CONDUCTING TICK-BORNE DISEASE RESEARCH IN TEXAS WITH A FOCUS ON *Rickettsia* spp.

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The field of vector-borne disease research uses multidisciplinary approaches to help understand complicated interactions. This dissertation, covers three different aspects of tick-borne disease research which all focus on exploring tick-borne diseases in the non-endemic areas of Denton, County Texas and the state of Texas with a focus on *Rickettsia* spp. These aspects include tick sampling, testing ticks for the presence of *Rickettsia* spp., and creating species distribution maps of the *Rickettsia* spp. *Rickettsia amblyommatis* and tick species *Amblyomma americanum*.

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CHAPTER 1

INTRODUCTION

The field of vector-borne disease research uses multidisciplinary approaches to help understand complicated interactions. This dissertation, covers three different aspects of tick-borne disease research which all focus on exploring tick-borne disease in the non-endemic areas of Denton, County Texas and the state of Texas with a focus on *Rickettsia* spp.

1.1 Background

Humans have been aware of the parasitic behavior of ticks for thousands of years. Descriptions of the behavior of ticks have even been found in writings by Greek authors such as Homer and Aristotle (Sonenshine 1991). Ticks are considered the most important disease vector in North America and second only to mosquitos worldwide. They take blood meals from every class of vertebrate, and are responsible for transmitting more types of disease causing microorganisms than any other group of arthropod vectors (Sonenshine 1991, Jongejan and Uilenberg 2004). Ticks can become infected with pathogenic microorganisms either by taking a blood meal from an animal that has the organism in its blood or, in some cases, having the infection passed down to it from the mother. Once it has been infected with one or more of these disease causing organisms, it may remain infected through all the stages of its life cycle and be able to pass these diseases on to humans (Parola and Raoult 2001). One tick is capable of transmitting multiple infections with one bite (Bratton and Corey 2005).

1.1.1 Tick-Borne Disease in Texas

In October of 2004, the Texas Department of State Health Services began a partnership

with the University of North Texas Health Science Center Tick-Borne Disease Research Laboratory (UNTHSC) to provide tick testing services to Texas residents bitten by ticks. Ticks that have attached themselves to Texas residents can be sent to the UNTHSC where they are tested for the disease causing agents *Borrelia, Ehrlichia* and *Rickettsia* (Williamson et al. 2010). The fact that the state has made this free service available shows that there is concern about tick-borne illnesses in Texas, even though tick-borne diseases are not found in high numbers. The lower incidence of these diseases in Texas has resulted in a lower level of tick survey data being collected when compared to states where tick-borne diseases are prevalent (Williamson et al. 2010).

Although not endemic, tick-borne diseases do present a risk to residents. Individuals who become infected with these diseases in Texas may be at additional risk as doctors in the state may not consider tick-borne diseases when diagnosing patients. Delays in correct diagnosis can lead to delays in receiving appropriate treatment (Williamson et al. 2010).

1.1.2 Tick Collection

The first aspect of tick-borne disease research covered in this dissertation is the process of tick collection or sampling. There are four common methods used to collect ticks: walking, dragging, using traps, and collecting ticks from hosts. The walking method involves an investigator walking through the sampling area in light colored cotton clothing and then gathering the ticks that are found on his or her clothing. The dragging method of sampling involves dragging a piece of cloth over leaf litter and low vegetation. The ticks that become attached to the cloth are then removed at regular intervals. When using this method, dense ground cover may cause it to be difficult to drag the cloth through it (Ginsberg and Ewing 1989,

Falco and Fish 1992). The third method is the use of carbon dioxide (CO_2) traps. CO_2 traps can be used because many tick species are attracted by CO_2, but there are also species that do not respond to these traps, or that can escape from them. The final method involves the trapping of tick hosts and removing ticks from these hosts (Ginsberg and Ewing 1989). The process of tick collection does appear very straight forward. However, the process of performing tick collection and making sure your approach will meet the goals of your research can be more complex. There can be issues related to the environment or the tick species in the study area that may make certain methods more appropriate than others. There is not currently adequate information that assists new researchers with determining what methods may be most appropriate in different situations.

