SURVEILLANCE OF TICKS AND ASSOCIATED PATHOGENS IN CENTRAL TEXAS

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Master's Program in Public Health

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DEDICATION

To my grandpa, Lucio Reyes Mendoza

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by

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THESIS

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ABSTRACT

Background: Ticks are the second most common vector of human and veterinary pathogens, after mosquitoes, which results in a number of different diseases around the world. These diseases, referred to as tick-borne diseases (TBDs), are increasing in their prevalence around the world as the geographical distribution of ticks change due to environmental and human factors. Increased testing for pathogens and a better comprehension of their relationship to different tick species will be crucial in deepening our understanding of TBDs and protecting our human and animal populations. Increasing surveillance of ticks and the potential pathogens they may be vectors for and understanding the current and future projections of their geographic distribution across the United States and along the United States – Mexico border is crucial to understanding the disease risk posed by ticks from a public health standpoint. **Objective:** To identify *Rickettsia rickettsii* in ticks collected from feral swine (*Sus scrofa*) and white-tailed deer (*Odocoileus virginianus*) in Central Texas, this study will 1) identify the species of ticks collected in Central Texas using morphological keys and characteristics; 2) extract DNA; 3) use polymerase chain reaction (PCR) to amplify 3 Rickettsial genes to determine presence of *R. rickettsii* in extracted DNA samples. **Methods:** A cross-sectional study was used to analyze extracted DNA collected from a convenience sample of ticks taken from feral swine and white-tailed deer in Travis County, Texas. PCR was used to identify the bacteria *Rickettsia rickettsii* using the Rickettsia *17 kDA* antigen primer, *rOmpA* primer, and the *gltA* primer. PCR samples will be run in 1.5% agarose gels. Any positive samples for *R. rickettsii* were prepared for genetic sequencing and sent out to GENEWIZ to confirm the species of tick the positive sample came from. **Results:** Ticks collected from feral swine and white-tailed deer were identified as *Ixodes scapularis* (*I. scapularis*) and *Amblyomma*

americanum (*A. americanum*). In total, 41 ticks were collected from 13 different white-tailed deer and 35 ticks were collected from 15 different feral swine in Travis County, Texas. Only 74 ticks of the 76 were used in the DNA extraction because one tick sample from a feral swine was a molted exoskeleton and another tick from another feral swine was missing their head and some of their legs making it almost impossible to correctly identify their genus and species. Results from amplifying the rickettsial *gltA* gene indicated a rickettsial prevalence of *Ixodes scapularis* and *Amblyomma americanum* of 10/18 (56%) and 5/17 (29%), respectively, with an overall average among ticks being 15/35 (43%). Results from amplifying the *17 kDa* gene indicated a spotted fever group rickettsioses prevalence of *I. scapularis* and *A. americanum* of 8/18 (44%) and 2/17 (12%), respectively, with an overall average among ticks being 10/35 (29%). Results from amplifying the *rOmpA* gene indicated a *R. rickettsii* prevalence of *I. scapularis* and *A. americanum* of 8/18 (44%) and 1/17 (0.06%), respectively, with an overall average among ticks being 9/35 (26%). **Conclusion:** The vector competency of *A. americanum* and *I. scapularis* to acquire and transmit *R. rickettsii* has been understudied. Confirming the presence of a Spotted Fever Group Rickettsia, *R. rickettsii*, in these two species of ticks provides evidence of the ability of these two tick species present in Texas to acquire and transmit a pathogen that is not readily connected to them in the literature. This study provides a platform in which more research can be done to confirm the ability of these two species to transmit *R. rickettsii* and provides public health officials the ability to use this research to develop new plans for tick surveillance.

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CHAPTER 1: INTRODUCTION

Ticks are second only to mosquitoes in their ability to transmit human and veterinary pathogens and spread a substantial amount of infectious organisms (Nicholson et al., 2019). Of the nearly 900 known species of ticks, each one requires a blood meal to develop into the next life stage and reproduce and they can parasitize every class of vertebrates in almost any sector of the world (Socolovschi et al., 2009). Ticks have the ability to transmit protozoa, viral, bacterial, and fungal pathogens, which contributes to thousands of cases of tick-borne diseases every year in humans and the incidence of tick-borne diseases is increasing around the world (Nicholson et al., 2019). Tick bites can also result in allergic reactions, fatal paralysis, or toxic reactions. The global economic impact of ticks and the diseases they carry is hard to measure, but it is estimated that they contribute to billions of U.S. dollars in lost productivity (Jongejan and Uilenberg, 2004).

As the incidence of tick-borne diseases, emergence of new pathogens, and the changing geographical ranges of ticks increases around the world and the United States, there is a need for research on tick-borne diseases to escalate and focus on increasing the intrinsic knowledge of tick-borne diseases, catalyze research to improve the diagnosis, prevention, and treatment of tick-borne diseases, and invest in resources that allow additional research to develop novel vaccines or therapeutic options (NIH, 2019). Tick species and associated pathogens found in Central Texas have been reported, but most studies have focused on established parasitic relationships to a specific species of tick. The two most common species of tick found in Central Texas are *Ixodes scapularis* and *Amblyomma americanum*.

I. scapularis has been shown to be vectors of 16 human pathogens, the main ones being *Borrelia burgdorferi*, *Babesia microti*, and *Anaplasma phagocytophilum* (Nelder et al., 2016). *A.*

americanum is a known vector of several bacterial pathogens like *Francisella tularensis*, *Ehrlichia chaffeensis*, and *Rickettsia amblyommatis* (Levin et al., 2017). However, more research is needed to determine both ticks' species vector competence for other zoonotic diseases. One bacterial pathogen of significance, *Rickettsia rickettsii*, the causative agent of Rocky Mountain spotted fever (RMSF), has been minimally studied in connection to the aforementioned tick species. *I. scapularis* harbors a *Rickettsia* endosymbiont and has been shown to carry other *Rickettsia* taxa, some of which pose a threat to human health (Nelder et al., 2016). *R. rickettsii* specifically, and the vector competence of *I. scapularis* has been under investigated, but further research is justified in determining the exact role, if any, *I. scapularis* plays in transmitting *R. rickettsii*. Ticks from the genus *Amblyomma* are acknowledged vectors of RMSF in South America, but *A. americanum* and its vector competence for *R. rickettsii* hasn't been properly studied (Levin et al., 2017). This proposed study aims to further understand the potential vector role of *I. scapularis* and *A. americanum* for rickettsia by screening ticks collected from feral swine (*Sus scrofa*) for the presence of the bacterial pathogen *Rickettsia*.

CHAPTER 2: BACKGROUND AND SIGNIFICANCE

2.1 Ticks

Of the nearly 900 different species of ticks known, most belong to two main families, Argasidae (soft ticks) and Ixodidae (hard ticks). These two families of ticks have different morphological and physiological features, as well as life cycles, that differentiate them into their respective families. For this proposed study, only ticks from the Ixodidae family will be used, specifically, the genera *Amblyomma* and *Ixodes*. Ticks are able to feed anywhere from several days to weeks, are capable of ingesting more than 100 times their body weight in blood and secrete excess water from the blood meal back into the host through their salivary glands (Estrada-Peña, 2015). As obligate parasites, ticks require a blood meal from a vertebrate host to transition to the next stage in their life cycle – a process that can facilitate the transmission of a variety of pathogens to animals and humans (Estrada-Peña, 2015).

Life Cycle

A tick has four development stages in its life cycle – egg, larvae, nymph, adult – to achieve full maturity, each step complex in the timing and length of feeding. Ixodid larvae and nymphs typically feed once for several days before dropping off the host to molt into the next stage of development. Once an adult female is engorged, it will drop off the host to lay thousands of eggs and proceed to die shortly thereafter. Adult males are intermittent feeders and can remain on a host for weeks or months and mate with a multitude of females, or they can detach and go to another host in search for more females (Sonenshine, 1994; Tahir et al., 2020). Ticks either exhibit endophilous behavior or exophilous behavior when feeding. Ticks with endophilous behavior live closely to their hosts and are restricted to their host's shelters so their chances for survival are increased since they molt in a protected environment away from environmental factors. Ticks with

exophilous behavior wait for a host outside of a shelter, so weather variables can dictate the questing, when a tick climbs a piece of grass or structure and extends its front legs to latch onto a host when one walks by, behaviors of the tick (Estrada-Peña, 2015).

