, while the Ensenada site is rocky, steep, and used for low-density cattle grazing. Studies investigating the phenology of questing ticks have demonstrated clear relationships between elevation and climate and timing of activity, but the relationship between those factors and the ectoparasites described here is unknown (MacDonald, 2018; Perret et al., 2004). Differences in microhabitat (due to both biotic and abiotic factors) and species composition of the surrounding community may have impacted observed ectoparasite distributions. Because this study was a cross sectional in nature in only two locations, with a relatively small sample size, it is not possible to quantify which factors were responsible for the variation. However, the data presented here suggest that ectoparasite species distribution and phenology may vary markedly within a relatively small geographic area, which has implications for understanding the timing and probability of disease transmission risk even within a state or region.

The relationships between body mass and ectoparasite burden suggest that population structure may be an important determinant of vector-borne disease risk. Body mass in this case is likely to be, at least partially, a proxy for age, and younger (and therefore smaller) animals may be susceptible to higher tick burdens due to lack of acquired immunity (Njau et al., 1988). Acquisition of immunity to ticks following infestation appears to be dependent on both host and tick species (Évora et al., 2015), but the significant inverse relationship here between rabbit size and burden of *H. leporispalustris* and *D. parumapertus* suggests that younger, smaller animals may be more susceptible to ticks and potentially play a disproportionate role in supporting tick populations and tick-borne pathogens. A reverse causal relationship cannot be ruled out (higher infestation with ticks results in weight loss or reduced gain), although body mass variation seemed primarily associated with skeletal size and no rabbits were appreciated to be underweight or emaciated. Relationships between flea abundance and body size have been studied in a number of other host and flea species, and results are variable, with significant associations (both positive and negative) observed amongst for example rodents and their fleas (Kiffner et al., 2013; Pontifes et al., 2022). However, the relationship we found between greater rabbit body size and higher *E. glacialis* burden is consistent with another study that confirmed a greater flea burden amongst larger rabbits (Chekchak et al., 2000).

The only tick species and the vast majority of fleas found on lagomorphs in this study were highly specific to lagomorph hosts. For rickettsial spillover to occur to other species, the presence of a more generalist vector would be needed to act as a bridge between species. Lagomorphs in other regions are frequently infested with more generalist ticks, and while none were identified here, the presence of other vectors, incidentally or at other times of year, is plausible. Immature *D. variabilis* frequently parasitize small rodents and lagomorphs and while the western equivalent, *D. similis*, is not common in arid desert regions, there is evidence that it occurs in pockets where the microclimate can support its persistence in an otherwise inhospitable environment (López-Pérez et al., 2022). The most commonly identified flea in this study, *E. glacialis*, has been identified occasionally on non-lagomorph hosts but is not known to vector any rickettsial pathogens. The *E. gallinacea* flea which was identified on a few rabbits in Ensenada has a wide host range and geographic distribution, and is known to carry *R. felis*, so likely has the potential to transfer *Rickettsia* spp. between host species (Karpathy et al., 2009; Schloderer et al., 2006).

It remains unknown whether rabbits or hares are capable of maintaining or contributing to the cycles of *R. rickettsii* or other pathogenic rickettsial species in northern Mexico. The notable difference in ectoparasite species and serologic results, however, suggests important differences in regional ecologies that may and affect different populations' susceptibility to rickettsial infection. Further research in both the laboratory on pathogen susceptibility and in the field on ectoparasite variation year round are needed, and would be a valuable addition to the understanding of sylvatic rickettsial cycles.

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Table 2.1. Counts of lagomorph hosts (N), number of hosts that were female (#F) and number parasitized (n) with ticks and fleas in two locations (Mexicali and Ensenada).

			Ticks				Fleas			
	Hosts		*H. leporispalustris*		*D. parumapertus*		*E. glacialis*		*E. gallinacea*	
Mexicali	N	#F	n	Proportion [95% CI]	n	Proportion [95% CI]	n	Proportion [95% CI]	n	Proportion [95% CI]
S. audubonii	32	15	13	0.41 [0.24, 0.59]	0	-	23	0.72 [0.53, 0.86]	0	-
L. californicus	1	1	1	-	0	-	1	-	0	-
Ensenada										
S. audubonii	23	10	2	0.09 [0.01, 0.30]	15	0.47 [0.30, 0.65]	0	-	3	0.13 [0.03, 0.35]
L. californicus	1	1	1	-	1	-	0	-	0	-

Table 2.2. Total counts of ticks and fleas collected from two lagomorph hosts in Mexicali and Ensenada

	Ectoparasites								
	Ticks						Fleas		
	H. leporispalustris				*D. parumapertus*		*E. glacialis*	*E. gallinacea*	*C. inaequalis*
Mexicali	M	F	N	L	M	F			
S. audubonii	7	5	0	122	0	0	215	0	0
L. californicus	0	0	0	1	0	0	3	0	0
Ensenada									
S. audubonii	1	1	0	0	18	15	0	3	1
L. californicus	0	1	0	0	18	9	0	0	0
Total	8	7	0	123	36	24	218	3	1

M = Male, F = Female, N = Nymph, L = Larva

Table 2.3. Results of PCR and serology testing of host samples (blood, serum, and tissue) from both Mexicali and Ensenada

	Pan-*Rickettsia* PCR		*R. belli* PCR		Serology
Mexicali	Blood	Tissue	Blood	Tissue	SFG IFA
S. audubonii	0/30	0/28	0/30	0/28	6/28
L. californicus	0/1	0/1	0/1	0/1	0/1
Ensenada					
S. audubonii	0/22	0/22	0/22	0/22	11/21
L. californicus	0/1	0/1	0/1	1/1	1/1

