2022-12-01

Survey Of Fleas And Ticks For Rickettsia Rickettsii And Rickettsia Typhi And Surveys Of Humans And Wild Animals For Serological Evidence Of Infection By These Rickettsiae In Rural And Urban Areas Of El Paso, Texas And Other Areas Of Texas

Karen Rocio Valdez
University of Texas at El Paso

SURVEY OF FLEAS AND TICKS FOR *RICKETTSIA RICKETTSII* AND *RICKETTSIA TYPHI* AND SURVEYS OF HUMANS AND WILD ANIMALS FOR SEROLOGICAL EVIDENCE OF INFECTION BY THESE *RICKETTSIAE* IN RURAL AND URBAN AREAS OF EL PASO, TEXAS AND OTHER AREAS OF TEXAS

KAREN ROCIO VALDEZ

Master's Program in Biological Sciences

APPROVED:

Douglas M. Watts, Ph.D., Chair

Igor Almeida, Ph.D.

Donald Bouyer, Ph.D.

Stephen L. Crites, Jr., Ph.D.
Dean of the Graduate School

Dedication

I dedicate this thesis to my family for teaching me to believe in myself, in God and in my dreams. To my husband, Adam, for your love, friendship and patience listening to the same presentation over and over. To Dr. Watts, I am infinitely grateful for your guidance, leadership and encouragement in this journey.

SURVEY OF FLEAS AND TICKS FOR *RICKETTSIA RICKETTSII* AND *RICKETTSIA TYPHI* AND SURVEYS OF WILD ANIMALS FOR SEROLOGICAL EVIDENCE OF INFECTION BY THESE *RICKETTSIAE* IN RURAL AND URBAN AREAS OF EL PASO, TEXAS AND OTHER AREAS OF TEXAS

by

KAREN ROCIO VALDEZ, B.S.

THESIS

Presented to the Faculty of the Graduate School of

The University of Texas at El Paso

in Partial Fulfillment

of the Requirements

for the Degree of

MASTER OF SCIENCE

Department of Biological Sciences

THE UNIVERSITY OF TEXAS AT EL PASO

December 2022

Acknowledgements

After an arduous journey, I am nearing the completion of the academic requirements for my Master's degree. I would like to acknowledge several individuals who have helped make this achievement possible. I want to first and foremost acknowledge Dr. Douglas M. Watts, whose mentoring has had a profound effect on the outcome of my training and words cannot express my endless gratitude for his unconditional support and guidance. Secondly. I would like to acknowledge Nicole Mendell who was my mentor and laid the foundation for my success through her teaching and encouragement through the years. Likewise, I would like to thank my other committee members, Drs. Almeida and Donald Bouyer for their support and shared knowledge as co-mentors. Also, I am grateful to Dr. Maldonado who was the first to give me the opportunity to be part of Bridges to the Baccalaureate Program that changed my career path and opened many doors for me to pursue my career. Finally, I would like to thank the Western Gulf Center of Excellence for Vector Borne Diseases for the support provided to me on this dissertation.

Abstract

The ecology and epidemiology of Rocky Mountain Spotted fever and typhus fever pathogens are poorly understood along the United States–Mexico border, especially in the far Southwestern region of Texas. The objective of this dissertation was to understand the prevalence and distribution of *Rickettsia rickettsii* and *Rickettsia typhi* and associated tick and flea species and the prevalence of these Rickettsiae in domestic and feral mammals in urban and rural areas of the El Paso community and other areas of Texas. Also, the goal was to determine if humans are being infected by *R. rickettsii* and *R. typhi* in the El Paso community. The methods for the collection of ticks included dragging, flagging and direct collection by hand from the animals. Flea trapping consisted of a commercial adhesive trap with an intermittent green light and direct collections by hand from the animals. The collected ticks and fleas were identified morphologically using taxonomic keys. Ticks and fleas were sorted by species, sex and/ or life stage and prepared in pools of 7 specimens**.** The testing of ticks and fleas for Rickettsiae was performed by first grinding individual pools in phosphate buffered saline (PBS), 200 µl per pool. The nucleic acid was extracted using a Qiagen DNeasy blood and tissue kit and then tested for spotted fever group *Rickettsia* by a nested polymerase chain reaction (PCR) assay. The genus specific primers use in the PCR included the 17kDa lipoprotein gene which amplified a 434- bp DNA fragment, gltA and ompA genes. If samples were positive for *Rickettsia*, they were further tested by Sanger dideoxy sequencing to determine the Rickettsial species. A total of 56 pools consisting of 223 fleas were collected in and around the El Paso community, including 40 pools of *Pulex irritants*, 11 pools of *Echidnophaga gallinacea* and 5 pools of *Ctenocephalides felis*. All of these fleas were collected by hand from small mammals including, *Canis latrans* (coyotes), *Procyon lotor* (racoons), *Urocyon cinereoargenteus*(foxes), *Canis lupus familiaris*(dogs) and *Felis catus*(cats). On testing these pools, all were negative except one pool of 4 *C. felis* that was collected from a dog and was

positive for *R. typhi* A total of 48 pools, consisting of a total of 197 ticks were collected by hand from dogs in and around the El Paso community, including the sister city of Ciudad Juarez, Mexico, Doña Ana County, New Mexico, Canutillo, Texas, and San Elizario, Texas. In addition, ticks were collected from *Sus scrofa* (feral hogs) and *Odocoileus virginianus* (white-tailed deer) in Travis County, Texas. Only one species of ticks, namely *Rhipicephalus sanguineus* was collected from a dog in El Paso, Texas. In Culberson, Texas a single *Dermacentor albipictus* was collected from a *Odocoileus hemionus (*mule deer). In Travis County, *Ixodes scapularis and Amblyomma americanum* were collected from feral hogs *and I. scapularis and D. albipictus* were collected from white-tailed deer. A total of 197 *R. sanguineous* were tested for *Rickettsiae*, including 69 from Juarez, 117 From El Paso, 11 from Doña Ana, New Mexico for *Rickettsiae* and all were negative. Of a total of 26 pools, consisting of 83 *I. scapularis* and 27 pools consisting of 58 *A. americanum* collected from hogs in Travis County. Nine pools of *I. scapularis* were positive for Rickettsia and 4 pools of *A. americanum* were positive for *Rickettsia buchneri*, a non-pathogenic species. Among a total of 64 pools, consisting of 196 *I. scapularis* and 3 pools consisting of 11 *D. albipictus* collected from white tail deer in Travis County, 24 pools of I. scapularis were positive for *R. buchneri*, a non-pathogenic species. In addition, preliminary results of testing 375 human plasma samples collected in El Paso for antibody to *Rickettsia* indicated that they were negative, thus suggesting that humans were not being infected with these pathogens in the El Paso Community. The findings of these studies established the first surveillance program for RMSF and typhus fever pathogens in Central and Southwest Texas and suggested that rickettsial pathogens were not of major public health importance in Central and Southwest Texas. Also, the findings of this study provided the first description of the biology of the potential tick and flea vectors of rickettsiae species in the El Paso and surrounding communities, and in Travis County, Texas.

Table of Contents

List of Tables

List of Illustrations

Chapter 1: Background

1.1 Tick-borne Diseases in the United States

Globally, ticks and fleas are the most important vectors of human and animal pathogens caused by bacteria, viruses, protozoa and helminths (Nguyen et al., 2020). Moreover, these vectors are of public health concern because the incidence of associated diseases and population density is increasing drastically with the ability to transmit zoonotic vector-borne pathogens (Estrada-Peña, 2015). It is crucial to understand the relationship between vertebrate hosts, invertebrate vectors and pathogens due the geographical expansion of vectors that is leading to the emergence and increase of vector-borne diseases causing significant human morbidity and mortality (Nguyen et al., 2020).

Tick borne diseases are responsible for over 95% of all vector-borne diseases reported in the United States annually (Boulanger et al., 2019). The ecology and epidemiology are poorly understood with human cases nearly tripling since 2004 (Eisen et al., 2017; Eisen & Paddock, 2020; Nguyen et al., 2020). Lyme disease, babesiosis, ehrlichiosis, Rocky Mountain spotted fever (RMSF), and tularemia are a public health concern in the United States (Centers for Disease Control and Prevention, 2019b). According to the CDC, some of the most important tick species in the United States that serve as vectors of human and animal pathogens are *Dermacentor variabilis* (American dog tick), *Ixodes scapularis* (Blacklegged tick), *Rhipicephalus sanguineus* (Brown dog tick), *Amblyomma maculatum* (Gulf Coast tick), *Amblyomma americanum* (Lone Star tick), *Dermacentor andersoni* (Rocky Mountain wood tick) and *Ixodes pacificus* (Western Blacklegged tick) (Centers for Disease Control and Prevention, 2019b).