To further complicate the life cycle, different species of ticks are either one-host, two-host, or three-host specific, meaning they must stay on one host for all life stages or find a new host to proceed in their development. Ticks that can complete their life cycle in fewer molts away from a host increase their odds of surviving since they rely less on the environment and weather to influence their development and questing behavior (Estrada-Peña, 2015). According to Estrada-Peña (2015), these aspects are important in the transmission of different pathogens. For a pathogen to be successfully transmitted to a new host, it must be acquired from a host, passed along the life cycle of a tick, and successfully spread to a new host all the meanwhile depending on the survival and activity level of the tick and available hosts.

Amblyomma americanum

This species of tick is commonly known as the Lone Star tick and requires a three-host cycle. *A. americanum* are aggressive and non-specific feeders in each stage of their life cycle which means they have the potential to interact with a variety of infectious pathogens (Mangan et al., 2018). After hatching, an *A. americanum* larvae will quest to attach to the first host, usually a small vertebrate animal. Once the blood meal is complete, it will fall off the host to molt into a nymph. The nymph quests again and attaches to a slightly bigger vertebrate animal where it proceeds to feed, fall off, and molt into an adult. The adult tick quests to find its last host and depending on the sex of the tick, will either fall off to lay its eggs or remain on the host to mate with other ticks.

The larval and nymphal stages of this species prefer small or medium wild animals, ground-inhabiting birds, dogs. or rodents as hosts. Animals under these categories include quail, wild turkeys, raccoons, opossums, eastern cottontail rabbits, domesticated dogs, and coyotes (Bishop & Trembley, 1945; Kollars et al., 2000; Texas A&M AgriLife Extension, n.d.). Adults prefer larger animals for their blood meals, primarily livestock and white-tailed deer (Bishop & Trembley, 1945; Texas A&M AgriLife Extension, n.d.). However, host associations change based on the geographical range of the tick and host availability. Lone star ticks have nonspecific and aggressive biting habits and are known to use humans for a blood meal throughout all three stages of their life cycle (Kollars et al., 2000; Texas A&M AgriLife Extension, n.d.).

According to the Centers for Disease Control and Prevention (CDC) (2021), *A. americanum* is widely distributed throughout the southeastern and eastern United States. Their map only provides a rough picture of the distribution of *A. americanum*, so recent efforts have been made to better define the geographical distribution of this species with more precision beyond a general continental-scale map (Springer et al., 2014). Springer et al. (2014) compiled data from published literature and databases overseen by the USDA, U. S. National Tick Collection, and Walter Reed Biosystematics Unit to present a county-level distribution map for the United States. This resulted in 18,121 collections made between 1898 and 2012 showing established or reported presence of *A. americanum* in 1,300 counties across 39 states and the District of Columbia (Springer et al., 2014). These counties are primarily located amongst the southern states, but there were distributions of this species of tick further North along the Atlantic coast and Midwestern states (Springer et al., 2014). In Texas, 3,904 collection records were found between the 1900s-2010s, reaching as far west as Midland, Crockett, and Val Verde counties and into the southern counties along the United States – Mexico border. Springer's et al. (2014) spatial distribution map

provides a tool to better understand *A. americanum*'s geographical range, but still falls short since the collection data they used was from convenience sampling as opposed to systematic sampling and some states along the given periphery of their map perform little to no surveillance of tick species.

In Mexico, knowledge about the geographical distribution of *A. americanum* is also scarce. The Mexican states that lie along the border with the United States are Baja California, Sonora, Chihuahua, Coahuila, Nuevo Leon, and Tamaulipas. *A. americanum* has been found in Tamaulipas, Nuevo Leon, and Coahuila, but this does not mean they are not present in the other states, they may not have been identified due to the lack of surveillance (Guzmán-Cornejo et al., 2011). In the United States and Mexico, the geographical range is expected to keep changing in the coming years as factors such as climate change and increases in white-tailed deer populations drive *A. americanum* into new regions (Raghavan et al., 2019). Understanding the current and future projections for this tick species distribution across the United States and along the United States – Mexico border is crucial to understanding the disease risk posed by ticks from a public health standpoint.

This species of tick completes its life cycle in three active seasons. Hatching and feeding as larvae takes place in the summer of year one. Years two and three occur in the spring when nymphs are feeding and adults are mating, respectively (Mangan et al., 2018). These seasonal trends are influenced by environmental factors, mainly temperature and photoperiod. This particular species of tick seems to excel when the photoperiod is increased and temperatures are moderately warm and adverse changes in these factors can cause them to quiescence, go dormant, or diapause, suspended development, when there are unfavorable conditions (Mangan et al., 2018). Winter typically kills all unfed larvae which means there is usually only one generation of ticks

per year. Hence, these ticks survive in distinct clusters throughout their life cycles due to seasonal changes.

The host-seeking activity period differs slightly for each life cycle stage. Larvae experience their highest host-seeking activity period in July, and it lasts through September. Nymphs activity period starts in May and goes through August. Adult Lone Star ticks reach their highest host-seeking activity from April through July, and their activity starts to slow down as summer continues (Childs & Paddock, 2003; Springer et al., 2015). This active host-seeking period is not set and in some southern states, this species of tick in all life cycle stages has been found throughout the year, though in smaller numbers during the winter (Bishopp & Trembley, 1945).

Ixodes scapularis

Otherwise known as the black-legged tick, *I. scapularis,* is considered to be the most fatal tick vector in the United States (Wolf et al., 2020). This tick also requires a three-host cycle, but the entire cycle typically takes two years compared to the three-year length for the Lone Star tick. According to Wolf et al. (2020), the first summer after hatching is when the larvae take their first blood meal, nymphs feed during the late spring/early summer of the second year, and adults then feed in the fall. Unlike the Lone Star tick, black-legged ticks are rather specific in the animals they choose as their hosts. Adult black-legged ticks prefer white-tailed deer (*Odocoileus virginianus*) and coyotes (*Canis latrans*) (Kollars et al., 1999). The white-tailed deer is essential for the black-legged tick's survival and reproduction – about 50% to 95% of adult female ticks feed on white-tailed deer (Wolf et al., 2020). Nymphs and larvae preferred hosts are broad-headed kinks (*Eumeces laticeps)* and the white-footed mouse *(Peromyscus leucopus*), the latter being the most important and abundant host for pathogens transmitted by this species of tick (Kollars et al., 1999;

Wolf et al., 2020). Although not as aggressive biters of humans like the Lone Star tick, black-legged ticks will feed on humans if available. The greatest risk of being bit by a black-legged tick is in the spring, summer, fall, and in winter if temperatures are above freezing (CDC., 2021). Like the Lone Star tick, the most common stages of black-legged tick to bite a human are nymphs and adult females.

Aside from the number of hosts available, the black-legged tick's life cycle relies heavily on the landscape, vegetation, and climate (Wolf et al., 2020). They prefer to live in forested areas, particularly where forests have been regrown on old farmland or timber operations. Black-legged ticks also require relatively high humidity to increase their chances of survival and high temperatures provide more opportunities for larvae to feed which increases their chances of becoming an adult and spreading to new geographical locations (Ogden et al., 2014). In the United States, they are endemic in parts of the Midwest, Northeast, West, Southeast, and South (CDC, 2021; Wolf et al., 2020).

Since the connection between *I. scapularis* and the causative agent of Lyme disease, *Borrelia burgdorferi*, was determined, there have been efforts made throughout the years to understand and update the geographical distribution of this species frequently. Much more systematic surveillance in *I. scapularis* geographical distribution has been done compared to *A. americanum* and its geographical distribution, but it is still difficult to observe trends in distribution since surveillance efforts are lacking in general. *I. scapularis* has been documented in 1,420 counties across 43 states in the United States, which marks a 44.7% increase in the number of counties that reported presence of this species compared to one of the first distributional maps of *I. scapularis* published in 1998 by Dennis et al. (1998) (Eisen et al., 2016). This updated geographical distribution by Eisen et al. (2016) indicates several counties in the south of Texas

along the international border with Mexico acquired populations of *I. scapularis* since the 1998 distribution map. The were no changes in counties west of the western edge of the distribution map from 1998.

The geographical distribution and taxonomic knowledge of *Ixodes* ticks in Mexico has been limited (Guzmán-Cornejo et al., 2007). Only birds and mammals have been reported as hosts to *Ixodes* ticks in Mexico unlike in the United States where *Ixodes* are known to parasitize reptiles along with birds and mammals (Guzmán-Cornejo et al., 2007). *I. scapularis* has been found in the states throughout Mexico, but in states along the international border with the United States, *I. scapularis* has been documented in Baja California, Coahuila, Nuevo Leon, and Tamaulipas ((Guzmán-Cornejo & Robbins, 2010).