1.1.3 Next-Generation Sequencing

The second aspect of tick-borne disease research covered involves using sequencing to identify *Rickettsia* spp. in collected ticks. Sequencing technologies first appeared in the 1970s, but remained limited to research environments due to cost, difficulty, and that the process required the use of dangerous reagents. Sanger sequencing followed this initial sequencing technology and it became the basis for the initial automated sequencers. The Sanger sequencing method was used to sequence the first complete genome of a free-living microorganism in 1995. Next-generation sequencing (NGS) was first introduced in the early 2000s and reduced both sequencing time and cost (Besser et al. 2018). It is a very useful technology that can be applied to disease causing organisms, vectors that carry them and even human hosts. (Gwinn et al. 2019). There are multiple different NGS platforms that can all determine the DNA sequence of sections of DNA that can then be mapped to reference

sequences (Behjati and Tarpey 2013, Gwinn et al. 2019). In general, sequencing has become more affordable and now can be accessed by individual researchers. It is also becoming a common tool in the field of vector-borne disease (Rinker et al. 2016).

1.1.4 Disease Ecology and Species Distribution Mapping

The last aspect of tick-borne disease research covered in this dissertation involves creating species distribution maps. There are multiple things that can make it difficult to map human risk of vector-borne disease. Trying to provide risk maps based on vector habitat, reservoir habitat, or human incidents all pose their own problems. Risk maps based on human incidents can be flawed due to inconsistencies in the data. Often the location provided for each human incident is not the same as the location where the individual contracted the disease. There may also be differences between the number of cases reported and the actual number of individuals who contracted the disease. Maps that attempt to show disease risk based on likely vector distribution also fall short of being able to adequately show risk, because the risk of disease correlates less with vector presence than it does with density of infected vectors (Ostfeld et al. 2005). Beyond these issues with looking at areas of potential risk, an important factor that has often been over-looked is the role of environment in disease mapping. In public health, disease mapping has often treated environment as the location of exposure to an infectious agent. However, the occurrence of disease transmission, as seen through an ecologist's point of view, is merely the existence of species in a location whose presence is dependent on the environmental conditions of that location. Although change can occur slowly, there has been an increase in frequency of space-and-environment modeling from previous modeling approaches for disease transmission that utilized space-only (Peterson 2014).

Maxent software has become very popular, with more than 1,000 applications of it being published between 2006 and 2013. Maxent is used to create species distribution models (SDM) using species presence data in conjunction with environmental information. The software package itself is easy to use and it has been shown to outperform other methods based on predictive accuracy (Merow et al. 2013). Maxent uses the concepts of maximum entropy along with environmental variables and species presence-only data to make predictions concerning species distribution. Although Maxent does hold the fundamental assumption that the entire area being analyzed has been systematically sampled, frequently this is not the case. Because the software is regularly being used with datasets that do not meet this fundamental assumption, there have been articles that look at its performance under these circumstances. These articles have found that Maxent is able to cope with small sample size, irregularly sampled datasets and datasets with minor location errors (Elith et al. 2006, Elith et al. 2011a, Kramer-Schadt et al. 2013a, Merow et al. 2013, Fourcade et al. 2014). Maxent has also been used in multiple projects where species distributions of disease vectors were created (Atkinson et al. 2012, Illoldi-Rangel et al. 2012a, Slater and Michael 2012, Conley et al. 2014, Dicko et al. 2014, Garza et al. 2014).

1.2 Research Goals and Organization of Dissertation

With the understanding that tick-borne diseases do present a risk to the population of Texas, there is value in increasing our understanding of these diseases in this area. Additionally, furthering our understanding of the current state of these diseases may help us to understand why they are not endemic in this area or, if that changes, may give us a base from which to analyze the change.