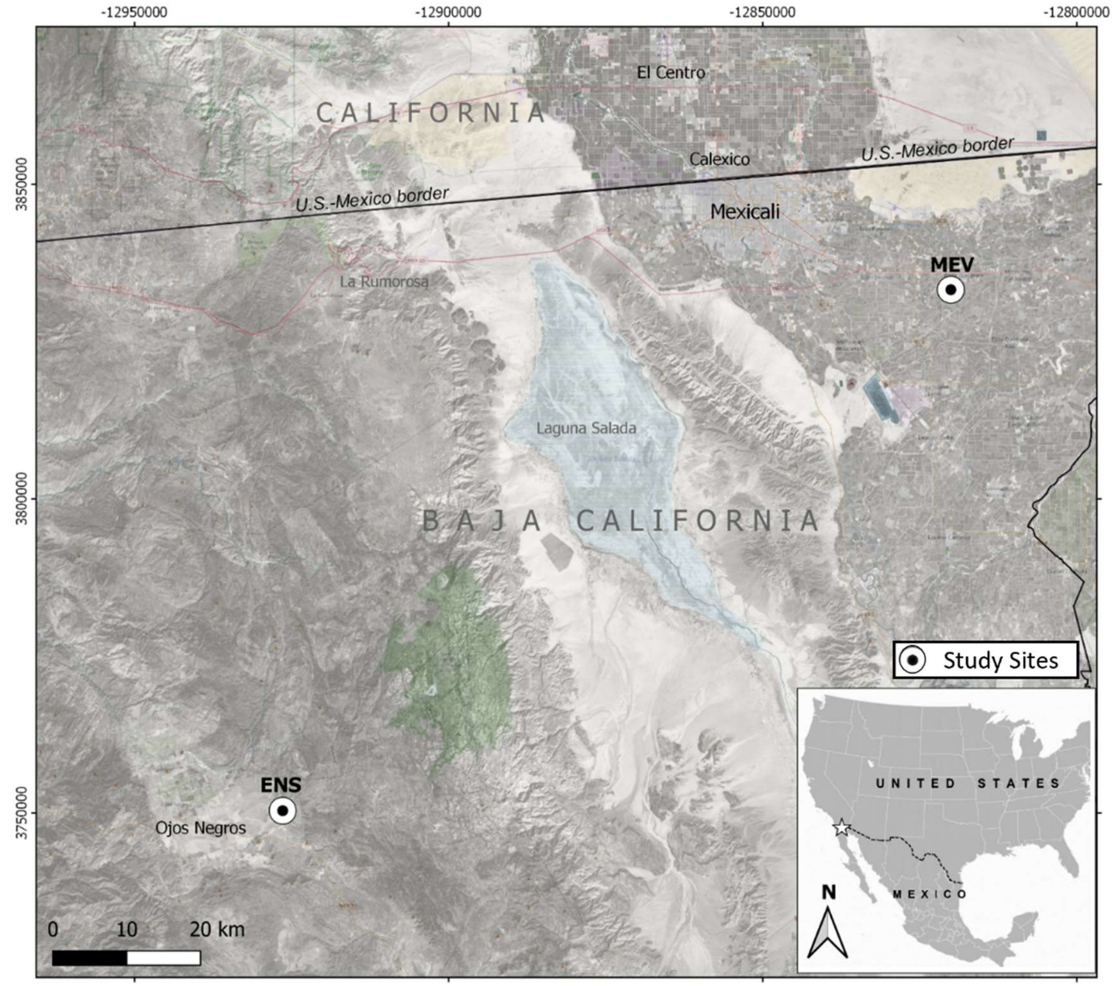
Table 2.4. Results of rickettsial PCR for ectoparasites collected from lagomorphs by pan-*Rickettsia* RT-PCR and *R. belli*-specific PCR.

Species	***Pan-Rickettsia* RT-PCR**		***R. bellii* RT-PCR**	
	Number Pos	Prop (95% CI)	Number Pos	Prop (95% CI)
H. leporispalustris	0/138	-	2/138	0.01 (0.00, 0.06)
D. parumapertus	0/60	-	53/60	0.88 (0.77, 0.95)
E. glacialis	2/108	0.19 (0.00, 0.07)	-	-
E. gallinacea	0/3	-	-	-
C. inaequalis	0/1	-	-	-

Table 2.5. Influence of body mass (per 100g body mass) and sex on burden (count per host individual) of selected ectoparasites by location. The effect estimate is the exponentiated β coefficient.

		Weight (per 100g)			Sex		
Location	Ectoparasite	B coefficient	Effect Estimate (95% CI)	*P*-value	B coefficient	Effect Estimate (95% CI)	*P*-value
Mexicali	*H. leporispalustris*	-0.32	0.73 (0.64, 0.83)	**<0.001**	-1.19	0.30 (0.17, 0.51)	**<0.001**
Ensenada	*D. parumapertus*	-0.33	0.72 (0.54, 0.95)	**0.021**	-0.14	0.87 (0.40, 1.85)	0.717
Mexicali	*E. glacialis*	0.19	1.13 (1.01, 1.26)	**0.039**	0.48	1.62 (1.170, 2.26)	**0.004**

Figure 2.1. Map showing the two study sites in the Mexicali Valley (MEV) and near Ensenada (ENS) in northern Baja California where lagomorph sampling was performed.