Similar to *A. americanum*, the host-seeking activity period for *I. scapularis* differs for each life cycle stage. *I. scapularis* larvae's peak host-seeking activity period occurs from July through September (Spielman et al., 1985). Nymphs are active during May through July and adults reach peek host-seeking activity throughout the fall and winter months (Spielman et al., 1985). An interesting aspect of this host-seeking activity is that *I. scapularis* nymph's activity period occurs before the larval activity period. This "reversed" pattern of activity enables effective transmission of non-inherited pathogens since larvae can acquire pathogens that have been transmitted to a host from nymphs that fed on that same host earlier in the year (Spielman et al., 1985). Once those larvae molt into nymphs, they will continue the transmission of the pathogen.

2.2 Tick-Borne Diseases

In the last few decades, interest in ticks and tick-borne diseases (TBDs) has continued to increase due to the changes in the geographical distribution of ticks (Estrada-Peña, 2015). This has led to an increase of tick-borne diseases (TBDs) being diagnosed (Eisen & Eisen,

2018). TBDs accounted for more than 75% of all vector-borne cases reported in the United States between 2004 and 2016 (NIH, 2019). According to the Centers for Disease Control and Prevention (CDC), the number of people diagnosed with Lyme disease in the United States each year is approximately 300,000 making it the most common vector-borne disease in the United States (Hodo et al., 2019). Other TBDs have become some of the most common diseases transmitted by arthropods and the number of reported cases continue to increase. Of the approximately 100 known arthropod-borne infections, most are associated with 116 tick species (Brites-Neto et al., 2015).

There are more than 20 different bacteria, viruses, and parasites that can cause severe illness known to be transmitted to humans through a tick bite in the United States (NIH, 2019). However, many of these TBDs were only discovered during the past ten years reflecting the emerging threat ticks pose and the new frontier of vector-borne diseases faced by the global community. The fluctuations in geographic ranges of ticks and the introduction of species into new habitats has been one reason TBDs are starting to receive more attention amongst the scientific community. Some researchers believe this spread is due to uncontrolled movements of domestic or wild animals, changes to the climate, and changes in the use of land resources which all allow for an increase in the abundance of hosts (Estrada-Peña, 2015). The climate changes, in particular, contributes to the spread of tick species to new geographical locations since warmer conditions allow them to survive and continue their life cycle unheeded by low temperatures causing concern to regions that have since been deemed free of TBDs (Ostfeld & Brunner, 2015).

Rickettsia rickettsii

As we have seen with the recent COVID-19 outbreak, of zoonotic origin, far too little is known about diseases that can be passed from animals to humans. Ticks are known to pass along

a variety of diseases that can be crippling and cause chronic medical issues such as Lyme disease and Rocky Mountain spotted fever (RMSF). This study used the ticks collected from feral swine and white-tailed deer to test for spotted fever group rickettsioses, specifically *R. rickettsii*, with the aim to further define the vertebrate host of this pathogen and the vector competency of *A. americanum* and *I. scapularis*.

RMSF belongs to a family of diseases referred to as spotted fever group rickettsioses (SFGR). The bacteria that cause it, *R. rickettsii*, is a gram-negative, obligate intracellular bacterium and RMSF is recognized as one of the most common and severe rickettsial illnesses in the United States (McDade & Newhouse, 1986; Yaglom et al., 2018). Humans acquire the bacterium once an infected tick bites them. Humans act as dead-end host for *R. rickettsii* since they are not natural reservoir hosts for the pathogen, but the disease has a 20 to 30 percent mortality rate for humans without quick antibiotic treatments given soon after a bite (Snowden & Simonsen, 2020). Rickettsia infects the vascular endothelial cells lining vessels throughout the body which results in the infected person experiencing fever, myalgia, headaches, rash, and cardiovascular instability (Snowden & Simonsen, 2020). There are no treatments for RMSF, only ways to manage it. Doxycycline is the primary drug used to manage the disease, but the best prevention is to prevent ticks from getting on you by wearing insect repellant, protective clothing, and examining your body for ticks after having been in an environment that is conducive to tick populations (Biggs et al., 2016).

R. rickettsii is passed mainly through transovarial and transstadial transmission, but ticks can also become vectors when feeding from an infected host or feeding alongside ticks who are already infected (McDade & Newhouse, 1986). In order for a tick to become infected with *R. rickettsii* from the host, it must be attached a minimum of 3-24 hours to have effective transmission

from the host to the tick (Tahir et al., 2020). The transmission time fluctuates for different pathogens based on the number of pathogens present in the salivary glands of unfed ticks at the time of a blood meal (Lejal et al., 2019). However, interrupted feeding, a phenomenon where a tick partially feeds from one host, falls off and survives long enough to attach to a new host and resume feeding, all in the same life stage, can result in reduced transmission time of *R. rickettsii* (Tahir et al., 2020). Interrupted feeding can be caused by the death of the host, the host's immune response, or from the host shaking or scratching and one tick that has been shown under laboratory conditions to successfully reattach to a new host is *I. scapularis* (Tahir et al., 2020; Shih & Spielman, 1993). Tahir et al. (2020) proceeds to claim that based off the evidence of ticks reattaching to new hosts, it can be assumed that ticks will reattach to a new host to complete their blood meal, regardless of their species, in nature.

The ticks in the United States that are the primary vectors for *R. rickettsii* are the American dog tick (*Dermacentor variabilis*), the Rocky Mountain wood tick (*Dermacentor andersoni*), and the brown dog tick (*Rhipicephalus sanguineus*). However, a tick's vectorial capacity is determined by their co-evolution with pathogens they carry, their ability to survive and reproduce, the range of hosts they use for meals, and the ability to drink copious amounts of blood over a significant period of time (Tahir et al., 2020). Although *I. scapularis* and *A. americanum* are not primary vectors, there is literature to support the idea that these particular species may have the vector competency needed to be vectors of *R. rickettsii* and further research is warranted.

Levin et al. (2017) indicates the Lone Star tick's vector competence has not been appropriately studied. Whereas *Amblyomma* ticks are recognized vectors of RMSF in Central and South America, they are not recognized as vectors in the United States. *A. americanum's* geographical range in the United States extends throughout the southern states and overlaps with

areas of reported RMSF cases and the known distribution of *D. variabilis*. *A. americanum* also feeds on the same species of vertebrate hosts as *D. variabilis,* increasing the chances for *A. americanum* nymphs or larvae to be exposed to *R. rickettsii*, either from feeding on an infected animal or feeding side by side with an infected *D. variabilis* adult (Levin et al., 2017). Under laboratory conditions, *A. americanum* was shown to have vector competence for two different isolates of *R. rickettsii* and it was transmitted transovarially, indicating *A. americanum* was capable of contracting, maintaining, and disseminating the pathogen (Levin et al., 2017). As noted earlier, their penchant for feeding on a variety of hosts, being aggressive biters, attaching to hosts in large numbers, and having been shown to contain blood meals from different hosts (Allan et al., 2010) increases their vector competence as well. As the geographical distribution of *A. americanum* changes, their populations may be introduced to *R. rickettsii* for the first time as a result of interspecies spill-over events like feeding from an infected host or next to infected ticks.

The most well-known pathogen associated with *I. scapularis* is *Borrelia burgdorferi*, the causative agent of Lyme disease. *I. scapularis* is capable of being a vector for a variety of other pathogens including species of *Anaplasma, Babesia, Ehrlichia,* and *Rickettsia* to name a few. One literature review found that blacklegged ticks contained 12 *Rickettsia* taxa and the *Rickettsia* endosymbiont of *I. scapularis* (Nelder et al., 2016). Although *R. rickettsii* was not detected in the samples examined in the literature review, Nelder et al. (2016) is quick to point out that research in establishing a relationship between *I. scapularis* and *R. rickettsii* is lacking and that more investigation is needed. One of the other *Rickettsia* taxa identified in *I. scapularis, R. parkeri*, has been identified as a human pathogen and more clinical and epidemiological studies are needed to determine what risk, if any, these other *Rickettsia* taxa pose. Blacklegged ticks are liberal in their feeding patters and, like *A. americanum*, bite humans often. Due to these factors, continuing to

surveille and identify any relationships between the blacklegged tick and pathogens of human importance is necessary.