Rocky Mountain spotted fever was identified as the first tickborne human pathogen in the United States in 1909 and is transmitted primarily by *R. sanguineus* and is caused by the bacteria

Rickettsia rickettsii. This tick originated in Africa and is believed to have been introduced into the Americas by humans who migrated from Asia and Africa and brought domestic dogs to America through the Bering land bridge (Drexler et al., 2017a; Moraes-Filho et al., 2011). *Ixodid* spp. ticks are the vectors of the pathogens that are the cause of more human cases than other genera of ticks. *Borrelia burgdorferi* is the cause of Lyme disease and is transmitted by the bite of *Ixodes scapularis*, this disease has increased by more than 300% between 1993 and 2012 and is one of the most frequently diagnosed tick-borne diseases in United States, especially in Northeast U.S.A., but rarely reported as a cause of disease in Texas. (Centers for Disease Control and Prevention, 2020). Babesiosis is caused by the parasite *Babesia microti* transmitted by *Ixodes scapularis*, it is endemic in northeastern and mid-western United States, but cases of babesial infection are rarely reported because the illness some people do not present with clinical manifestations (Krause, 2002). Ehrlichiosis is a bacterial disease caused by *Ehrlichia chaffensis*, *E. ewingi and E.muris and* can be transmitted to dogs and humans, it is commonly transmitted by *A. americanum, D. variabilis* and *R.sanguineus* (Murphy et al., n.d.)

1.2 Taxonomy and Biology of Tick Species

Approximately 900 known tick species are distributed among three families, including the: *Argasidae* (soft ticks), *Ixodidae* (hard ticks) and *Nuttallellidae* (monotypic) (Francisco, 2013). While *Argasidae* and *Ixodidae* ticks are distributed globally, *Nuttallellidae* are restricted to South Africa and Tanzania (Brites-Neto et al., 2015). While all ticks are obligate blood feeders, just 10% are of concern due to their ability to transmit pathogens to humans and livestock (Oliver, 1989b). There are several differences in the biology and morphology of the *Argasidae* and *Ixodidae* families; however, the *Nuttallellidae* family has characteristics of both *Argasidae* and *Ixodidae* (Francisco, 2013). Some of the main differences is that soft ticks do not show sexual dimorphism,

feed faster and leave their host promptly without attaching for long periods, can lay eggs several times during their life time and are more resistant to temperature and host changes that allow them to survive without a blood meal for several years. On the other hand, sexual dimorphism occurs in the adult stage of hard ticks, and they need to attach to their host for several days to weeks, depending on the species, host type and life stage. The female hard ticks lay about 2,000 to 18,000 eggs followed by their death. The survival of hard ticks is influenced by the temperature and humidity in their habitat and host availability (Brites-Neto et al., 2015; Estrada-Peña, 2015a; Francisco, 2013). The *Argasidae* family is comprised of about 170 species while the *Ixodidae* family contains more than 650 species, with the latter family being medically and veterinary more important because they affect both animal and human health globally. (J. H. Oliver, 1989). Little is known about the Nuttallellidae family and their medical importance, if any, remains uncertain (J. H. Oliver, 1989).

Ixodid tick life cycle differs from that of argasids in both development and duration, including three development stages: larva, nymph and adult (Estrada-Peña, 2015b). Usually, larvae and nymphs feed on small hosts such as rodents and birds while adults feed on large animals. Some species of ticks are host specific while others are less selective. These ticks are more commonly found on mammals rather than birds or reptiles (Estrada-Peña, 2015b). Ixodid ticks feed on hosts at least one time during every developmental stage. They inject saliva while they are feeding facilitating pathogen transmission since they complete engorgement by salivating and sucking through the same canal (Tahir et al., 2020) Some species of ticks feed on a different host during each stage while others complete their cycle by feeding only on two hosts or even just on one. Moreover, females die after producing and depositing eggs. (Estrada-Peña, 2015b) Each stage of ixodid ticks require large blood meals; larval and nymphal stages usually need 3 to 7 days and 4

to 8 days to feed respectfully. While adult females require 7 to 12 days, more time is needed when feeding on reptiles (Estrada-Peña, 2015b).

1.3 Taxonomy and Biology of Flea Species

Fleas are holometabolous arthropods that belong to the Order *Siphonaptera* including about 2,574 species that belong to 16 families and 238 genera that survive as external parasites on mammals and birds, but are found principally on mammals (Bitam et al., 2010). Fleas are small, wingless insects that have four life stages prior to becoming adults: egg, larvae, pupae and adult. Female fleas must take blood meals in order to complete ovary maturity. The number of eggs and the fecundity period varies depending on the species. Larvae development depends on environmental variations, humidity and food accessibility. In this stage they are free moving, have their own biting mouthparts, avoid light and are found in low vegetation, mattresses or carpet fibers. Pupae is the third stage that develops to adults, but if they are not in the right conditions or proper stimulus, they can stay in diapause (Bitam et al., 2010). In the last stage of the life cycle, fleas seek hosts for a blood meal that are attracted through the stimulus created by the hosts such as body heat, movement and exhaled carbon dioxide. (Bitam et al., 2010).

Fleas seek hosts in two different ways, either restricted or permissive. Restricted species search for specific hosts while the permissive species have a wide host range meaning that they can spread infectious disease pathogens to their multiple hosts (Maleki-Ravasan et al., 2017) Some of the most important synanthropic fleas are: *Pulex irritants* (human flea), *Cthenocephalides felis* (cat flea), *Ctenocephalides canis* (Dog flea); *Xenopsylla cheopis* (oriental rat flea), *Nasopsyllus fasciatus* (northern rat flea), *Echidnophaga gallinacea* (sticktight flea) and *Tunga penetrans* (sand flea, jigger, chigoe) (Bitam et al., 2010).

1.4 Flea-borne Diseases in the United States

Fleas are insects of medical and veterinary importance, they function as vectors and intermediate hosts of pathogens and are distributed widely throughout the world (Bitam et al., 2010). Flea-borne typhus has been enzootic in United States for years and is one of the most prevalent rickettsial infections that is a caused by *Rickettsia felis* and *Rickettsia typhi* (Anstead, 2020). This disease was first described in the Southeastern United States and in Texas in 1913 but was not recognized as a health concern until 1920 in the Southwestern United States where human cases kept increasing and in between 1931- 1946, 42,000 cases were reported (Anstead, 2020). It is believed that flea- borne typhus was first introduced to the United States through the Southern and Gulf Coast seaport and through the Texas-Mexico border in the early 20th century (Anstead, 2020).This disease is resurging in United States, and during the past decade the incidence of flea-borne typhus has more than double in California and Texas (Anstead, 2020). Some of the most medically important flea species in the United States are *Xenopsylla cheopis* (oriental rat flea), *Leptopsylla segnis* (European mouse flea), *Echidnophaga gallinacea* (chicken flea), and *Nosopsyllus fasciatus* (Northern rat flea), and *Ctenocephalides felis* (cat flea) (Anstead, 2020). Vector and host geographic distributions are expanding due to environmental and human behavior modifications, thus increasing the human exposure to vectors and transmittable pathogens (Bitam et al., 2010).

1.5 Ticks and Fleas in Texas

In Texas, tick and flea- borne diseases are not commonly diagnosed, and the public, in general are not aware of tick and flea species and pathogens they transmit. Moreover, feral swine plays an important role as a competent host for ticks and they have become a concern because their population and range have expanded rapidly and therefore, poses a high risk to transmit disease to

animals and humans (Mapston, n.d.). Their geographical distribution has increase over the years in Texas with an estimated population of 2.6 million in all ecological zones, leading to expansion and introduction of pathogens such as tularemia (*Francisella tularensis*) and the African swine fever virus (Sanders et al., n.d.). At least seven important species of ticks have been collected from feral swine in Texas: *Amblyomma americanum, Amblyomma cajennense, Amblyomma maculatum*. *Dermacentor albipictus, Dermacentor halli, Dermacentor variabilis* and *Ixodes scapularis*(*Wild Pigs and Ticks: Implications for Livestock Production, Human and Animal Health Wild Pigs and Ticks: Implications for Livestock Production, Human and Animal Health Ii*, 2016). Moreover, *Riphicephalus annulatus* and *Rhiphicephalus microplus* (the cattle fever ticks), were introduced and spread to California, Texas and across the southeastern United States by the early 1900s but they were eradicated. However, they were detected again in Cameron County, Texas in 2014 on White-tailed deer, nilgai antelope and feral swine (Corn et al., 2016).As animal population and their range expands, the tick distribution is increasing and adapting to new environments increasing the potential exposure to pathogens.