The field of vector-borne disease research, under which tick-borne disease research falls, is one that that would benefit from multidisciplinary approaches (Moore 2008, Estrada-Pena and Garcia 2014, Jamison et al. 2015). The overall goal of this project is to expand on the knowledge of ticks and tick-borne disease in the non-endemic areas of Denton County, Texas and the State of Texas using multiple disciplines. Each chapter (2, 3 and 4) contain their own goals, with chapter 1 providing an introduction and chapter 5 providing a summary of results, contributions and future research possibilities.

In chapter 2, the process of tick collection is reviewed. There is not a wealth of information to help new researchers work through the available options. There are also pitfalls that can impact this practice that may not be initially obvious. The goal of this chapter was to provide a background of information and what may need to be addressed or considered when planning a tick collection survey.

Chapter 3 explores the species of ticks located in two recreational areas in Denton County, Texas to determine if they were carrying any spotted fever group rickettsia (SFGR) bacteria. Being the first tick collection project in these two areas, the information is useful as no pre-existing data on these areas exists. Additionally, it provides information that might help to risk to individuals who use these areas for recreational purposes.

Chapter 4 expands the research area to the entire state of Texas. Here I compare species distribution maps of *Amblyomma americanum* ticks and *A. americanum* ticks infected with *Rickettsia amblyommatis*. The goal was to see if there were difference in the expected distribution of these. Finding a difference could support using infected ticks to map distributions of tick-borne infectious agents, possibly allowing us to identify areas of increased

risk. It may also provide information that could be used to better understand the environmental differences between the tick distribution and the distribution of the infected tick.

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CHAPTER 2

CONSIDERATIONS WHEN CHOOSING A TICK SAMPLING METHOD AND A TICK COLLECTION PROJECT IN DENTON COUNTY, TEXAS

2.1 Abstract

Determining appropriate sampling methods for tick research projects can be complicated. Many factors need to be considered when choosing a method or methods. Here, I look at the common methods used, what needs to be considered when choosing methods, what methods are appropriate for certain research goals, and the pros and cons for each method. I also examine the difficulty experienced while gathering ticks in the low tick density area of Denton County, Texas.

2.2 Introduction

The collection of ticks for research purposes is important in both tick and tick-borne disease research. Tick collection has been a part of research related to the presence, or prevalence, of tick-borne disease in tick populations, the study of different species as possible tick-borne disease reservoirs, migration of tick-borne disease carrying ticks on migratory birds, ecology of pathogens that carry tick-borne disease and tick ecology, just to name a few (Giardina et al. 2000, Randolph 2000, de la Fuente et al. 2004, Pichon et al. 2006, Brinkerhoff et al. 2011, Estrada-Pena et al. 2011, Hersh 2012, Salkeld et al. 2015). With many research goals benefitting from tick sampling, understanding the available sampling options and how they may relate to research goals is critical. Many things need to be taken into consideration when choosing a sampling method, including the goal of the research project, the species to be sampled and how they seek out their hosts, the environment the sampling will be done in, tick

density, available budget, and available personnel. Choosing how the ticks are collected for a research project can impact study results and conclusions (Holscher et al. 1980, Ginsberg and Ewing 1989, Falco and Fish 1991, Schulze et al. 1997, Petry et al. 2010, Tack et al. 2011, Rynkiewicz and Clay 2014). Being aware of how sampling methods impact research and what is involved in different methods can help in choosing the best sampling approach for a project.

2.2.1 Considerations When Starting a Tick Sampling Project

Following is a review of multiple items that should be considered when starting a tick collection project.