2.3 Vertebrate Hosts

Although *R. rickettsii* is sustained in ticks through transstadial and transovarial transmission, the efficiency of this vertical transmission differs based on the rickettsial and tick species (Tomassone et al., 2018). Therefore, vertebrate animals play a crucial role in the maintenance and perpetuation of the *R. rickettsii* life cycle. Knowledge of the transmission of the pathogen to a tick during the feeding process is low as there are few studies that provide evidence that specific animal species are competent reservoir hosts for *R. rickettsii.* A 'good' host is one that can permanently maintain a pathogen and transmit it to the target population or vector and there are few studies investigating natural infection of vertebrate species by *Rickettsia* species (Haydon et al., 2002; Tomassone et al., 2018). The results of this study could spur more research that could better define the vertebrate hosts of *R. rickettsii* and white-tailed deer and feral swine should be at the top of that research.

White-Tailed Deer

I. scapularis ticks require one blood meal to transition to the next stage of their life cycle. Larval and nymphal ticks usually use smaller vertebrate hosts for their meals, but they have also been known to use white-tailed deer. Adult ticks depend mostly on white-tailed deer to mate and use as a blood meal for the females before they lay their eggs and die, so they are closely adapted to the lifestyle of white-tailed deer (Carroll et al., 1998). The presence of *I. scapularis* in a geographical area seems to be dependent on the presence of white-tailed deer – the geographical range of *I. scapularis* represents the geographical range of white-tailed deer and there also seems

to be a temporal correlation where when new populations of white-tailed deer pop up, so do *I. scapularis* ticks (Spielman et al., 1985).

White-tailed deer can be found all over the United States and in Texas, it is estimated that there are around four million deer, primarily in the central and eastern parts of the state (Texas Parks and Wildlife (TPW), n.d.). They mainly eat weedy plants and shrubs, are typically found in woody and brushy areas of the state, and do not migrate unless pressured to do so by external forces (TPW, n.d.). According to Texas Parks & Wildlife (n.d.), white-tailed deer populations in west Texas are slowly increasing in numbers and expanding in their distribution. They are pushing further west into Pecos and Brewster counties and expanding south along the Rio Grande River. The main drivers for their expansion in West Texas are the increasing density of woody plants, like mesquite, and improved water distribution due to livestock management (TPW, n.d.).

White-tailed deer require further investigation into their competency as a vertebrate host for *R. rickettsii*. They are host to several species of ticks including *I. scapularis* and *A. americanum* and act as reservoir hosts for pathogens like Ehrlichiosis and Lyme disease. This indicates they may be capable vertebrate hosts for *R. rickettsii*, but determining this is beyond the scope of this study.

Feral Swine

Feral swine, otherwise known as wild boars or wild hogs, are indigenous in many countries around the world and are a known reservoir for a number of pathogens, including viruses, bacteria, and parasites (Meng et al., 2009). Exposure of feral swine to humans and domesticated animals has increased due to continued human encroachment of lands for development and agricultural purposes and increased hunting activities (Meng et al., 2009). Feral swine are known hosts for

several zoonotic bacterial pathogens like *Francisella tularensis* and *Yersinia pestis*, causative agents of Tularemia and Plague, respectively, and are known to host a variety of tick species, particularly ticks from the genus *Amblyomma*. However, their competency as reservoir hosts for *R. rickettsii* has been understudied.

Feral swine are not native to the Americas and were introduced to the Americas by European travelers during the sea exploration age to be used for domesticated purposes. Decades later, the Eurasian wild boar would be introduced to parts of the Unites States where they quickly mated with the domestic pigs already here and resulted in the hybrid population of feral swine that is present across the United States now. It is estimated that in the United States, their population is around seven million, with 2.6 million estimated to reside in Texas alone (TPW, n.d.). As of 2019, feral swine were present in all counties in Texas except El Paso county, but that has changed. Some research has been done indicating the presence of Rickettsia species in ticks collected from feral swine, but much more research is needed to further define their competency as vertebrate hosts for *R. rickettsii*, but determining this is beyond the cope of this study (Cleveland et al., 2019; Kmetiuk et al., 2019).

2.4 Environmental Factors

The major aspect that is changing the traditional knowledge about ticks and TBDs are environmental factors, specifically climate change. As climate change has become recognized more and more by the scientific community as changing infectious disease and vector dynamics, attention is shifting into researching how it is affecting the geographical distribution of ticks and the diseases they disseminate. As the weather changes due to climate change, the prevalence, range, and activity of ticks and their associated pathogens is going to increase (Bouchard et al., 2019). Raghavan et al. (2019) assessed the spatial distribution of *A. americanum* in North America

using current climate conditions to understand and anticipate potential changes in distribution using different climate scenarios: climate changes under medium-to-low greenhouse gas emissions and climate changes under high greenhouse gas emissions. Under both scenarios, northward and westward expansion of *A. americanum* in the United States can be expected to occur (Raghavan et al., 2019). This phenomenon will be aided by an increase in the range of potential animal hosts and changes to human behaviors which will result in bringing animals and humans into closer contact with ticks for a longer period of time (Bouchard et al., 2019). The increased abundance of ticks and their appearance in new locations presents a severe public health risk to humans, livestock, and companion animals in areas where ticks were previously unknown or considered to be insignificant to the health of the local human and animal populations (Sonenshine, 2018).

A tick's distribution used to be confined to a specific geographic range since they were restricted by their adaptations to local environmental factors. These factors consisted of relative humidity, temperature fluctuations, soil moisture, dense vegetation, humid leaf litter, and forests providing shade (Sonenshine, 2018). Many species also face restrictions due to their specific host preference. Other factors besides climate change that must be taken into account when considering changing geographic distributions are host availability, host specificity, habitat suitability, relative humidity tolerance, extent and duration of freezing temperatures, and habitat modification due to human impact (Sonenshine, 2018).

Amblyomma americanum

A. americanum are rare in that they are considered to hunter ticks, meaning they will crawl meters to attach to a host when they catch a host's odor. This willingness to travel across larger distances than other ticks increases their host range since they don't have to wait for a host to pass

by like other ticks do (Sonenshine, 2018). According to Monzón et al. (2016), *A. americanum* has been expanding their zoogeographic range as far north as the Canadian border and as far west as Oklahoma, Nebraska, and South Dakota in the United States which is consistent with climate change. The ticks found in Oklahoma and New York, new geographical ranges for them, were genetically distinct from ticks in the historic ranges which suggests the possibility of adaptive evolution in these specific populations which has major implications for their transmission of pathogens (Monzón et al., 2016).

These ticks are sensitive to changes in ground level moisture, which is affected by atmospheric relative humidity, so dry conditions could hamper the further westward extension of this tick and decrease their population densities. However, Texas has massive variability in its climate due to its unique position in North America and the variety of factors that affect its climate. Movements of seasonal air masses, subtropical west winds from the Pacific Ocean and northern Mexico, tropical cyclones/hurricanes from the Gulf of Mexico, a high-pressure system in the Atlantic ocean known as the Bermuda high, and the movement of jet streams all play a role in the different climates seen in Texas and climate change is causing these variables to change (Texas Water Development Board [TWDB], 2012).

West Texas's weather is primarily affected in the summertime by the North American Monsoon and the El Niño Southern Oscillation (TWDB, 2012). A weak oscillation, known as the La Niña phase, results in lower precipitation, whereas the El Niño phase, a strong oscillation, results in higher-than-normal precipitation (TWDB, 2012). Climate models are inherently complex and there is some uncertainty when planning a climate model at a local scale, but climate scientists have analyzed El Niño patterns and have noted increased stronger El Niño events due to warming in the western Pacific, which suggests more frequent and extreme El Niño events in the future

(Wang et al., 2019). So, although West Texas is primarily an arid region, increased and stronger El Niño events could lead to increased precipitation allowing for a more habitable environment for *A. americanum*.

Ixodes scapularis

I. scapularis is the primary vector for a majority of TBDs than any other human/animal biting tick in North America and its geographic range has significantly expanded (Sonenshine, 2018). Due to warmer winters and the proliferation of white-tailed deer, these ticks have spread into Southern Canada and into the Midwest and all of the eastern United States (Sonenshine, 2018). According to Hahn et al. (2016), there are reported, but not established, populations of I. scapularis in Central Texas and no records available for West Texas.