Flea-borne rickettsia include *Rickettsia typhi* and *Rickettsia felis* which are the cause of murine typhus (endemic typhus) and flea-borne spotted fever, respectively. Murine typhus is endemic in south Texas and is transmitted by the bite of the cat flea (*Ctenocephalides felis*), however, murine typhus continues to emerge outside its enzootic range (Adams et al., 1970; Adjemian et al., 2010; Blanton & Walker, 2017b; Schriefer et al., 1994). The enzootic cycle of *R. typhi* also involves rat to rat transmission by the Oriental rat flea (*Xenopsylla cheopis)* (Francisco, 2013). The cat flea is abundant in Texas and California where it feeds on humans, dogs, cats, opossums, skunks and racoons and transmits infections to humans by the inoculation of feces or by the bite of fleas infected with rickettsiae (Anstead, 2020). In Galveston, Texas murine typhus

cases reemerge after years and opossums are the reservoir animals that have been implicated in the transmission cycle of *R. typhi* (Blanton et al., 2016). In addition, Texas has been reporting the highest number of flea-borne typus cases, annually in lower Rio Grande Valley, the Coastal Bend area, Bexar, Harris, and Travis counties, among others(Flea-Borne Typhus | Home, 2021; Pieracci et al., 2017)

1.6 TICKS AND FLEAS IN EL PASO, TEXAS

The prevalence, ecology and epidemiology of both tick and flea species and associated pathogens in El Paso County, Texas is not understood due to inadequate surveillance and although tick-borne diseases are reportable, cases have not been reported in the county. However, Rocky Mountain spotted fever is an emerging public health concern along the US- Mexico border where hundreds of deaths have occurred. The only reported hard ticks that bite humans in this region is *R. sanguineus* which can transmit *R. rickettsii,* a member of the Spotted Fever Group that causes RMSF (Centers for Disease Control and Prevention, 2019). Of the Spotted Fever Group, *R. rickettsii* is the cause of the most lethal disease (Álvarez-Hernández et al., 2016). The distribution of *R. rickettsii* along the USA Mexico border is unknown. However, the brown dog tick has been implicated in the reemergence of RMSF in Baja California, Sonora, Chihuahua and Coahuila (Drexler et al., 2017b). In 2004 the brown dog tick was considered important for the first time in the epidemiology of RMSF in Arizona after an outbreak that affected the population with free roaming dogs the most likely amplifying host of *R. rickettsii* (Drexler et al., 2017a) In addition, *Ehrlichia* spp. and *Anaplasma phagocytophilum* were reported in dogs from Ciudad Juarez, Chihuahua in 2018 (Escárcega Ávila et al., 2018). In contrast to El Paso, Chihuahua State confirmed 98 Rickettsiosis cases, including 39 that were caused by Rocky Mountain spotted fever (Maynez-Prieto et al., 2021).

The biology and ecology of fleas is poorly understood in the El Paso and surrounding urban and rural communities. Moreover, the incidence of flea-borne cases along Juarez-El Paso border is unknown. However, recently cases were reported in the borders of the southern region of Texas (Flea-Borne Typhus | Home, 2021).

Chapter 2: Species Diversity of Ticks and Fleas and the Geographic Distribution of Potential Vectors of Rickettsiae in the El Paso Community.

2.1 Study Site and Collection of Ticks and Fleas from Wild and Domestic Animals

This study was performed in rural and urban areas in and surrounding El Paso and Travis County, Texas. Wild animals were trapped by Dr. Kenneth Waldrup, the Texas State veterinarian who provided ticks and fleas that were collected from wild and domestic animals. The wild animals were trapped at selected sites in the Franklin Mountains State Park of El Paso which is part of the Chihuahuan Desert and largest urban park in the nation covering about 24,000 acres with hot and dry desert climate. The more common plants in the park includes *Echinocactus grusonii* (barrel cactus), *Yucca filamentosa* (yucca), *Argemone mexicana* (Mexican poppies), along with trees, including *Populus deltoides* (cottonwood), *Celtis occidentalis* (hackberry), and *Quercus robur* (oak) that are found along the springs on the mountain slopes. Mammals in the park include: *Odocoileus hemionus* (mule deer), *Puma concolor* (mountain lions), *Ursus americanus (*black bear), *Bassariscus astutus* (ring-tailed cat) and a variety of rodent species. Another desert site was in 1000 acres of undeveloped land located at the eastern entrance to the El Paso international airport that has flora and fauna similar to that described for the Franklin Mountains State Park. In addition, ticks were collected in the surrounding areas of El Paso, Texas and Doña Ana County, Anthony, New Mexico, and in Presidio, Ft. Hancock and San Angelo and Travis County, Texas and in Mexico. The domestic animals were collected in El Paso. Also, Dr. John Morrill, a Texas State contractor who was employed to control the population density of white-tailed deer and feral hogs in Travis County, Texas provided tick and fleas collected directly by hand from these animals. In addition, staff of private veterinarian clinics and grooming business and the El Paso Animal Services provided ticks and fleas collected directly by hand from domestic

animals. The field methods for the collection of ticks included dragging and flagging cheesecloth mess for questing ticks in open field sites, and the use of dry ice as a source of CO_2. (Carroll & Schmidtmann, 1992; Tietjen et al., 2019). Flea trapping was performed with a commercial adhesive trap with an intermittent green light source to attract them. Each collection using field methods and trapped animals was given a number in consecutive order and the number was assigned to the tick and/or flea samples. The information included the location of capture, date and animal species as well as habitat type.

2.1.2 Identification of Ticks and Fleas

Ticks and fleas were transported alive in containers on ice-packs to the UTEP Biosafety Level 2 laboratory located in room 222 in the UTEP Biology building where they were euthanized by exposure to -20°C for a minimum of 2 hours. The ticks or fleas were transferred to a chill table and identified morphologically using a taxonomic key (Center for Disease Control, n.d.; *EHS Pictorial Keys | EHS | CDC*, n.d.; Keirans & Litwak, n.d.) Ticks and fleas were washed and disinfected with 3% bleach, followed by 70% ethanol and then rinsed with sterile water. Fleas were pooled, 7 per pool according to species, date of collection, and animal species. Ticks were pooled, 7 per pool according to species, sex and/ or life stage.

2.2 RESULTS

A total of 56 pools, consisting of 223 fleas were collected by hand from *Canis latrans (*coyotes), *Procyon lotor* (racoons), *Urocyon cinereoargenteus (*gray foxes), *Canis lupus familiaris* (dogs), and *Felis catus (*cats) in and around the El Paso community. Table 2.1 shows the total of fleas collected, capture sites and quantity of fleas collected at each location during 2020-2022 from animals in El Paso, TX, and surrounding areas, and in San Angelo and Travis County, Texas. Fleas were classified and pool into 3 genera and 3 species, including 40 pools of *Pulex irritants*, 11 pools

of *Echidnophaga gallinacea* and 5 pools of *Ctenocephalides felis*. Table 2.2 shows the frequency of the different flea species collected in El Paso, Texas and surrounding areas. *Pulex irritants* was the predominant flea species that was collected from racoons, coyotes, foxes, dogs and cats and accounted for a total of 148 fleas (68.52%) followed by *Echidnophaga gallinacea* 54 fleas (25%) collected from a woodrat, coyotes, a racoon, a gray fox and a cat and *Ctenocephalides felis* 13 fleas (6.01%) collected from a racoon and dogs. Table 2.3 shows the frequency of the different flea species collected in Travis County, Texas, with *Pulex irritants* being the most commonly collected species.

Cat flea Human flea Sticktight flea

Illustration 2.1: Flea species collected during 2020-2022 from animals in El Paso, TX, San Angelo and Travis County, TX.

Table 2.1. Summary of fleas collected during 2020-2022 from animals in El Paso, TX, surrounding areas, San Angelo and Travis County

Capture site	*P. irritants*	*C. felis*	*E. gallinacea*	Total of fleas collected	Animals
Anthony, New MX	0	1	0	1	Dog
El Paso County, TX	123	12	33	168	Racoon, Coyote, Fox, Dog, Cat
Fort Hancock, TX	4	0	0	4	Coyote
Presidio, TX	15	0	0	15	Coyote
San Angelo, TX	4	0	0	4	Dog
Travis County, TX	7	1	0	8	Racoon
Unknown	2	0	21	23	Woodrat and Coyote
Total Fleas	155	14	54	**223**	

Table 2.2. The frequency of collecting different flea species in El Paso, Texas and surrounding areas

Species	**Number**	**Percentage**	**Animals**
Human flea/*P. irritants*	148	68.52%	Racoon, Coyote, Fox, Dog, Cat
Sticktight flea/*E. gallinacean*	54	25%	Coyote, Racoon, Fox, Cat
Cat Flea/*C. felis*	13	6.01%	Dog, Racoon
Total	215		

Table 2.3. The frequency of collecting different flea species in Travis County, Texas

Species	Number	Percentage	
Human flea/ *P. irritants*	7	88%	Racoon
Cat flea/*C. felis*	1	12.50%	Racoon
Total	8		

A total of 168 pools, consisting of 546 ticks were collected from July 2020 to January 2022 in El Paso, Texas, and surrounding areas, and in Travis County and Culberson County, Texas (Table 2.4). A total of 48 pools, consisting of 197 ticks were collected in and around the El Paso community, including the sister city of Ciudad Juarez, Mexico, Doña Ana County, New Mexico, Canutillo, Texas, and San Elizario, Texas. In El Paso only *Rhipicephalus sanguineus* ticks were collected from *Canis lupus familiaris* (dogs) (Illustration 2.2). In Culberson County, Texas located 4 hours away from El Paso *Dermacentor albipictus* was collected from a mule deer (*Odocoileus hemionus)*. In addition, A total of 120 pools, consisting of 348 ticks were collected in Travis County, from white-tailed deer (*Odocoileus virginianus)* and from feral swine (*Sus scrofa)* including three different species, *Ixodes Scapularis (80% (279/546), Amblyomma Americanum (16.66% (58/546),* and *Dermacentor Albipictus 3.16% (11/546)*. 83 *I. scapularis and 58 A. americanum* were collected from feral swine *and 196 I. scapularis and 11 D. albipictus* were collected from white-tailed deer.

Brown dog tick

Illustration 2.2: Tick specie collected during 2020-2022 from animals in El Paso, TX, and surrounding areas.