2.2.1.1 The Goal of the Project

The goal of the research project is a major consideration when selecting possible sampling methods. If the only interest in gathering ticks is to use them for experiments in the laboratory and the primary concern is gathering specific species or life stages, then your method options will be much different than if the research goals involve looking at the tick densities or infected tick densities within your study area. If determining tick density in the study area is a goal then the sampling method must allow for the quantification of the ticks sampled by distance or time (Falco and Fish 1989, Ginsberg and Ewing 1989, Schulze et al. 1997). Once the research goals are understood, the methods can be limited to those that accomplish these goals. Understanding each collection method, which is covered later, is necessary to determine what methods may fit the goals.

2.2.1.2 Species and Life Stage of Ticks

Understanding the tick species or life stage of a tick species that will be sampled in the

study area can also impact which methods may be appropriate. Host seeking behavior varies between tick species, with some species such as the Lone Star tick responding well to CO_2traps with others being ambush specialists (Wilson et al. 1972, Ginsberg and Ewing 1989). Additionally, different methods have even shown variability in effectiveness between different life stages within species (Kinzer et al. 1990, Falco and Fish 1992, Petry et al. 2010, Kensinger and Allan 2011, Rynkiewicz and Clay 2014). Looking at previous research on sampling the ticks and life stages of ticks that are to be gathered can help to determine what methods may work or if there are any to avoid.

2.2.1.3 Environment

Once it is determined which sampling methods will provide the information required for the goals of the project and which ones will work with the ticks to be sampled it is also necessary to explore the study area where the sampling will be done. This can help to determine if certain methods may work better than others in that site. Some environmental features of the study site may make certain methods more difficult than others or may require certain materials be used. For example, heavier fabric such as denim may be required when making dragging or flagging equipment if there are briars or plants that might catch and tear the material (Sonenshine 1993). Weather changes can also impact the environment and the method of sampling (Schulze et al. 1997).

2.2.1.4 Tick Density

Tick research can be important in areas of high tick density as well as low tick density. Understanding the ecology of ticks, the diseases they carry and what might influence tick

populations and infection prevalence both positive and negative can be beneficial. However, tick sampling in areas of low tick density can pose different challenges compared to sampling ticks in high-density areas. In my project, sampling in a low-density area where there was a majority of *Amblyomma americanum* (lone star) ticks, it was found that drag sampling did not provide an adequate number of ticks. In lower density areas, it may be more critical to focus on a method that will work with the available personnel time as well as a method specifically suited for the target tick species or life stage. In some low-density situations, it may be necessary to set the research goals based on what data can be acquired, as not all methods may be suitable for the environment, which can limit research goals.

2.2.1.5 Budget

Budget is always a consideration. Some techniques such as walking simply require a researcher with appropriate clothing, while others may require special items such as drag apparatus, small animal traps or CO_2 tick traps. There can also be considerations involved in the distance required to travel to the study site, available vehicles and gas (Schulze et al. 1997).

2.2.1.6 Available Human Resources

When planning the project, it is necessary to know how much time per researcher will be required to complete the sampling. Based on research goals it may require one or multiple years to complete the project. For some methods it is best to have the same researcher or researchers perform the sampling between study sites, if data between sites will be compared. For multiple year projects it would be optimal to have the same researchers available throughout sampling (Ginsberg and Ewing 1989). Some sampling methods may also require

more time to perform or simply be less productive. For example, in my research project, each hour of drag sampling collected far fewer ticks than each hour of using tick traps. Knowing the requirements of each method can help determine what the human resource expectations will be for that method. Additionally, during project planning, knowing what human resources are available and for what period can help determine what sampling methods will be feasible.