Chapter 3

Tick-borne pathogens in sheltered dogs at the California-Mexico border

Laura Backus[1], Janet Foley[1], Claire Chung[1], Sophia Virata[1], Oscar Éfren Zazueta[2], Andrés López-Pérez[1]*

[1]Department of Veterinary Medicine and Epidemiology, School of Veterinary Medicine, University of California, Davis, CA, USA

[2]Dirección de Enseñanza y Vinculación, Instituto de Servicios de Salud Pública del Estado de Baja California, Mexicali, México

*Corresponding author

Abstract

Dogs may serve as sentinels for zoonotic pathogens that pose a threat to human health. Sheltered dogs in particular may be at high risk of pathogen exposure and experience a heavy burden due to these diseases. The emergence of Rocky Mountain spotted fever (RMSF) in the southwestern United States and northern Mexico has resulted in exceptionally high human case fatality rates, reaching 10-30% in some areas. The cycle of RMSF transmission in this region highlights the shared risk of tick-borne disease between humans and canines. Two species (currently designated as lineages) of brown dog ticks vector *Rickettsia rickettsii*, the causative agent of RMSF, as well as pathogens that primarily affect dogs but are of increasing importance to human health, including *Ehrlichia canis* and *Anaplasma platys*. In this cross-sectional study, we sampled dogs in shelters from four locations along the US-Mexico border: San Diego County and Imperial County in California, and Mexicali and Tijuana in Baja California. Blood samples from 239 dogs were tested by PCR indicating active infection and for antibodies indicating exposure to *Ehrlichia, Anaplasma*, and *Rickettsia* spp. Two of 78 dogs sampled in Tijuana (2.6%) were actively infected with *R. rickettsii*. Infection with *E. canis* and *A. platys* ranged across shelters from 0 to 27% and 0 to 33%, respectively. Dogs in all four locations demonstrated exposure to all three pathogens, though *Rickettsia* and *Ehrlichia* seropositivity was highest in Mexicali (81% and 49%, respectively) and *Anaplasma* seropositivity was highest in Tijuana (45%). Both temperate and tropical lineage ticks were found on the dogs, and both appear to be responsible for spread of pathogens in the region. While infection and exposure were highest in sheltered dogs in the southern locations, dogs in all locations demonstrated exposure to all pathogens, demonstrating the potential for emergence and spread of zoonotic pathogens with significant public health consequences in southern California and northern Baja California.

Keywords: *Anaplasma platys*, dogs, *Ehrlichia canis*, Rocky Mountain spotted fever

Introduction

The expansion of tick-borne disease in populations of domestic dogs represents a significant health threat to canids and is associated with increasing risks to humans (Dantas-Torres and Otranto, 2016). Dogs are central to cycles of numerous tick borne zoonoses, acting as both sentinels due to their close interaction with humans and as key players in pathogen spread (Morand et al., 2020). Dogs may also support populations of ticks that may bite and infect humans. This is particularly true for the brown dog tick, which comprises multiple species within the *Rhipicephalus sanguineus* complex, all of which are dependent on dogs to complete their life cycle (Dantas-Torres, 2010; Nava et al., 2018).

Brown dog ticks are found worldwide and are responsible for maintenance and transmission of multiple canine pathogens, including *Rickettsia rickettsii*, the cause of Rocky Mountain spotted fever (RMSF), *Ehrlichia canis*, the cause of canine ehrlichiosis, and *Anaplasma platys*, the cause of infectious cyclic thrombocytopenia in dogs (Bremer et al., 2005; Demma et al., 2005; Snellgrove et al., 2020). In North America, the two brown dog tick species are referred to as the tropical and temperate lineages, and both are capable of pathogen transmission, though they have not been directly compared for transmission efficiency (Nava et al., 2015). The brown dog tick thrives in peridomestic environments in and around homes and kennels, and, while it typically feeds on dogs at all life stages, incidental human feeding occurs especially when ticks are at high density and possibly at elevated temperatures (Dantas-Torres, 2010; Parola et al., 2008). Of the diseases spread by the brown dog tick, RMSF poses the greatest threat to human health in the southwestern United States and northern Mexico, where epidemics have had case fatality rates ranging from 10 to 30% (Álvarez-López et al., 2021; Biggs, 2016). The emergence of the disease is associated with heavy environmental burdens of ticks resulting from large numbers of dogs, particularly free-roaming dogs (Alvarez-Hernandez et al., 2020; Nicholson et al., 2006). Dogs and brown dog ticks support other spotted fever group rickettsial zoonoses as well, including *R. massiliae*, which may represent an emerging threat in the same regions at risk of RMSF outbreaks (Beeler et al., 2011; Eremeeva et al., 2006).

Both *E. canis* and *A. platys* were historically regarded as strictly canine diseases, but both have now been proven to infect humans as well (Arraga-Alvarado et al., 2014; Maggi et al., 2013; Perez et al., 2006). In addition, because they are transmitted by the same tick, their presence may be a surrogate indicator of risk for spotted fever group rickettsiosis (Diniz et al., 2010). There is significant overlap in the clinical signs of RMSF, ehrlichiosis, and anaplasmosis in dogs, with nonspecific signs like fever and thrombocytopenia common to all three diseases (Greene, 2012). Infection with *R. rickettsii* may lead to severe RMSF and death in dogs, or infection may be subclinical (Levin et al., 2014). Monocytic ehrlichiosis and anaplasmosis often progress to chronicity, and chronic *E. canis* infection may manifest in multiple-organ disease impacting kidneys, eyes, and joints among other tissues, and result in immune suppression and increased susceptibility to other infections (Burton et al., 2020). In addition, coinfection with both *A. platys* and *E. canis* results in greater hematologic abnormalities and duration of infection than infection with either alone (Gaunt et al., 2010).