Table 2.4. Summary of ticks collected during 2020-2022 from animals in El Paso, County TX, and other areas of TX, and New Mexico, and Mexico

Capture Site	*R. sanguineus*	*I. scapularis*	*A. americanum*	*D. albipictus*	Total of Ticks Collected	Animals
Ciudad Juarez, MX	69	0	0	0	69	Dog
Culberson, TX	0	0	0	1	1	Mule Deer
Doña Ana County, New MX	11	0	0	0	11	Dog
El Paso County, TX	114	0	0	0	114	Dog
Travis County, TX	0	83	58	0	141	Feral swine
Travis County, TX	0	196	0	11	207	White-tailed deer
Unknown	3	0	0	0	3	Dog
Total Ticks	197	279	58	12	**546**	

Table 2.5. Frequency of different tick species collected in El Paso, TX and surrounding areas

Species	Number	Percentage	Animals
Rhipicephalus sanguineus	197	99%	Dog
Dermacentor albipictus	1	1%	Deer
	198		

Table 2.6. Frequency of different tick species collected in in Travis County, Texas (include animals)

Species	Number	Percentage	Animals
Ixodes scapularis	279	80%	Deer, Hog
Amblyomma americanum	58	16.66%	Hog
Dermacentor albipictus	11	3.16%	Deer
Total	348		

2.3 DISCUSSION

The data generated by this study provided a preliminary understanding of the potential public health risk of ticks and fleas species in the Central and Southwest Texas region. These results showed that dragging, flagging and CO2 traps were not successful in this area for collecting ticks even though these methods have been successful in other places in Texas and United States (Mendell et al., 2019). Also, adhesive traps with intermittent light source were not successful for collecting fleas in this area and the best collection method for collecting ticks and fleas was by direct removal from animals by hand. All ticks were collected by hand directly from the animals showing that the weather in this area was too hot for ticks and that they preferred to stay on the animals for survival. Ticks and fleas collected from August 2019 through January 2022 indicated that only a single tick species was captured and identified as *Rhipicephalus sanguineus* in El Paso, Texas region. Meanwhile, for the fleas, three species were captured and identified as

Echidnophaga gallinacea, Pulex irritants, and *Ctenocephalides felis* in El Paso, Texas region and surrounding areas. Also, these results showed that human flea and stick-tight flea were most prevalent in El Paso and surrounding community. Overall, progress thus far suggested that only one tick and 2 flea species that are known to feed on humans was found in the El Paso community

A total of 141 ticks were recovered from feral swine in Travis County, Texas from 2018 to 2020, including two different species identified as Ixodes *Scapularis (n=279) and Amblyomma americanum (n=58*).These results are consistent with another study that was performed by Texas A&M on ticks collected from feral hogs in different ecoregions of Texas. , Seven different species of ticks were collected from the hogs in Bexar County, Bell County, Coryell County, Brazos County and Sinton, Texas including *Amblyomma americanum, Ixodes scapularis, A. cajennense, Dermacentor albipictus, Dermacentor halli and Dermacentor variabilis*.(Sanders et al., n.d.). In Travis County, Texas, two different species were collected, including *Amblyomma americanum* and *Ixodes scapularis* showing that feral swine is a competent host for ticks that are a public health concern for humans and animals. On the other hand, from 2018 to 2020, 207 ticks were obtained from white tail deer in Travis County, Texas, including *Ixodes scapularis* (196) and *Dermacentor albipictus (n=11)*. These results were also consistent with another study in Travis County, Texas from October 2001 to 2015 where 1493 *Ixodes Scapularis* were collected from white-tail deer in different counties of Texas including Bee, Gonzales, Guadalupe, Hamilton, Karnes, Kerr, Medina, Real, Travis, Uvalde, Webb and Williamson (Adetunji et al., 2016). The findings of this study provided an understanding of the diversity of tick species associated with different animal host as potential vectors and vertebrate host of tickborne pathogens in New Mexico, Mexico and Texas. (Yu et al., 2020).

Chapter 3: The Prevalence and Distribution of *R. rickettsii* and *R. typhi* in Different Tick and Flea Species.

3.1 Medical Importance of Hard Ticks

Globally tick-borne diseases have emerged and increased in the past decades causing enormous health and economic loses affecting the health of both human and animals. Ticks can transmit a variety of arthropod-borne pathogens and therefore, plays an important role in the transmission of disease pathogens globally (Dantas-Torres et al., 2012). Hard ticks are ectoparasites of mammals, birds and reptiles that can have an affect directly or indirectly by transmitting disease pathogens or by taking blood from animals that can cause anemia and a reduction in weight among the animals as well as severe dermatitis or even tick-bite paralysis (Rajput et al., 2006). Worldwide, the genera of *Hyalomma, Boophilus, Rhipicephalus, Amblyomma* and *Ixodes* are of medical and economical importance with the ability to transmit important zoonotic tick-borne disease pathogens that cause such diseases as Crimean-Congo hemorrhagic fever, Rocky Mountain Spotted fever, Anaplasmosis, Babesiosis, Ehrlichiosis and Lyme borreliosis (Dantas-Torres et al., 2012). Tick species and associated pathogens vary from region to region. The emergence of these diseases has been attributed to climate changes, land use, host availability and people's behavior (Diuk-Wasser et al., 2020).Some vertebrate hosts serve as reservoirs while others serve as accidental hosts for several tick species such as: *A. americanum*, *I. ricinus*, and *R. sanguineus* (Dantas-Torres et al., 2012).

Tick transmission of pathogens is vertical, transstadial and horizontal. Vertical transmission occurs when the vector transmits the pathogen from the infected female to her offsprings, and transstadial transmission is from one life stage to the other and horizontal transmission is from an infected tick to a vertebrate, including humans. (Boulanger et al., 2019). For instance, the bacteria

Borrelia burgdorferi and *Rickettsia* species are transmitted transovarially and transstadially (Barker & Reisen, 2018; Day | & Newton, n.d.). In addition, *Rickettsia* species are also maintained within tick population by transovarial transmission from female to offspring transstadially from one life stage to the next(Blanton, 2019).

3.2 Medical Importance of Fleas

Fleas are vectors and reservoirs of human and animal pathogens and they have a worldwide distribution (Maleki-Ravasan et al., 2017). The number of diseases caused by mosquitos, fleas and ticks has been tripling over the years. ("Centers for Disease Control and Prevention," 2019a). Fleas have been increasing their geographic distribution and host ranges due to the destruction of wild habitats and climate change (Bitam et al., 2010; Blanton & Walker, 2017a). There is an array of pathogens transmitted by fleas that have not been studied and may emerge or re- emerge (Bitam et al., 2010). Fleas are intermittent feeders that go from host to host to feed and facilitate disease pathogen transmission. Some of the most common human pathogens are *Yersinia pestis* (plague), *Rickettsia typhi* (murine typhus), *Francisella tularensis* (tularemia) and *Bartonella henselae* (cat scratch disease) (Maleki-Ravasan et al., 2017). Flea-borne rickettsiae are transmitted to humans by infected feces after fleas bite their host or by oral route through regurgitation of blood meals (Azad & Beard, 1998; Bitam et al., 2010). Murine typhus or endemic typhus is an illness that has been present for years and is caused by *Rickettsia typhi*, the brown and black rats is the suggested reservoirs and fleas are the vectors, with more than eleven species of fleas having been implicated as vectors of Flea- borne typhus (Anstead, 2020). Humans become infected though inoculation of an infected flea feces when they are scratched onto the wound (Blanton, 2019).

In the United States murine typhus is endemic in southern California and in south Texas where opossums are the hosts and cat fleas the vectors, Flea- borne spotted fever is another disease transmitted by cat fleas and is caused by *Rickettsia felis* globally (Blanton, 2019)

The species of the genus Rickettsia are classified into four groups including a basal ancestral group, the spotted fever group (SFG), the typhus group and the transitional group (Blanton, 2019). The spotted fever group rickettsiae are transmitted by several species of ticks transstadially and transovarially. *R. rickettsii* is the most pathogenic rickettsial species of this group and can cause severe and fatal disease when not recognized and treated properly (Blanton, 2019). In United States Rocky Mountain Spotted Fever is common in the southeast and south-central states where the vectors are *Dermacentor variabilis, Dermacentor andersoni* and *Rhipicephalus sanguineus* and other *Amblyomma* species (Blanton, 2019)