These ticks prefer forest and brushy habitats where there is humid ground cover and they are sensitive to desiccation that limits their ability to quest for hosts, but they've been found to establish themselves in temperate shrublands (Sonenshine, 2018). Hosts do not limit their ability to spread since they can feed on a variety of medium or large sized mammals and humans besides their preferred white-tailed deer. Larvae and nymphs of this species feed on a variety of small mammals like white-footed mice, but they can also feed on migratory birds and lizards, meaning they can adapt to find sufficient hosts as their geographic range changes (Sonenshine, 2018).

As climate change increases, these species of ticks are moving northward and further west in the United States into areas that were once considered too arid for their establishment. As both species are not confined by their hosts preferences, as animals continue to spread in their distribution, and as weather patterns, like El Niño, could bring more hospitable weather patterns to West Texas, surveillance will be important to determine if these species of ticks are establishing

populations further west of Central Texas into the El Paso region and along the United States-Mexico border. Research of tick populations along this border region are severely lacking, but one study completed has indicated that under certain climate conditions, *I. scapularis* populations can be supported along the Texas-Mexico transboundary region (Feria-Arroyo et al., 2014).

Humans also have a large role to play in the changing distributions of ticks. Globalization has resulted in increased travel and a constant flow of goods, products, and pathogens between borders. As humans continue to encroach into new regions and develop it for use, they come into closer contact with animal hosts that can transmit diseases and their parasites to them. As has been noted, *I. scapularis* and *A. americanum* are willing to use humans as hosts so as urbanization increases and brings humans closer to these tick populations, so does the risk of transmission of the pathogens they carry. As the climate warms up, humans could increase the time they spend outdoors which brings them into closer contact with ticks during the time of year they are most active like the spring and summer. There is also very little knowledge about TBDs and how to protect oneself against ticks, which increases the risk to humans of acquiring a TBD. In this border region, human migration and trade can introduce the establishment of tick species and as Texas has a legacy of neglected tropical diseases and is vulnerable to outbreaks of vector-borne diseases, surveillance programs are urgently needed (Esteve-Gassent et al., 2014).

CHAPTER 3: STUDY RATIONALE

Environmental conditions and human behavior have been known to cause changes in the geographical distribution of vectors and pathogens (Esteve-Gassent et al., 2014). In Central Texas, ticks and their associated pathogens are being researched, but more research is needed to explore the possibility that ticks endemic to Central Texas are vectors for pathogens that are not readily associated with them. One of the most well-known and medically significant commensalism relationships between a tick and a pathogen is that of *Ixodes scapularis* and *Borrelia burgdorferi*, the causative agent of Lyme disease. Although this relationship is well documented, research into investigating if specific species of ticks can acquire and become vectors for other pathogens has been historically understudied. Due to the exploratory nature of this research, the act of investigating new relationships between pathogens and the tick species that act as vectors for them would require a substantial amount of manpower and funding.

However, as climate change and its effects on the planet increase and as humans encroach onto and develop untouched land, it's feasible that the relationships between specific pathogens and species of ticks are changing. Increased surveillance will be needed to determine these relationships and observe the changes in the geographical distribution of ticks. The initial step of identifying a pathogen in a species of tick not known to be a vector for said pathogen would provide opportunities for future research. This could encompass further research into the new tick-pathogen relationship, studies on vector competency of tick species for the specific pathogen, and could further define the vertebrate hosts of specific pathogens. As Texas sits on the United States-Mexico border, any changes to the geographical distribution of ticks that brings new tick species and their associated pathogens to the border could pose a significant public health threat to the surrounding populations.

3.1 Problem Statement

Ixodes scapularis is well known as a tick of medical significance due to the variety of pathogens its known to transmit, the most notorious being the causative agent for Lyme disease. *Amblyomma americanum* is another tick of medical significance since it is a vector for a variety of pathogens like the causative agents of human ehrlichiosis (*Ehrlichia ewingii*) and tularemia (*Francisella tularensis*). This tick has received more attention in the last few years as it has been connected to alpha-gal syndrome, which results in people who have been bit by it developing an allergy to red meat. Since both of these species are well documented vectors for a variety of pathogens, more surveillance and testing needs to be done to determine if they can acquire and become vectors for other medically important pathogens like *R. rickettsii*.

3.2 Research Question

To identify the presence of *Rickettsia rickettsii* in ticks collected from feral swine (*Sus scrofa*) and white-tailed deer (*Odocoileus virginianus*) in Central Texas.

3.3 Aim

The aim of this study is to identify the presence of *R. rickettsii* in two species of ticks that are not well-established vectors of the pathogen.

3.4 Objectives

To identify *R. rickettsii* in ticks collected from feral swine (*Sus scrofa*) and white-tailed deer (*Odocoileus virginianus*) in Central Texas, this study will 1) identify the species of ticks collected in Central Texas using morphological keys and characteristics; 2) extract DNA; 3) use polymerase chain reaction (PCR) to amplify 3 Rickettsial genes to determine presence of *R. rickettsii* in extracted DNA samples.

CHAPTER 4: MATERIALS AND METHODS

4.1 Tick Collection and Identification

Ticks were collected from free ranging white-tailed deer and feral swine taken by experienced sharpshooters in accordance with Scientific Permit SPR-0801-168 issued by the Texas Parks and Wildlife Department, Austin, Texas in several areas around Travis County, Texas. The tick collection from white-tailed deer occurred between November and December of 2019. The tick collection from feral swine occurred between June and September of 2020. Specimens were stored in glass vials containing 90% ethanol. Species and life stage of ticks were identified morphologically based on standard taxonomic keys (Keirans & Litwak, 1989; Keirans & Durden, 1998; Mathison & Pritt, 2014; Pratt & Stojanovich, 1946).

4.2 DNA Extraction

To remove any external contaminants, ticks were washed sequentially for one minute each with distilled water, 10% bleach, 1x PBS, 70% ethanol, 1x PBS and distilled water. Unfed ticks were bisected longitudinally using sterile scalpels with one half used for extraction and the other half placed in a sterile microcentrifuge tube that was stored at -20°C. For bloated ticks, four legs were removed to be processed for DNA extraction and the rest of the tick was placed in a sterile microcentrifuge tube that was stored at -20°C. The use of only half of the body or legs allows the remaining specimen to be preserved should further identification or testing of pathogenic organisms outside the purview of this study be needed. The bisected piece of tick or legs were placed in a Biomasher II® Closed System Disposable Tissue Homogenizer (Kimble) containing 100 µl of PBS and homogenized manually with the attached pestle.

DNA was extracted using a DNeasy Blood and Tissue Kit (Qiagen, Inc., Valencia, CA, USA) as specified by the manufacturers' instructions with some modifications. After the sample was completely homogenized, 20 µl of proteinase K and 200 µl of AL Buffer (Qiagen) was added to the sample, vortexed for 8 seconds, and incubated at 56°C for one hour. Afterwards, 200 µl of 100% ethanol was added and vortexed for 8 seconds. The sample was pipetted into a spin column placed inside a 2 ml collection tube and centrifuged at 6,000 x g for 1 minute at room temperature. The flow through was disposed of and the spin column was placed into a new 2 ml collection tube. 500 µl of AW1 Buffer (Qiagen) was added and the sample was centrifuged at 6,000 x g for one minute at room temperature. The flow through was disposed of again and the spin column was placed into a new 2 ml collection tube. 500 µl of AW2 Buffer (Qiagen) was added and centrifuged for 3 minutes at 20,000 x g to dry the membrane. The sample was centrifuged once more for 1 minute at 20,000 x g to ensure dryness. The spin column was then placed into a clean 2 ml microcentrifuge tube and 100 µl of AE Buffer (Qiagen) was added. The sample was incubated at room temperature for 1 minute and centrifuged for 1 minute at 6,000 x g to elute. The final eluted volume was 100 µl of extracted DNA and it was stored at -20°C. Concentrations and purity of extracted DNA were measured using the NanoDrop® ND-1000 and quality of genomic DNA was measured by running agrose gels.

4.3 PCR

PCR reactions content included 1 µL of MgCl, 1.25 µL of forward primer, 1.25 µL of reverse primer, 4 µL of extracted DNA, 7.13 µL of PCR master mix, and 10.37 µL of nuclease-free water to make a total volume of 25 µL per PCR tube. All PCR reactions were conducted on an iCycler thermal cycler (Bio-Rad, Hercules, CA). The three primers utilized for PCR were *gltA* (encoding for *Rickettsia* genus-specific citrate synthase gene), *17 kDa* (17 kDa lipoprotein

precursor antigen gene specific to spotted fever group (SFG)), and *rOmpA* (encoding the 190 kDa outer membrane protein A specific to *R. rickettsii*).