While *E. canis* and *A. platys* have been found in clinically ill dogs presenting to veterinarians in southern California (Kidd et al., 2017), locally acquired cases of RMSF have not been reported in dogs in California. However, the ongoing reporting of human RMSF cases in northern Baja California and Arizona, immediately adjacent to Imperial and San Diego counties in southern California, suggests that there may be risk of disease emergence in California as well (Herrick et al., 2016; Zazueta et al., 2021). Previous work has also demonstrated high levels of exposure to rickettsial pathogens in dogs in California, particularly those residing close to the border (Estrada et al., 2019). Dogs are recognized as sentinels for a number of vector borne diseases, and particularly for RMSF, there is a strong association between cases in dogs and humans in close proximity (Paddock et al., 2002). However, the social and ecological factors that drive disease transmission by the brown dog tick—large numbers of free roaming dogs with low rates of spay and neuter, with limited access to tick prevention and veterinary care—have a lower probability of diagnosis, and sheltered and stray dogs have been identified as a critical reservoir of zoonotic pathogens (López-Pérez et al., 2020; Otranto et al., 2017). The movement and re-homing of sheltered dogs is a documented risk factor for vector and pathogen spread (Wright et al., 2020). Surveillance of sheltered dogs, therefore, offers a potential opportunity to identify emerging zoonoses that threaten both human and canine health. The purpose of this study was to assess exposure to and infection with three pathogens (*R. rickettsii*, *A. platys*, and *E. canis*) vectored by brown dog ticks in sheltered dogs in southern California and northern Baja California, with the goal of determining a baseline for current and potential transmission of vector-borne diseases in this region.

Methods

Sample Collection

A cross-sectional survey was conducted at seven animal shelters in southern California and northern Baja California between October 2021 and May 2022. The study area was divided into four quadrants: San Diego County and Imperial County on the California side, and Tijuana and Mexicali in Baja California (Figure 3.1). Dogs at least 6 weeks of age of any size and breed were enrolled as a convenience sample based on presence in shelter at time of survey. Demographic data collected included sex, weight, breed, estimated age, body condition (scored out of 5), and, where possible, location of origin and shelter intake date. For analysis, dogs were grouped as small (<10kg), medium (10-20kg), or large (>20kg), and classified as puppy (<6 months), juvenile (6-12 months), or adult (>1 year) based on dentition. Body condition score (BCS) was assessed on a 1-5 point scale (Eastland-Jones et al., 2014). Dogs were examined for ticks which were collected when present and stored in 70% ethanol. If fewer than 10 ticks were found on a dog, all were collected; for dogs with more than 10 ticks, only the first 10 found were collected. Whole blood was collected from each dog and stored at -20 ºC until analysis. This research was approved by the UC Davis Institutional Animal Care and Use Committee (Protocol #22235).

Serology

The commercially available SNAP 4dx Plus was used to assess exposure to *Ehrlichia* and *Anaplasma* species (IDEXX Laboratories, Inc., Westbrook, Maine, USA). The assay does not differentiate between *E. canis* and *Ehrlichia ewingii*, or between *A. platys* and *Anaplasma phagocytophilum*. While not the focus of this study, in addition to detecting antibodies to *Ehrlichia* and *Anaplasma*, the 4dx Plus also tests for antibodies to *Borrelia burgdorferi* and heartworm (*Dirofilaria immitis*) antigen. Whole blood was mixed with kit-provided conjugate and tests read at eight minutes per manufacturer instructions. Indirect immunofluorescent assay (IFA) slides for Rocky Mountain spotted fever were used to detect antibodies to spotted fever group rickettsial species (VMRD, Inc., Pullman, WA, USA). Serum was diluted at a 1:64 ratio in phosphate buffered saline (PBS) and 25µL volumes spotted on slides which were incubated at 37ºC with saturated humidity for 35 minutes, then washed in PBS, spotted with 25µL volumes of 1:100 dilution fluorescent conjugated anti-dog immunoglobulin (LGC SeraCare, Gaithersburg, MD, USA), and incubated again for 35 minutes at 37ºC. Slides were then washed with PBS and counterstained with Eriochrome Black, dotted with 10% glycerol to fill wells, and covered with a glass coverslip. Slides were evaluated via fluorescent microscopy. A positive control (RMSF FA Positive Control from VMRD) and negative control (known negative sample) were used for each run, and samples were considered positive based on the presence of bright green fluorescence with the expected distribution pattern.

DNA Extraction and PCR

The Qiagen DNEasy Blood and Tissue extraction kit (Valencia, CA, USA) was used to extract DNA from blood samples and engorged ticks. Ticks were identified morphologically prior to extraction using dichotomous keys (Furman and Loomis, 1984). Engorged ticks were sterilely sliced in half and incubated overnight at 56°C in kit-provided ATL buffer and proteinase K, and remaining extraction steps were according to kit manufacturer directions. Unengorged ticks were extracted using a modified ammonium hydroxide protocol (Foley et al., 2019).

Ticks identified morphologically as brown dog ticks were classified as temperate lineage (*R. sanguineus* s.s.) or tropical lineage using a lineage-specific PCR protocol using two sets of primers that amplify each lineage as described by (Brophy et al., 2022). Amplicons were subsequently visualization on 1% agarose gel containing ethidium bromide.

All DNA samples from ticks and blood were screened for rickettsial organisms using a real-time pan-*Rickettsia* PCR (polymerase chain reaction) protocol that detects a 133 base pair conserved region of the citrate synthase (gltA) gene (Stenos et al., 2005). Positive samples were then tested using a *R. rickettsii* specific real-time PCR protocol (Kato et al., 2013). All samples were tested by real-time PCR for *E. canis* (Doyle et al., 2005) and for *A. platys* using proprietary 16S rRNA primers and probe designed and validated by the Real-time PCR Research and Diagnostics Core Facility at UC Davis, based on GenBank ID EU004823 (https://pcrlab.vetmed.ucdavis.edu/). Conventional PCR and sequencing was performed on samples that were positive by pan-Rickettsia PCR using a nested protocol for a 200bp portion of the 17kDa gene (Shapiro et al., 2010), 512 bp region of the ompA gene (Roux et al., 1996), and 800bp portion of the gltaA gene (Fournier et al., 2003). Sequencing was performed by the UC Davis DNA

Sequencing Facility by ABI Prism 3730 Genetic Analyzer (Thermo Fisher Scientific, Waltham, MA, USA).