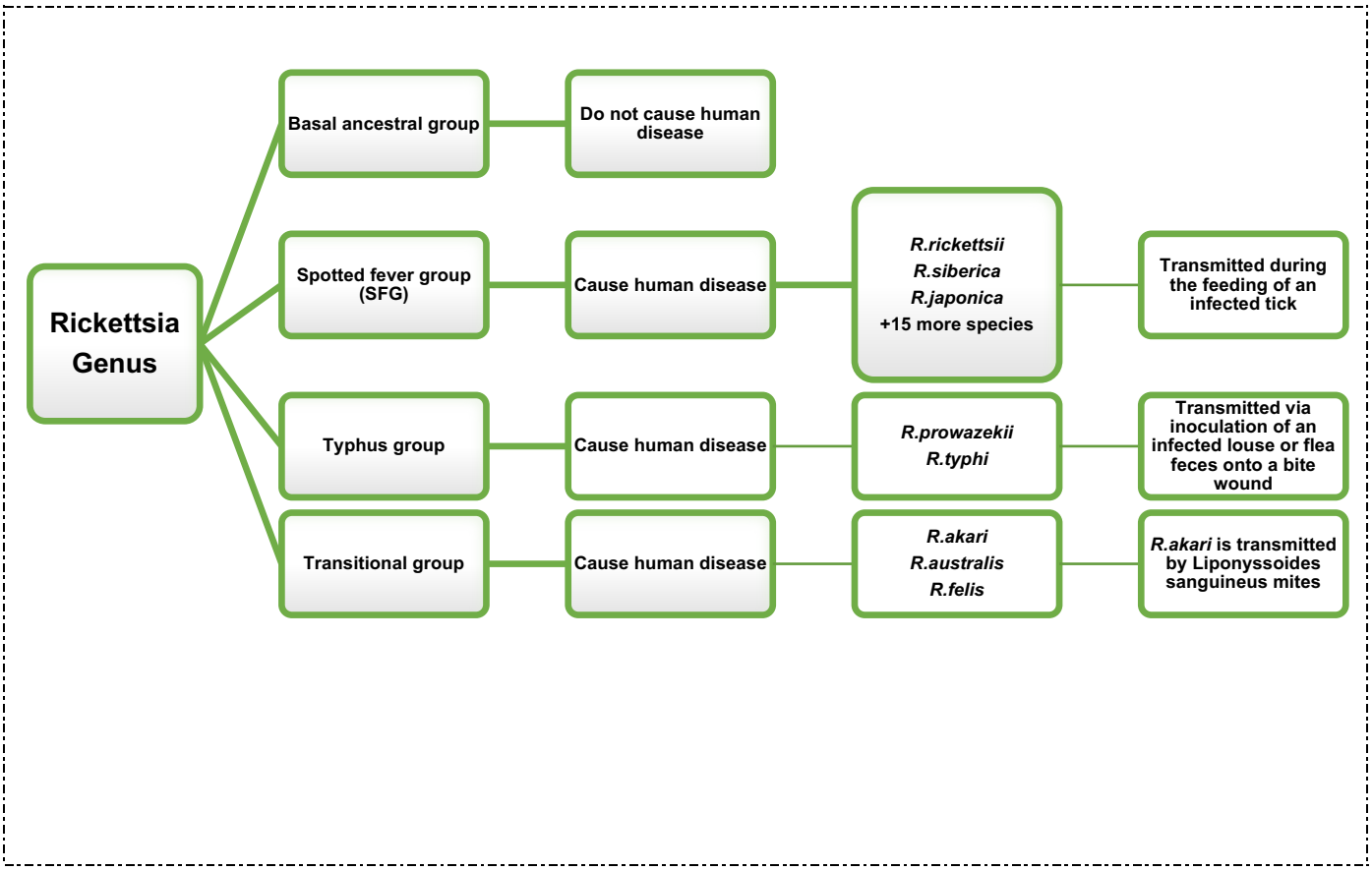

Illustration 3.1: Rickettsia genus

3.3 Materials and Methods

3.3.1 Testing of Fleas and Ticks for Rickettsial Pathogens

Ticks and fleas were sorted by species, sex and/ or life stage. If the ticks were blood feed, four legs from each tick were used for testing by PCR and the other four were pooled and stored at -80°C for future analysis. On the other hand, if they were not blood fed, ticks were cut in half for processing. Ticks and fleas were grouped into pools of up to seven ticks or fleas. After that they were homogenized manually with disposable and sterile pestles in the presence of 200 μl of PBS.

3.3.2 Polymerase Chain Reaction

Fleas and ticks were stored at -80 °C until nucleic acids were extracted for PCR testing using the Quiagen DNAeasy Extraction kit according to the manufacturer's recommendations. The quality and quantity of DNA and RNA was analyzed by NanoDrop 100 before pathogen detection. After that, spotted fever group Rickettsia were tested using genus specific primers through nested PCR of the 17kDa lipoprotein gene which amplified a 434- bp DNA fragment, gltA (citrate synthase gene) and ompA. Reaction mixes for PCR were prepared in 25 ul reactions utilizing GoTaq green master mix (Promega) and 800 ug/ml bovine serum albumin (BSA) water. In addition, we utilized DNA extracted from *R. sibirica* culture as a positive control All PCR thermal cycling conditions used were as published before. After that, products were run on a 1.5% agarose gel stained with gel red (Mendell et al., 2019).

3.3.3 Sequencing

If samples were positive for rickettsia and the species were unknown, further sequencing was performed. In order to identify the *Rickettsiae* species, amplicons were purified with the Qiagen purification kit according to the manufacturer's protocol and send outside the university to commercial laboratory (Genewiz) to be tested by Sanger dideoxy sequencing, resultant sequences were aligned and compared with published gene sequences available in GenBank. After that, the samples were shared with colleagues at University of Texas–Medical Branch (UTMB) for confirmation of the identity of any positive samples.

3.4 RESULTS

Table 3.1. Summary of the fleas collected during 2020-2022 from animals in El Paso, TX and surrounding areas, and in San Angelo and Travis Counties, Texas and results of testing fleas for Rickettsiae pathogens

Capture Site	*P. irritants*	*C. felis*	*E. gallinacean*	Total Number of Ticks Tested	Results
Anthony, New Mexico	0	1	0	1	Negative
El Paso County, Texas	123	12	33	168	1 pool positive
1 pool from *Ctenocephalides felis* was positive for *Rickettsia felis* (obtained from dog) from El Paso, Texas on April,2022, we targeted the rickettsial gene 17kda,gltA and ompA					
Fort Hancock, Texas	4	0	0	4	Negative
Presidio, Texas	15	0	0	15	Negative
San Angelo, Texas	4	0	0	4	Negative
Travis County, Texas	7	1	0	8	Negative
Unknown	2	0	21	23	Negative
Total	155	14	54	223	
Total Fleas Tested	223				

A total of 56 pools consisting of 223 fleas collected in and around the El Paso community were tested, including 40 pools of *Pulex irritants* consisting of 155 fleas, 11 pools of *Echidnophaga gallinacea* consisting of 54 fleas, and 5 pools of *Ctenocephalides felis* consisting of 14 fleas. All were negative for *Rickettsia spp*. except one pool containing 4 fleas of *C. felis* that was positive for *R. typhi*, collected from a dog in El Paso, Texas, Zip code 79935 on august 30, 2021.

Table 3.2. Summary of the ticks collected during 2020-2022 from animals in El Paso, TX, surrounding areas and Travis County and the results of testing the ticks for Rickettsiae pathogens

Capture Site	*R. sanguineus*	*I. scapularis*	*A. americanum*	*D. albipictus*	**Total number of ticks tested**	**Results**
Ciudad Juarez, Chihuahua, MX	69	0	0	0	69	Negative
Culberston, Texas	0	0	0	1	1	Negative
Doña Ana, Nuevo Mexico	11	0	0	0	11	Negative
El Paso County, Texas	114	0	0	0	114	Negative
Travis County, Texas	0	83	58	0	141	13 pools positive
13 pools from Ixodes scapularis were positive for Rickettsia they were obtained from feral swine we targeted for the rickettsial gene 17kda, glta and ompA. After sequencing *R. buchneri*						
Travis County, Texas	0	196	0	11	207	Positive
24 pools from Ixodes scapularis were positive for Rickettsia, they were obtained from white tail deer we targeted for the rickettsial gene 17kda, gltA and ompA. After sequencing *R. buchneri*						
Unknown	3	0	0	0	3	Negative
Total Ticks Tested	546					

A total of 197 *R. sanguineous in 55 pools* were tested, including 69 from Juarez, 117 From El Paso, 11 from Doña Ana, New Mexico for Rickettsiae and all were negative. Of a total of 26 pools, consisting of 83 I. scapularis and 27 pools consisting of 58 A. americanum collected from hogs in Travis County. 9 pools of *I. scapularis* were positive for Rickettsia and 4 pools of *A. americanum* were positive for *Rickettsia buchneri*, a non-pathogenic species. Among a total of 64 pools, consisting of 196 I. scapularis and 3 pools consisting of 11 *D. albipictus* collected from white tail deer in Travis County 24 pools of I. scapularis were positive for *R. buchneri*, a non-pathogenic species.

3.5 DISCUSSION

The ecology and epidemiology of vector -borne diseases are poorly understood in around the El Paso community and it is crucial to collect and test ticks and fleas to better understand any associated pathogens in the El Paso and surrounding communities to being prepared for future outbreaks.

Moreover, with this project the first laboratory with the capability in West Texas to identify Rickettsiae pathogens was established. The field-collected tick and flea samples were tested by Polymerase Chain Reaction (PCR) that detected *Rickettsia felis in* one pool of 4 *Ctenocephalides felis fleas* in El Paso, Texas. Our data showed that there is a human health threat and even though a very small percentage was positive for this bacterial pathogen that is enzootic the El Paso community. Of 198 brown dog ticks tested, all were negative for Rickettsia spp.