Primer *gltA* was used to amplify a 381 bp of a Rickettsia genus-specific citrate synthase gene. PCR conditions were as follows: initial denaturation at 95º C for 10 min, 35 cycles of denaturation at 95º C for 30 sec, annealing at 58º C for 30 sec, and extension at 72º C for 1 min. A final extension step was done at 72º C for 10 min and end with 4ºC for infinity. Primer *17 kDa* was used to amplify a ~ 442 bp of a 17 kDa lipoprotein precursor antigen specific to spotted fever group rickettsioses. PCR conditions were as follow: initial denaturation at 95º C for 2 min, 35 cycles of denaturation at 95º C for 30 sec, annealing at 55º C for 30 sec, and extension at 72º C for 45 sec. A final extension step was done at 72º C for 7 min and end with 4ºC for infinity. Primer *rOmpA* was used to amplify a 532 bp of a 190 kDa outer membrane protein A specific to *R. rickettsii*. PCR conditions were as follow: initial denaturation at 95º C for 5 min, 40 cycles of denaturation at 95º C for 30 sec, annealing at 55º C for 30 sec, and extension at 72º C for 1 min. A final extension step was done at 72º C for 10 min and end with 4ºC for infinity.

Each set of reactions used genomic DNA from *R. slovaca* as a positive control and nuclease-free water for a negative control. PCR products were run on a 1.5% agarose gel at 85 volts for 55 minutes. Amplicons were visualized using a benchtop UV Transilluminator (UVP Analytik Jena US LLC, Upland, CA) and then visualized using the GelDoc-It® Imaging System (UVP Analytik Jena US LLC, Upland, CA). Samples that tested positive were not sequenced due to time limitations and scope of study.

CHAPTER 5: RESULTS

From November 6, 2019 – December 18, 2019, 41 adult *Ixodes scapularis* ticks, comprising 31 females and 10 males, were removed from 13 white-tailed deer. From June 28, 2020 – September 18, 2020, 35 *Amblyomma americanum* ticks, comprising 20 nymphs, 14 adults (9 females and 5 males), and 1 molted exoskeleton were removed from 15 feral swine. The exoskeleton and one female tick were excluded from DNA extraction and PCR due to the inability to identify the species of tick on account of lacking identifying features such as the head and multiple legs missing and because the exoskeleton wasn't an actual specimen sample. All ticks that were removed from a specific deer or feral swine were kept together and pooled for the DNA extractions. Within each pool, specimens were separated by sex, and a maximum of 4 or 5 ticks were included per sample in a pool depending on size of bisected tick or how many legs were included to maximize DNA yield. Pooling the ticks resulted in 18 samples of extracted DNA representing the 13 white-tailed deer the ticks had been pulled from. Pooling the ticks from feral swine resulted in 17 DNA samples representing 14 wild swine; one sample from one feral swine had to be excluded due to poor quality of specimen which made it impossible to identify using morphological characteristics. Samples were only considered positive if the amplicon was able to be reproduced a second time.

5.1: *gltA* gene

Results from amplifying the rickettsial *gltA* gene from the extracted tick DNA are shown in Table 2. The rickettsial prevalence of *Ixodes scapularis* and *Amblyomma americanum* were 10/18 (56%) and 5/17 (29%), respectively, with an overall average among ticks being 15/35 (43%).

5.2: *17 kDa* gene

Results from amplifying the spotted fever group rickettsioses specific 17 kDa outer membrane antigen gene from the extracted tick DNA are shown in Table 2. The spotted fever group rickettsioses prevalence of *I. scapularis* and *A. americanum* were 8/18 (44%) and 2/17 (12%), respectively, with an overall average among ticks being 10/35 (29%).

5.3: *rOmpA* gene

Results from amplifying the 190 kDa outer membrane protein A specific to *R. rickettsii* from the extracted tick DNA are shown in Table 2. The *R. rickettsii* prevalence of *I. scapularis* and *A. americanum* were 8/18 (44%) and 1/17 (0.06%), respectively, with an overall average among ticks being 9/35 (26%)

CHAPTER 6: DISCUSSION

Second only to mosquitoes in their ability to transmit pathogens of human and veterinary importance, ticks transmit a wide range of protozoa, viral, bacterial, and fungal pathogens, which contributes to thousands of cases of tick-borne diseases (TBDs) every year (Nicholson et al., 2019). TBDs accounted for more than 75% of all vector-borne cases reported in the United States between 2004 and 2016 (NIH, 2019) and the incidence of TBDs continues to increase in the United States and around the world. Coupled with the emergence of new pathogens and the changing geographical ranges of ticks, there is a need for more research and surveillance on tick-pathogen relationships.

Nelder et al. (2016) performed a systematic review of the literature to identify human pathogens that were associated with *I. scapularis*. They found little evidence demonstrating *I. scapularis* as effective vectors of *Rickettsia*. This lack of evidence does not mean *I. scapularis* is incapable of being vectors for rickettsial species, rather it emphasizes the need for exploratory research into establishing the presence of new pathogens in *I. scapularis* and testing their vector competency for these pathogens once identified. Nelder et. al (2016) went on to say that the majority of pathogens, their maintenance in nature, preferred vertebrate hosts, and vector competency in different species of tick is poorly understood. They recommended longitudinal, ecological, and epidemiological studies in endemic and emerging areas to better understand the intricate epidemiology of pathogens transmitted by *I. scapularis* (Nelder et al., 2016).

Similarly, the search for new pathogens found in *A. americanum* and their vector competency has not been appropriately studied. The genus *Amblyomma* are recognized vectors of RMSF in Central and South America, but not in the United States, which could be indicative of

minimal research efforts to establish the genus as vectors for RMSF in the United States. Levin et al. (2017) tested the ability of *A. americanum* to acquire and transmit *R. rickettsii* under laboratory conditions. They found that under these controlled conditions, *A. americanum* demonstrated vector competence for *R. rickettsii* by successfully maintaining the pathogen throughout the tick's life cycle and transmitting it to vertebrate hosts during subsequent blood meals (Levin et al., 2017). Levin et al. (2017) concluded that the role of *A. americanum* in the ecology and epidemiology of RMSF in the United States deserves further investigation.

These studies support the research question proposed by this study. Although exploratory in nature, the importance of testing two tick species for a pathogen not readily associated with them is vital to increasing our comprehension of TBDs and how they are transmitted, maintained, and passed to humans in nature. To determine the presence of *R. rickettsii* in the ticks collected, the three primers used in the PCR are crucial for detection and diagnosis of the pathogen. The three genes typically targeted by PCR are citrate synthase (*gltA)*, the 17 kDa lipoprotein precursor antigen gene (*17 kDa)*, and a gene for outer membrane protein A (*ompA)* (Prakash et al., 2012). Detection of the *gltA* gene is confirmation that the amplicon belongs to the rickettsial genus that encompasses the spotted fever group rickettsia (SFGR) and the typhus group (TG) (Prakash et al., 2012). An amplicon resulting from the use of the *17 kDa* gene confirms the presence of SFGR specifically and amplification of the *ompA* gene is conclusive evidence for SFGR depending on what primer sequence is used (Prakash et al., 2012).

The *gltA* oligonucleotide primer sequences used were derived from two areas of the citrate synthase gene, between nucleotides 877 and 1258, from *R. prowazekii* (Regnery et al., 1991). These primers primed the synthesis of DNA products from all species of Rickettsiae, meaning these primers were good at selecting Rickettsiae DNA, whether SFG or TG, and not other DNA

from different species of bacteria like *E. coli* or *Mycobacterium* species (Regnery et al., 1991). This means any amplicons observed using these two primer sequences can confidently be determined to be either a SFG or TG Rickettsiae, which was the first step in confirming whether the genomic DNA extracted from the ticks contained *R. rickettsii*. The number of DNA samples that had amplicons using the *gltA* primers among all the ticks was 15/35 (43%). Of the samples containing DNA extracted from *I. scapularis* ticks, 10/18 (56%) amplified and the *A. americanum* DNA resulted in 5/17 (29%) samples amplifying.