Data Analysis

Data were analyzed using R Studio (RStudio Team, 2019). Generalized linear mixed models, using location as a random intercept, were used to assess the relationship between odds of infection or pathogen exposure and potential risk factors. Each risk factor (age class, weight class, sex, underweight yes/no, and presence of ticks) was tested in a univariable model, then forward stepwise model building was performed, and covariates were retained in the final multivariate model if they were significantly associated with outcome. Coinfection was assessed via chi-square test.

Results

A total of 239 dogs were sampled across the four quadrants at seven shelters. In total, 114 of 239 were female (47.7%). Of the Mexicali and San Diego samples, approximately two thirds were male, while two thirds were female in Tijuana (Table 3.1). The majority (82.4%) of dogs across all sites were medium or large (>10kg). Most dogs (84.9%) were adults, with a similar age structure between study sites though no puppies were sampled in San Diego.

A total of 200 ticks, all brown dog ticks, were collected from 36 dogs in Mexicali and 8 dogs in Tijuana. No ticks were found on dogs in Imperial County or San Diego County in California. The majority of ticks were adults (66/200 or 33% female, 75/200 or 37.5% male) and nymphs (57/200, 28.5%). Lineage testing showed that ticks collected from Mexicali were all tropical lineage, while all ticks collected from all shelters in the Tijuana region were temperate lineage.

Two dogs were positive on pan-*Rickettsia* PCR, and both were subsequently positive on *R. rickettsii*-specific PCR (Table 3.2). The two positive dogs were from two different shelters in the Tijuana area sampled in December. Both were adult males; one was a mid-sized shepherd mix, and the other a Labrador retriever mix. Neither showed overt signs of illness at time of sampling, though one was also seropositive for *Ehrlichia* and *Anaplasma*, while the other was coinfected with *A. platys*. *R. rickettsii* was further confirmed via DNA sequencing of the 17kDa, gltA, and OmpA genes for each. Rickettsial seroprevalence differed significantly (P<.001) among the shelters, ranging from 15.2% of dogs in Imperial up to 81% in Mexicali. Two ticks collected from different dogs in one shelter in Tijuana were positive for *Rickettsia* spp.; one was positive for *R. rickettsii,* while the other rickettsial agent could not be identified to species (Table 3.3). The *R. rickettsii* positive sample could only be successfully sequenced using the 17kDa gene, but was 100% homologous to the *R. rickettsii* 17kDa sequences obtained from the dogs. The single tick from Mexicali that was positive by pan-*Rickettsia* screening was determined to be infected with *R. felis* via sequencing of the 17kDa gene.

E. canis and *A. platys* seroprevalence and PCR-prevalence were significantly different among locations (Table 3.2). Both PCR (32.9%) and antibody (44.9%) prevalence for *Anaplasma* were considerably higher in Tijuana than the other locations (Figure 3.2). For *Ehrlichia,* rates were much higher in Mexicali than other locations (27.0% PCR positive and 49.2% seropositive). No dogs were positive by serology for *B. burgdorferi*.

Both *A. platys* and *E. canis* can cause chronic infection, meaning a positive PCR result is not necessarily indicative of recent exposure. Because recent exposure is likely to be a better proxy of current tick-borne disease risk, we identified PCR-positive cases of *E. canis* and *A. platys* that were negative on serology, indicating that they had not yet mounted an immune response. Out of the 30 total dogs PCR-positive for *A. platys*, the majority (25) were from Tijuana, of which 12 were considered acute because they were seronegative (48.0%, 95% CI [28.3-68.2]), suggesting widespread recent infection. This is in contrast to Mexicali, where only five individuals were PCR-positive for *A. platys*, but four of those were acute (80%, 95% CI [29.9-98.9]). Only three acute cases of *E. canis* were identified, and all three were from Mexicali.

Generalized linear mixed modeling was used to assess whether there was an association between any dog-level risk factors (demographic characteristics or presence of ticks) and pathogen presence (PCR) or exposure (serology). Being severely underweight (BCS of 1.5 out of 5 or below) was associated with a 7.24 (95% CI: 1.77-32.63) times higher odds of being positive by PCR for *E. canis.* Puppies had a 4.07 (95% CI: 1.16-12.92) times higher odds of being positive than adult dogs in a univariable model, though age was no longer significant when included in the model with underweight status. For *Anaplasma* spp. antibodies, small size (<10kg) was associated with a 77.6% reduction in odds. Infestation of ticks was associated with a 4.05 times increase in the odds of being PCR positive for *A. platys* (95% CI: 1.21-15.36, P<.001). Being seropositive for *Ehrlichia* was associated with a 3.42 times increase in the odds of being seropositive for Rickettsia (P<.001, 95% CI 1.63-13.31), while *Anaplasma* seropositivity was not significantly associated with *Rickettsial* seropositivity (P=0.20). No other demographic characteristics were associated with positivity by serology or PCR for any of the three pathogens.

Coinfection and co-exposure between *Ehrlichia* and *Anaplasma* spp. were assessed by chi-square for both PCR and serological results. Results were not significant for active infection detected by PCR (chi-square

0.368, df=1, P=0.554), suggesting that co-infection was not more likely than infection with either pathogen individually. However, there was significant evidence of co-exposure based on serology (chi-square 51.79, df = 1, P<.001).

Discussion

Shelter-based surveillance is a valuable tool to explore community risk for RMSF or other zoonotic or canine diseases. In our study, we found extreme regional variability in rates of infection and exposure to tick-borne pathogens, indicating that risk of infection is markedly different even in adjacent locations. Even where we did not find active infections, there was considerable exposure to *Ehrlichia*, *Anaplasma*, and *Rickettsia* species, suggesting that pathogens are circulating in those areas. While they may frequently go undetected, there is likely a greater burden of disease in dogs than is currently recognized, as well as potential for transmission of tick-borne zoonoses. The discovery that two different species of ticks in the *R. sanguineus* s.l. group are responsible for transmission of pathogens in directly adjacent regions is also notable, and raises questions about how these two species may differ in behavior, ecology, and disease transmission risk.