A total of 141 ticks were collected from feral swine in Travis County, Texas from 2018 to 2020 (83 *Ixodes Scapularis*, 58 *Amblyomma americanum*), from which 13 of 53 pools were positive for *Rickettsia* using PCR assay. From 2018 and 2020, 207 ticks were obtained from white-tailed deer in Travis County, Texas (196 *Ixodes scapularis* and 11 *Dermacentor albipictus*), from which 24 of 67 *Ixodes scapularis* pools tested positive for Rickettsia using the PCR assay. After sequencing, the results showed they were positive for *Rickettsia buchneri*, a non-pathogenic endosymbiotic bacterium not representing a public health concern. These findings of this study, although preliminary, suggested that the risk of human disease associated with tick- and flea-borne pathogens was very low in the El Paso community and in Travis County, Texas.

Chapter 4: Serosurveys for Evidence of Infection by *R. rickettsii* and *R. typhi* in Humans in El Paso, Texas

4.1 RICKETTSIOSES

Rocky Mountain Spotted fever (RMSF), murine typhus, Q fever and ehrlichiosis are the most prevalent rickettsial diseases transmitted by tick and fleas and in the United States. RMSF that belongs to the Spotted Fever Group rickettsiae is caused by *Rickettsia rickettsii* (Biggs et al., 2016; Vaughn et al., 2014). Murine typhus cases have been reported in the United States since 1944 and the cause of this disease, R. typhi remains enzootic in California and south Texas (Blanton & Walker, 2017b). Seroprevalence studies of Spotted Fever Group rickettsial infection in El Paso, Texas will help to determine if *R. rickettsii* or *R. typhi* are contributing to the increase incidence of Spotted Fever Group rickettsiosis in this area.

Rickettsioses are difficult to diagnose due that they share similar clinical manifestations to other febrile infections, the most effective and widely diagnostic method is through serological assays based on antibody detection (Blanton, 2019). The first serological assay utilized to diagnose rickettsial diseases was the Weil-Felix test, however it lacked specificity and sensitivity (Parola et al., 2005). There are enzyme linked immunosorbent assays (ELISA) commercial kits available to test for rickettsial testing. However, these kits are only a qualitative method and not a quantitative method to demonstrate if the antibody levels increased or decreased (Frieden et al., 2016). Indirect immunofluorescence antibody assay (IFA) is the gold standard serologic test for the diagnosis of rickettsial infection and even though, it is not sensitive to detect antibodies during the first week of infection, IFA is highly reactive for several months after initial infection (Frieden et al., 2016). The IFA assay offer qualitative results showing the presence or absence of the antibody and also quantitative results that demonstrate whether the antibody levels increased or decreased, which is

crucial to confirm positive results (Frieden et al., 2016). Moreover, this assay includes all the rickettsial protein antigens and group-shared lipopolysaccharide antigen offering group-reactive serology, for the detection of IgG antibodies with the standard titer for IgM (Preto, 2005.) According to Kostman IFA have a sensitivity and specificity index of 94% for the detection of antibodies to *R. rickettsii* while for *R.typhi* other studies have shown 85 to 94% (Kostman, n.d.) Serology is the best method for clinical diagnosis. However, molecular methods should be used in combination with serological methods to be able to diagnose acute disease (Abdad et al., 2018) Moreover, Studies have shown that at least 10% of the US population has been infected with a spotted fever group *Rickettsia* (SFGR) species at any point of their life (Alugubelly et al., 2021).

4.2 Materials and Methods

4.2.1 Blood Collection Methods

Cord-blood plasma samples were collected during 2017 and 2018 from child-bearing mothers at 3 local hospitals in the City of El Paso, Texas. A questionnaire was completed by each participant to include important data such as travel history in the last 5 years, occupation, age and collection date was recorded. Samples were collected aseptically in SST tubes then centrifugated at 3000xg for 10 minutes and then serum was transferred to a closed sterile container. All blood samples were treated as potentially infectious. After that, samples were stored at -20 C until testing.

4.2.2 Immunofluorescence Assay (IFA)

To determine if humans were infected by *R. rickettsii* and *R. typhi* in the El Paso community and other areas of Texas, human and animal serum samples were tested by Indirect Immunofluorescence Assay (IFA) for antibodies to *R. rickettsii* and *R. typhi* using multiple rickettsial antigens. 12- well slides were prepared at the UTMB BSL3 lab with antigen suspensions of intact Vero cells infected with *Rickettsia rickettsii* Sheila Smith, and *R. typhi* Wilmington strain,

in PBS with 3% bovine serum albumin (BSA), allowed to dry, and fixed and permeabilized with acetone, slides were stored at -80°C until they were used. For antigen preparation they utilized cells until they were over 70% infected (Blanton et al., 2015; Olano et al., 2003). Both IgM and IgG antibodies can be detected, while the detection of specific IgM antibodies can identify various species of *Rickettsiae* as well as provide evidence of recent infection. The IFA reagents were available commercially for both the spotted fever group and the typhus group from Pan Bio, Inc and Cypress, CA (Preto, 2005). *R. rickettsii* and *R. typhi* positive and negative controls were provided by the Rickettsial and Ehrlichial Diseases Research Laboratory at UTMB, Galveston, Texas. Phosphate Buffered Saline (PBS) was prepared according to manufacturer's instructions. Blocking solution contained PBS with bovine serum albumin 1% and 0.01% sodium azide. Sample/conjugate diluent contained PBS with 1% BSA and 0.1% Tween 20. Wash buffer contained PBS with 0.1% Tween 20. After slides were removed from the freezer, they were submerged in PBS for 10 minutes at room temperature then submerged for 15 minutes in blocking solution to later be removed and blotted dry. For patient samples, positive control and negative control were diluted 1:128, I made two-fold serial dilutions of the diluted positive control and I tested four serial dilutions of the positive control with each run to assure sensitivity of the assay. The titer of the positive control was 1:128. In the second stage, fluorescein-labeled antibody to human heavy-chain IgG was added to each antigen well on the slides. I utilized a fluorescein-labeled conjugate to detect serum antibodies specific to *Rickettsia rickettsii* and *R. typhi*. After incubation to allow the antigen-antibody to react with the fluorescein-labeled anti-human IgG, the slides were washed with PBS and Tween 20 and counterstained with Evans Blue, dried, mounted and examined using fluorescence microscopy (Caravedo Martinez et al., 2021). Slides were stored away from light at 2-8°C until ready to read. First, the negative control was read, then the positive

control and after that the test samples. If positive, antigen–antibody reactions appear as rickettsial bodies exhibiting bright apple-green cytoplasmic fluorescence (Caravedo Martinez et al., 2021).For positive samples I prepared a serial two-fold dilutions of the 1:64 screening dilutions of the positive control and any positive sample utilizing sample/conjugate diluent. I added the positive control, negative control and positive patient samples 1:128 to 1:4096 dilutions to each well on the slide (Premaratna et al., 2012).

4.3 RESULTS

A total of 375 cord-blood plasma samples were tested for *R. rickettsia* and *R. typhi* antibodies to determine if humans were being infected by *R. rickettsia* in the El Paso community. The results indicated that all the samples were negative. However, when tested for *R. typhi,* 0.53% (2/375) were positive at a screening titer of 1:128 with a reciprocal endpoint titer of 2048, both of the patients had a history of travel to Mexico. The seroprevalence rate was 0.53% for *R. typhi* while all were negative for *R. rickettsii*. All slides included both a positive and a negative control. The control wells were read first to ensure correct interpretation. The negative control never produced fluorescence.

4.4 DISCUSSION

The purpose of this study was to determine if humans were infected previously with *R. rickettsia* and *R. typhi* in the El Paso community. Even though only two samples were positive for *R. typhi* and remained uncertain if the patients were infected in United States or Mexico, due to their travel history to Mexico. Continued surveillance remains necessary in this region. The findings suggested that *R. rickettsii* and *R. typhi* are not a threat to human health in the El Paso and surrounding communities in West Texas but due to the fact that rickettsial diseases continuing to emerge and expand, surveillance remains necessary. Moreover, outbreaks of Rocky Mountain

Spotted Fever (RMSF) in Ciudad Juárez, Mexico poses a threat of the spread of *R. rickettsii* to the El Paso community. According to the Chihuahua Health Department in Ciudad Juárez, from (Emite Secretaría de Salud Recomendaciones de Prevención Ante La Rickettsiosis | Portal Gubernamental Del Estado de Chihuahua, 2022). January to October 2022 a total of 82 Rickettsiosis cases have been reported throughout the state of Chihuahua, MX, including 20 fatal cases, 24.39% (20/82), and of the 82 non-fatal cases, 38 occurred in the city of Ciudad Juárez and included 10 fatal cases. Observable differences between the City of El Paso and Ciudad Juárez include the larger population of feral dogs found in Ciudad Juárez. This increased population of feral dogs provides ample feeding opportunities for ticks and fleas and the opportunity to support and maintain an enzootic and transmission cycle involving the brown dog tick, *R. rickettsii* and dogs. Also, suitable tick questing environments of overgrown vegetation in Ciudad Juárez through neglected maintenance along public roads, near and around houses support the proliferation of tick and flea populations The City of El Paso enforces the maintenance of overgrown vegetation on and near both public roads and houses through citations and routine maintenance. This routine maintenance reduces and eliminates suitable habitat for ticks and fleas during questing periods of host seeking. These preliminary findings suggested that murine typhi and RMSF are not a major human health threat in the El Paso community, however, the occurrence of RMSF human cases in nearby Ciudad Juárez poses a serious threat of the introduction of the *R. rickettsii* into the El Paso community.