Once samples presented amplicons identifying as either SFG or TG Rickettsiae, the *17kDa* primer was used to single out SFG Rickettsiae since *R. rickettsii* is a SFG Rickettsiae. The specific sequence for the *17kDa* primer was used due to a study that collected data on the DNA sequence of the gene encoding the 17-kilodalten protein antigen from *R. rickettsii* (Anderson et al., 1987; Webb et al., 1990). Anderson et al. (1987) used molecular cloning techniques to express the surface protein antigen from *R. rickettsii* in *E. coli.* These specific sequences are from the 17K protein that is a surface protein of *R. rickettsii* (Anderson et al., 1987). This means the primer sequences are good diagnostic tools to identify SFG Rickettsiae, specifically *R. rickettsii*, in PCR. The number of DNA samples that had amplicons using the *17kDa* primer during PCR for *I. scapularis* and *A. americanum* were 8/18 (44%) and 2/17 (12%), respectively, with an overall average among ticks being 10/35 (29%).

The *rOmpA* primers are from regions of the 190-kDa SFG antigen gene derived from the *R. rickettsii* sequence that amplifies products from between nucleotides 70 and 602 (Regnery et al., 1991). This is the last set of primers to be used during PCR, because depending on the sequence used, it can further substantiate the results from the first two primers and provide confidence in determining the presence of *R. rickettsii* in the extracted DNA samples. These two *rOmpA* primers

were considered to be the most useful in their ability to prime products of appropriate lengths, produce a minimum of nonspecific products, and differentiate between closely related SFG species (Regnery et al., 1991). The number of DNA samples that had amplicons indicating presence of *R. rickettsii* from *I. scapularis* and *A. americanum* were 8/18 (44%) and 1/17 (0.06%), respectively, with an overall average among ticks being 9/35 (26%).

Regnery et al. (1991) deduced that the *gltA* gene is the most stringently conserved of the three rickettsial genes, followed by the *17kDA* gene, and finally the *ompA* gene. This means the *gltA* gene is the most limiting in determining species since it encompasses both groups of Rickettsiae while the *ompA* gene is better at identifying at a species level depending on what exact primer sequence is used. Furthermore, the PCR patterns were almost completely free of intraspecies genetic variation meaning the absence of observed sequence divergence among isolates of a particular species is why this PCR technique using these three primers works for identifying rickettsial species (Regnery et al., 1991). It is for this reason the results produced by the PCR in this study should be counted as evidence towards the presence of *R. rickettsii* in *I. scapularis* and *A. americanum*.

The DNA samples were slowly whittled down to positive *R. rickettsii* samples using these three primers. Of the 15 DNA samples that had amplicons with the *gltA* gene, only 10 displayed amplicons using the *17kDa* gene primer. This would indicate that 5 of the 35 samples were TG Rickettsiae, but DNA sequencing would be needed to confirm this. Of the 10 DNA samples that had amplicons with the 17kDa gene primer, only 9 displayed amplicons with the *rOmpA* gene primer. However, this is where some results didn't match with what the expected results should have been. Extracted DNA sample L1, as seen in Table 3, had amplicons for the *gltA* gene and the *rOmpA* gene, but not the *17 kDa* gene. Seeing as how the *rOmpA* gene is meant to identify *R.*

rickettsii, after the *17 kDa* gene identifies the sample as a SFG Rickettsiae, there should've been an amplicon with the *17kDa* gene. One factor that could explain this result is that when the PCR samples were being prepared, the L1 DNA sample was not properly mixed before placing the 4 µl of DNA into the designated PCR tube which would've resulted in little to no DNA being present to be amplified during PCR. It is also possible that the L1 sample could have shown up as a false positive on the *rOmpA* primer if the DNA sample had been contaminated at some point during the process. The only way to confirm what the L1 sample represents would be to sequence the DNA or perform a new DNA extraction of the left-over specimen and redo all three sets of primers. Due to time constraints, neither of these were done. DNA samples H1 and AB did not amplify with the *rOmpA* primer indicating they were SFG Rickettsiae, but not *R. rickettsii*. Again, only genetic sequencing of this DNA could confirm this.

6.1: Limitations

This study was exploratory in scope and the results found are intended to be used as evidence to advocate for further research into the presence of *R. rickettsii* in these two tick species endemic to Texas. However, some limitations did present themselves throughout the duration of the study. The main limitation was that the collection of ticks was a convenience sample and was relatively small in sample size meaning it may not accurately depict the ecological reality in Central Texas. A more systematic collection method of ticks from different sectors of Travis County would provide a better idea of whether *I. scapularis* and *A. Amblyomma* are capable of being recognized as vectors of *R. rickettsii* as indicated by the results of this study. Another limitation is that DNA sequencing was not performed to verify if the samples that came back as positive for *R. rickettsii* were indeed *R. rickettsii* since that was beyond the scope of this study. Human error such as improper mixing, pipetting, or cross contamination could explain the L1

sample not showing an amplicon with the *17kDa* primer. Another limitation is that due to time constraints, this matter was not followed up on to determine the source of the discrepancy. Finally, this study was limited in scope, but provides opportunities for future research.

6.2: Future Research

The results obtained from this study provide a good framework on which to build future research off of. Continued research needs to be done to further investigate the relationship between tick species and pathogens not readily attributed to them. These results provide evidence of the ability of *I. scapularis* and *A. americanum* to be infected with *R. rickettsii*, but more research is needed to further certify these results. This study could provide the opportunity for vector competency of *R. rickettsii* to be further investigated in these two tick species, both in a controlled setting in the laboratory and in the environment using systematic collection techniques. Future research could define the vertebrate host of the pathogen. Conclusions on vertebrate hosts for *R. rickettsii* were not reached in this study because although the ticks used were taken from white-tailed deer and feral swine, the scope of this study did not prove that the ticks had contracted *R. rickettsii* through a blood meal from these two vertebrate animals.

Due to their life cycle, these two species use three different vertebrate hosts to take blood meals from. *R. rickettsii* can be passed along through transovarial and transstadial transmission or by feeding from an infected host or feeding alongside ticks who are already infected (McDade & Newhouse, 1986). In order to conclude that the white-tailed deer and feral swine were the hosts for *R. rickettsii* and had passed it to the ticks rather than the ticks acquiring the pathogen through a prior life stage, blood and serum samples would have had to have been collected and tested for the presence of *R. rickettsii*. Testing the blood and serum samples of the animals the ticks were

collected from would determine if the ticks obtained the pathogen from the host they were pulled from or a different host.

CHAPTER 7: CONCLUSION

In the last few decades, interest in ticks and tick-borne diseases (TBDs) has continued to increase due to the changes in the geographical distribution of ticks and their ability to transmit a variety of pathogens (Estrada-Peña, 2015). This has led to an increase of tick-borne diseases (TBDs) being diagnosed (Eisen & Eisen, 2018). Many of these TBDs were only discovered during the past ten years reflecting the emerging threat ticks pose and the new frontier of vector-borne diseases faced by the global community. The fluctuations in geographic ranges of ticks and the introduction of species into new habitats has been one reason TBDs are starting to receive more attention amongst the scientific community. Some researchers believe this spread is due to uncontrolled movements of domestic or wild animals, changes to the climate, and changes in the use of land resources which all allow for an increase in the abundance of hosts (Estrada-Peña, 2015). The climate changes, in particular, contributes to the spread of tick species to new geographical locations since warmer conditions allow them to survive and continue their life cycle unheeded by low temperatures causing concern to regions that have since been deemed free of TBDs (Ostfeld & Brunner, 2015).

The results from this study indicate the ticks in Texas can acquire and potentially transmit *R. rickettsii* to other animals and humans. Although not aggressive biters like *A. americanum*, *I. scapularis* also frequently uses humans for their blood meals. Their somewhat liberal feeding habits and the aggressive behavior of *A. americanum* justifies the continued identification of relationships with new pathogens and assessing their implications to public health and the health of animals, wild and domestic. As the climate continues to warm and vertebrate hosts expand their geographical ranges, ticks will also expand their spatial distribution across Texas and the Unites States. There are established populations of both tick species in some counties along the Unites

States – Mexico border and some studies predict their range expanding further west. Continued surveillance of pathogens found in both tick species can help establish baseline data and inform local public health officials about the local risk assessment.

CHAPTER 8: STRATEGIC FRAMEWORKS

8.1 Healthy People 2020

Healthy People 2020 (HP2020) provides 10-year national objectives based in science to improve the health of all Americans. The mission is to create a society where all people live longer and healthier lives by identifying health areas needing improvement, increasing public awareness and understanding of determinants of health, and identifying areas of increased research among other goals.