Detection of *R. rickettsii* in blood samples from multiple dogs in Tijuana suggests that the infection prevalence at the time sampling occurred was likely very high. Rickettsemia is transient, lasting only three to fourteen days in infected dogs (Levin et al., 2014; Piranda et al., 2008). Thus studies in people demonstrate that the diagnostic sensitivity of PCR on blood is low (Dantas-Torres, 2007; Renvoisé et al., 2012), even though PCR has excellent analytic specificity for *R. rickettsii* and can detect very low quantities of bacterial DNA. Notably, the two dogs that were *Rickettsia* positive by PCR were seronegative by IFA. Seroconversion is expected to occur 10-14 days post-inoculation, suggesting they were early in the course of disease (Levin et al., 2014). Neither dog that was positive for *R. rickettsii* appeared clinically ill on brief observation, though subtle clinical signs such as changes in appetite or behavior are difficult to appreciate in the shelter setting. While experimental and clinical data on *R. rickettsii* infections dogs have confirmed that fever, petechiation, anorexia, and lethargy are the primary clinical signs, it is unknown whether subclinical infections occur (Greene, 2012; Levin et al., 2014). Given that RMSF is likely to occur in the same populations where other tickborne pathogens are prevalent, clinical signs in the absence of fulminant infection or death may not be adequately sensitive in the shelter setting to detect RMSF (Cameron et al., 2020). In addition, shelter staff and veterinarians should be aware that positive results—either via serology or PCR—for *Anaplasma* or *Ehrlichia* do not preclude *R. rickettsii* infection, as was the case here where one *R. rickettsii* positive dog was seropositive for *Ehrlichia* and *Anaplasma*, while the other was co-infected with *A. platys*. Because tetracycline antibiotics are used for all three pathogen genera, dogs with clinical signs may be diagnosed and treated, with subsequent resolution, without any recognition of *R. rickettsii* infection. This combination of factors suggests that pathogen-specific testing for *R. rickettsii* is an important element of surveillance, but is not independently adequate.

Reliance on PCR for detection of tick-borne infections can yield an incomplete picture of the community's risk if acute infection prevalence is low, or for example with a low sample size or outside of the most active tick-transmission season. In contrast, there is considerable benefit to using serology to detect risk, given that it provides evidence for exposure over multiple months. In the US-Mexico border region, rickettsial serological positivity ranged from 15% in Imperial County, 29% in San Diego, 60% in Tijuana, to 80% in Mexicali. Serology has the added benefit of detecting population level susceptibility; while high levels of seropositivity suggests a history of high level of exposure, lower levels may indicate that a population has minimal immunity, and is therefore at higher risk of disease emergence with rapid spread. Because of the lag in seroconversion, local population level changes in seroprevalence with ongoing monitoring may be a better indicator of risk than a single time point.

Serology also confirmed circulation of *E. canis* and *A. platys* at all locations, confirming widespread exposure to tick-borne pathogens and to brown dog ticks, and *E. canis* exposure was strongly associated with rickettsial exposure. However, there are several limitations to using serology for surveillance in such a context. Firstly, the lag in time to seroconversion means that active infections may be missed. Secondly, serology is not specific for any of the three target pathogens in this study. Serology using *R. rickettsii* template slides captures exposure to all members of the spotted fever group (Raoult and Paddock, 2005; Valbuena et al., 2004). Likewise, the SNAP 4Dx Plus test does not discriminate between exposure to *A. phagocytophilum* and *A. platys* or between *E. canis* and *E. ewingii*. However, *E. ewingii* is not thought to occur in western North America, and *A. phagocytophilum* is transmitted by *Ixodes* species ticks (Fleshman et al., 2022; Harris et al., 2016). *Ixodes pacificus* occurs very sparsely in temperate areas of San Diego and northern Baja California (Furman and Loomis, 1984), so it is possible that *Anaplasma* seropositive dogs had been exposed to *A. phagocytophilum,* although the majority were likely exposed to *A. platys*, supported by the findings of PCR-positive dogs for that pathogen.

Rickettsial seropositivity could be due to a wide variety of pathogens. While some subset is almost certainly in response to *R. rickettsii*, other circulating rickettsial organisms may be responsible for seropositivity, particularly in San Diego, where nearly 30% of dogs were seropositive despite no reports of RMSF so far in the region. *Rickettsia felis* is an emerging zoonotic pathogen most commonly transmitted by fleas and is increasingly associated with murine typhus cases in the United States and Mexico (Abramowicz et al., 2010; Brown and Macaluso, 2016; Zavala-Velazquez et al., 2006). Serologic cross-reactivity may occur between *R. felis* and SFGR species (Pérez-Arellano et al., 2005). *Rickettsia* *felis* has been identified in the brown dog tick previously in both North and South America, and in this study it was identified in a single tick, but the tick's role in transmission is unknown (Abarca et al., 2013; de Oliveira et al., 2020). While no fleas were observed or collected during this study, dogs may also be exposed when bitten by the cat flea, *Ctenocephalides felis* (Abramowicz et al., 2012). Both *R. massiliae* and *R. parkeri* are human pathogens carried by the brown dog that are present in the study region, and while both are believed to cause milder illness than RMSF, may cause diagnostic confusion when serology is used in both humans and dogs to diagnose illness (Beeler et al., 2011; Grasperge et al., 2012; López-Pérez et al., 2022). Surveillance for these pathogens is warranted for both their impacts on human and canine health, as well as to inform diagnosis and detection of more pathogenic species.

There is evidence that risk of diseases transmitted by the brown dog tick is increasing in the border region studied here. A 1994 study found no evidence of *E. canis* in shelter dogs in southern California (Yamane et al., 1994), and the historical extent of A. platys in Mexico is unclear as it was only detected there in the last decade (Almazán et al., 2016). However, data from private commercial laboratories show a marked, steady increase in seroprevalence for *Anaplasma* and *Ehrlichia* antibodies in dogs from both San Diego and Imperial County since 2012 (Companion Animal Parasite Council, 2022).