References

Abdad, M. Y., Abdallah, R. A., Fournier, P.-E., Stenos, J., & Vasoo, S. (2018). *A Concise Review of the Epidemiology and Diagnostics of Rickettsioses: Rickettsia and Orientia spp*. https://journals.asm.org/journal/jcm

Adams, W. H., Emmons, R. W., & Brooks, J. E. (1970). The changing ecology of murine (endemic) typhus in Southern California. *The American Journal of Tropical Medicine and Hygiene*, *19*(2), 311–318. https://doi.org/10.4269/ajtmh.1970.19.311

Adetunji, S. A., Krecek, R. C., Castellanos, G., Morrill, J. C., Blue-McLendon, A., Cook, W. E., & Esteve-Gassent, M. D. (2016). Seroprevalence of Borrelia burgdorferi antibodies in white-tailed deer from Texas. *International Journal for Parasitology: Parasites and Wildlife*, *5*(2), 168–174. https://doi.org/10.1016/j.ijppaw.2016.06.002

Adjemian, J., Parks, S., McElroy, K., Campbell, J., Eremeeva, M. E., Nicholson, W. L., McQuiston, J., & Taylor, J. (2010). Murine typhus in Austin, Texas, USA, 2008. *Emerging Infectious Diseases*, *16*(3), 412–417. https://doi.org/10.3201/eid1603.091028

Alugubelly, N., Stokes, J. v., Cross, C. E., Ross, A. M. L., Crawford, A. E., Fiihr, G. F., & Varela-Stokes, A. S. (2021). Beyond the ifa: Revisiting the elisa as a more sensitive, objective, and quantitative evaluation of spotted fever group rickettsia exposure. *Pathogens*, *10*(2), 1–9. https://doi.org/10.3390/pathogens10020088

Álvarez-Hernández, G., Candia-Plata, M. D. C., Delgado-de la Mora, J., Acuña-Meléndrez, N. H., Vargas-Ortega, A. P., & Licona-Enríquez, J. D. (2016). Fiebre maculosa de las Montañas Rocosas en niños y adolescentes mexicanos: cuadro clínico y factores de mortalidad. TT - [Rocky Mountain spotted fever in Mexican children: Clinical and mortality factors]. *Salud Publica Mex*, *58*(3), 385–392. http://www.scielo.org.mx/scielo.php?script=sci_arttext&nrm=iso&lng=pt&tlng=pt&pid=S0036-36342016000300385%0Ahttp://www.scielosp.org/scielo.php?script=sci_arttext&nrm=iso&lng=pt&tlng=pt&pid=S0036-36342016000300385

Anstead, G. M. (2020). History, rats, fleas, and opossums: The ascendency of flea-borne typhus in the United States, 1910-1944. In *Tropical Medicine and Infectious Disease* (Vol. 5, Issue 1). https://doi.org/10.3390/tropicalmed5010037

Azad, A. F., & Beard, C. B. (1998). Rickettsial pathogens and their arthropod vectors. *Emerging Infectious Diseases*, *4*(2), 179–186. https://doi.org/10.3201/eid0402.980205

Biggs, H. M., Behravesh, C. B., Bradley, K. K., Dahlgren, F. S., Drexler, N. A., Dumler, J. S., Folk, S. M., Kato, C. Y., Lash, R. R., Levin, M. L., Massung, R. F., Nadelman, R. B., Nicholson, W. L., Paddock, C. D., Pritt, B. S., & Traeger, M. S. (2016). Diagnosis and Management of Tickborne Rickettsial Diseases: Rocky Mountain Spotted Fever and Other Spotted Fever Group Rickettsioses, Ehrlichioses, and Anaplasmosis — United States. *MMWR. Recommendations and Reports*, *65*(2), 1–44. https://doi.org/10.15585/mmwr.rr6502a1

Bitam, I., Dittmar, K., Parola, P., Whiting, M. F., & Raoult, D. (2010). Fleas and flea-borne diseases. *International Journal of Infectious Diseases*, *14*(8), e667–e676. https://doi.org/10.1016/j.ijid.2009.11.011

Blanton, L. S. (2019). The Rickettsioses: A Practical Update. In *Infectious Disease Clinics of North America* (Vol. 33, Issue 1, pp. 213–229). W.B. Saunders. https://doi.org/10.1016/j.idc.2018.10.010

Blanton, L. S., Idowu, B. M., Tatsch, T. N., Henderson, J. M., Bouyer, D. H., & Walker, D. H. (2016). Opossums and cat fleas: New insights in the ecology of murine typhus in Galveston, Texas. *American Journal of Tropical Medicine and Hygiene*, *95*(2), 457–461. https://doi.org/10.4269/ajtmh.16-0197

Blanton, L. S., Vohra, R. F., Bouyer, D. H., & Walker, D. H. (2015). Reemergence of murine typhus in galveston, texas, usa, 2013. *Emerging Infectious Diseases*, *21*(3), 484–486. https://doi.org/10.3201/eid2103.140716

Blanton, L. S., & Walker, D. H. (2017a). Flea-Borne Rickettsioses and Rickettsiae. *American Journal of Tropical Medicine and Hygiene*, *96*(1), 53–56. https://doi.org/10.4269/ajtmh.16-0537

Blanton, L. S., & Walker, D. H. (2017b). Flea-Borne Rickettsioses and Rickettsiae. In *American Journal of Tropical Medicine and Hygiene* (Vol. 96, Issue 1, pp. 53–56). American Society of Tropical Medicine and Hygiene. https://doi.org/10.4269/ajtmh.16-0537

Boulanger, N., Boyer, P., Talagrand-Reboul, E., & Hansmann, Y. (2019). Ticks and tick-borne diseases. In *Medecine et Maladies Infectieuses* (Vol. 49, Issue 2, pp. 87–97). Elsevier Masson SAS. https://doi.org/10.1016/j.medmal.2019.01.007

Caravedo Martinez, M. A., Ramírez-Hernández, A., & Blanton, L. S. (2021). Manifestations and Management of Flea-Borne Rickettsioses. *Research and Reports in Tropical Medicine*, *Volume 12*, 1–14. https://doi.org/10.2147/rrtm.s274724

Carroll, J. F., & Schmidtmann, E. T. (1992). Tick Sweep: Modification of the Tick Drag-Flag Method for Sampling Nymphs of the Deer Tick (Acari: Ixodidae). In *J. Med. Entomol* (Vol. 29, Issue 2). https://academic.oup.com/jme/article/29/2/352/2221076

Centers for Disease Control and Prevention. (2019a). Borrelia Mayonii | Ticks | CDC. https://www.cdc.gov/ticks/mayonii.html

Centers for Disease Control and Prevention. (2019b). Tickborne Diseases of the United States | Ticks | CDC. *Tickborne Diseases of the United States*. https://www.cdc.gov/ticks/diseases/index.html

Centers for Disease Control and Prevention. (2020). *Regions Where Ticks Live*. Ticks. https://www.cdc.gov/ticks/geographic_distribution.html

Corn, J. L., Duhaime, R. A., Alfred, J. T., Mertins, J. W., Leland, B. R., Sramek, R. L., Moczygemba, J. D., & Shaw, D. W. (2016). Survey for ticks on feral swine within a cattle fever tick-infested landscape in Texas, U.S.A. *Systematic and Applied Acarology*, *21*(11), 1564–1570. https://doi.org/10.11158/saa.21.11.11

Dantas-Torres, F., Chomel, B. B., & Otranto, D. (2012). Ticks and tick-borne diseases: A One Health perspective. *Trends in Parasitology*, *28*(10), 437–446. https://doi.org/10.1016/j.pt.2012.07.003

Diuk-Wasser, M. A., VanAcker, M. C., & Fernandez, M. P. (2020). Impact of Land Use Changes and Habitat Fragmentation on the Eco-epidemiology of Tick-Borne Diseases. *Journal of Medical Entomology*. https://doi.org/10.1093/jme/tjaa209

Drexler, N. A., Yaglom, H., Casal, M., Fierro, M., Kriner, P., Murphy, B., Kjemtrup, A., & Paddock, C. D. (2017a). Fatal rocky mountain spotted fever along the United States–

Mexico border, 2013–2016. *Emerging Infectious Diseases*, *23*(10), 1621–1626. https://doi.org/10.3201/eid2310.170309

Drexler, N. A., Yaglom, H., Casal, M., Fierro, M., Kriner, P., Murphy, B., Kjemtrup, A., & Paddock, C. D. (2017b). Fatal rocky mountain spotted fever along the United States–Mexico border, 2013–2016. *Emerging Infectious Diseases*, *23*(10), 1621–1626. https://doi.org/10.3201/eid2310.170309

Eisen, R. J., Kugeler, K. J., Eisen, L., Beard, C. B., & Paddock, C. D. (2017). Tick-borne zoonoses in the United States: Persistent and emerging threats to human health. *ILAR Journal*. https://doi.org/10.1093/ilar/ilx005