One topic of HP2020 that relates to this proposed study is public health infrastructure. The goal of this topic is to insure every Federal, State, Tribal, territorial, and local health agency have the necessary infrastructure to effectively provide essential public health services (Healthy Border 2020, n.d.). Public health infrastructure is critical in providing communities the ability to prevent disease, promote health, and prepare for and respond to acute or chronic challenges to health. It is also critical in establishing the foundation for planning, delivering, evaluating, and improving public health. The objective that relates to this proposed study under "Public Health Infrastructure" include:

- PHI-11 Increase the proportion of Tribal and State public health agencies that provide or assure comprehensive laboratory services to support essential public health services
    - PHI-11.1 Increase the proportion of tribal and state public health agencies that provide or assure comprehensive laboratory services to support disease prevention, control, and surveillance
    - PHI-11.3 Increase the proportion of tribal and state public health agencies that provide or assure comprehensive laboratory services that support reference and specialized testing

    - PHI-11.9 Increase the proportion of tribal and state public health agencies that provide or assure comprehensive laboratory services in support of public health-related research
- PHI-12 Increase the proportion of public health laboratory systems (including State, Tribal, and local) which perform at a high level of quality in support of the 10 Essential Public Health Services
    - PHI-12.2 Increase the proportion of public health laboratory systems (including State, Tribal, and local) that perform at a high level of quality in support of diagnosing and investigating health problems and health hazards in the community
    - PHI-12.4 Increase the proportion of public health laboratory systems (including State, Tribal, and local) that perform at a high level of quality in mobilizing community partnerships and action to identify and solve health problems

These objectives can be applied to this proposed study since the need for increased surveillance programs that examine tick-borne diseases is clearly demonstrated. This begins with an investment of infrastructure, such as laboratory capabilities, to promote research and testing of tick-borne diseases and establishing relationships with different local, state, and national entities to increase the surveillance of ticks in this region.

8.2 Healthy Border 2020

Healthy Border (HB) 2020 is a binational strategy of the U.S.-Mexico Border Health Commission to highlight public health issues prevalent among border populations along both sides of the border and aims to provide a framework for border region public health goals and actions needed to improve the health of the people who live along both sides of the border (Healthy Border,

n.d.). Healthy Border focuses on five public health concerns: chronic and degenerative diseases, infectious diseases, maternal and child health, mental health and addiction, and injury prevention.

Healthy Border 2020 acknowledges the risk of infectious diseases along the border, but does not include infectious diseases of a zoonotic nature in its focus of health issues of the binational communities. For future Healthy Border objectives, infectious diseases of a zoonotic nature should be considered since the border region shares an epidemiological profile and an outbreak could lead to mass exposure due to the high volume of traffic across the border.

8.3 Paso del Norte Regional Strategic Health Framework

The Paso del Norte Regional Strategic Health Framework was established by members of the Coalition for a Healthy Paso del Norte to select six health priorities that affect residents who reside in the Paso del Norte region. This region consists of El Paso and Hudspeth counties in Texas and Doña Ana, Luna, and Otero counties in New Mexico (Healthy Paso del Norte, n.d.). The six health priorities chosen were: obesity/diabetes/fitness/nutrition, mental health and behavioral health/wellness, substance abuse/chemical dependency/drug abuse, health sexuality/teen pregnancy, access to healthcare, and violence and injury prevention and reduction. The Paso del Norte Regional Framework makes no mention of infectious or zoonotic diseases.

CHAPTER 9: MPH CORE COMPETENCIES

The Masters of Public Health program and curriculum at the University of Texas at El Paso is centered on nine foundational competencies. These nine competencies are evidence-based approaches to public health, public health and health care systems, planning and management to promote health, policy in public health, leadership, communication, interprofessional practice, and systems thinking; each with multiple sub-competencies. The program has also included five Hispanic and border health concentration competencies.

Evidence-based Approaches to Public Health

At its core, evidence-based approaches to public health means employing evidence-based medicine, a process of using the best and most up to date evidence in caring for patients, to the public health field (Lhachimi et al., 2016). This proposed study utilizes a couple sub-competencies of evidence-based approaches to public health:

A.1 Apply epidemiological methods to the breadth of settings and situations in public health practice

A.4 Interpret results of data analysis for public health research, policy or practice

This proposed study will use descriptive epidemiology to characterize the distribution of health-related events related to tick borne diseases. The DNA extraction obtained from this proposed study will be used to extrapolate information about pathogens present within ticks and be resulted and presented for use in public health research.

Systems Thinking

Increasingly, systems thinking approaches are being incorporated into public health practices. Systems thinking focuses on the interrelationships between parts and how their relationships to a functioning whole work (Trochim et al., 2006). Systems thinking is dynamic and complex and must deal with competing interests, stakeholders, and variables. It will require a transdisciplinary group of individuals from public health, human medicine, veterinary medicine, scientists, and policymakers to contribute to the field of tick and tick-borne diseases research and how it can affect human and animal populations. As tick-borne diseases become more prevalent throughout the world, a systems thinking approach will be needed to ensure the threat tick-borne diseases pose will be handled in a competent manner that diminishes the threat while protecting human and animal populations as much as possible. This proposed study will:

H.1 Apply systems thinking tools to a public health issue

This systems thinking approach will hopefully lead to increased surveillance and push for more research of ticks and their associated pathogens to occur in Texas.

Hispanic/Border Health Concentration

The United States-Mexico border area is a territory that extends 3,141 kilometers from the Pacific Ocean to the Gulf of Mexico and includes the area 100 kilometers north and south of the international border (Healthy Border 2020, n.d.). This area encompasses numerous counties and municipalities that is home to approximately 15 million inhabitants on both sides of the border (Pan American Health Organization, 2014). This border region along the Rio Grande River should be viewed as a dynamic landscape where, irrespective of a man-made geopolitical border, zoonoses circulate. This proposed study will:

1. State and discuss the current major communicable, non-communicable, and environmental public health threats in Hispanic and border communities

Zoonoses and the pathogenic landscape in this region have long been understudied and as the threat of tick-borne diseases continue to rise, more understanding and research will be needed on how to combat them.

Table 1. Genes and Primers Utilized for PCR

Gene	Target detected	Primer	Sequence (5'-3')	Product Size (bp)
gltA	All rickettsiae (typhus group and SFGR)	RpCS.877p	5'-GGGGGCCTGCTCACGGCGG-3'	381
		RpCS.1258n	5'-ATTGCAAAAAGTACAGTGAACA-3'	381
17kDa	All SFGR	Rr17k.90p	5'- GCTCTTGCAACTTCTATGTT-3'	450
		Rr2608R	5'-CATTGTTCGTCAGGTTGGCG-3'	434
rOmpA	SFGR – *R. rickettsii*	Rr190.70p	5'-ATGGCGAATATTTCTCCAAAA-3'	532
		Rr190.602n	5'-AGTGCAGCATTCGCTCCCCCT-3'	532

Table 2. Extracted DNA samples and amplification of *gltA*, *17 kDa*, and *rOmpA* genes by PCR

Host	**Extracted DNA Samples**	**Number of Ticks/Sex**	**Amplification**		
White-tailed deer			*gltA* gene	*17 kDa* gene	*rOmpA* gene
			Yes	Yes	Yes
19036	A1	2*/F			
19037	B1	1/F	x	x	x
19041	C1	5/F	x	x	x
19043	D1	4/M			
	D2	3/F	x	x	x
	D3	2*/F			
19049	E1	1/F	x	x	x
19052	F1	1*/F			
	F2	1/M			
19054	G1	2*/F			
19061	H1	3*/F	x	x	
	H2	3/M			
19062	I1	3/F	x	x	x
19065	J1	2/F	x	x	x
19070	K1	1*/F			
19071	L1	1/F	x		x
19073	M1	4*/F	x	x	x
	M2	2/M	x		
Feral Swine					
19-25	N1	2/F			
	N2	1/M			
19-28	O1	2/F			
	O2	1/M	x		
19-29	P1	1/F			
19-33	Q1	2/F			
19-61	R1	1/M			
19-67	T1	5 nymphs			

19-68	U1	4 nymphs			
19-71	V1	1/F			
19-73	W1	1 nymph			
19-74	X1	2/F	x		
	X2	1/M			
19-77	Y1	1/M	x		
19-79	Z1	2/Z			
19-80	AB	4 nymphs	x	x	
19-81	AC	2 nymphs	x	x	x

* legs used in extracted DNA sample

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VITA

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