The differences in pathogen exposure and distribution between the eastern (Mexicali and Imperial County) and western (San Diego and Tijuana) study sites may be partly attributable to the presence of the two different species of brown dog tick. Analysis of the worldwide distribution of the tropical and temperate lineages originally suggested that they each has a discrete, non-overlapping distribution, based on latitude, that correlates with ambient temperature (Zemtsova et al., 2016). Subsequent phylogenetic analyses, however, indicated that the tropical lineage, which is dominant in Mexicali, overlaps in range with the temperate lineage in California and Arizona, and is moving northward (Brophy et al., 2022;

Villarreal et al., 2018). The lineage distribution in Tijuana had not previously been studied, and while the tropical lineage was previously found in San Diego, temperate and tropical lineages were found in overlapping range in adjacent counties to the north (Villarreal et al., 2018). While the RMSF cases in Mexicali were associated with the tropical lineage, the temperate lineage was responsible for Arizona outbreaks (Demma et al., 2005; Eremeeva et al., 2011). It is well established that both lineages are capable of spreading RMSF, but whether the differences in *Ehrlichia* and *Anaplasma* distribution between Tijuana and Mexicali could be due to variation in vectorial capacity between the species for those pathogens is unclear (Demma et al., 2005; Foley et al., 2019). The two lineages may also demonstrate different host seeking and biting behavior, which may result in pathogen transmission cycles that differ between even these close geographic locations (Backus et al., 2021). Further phylogeographic analysis and laboratory studies are indicated to clarify the impacts of brown dog tick species distribution on epidemiology of RMSF and other tick-borne diseases in this region.

We acknowledge several study limitations which impede direct comparison of risk of disease between locations. The first is that shelter populations were not directly comparable between locations. Intake date was not be determined for all dogs, but it was apparent from data that were available and discussion with shelter staff that turnover in Mexican shelters typically was higher than in California shelters, and dogs in Mexicali and Tijuana were being sampled closer to their date of intake. Sampling close to time of intake is likely to be a more sensitive indicator of disease risk within a region. Because sampling was cross sectional, seasonality may have impacted the prevalence of infection, particularly because shelters were not all sampled at the same time. Repeated sampling through the course of the year would help determine whether there is temporal variation that would impact surveillance. While pathogen-specific surveillance over time is likely to have the highest utility for detecting disease emergence, this study was a cross sectional assessment of a shelter population, representing only a small percentage of the total number of dogs in the shelter in a year, so the results obtained here may not represent either total annual shelter population or dog population as a whole. Data were not available on whether dogs were strays or relinquished to shelters by owners; this may have been unequal among regions, and owned vs. stray dogs typically differ in rates of exposure to ticks and tick borne pathogens, as well as overall health (Otranto et al., 2017). Finally, travel history—and therefore whether exposure occurred outside the study area or in one of the other quadrants—could not be ascertained.

Sheltered dogs provide a valuable source of sentinel data on tick-borne pathogens. The presence of pathogens that are vectored by brown dog ticks, combined with seropositivity to rickettsial organisms and proximity to Mexican cities experiencing epidemic disease, suggests that both Imperial and San Diego counties are at risk of RMSF emergence. PCR-based surveillance, even though duration of rickettsemia is short, may be a sensitive detector for *R. rickettsii* in an area where RMSF is emerging, as indicated by the finding of dogs infected with *R. rickettsii* in two different Tijuana shelters. Sheltered dogs represent a wide geographic cross section, likely overlapping with human tick-borne disease risk, and increased surveillance serves to benefit both canine and human health.

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Table 3.1. Demographic characteristics of dogs sampled across 7 shelters in 4 quadrants on either side of the US/Mexico border.

	Imperial (N=46)	Mexicali (N=63)	San Diego (N=52)	Tijuana (N=78)	Total (N=239)
Sex					
Female	24 (52.2%)	21 (33.3%)	17 (32.7%)	52 (66.7%)	114 (47.7%)
Male	22 (47.8%)	42 (66.7%)	35 (67.3%)	26 (33.3%)	125 (52.3%)
Weight					
<10 kg	9 (19.6%)	20 (31.7%)	2 (3.8%)	11 (14.1%)	42 (17.6%)
10-20 kg	11 (23.9%)	24 (38.1%)	10 (19.2%)	44 (56.4%)	89 (37.2%)
>20 kg	26 (56.5%)	19 (30.2%)	40 (76.9%)	23 (29.5%)	108 (45.2%)
Age					
<6 months	3 (6.5%)	8 (12.7%)	0 (0.0%)	5 (6.4%)	16 (6.7%)
6-12 months	5 (10.9%)	7 (11.1%)	4 (7.7%)	4 (5.1%)	20 (8.4%)
>12 months	38 (82.6%)	48 (76.2%)	48 (92.3%)	69 (88.5%)	203 (84.9%)

Table 3.2. Number and proportion of dogs positive by serology and PCR for tick-borne pathogens. Seropositivity for *Anaplasma* and *Ehrlichia* spp. was tested using the SNAP 4dx Plus, and IFA (indirect immunofluorescent assay) was used to determine rickettsial seropositivity.