Eisen, R. J., & Paddock, C. D. (2020). Tick and Tickborne Pathogen Surveillance as a Public Health Tool in the United States. *Journal of Medical Entomology*. https://doi.org/10.1093/jme/tjaa087

Emite Secretaría de Salud recomendaciones de prevención ante la rickettsiosis | Portal Gubernamental del Estado de Chihuahua. (2022). https://chihuahua.gob.mx/prensa/emite-secretaria-de-salud-recomendaciones-de-prevencion-ante-la-rickettsiosis

Escárcega Ávila, A. M., Luna Flores, B. S., de la Mora Covarrubias, A., & Jiménez, F. (2018). Análisis exploratorio de enfermedades Rickettsiales transmitidas por garrapatas en perros de Ciudad Juárez, Chihuahua, México. *Acta Universitaria*, *28*(3), 72–78. https://doi.org/10.15174/au.2018.1678

Flea-borne Typhus | Home. (n.d.). Retrieved September 5, 2022, from https://www.dshs.state.tx.us/IDCU/disease/murine_typhus/Flea-borne-Typhus.aspx

Francisco, A. R. L. (2013). 済無No Title No Title. In *Journal of Chemical Information and Modeling* (Vol. 53, Issue 9). https://doi.org/10.1017/CBO9781107415324.004

Frieden, T. R., Jaffe, H. W., Rasmussen, S. A., Leahy, M. A., Martinroe, J. C., Spriggs, S. R., Yang, M., Yang, T., Doan, Q. M., King, P. H., Starr, T. M., Jones, T. F., Boulton, M. L., Caine, V. A., Daniel, K. L., Fielding, J. E., Fleming, D. W., Halperin, W. E., Holmes, K. K., … Schaffner, W. (2016). *Diagnosis and Management of Tickborne Rickettsial Diseases: Rocky Mountain Spotted Fever and Other Spotted Fever Group Rickettsioses, Ehrlichioses, and Anaplasmosis-United States A Practical Guide for Health Care and Public Health Professionals Morbidity and Mortality Weekly Report Recommendations and Reports Centers for Disease Control and Prevention MMWR Editorial and Production Staff (Serials) MMWR Editorial Board*. http://www.cdc.gov/mmwr/cme/conted.html.

Kostman, J. R. (n.d.). *Laborato y Diagnosis of Rickettsial Diseases*.

Maleki-Ravasan, N., Solhjouy-Fard, S., Beaucournu, J. C., Laudisoit, A., & Mostafavi, E. (2017). The Fleas (Siphonaptera) in Iran: Diversity, Host Range, and Medical Importance. *PLoS Neglected Tropical Diseases*, *11*(1). https://doi.org/10.1371/journal.pntd.0005260

Mapston, M. E. (n.d.). *Feral Hogs in Texas in Texas*.

Maynez-Prieto, C., Rodríguez-Alarcón, C. A., Garza-Hernández, J. A., Laredo-Tiscareño, S. V., Espino-Solís, G. P., & Adame-Gallegos, J. R. (2021). Rickettsioses and Other Tick-Borne Diseases across the Border: Regional Focus in Chihuahua. *Southwestern Entomologist*, *46*(1), 167–178. https://doi.org/10.3958/059.046.0116

Mendell, N. L., Reynolds, E. S., Blanton, L. S., Hermance, M. E., Londoño, A. F., Hart, C. E., Quade, B. R., Esterly, A. T., Hendrix, C. B., Teel, P. D., Bouyer, D. H., &

Thangamani, S. (2019). Detection of rickettsiae, borreliae, and ehrlichiae in ticks collected from Walker County, Texas, 2017-2018. *Insects*, *10*(10). https://doi.org/10.3390/insects10100315

Moraes-Filho, J., Marcili, A., Nieri-Bastos, F. A., Richtzenhain, L. J., & Labruna, M. B. (2011). Genetic analysis of ticks belonging to the Rhipicephalus sanguineus group in Latin America. *Acta Tropica*, *117*(1), 51–55. https://doi.org/10.1016/j.actatropica.2010.09.006

Nguyen, V. L., Colella, V., Greco, G., Fang, F., Nurcahyo, W., Hadi, U. K., Venturina, V., Tong, K. B. Y., Tsai, Y. L., Taweethavonsawat, P., Tiwananthagorn, S., Tangtrongsup, S., Le, T. Q., Bui, K. L., Do, T., Watanabe, M., Rani, P. A. M. A., Dantas-Torres, F., Halos, L., … Otranto, D. (2020). Molecular detection of pathogens in ticks and fleas collected from companion dogs and cats in East and Southeast Asia. *Parasites and Vectors*, *13*(1). https://doi.org/10.1186/s13071-020-04288-8

Olano, J. P., Hogrefe, W., Seaton, B., & Walker, D. H. (2003). Clinical manifestations, epidemiology, and laboratory diagnosis of human monocytotropic ehrlichiosis in a commercial laboratory setting. *Clinical and Diagnostic Laboratory Immunology*, *10*(5), 891–896. https://doi.org/10.1128/CDLI.10.5.891-896.2003

Parola, P., Paddock, C. D., & Raoult, D. (2005). Tick-borne rickettsioses around the world: Emerging diseases challenging old concepts. In *Clinical Microbiology Reviews* (Vol. 18, Issue 4, pp. 719–756). https://doi.org/10.1128/CMR.18.4.719-756.2005

Pieracci, E. G., Evert, N., Drexler, N. A., Mayes, B., Vilcins, I., Huang, P., Campbell, J., Behravesh, C. B., & Paddock, C. D. (2017). Fatal flea-borne typhus in Texas: A retrospective case series, 1985-2015. *American Journal of Tropical Medicine and Hygiene*, *96*(5), 1088–1093. https://doi.org/10.4269/ajtmh.16-0465

Premaratna, R., Weerasinghe, S., Ranaweera, A., Chandrasena, T. N., Bandara, N. W., Dasch, G. A., & de Silva, H. J. (2012). Clinically helpful rickettsial disease diagnostic IgG titers in relation to duration of illness in an endemic setting in Sri Lanka. *BMC Research Notes*, *5*. https://doi.org/10.1186/1756-0500-5-662

Preto, O. (n.d.). *PAHO/WHO EXPERTS CONSULTATION ON RICKETTSIOSIS IN THE AMERICAS FINAL REPORT*.

Rajput, Z. I., Hu, S. hua, Chen, W. jun, Arijo, A. G., & Xiao, C. wen. (2006). Importance of ticks and their chemical and immunological control in livestock. *Journal of Zhejiang University: Science B*, *7*(11), 912–921. https://doi.org/10.1631/jzus.2006.B0912

Sanders, D. M., Schuster, A. L., Wesley Mccardle, P., Strey, O. F., Blankenship, T. L., & Teel, P. D. (n.d.). *Ixodid ticks associated with feral swine in Texas* (Vol. 38, Issue 2).

Schriefer, M. E., Sacci, J. B., Taylor, J. P., Higgins, J. A., & Azad, A. F. (1994). Murine typhus: updated roles of multiple urban components and a second typhuslike rickettsia. *Journal of Medical Entomology*, *31*(5), 681–685. https://doi.org/10.1093/jmedent/31.5.681

Tietjen, M., Esteve-Gassent, M. D., & Medina, R. F. (2019). Searching for the Immature Stages of Ixodes scapularis (Acari: Ixodidae) in Leaf Litter and Soil in Texas. *Journal of Medical Entomology*, *56*(1), 35–39. https://doi.org/10.1093/jme/tjy157

Vaughn, M. F., Delisle, J., Johnson, J., Daves, G., Williams, C., Reber, J., Mendell, N. L., Bouyer, D. H., Nicholson, W. L., Moncayo, A. C., & Meshnick, S. R. (2014). Seroepidemiologic study of human infections with spotted fever group rickettsiae in

North Carolina. *Journal of Clinical Microbiology*, *52*(11), 3960–3966. https://doi.org/10.1128/JCM.01733-14

Wild Pigs and Ticks: Implications for Livestock Production, Human and Animal Health Wild Pigs and Ticks: Implications for Livestock Production, Human and Animal Health ii. (2016).

Yu, S., Modarelli, J., Tomeček, J. M., French, J. T., Hilton, C., & Esteve-Gasent, M. D. (2020). Prevalence of common tick-borne pathogens in white-tailed deer and coyotes in south Texas. *International Journal for Parasitology: Parasites and Wildlife*, *11*, 129–135. https://doi.org/10.1016/j.ijppaw.2020.01.005

Vita

Karen R. Valdez

Karen Valdez earned an A.A. in General Studies from El Paso Community College followed by a B.S. in Biological Sciences with a Biomedical Concentration from The University of Texas at El Paso followed by pursuing a Masters in Biological Sciences working with Dr. Douglas Watts at The University of Texas at El Paso. She did a survey of fleas and ticks for *rickettsia rickettsii* and *rickettsia typhi* and surveys of humans and wild animals for serological evidence in El Paso, Texas.

While pursuing her education, Adam has worked as a Research Assistant working with Dr. Douglas Watts at The University of Texas at El Paso while working on ticks and fleas. She then worked as a Teaching Assistant at The University of Texas at El Paso while pursuing her masters.

Contact Information: karenrocio22@hotmail.com