	Imperial (N=46)		Mexicali (N=63)		San Diego (N=52)		Tijuana (N=78)		
	# Pos	% positive (95% CI)	# Pos	% positive (95% CI)	# Pos	% positive (95% CI)	# Pos	% positive (95% CI)	P-value
Serology									
Anaplasma spp.	1	2.2 (0.1, 11.5)	12	19.0 (10.2, 30.9)	1	1.9 (0.0, 10.3)	35	44.9 (33.6, 56.6)	**<.001**
Ehrlichia spp.	4	8.7 (2.4, 20.8)	31	49.2 (36.4, 62.1)	3	5.8 (1.2, 15.9)	31	39.7 (28.8, 51.5)	**<.001**
Rickettsia spp.	7	15.2 (6.3, 28,9)	51	81.0 (69.1, 89.8)	15	29.4 (17.5, 43.8)	46	59.7 (47.9, 70.8)	**<.001**
PCR									
A. platys	0	--	5	8.1 (2.7, 17.8)	1	1.9 (0.0, 10.4)	25	32.9 (22.5, 44.6)	**<.001**
E. canis	0	--	17	27.7 (16.6, 39.7)	0	--	10	12.8 (6.3, 22.3)	**<.001**
R. rickettsii	0	--	0	--	0	--	2	2.6 (0.8, 10.8)	.099

Table 3.3. Number of ticks and proportion testing positive by PCR from locations where ticks were identified.

	Mexicali (N=184)		Tijuana (N=16)		Total (N=200)	
	# Pos	% Positive (95% CI)	# Pos	% Positive (95% CI)	# Pos	% Positive (95% CI)
A. platys	5/154	3.2 (1.1, 7.4)	10/16	37.5 (15.2, 64.6)	15/170	6.5 (3.3, 11.3)
E. canis	18/155	11.6 (7.0, 17.7)	0/16	--	18/171	10.5 (6.4, 16.1)
Pan-*Rickettsia*	1/155	1.3 (0.2, 4.6)	2/16	6.2 (0.2, 30.2)	3/171	1.8 (0.4, 5.0)
R. rickettsii	0/1	--	1/2	--	1/3	--

Figure 3.1. Map showing locations of shelters in the four study quadrants (San Diego County, Imperial County, Tijuana, and Mexicali) along the western US-Mexico border.

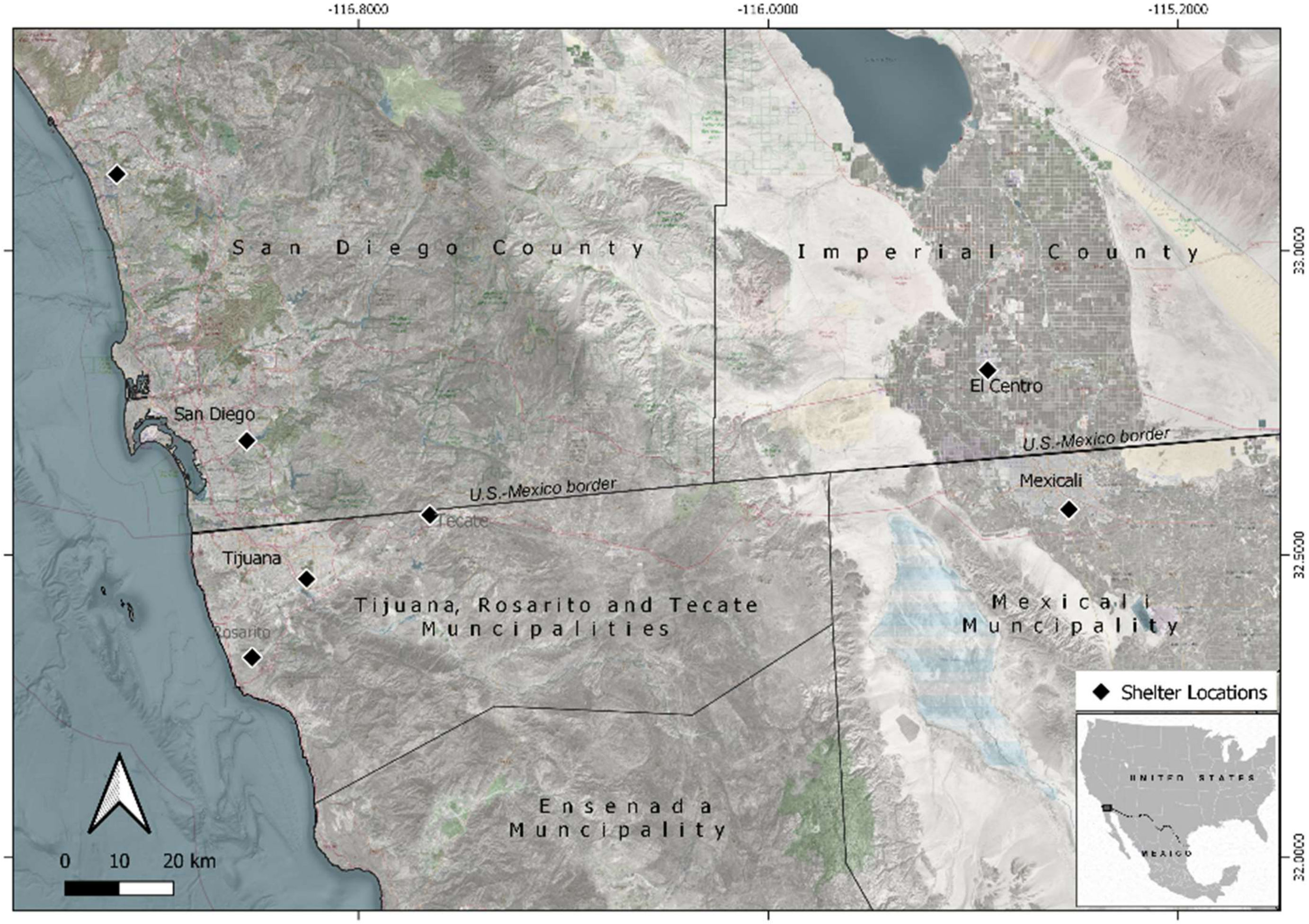

Figure 3.2. Proportion with 95% confidence interval of dogs positive by serology and PCR for tick-borne pathogens at each location.