2.2.2 Tick Collection Methods Review

Dragging, flagging, and walking are often grouped together as their approach and application are similar. Although, there have been documented differences in the ticks they catch and the environment they are used in, all three of these methods are applicable when looking at the prevalence of disease-causing organisms in tick populations and the risk of disease to humans and other species. This is because these methods are sampling questing ticks that are not actively feeding and are searching for their next blood meal. Infected ticks gathered in these methods are ticks that could pass a tick-borne disease they carry onto any host they may feed on (Ginsberg and Ewing 1989). These methods can also be standardized by the length of the flag, drag or walk or by the duration of time that each is performed (Falco and Fish 1989, Rulison et al. 2013). Although, CO_2 tick traps use a different approach, this method also gathers questing ticks and is applicable to studies looking at disease risk. However, length or time cannot be standardized using this method. Gathering ticks off hosts is often a good way of gathering ticks but this method does not gather questing ticks and is impacted by the fact that the animals the ticks are gathered off may be found in areas the ticks would not inhabit separately. It is also possible that the tick may have acquired a disease-causing bacterium from the current host and did not have that disease-causing organism prior to attaching to the

current host (Ginsberg and Ewing 1989). Following are some details on each of these sampling methods.

2.2.2.1 Dragging

Sampling by tick dragging or flagging involves pulling a white or light-colored cloth; typically flannel, canvas, denim, or corduroy, and typically 1 m^2 over vegetation to collect ticks that are seeking a host. In the case of dragging the material is connected to the entire length of a pole that has a cord at either end of it. The cord is held by the researcher who then drags the material behind them (Sonenshine 1991, Carroll and Schmidtmann 1992, Falco and Fish 1992, Schulze et al. 1997, Estrada-Pena et al. 2013, Rulison et al. 2013). Regular stops must be made to gather the ticks off the material to minimize the number of ticks that might be knocked off during the drag (Estrada-Pena et al. 2013, Nelson et al. 2015). This method is applicable when looking at the prevalence of disease-causing organisms in tick populations and the risk of disease to humans and other species but is also applicable in research where standardization is not a requirement. This method can be used to study tick densities, as it can be standardized by distance or duration (Falco and Fish 1989, Ginsberg and Ewing 1989, Estrada-Pena et al. 2013). Dragging is considered a more suitable method for flat surfaces such as the top of vegetation, leaf litter or open areas with lower uniform vegetation (Sonenshine 1993, Schulze et al. 1997). It is an inexpensive and easily performed method (Schulze et al. 1997). In areas with briars or vegetation that may hook onto the dragging device a stronger material such as denim may be more appropriate (Sonenshine 1993).

Dragging, as a sampling method, can be negatively impacted by multiple factors such as high winds or vegetation that has become wet by rain or dew (Sonenshine 1993, Schulze et al.

1997). It is also possible to lose ticks as they can be scraped off by vegetation or may drop off when they determine the dragging material is not a host. Minimizing dragging distance in between checking the dragging apparatus can help diminish these kinds of losses. Different distances have been referenced between 10 m to 20 m at which researchers have stopped to check the dragging apparatus (Falco and Fish 1992, Estrada-Pena et al. 2013, Rulison et al. 2013, Nelson et al. 2015). When using this method sampling bias can be introduced by variation in sampling technique that may exist between different researchers. It is best to try to keep the researcher(s) the same throughout sites (Ginsberg and Ewing 1989).

2.2.2.2 Flagging

In the case of flagging a piece of material is connected to a small pole at one end so that the apparatus resembles a flag. These can be made using the same materials with the same dimensions as a dragging apparatus. Weights can also be placed at the end of the flag material to help it sweep through the vegetation instead of just over it (Sonenshine 1991, Rulison et al. 2013). Flagging is considered appropriate for environments where you cannot drag through the area such as dense bushes (Sonenshine 1993). In some cases the terms dragging and flagging have been used interchangeably, but there are differences and researchers need to use the correct term when describing their methods (Ginsberg and Ewing 1989, Sonenshine 1993). Just as in dragging, flagging can be biased by variations in technique between researchers (Ginsberg and Ewing 1989, Sonenshine 1993). As this method is very similar to dragging, it can also be standardized by distance or time (Estrada-Pena et al. 2013).

2.2.2.3 Walking

Sampling ticks by walking is exactly what it sounds like. An investigator walks through

the study area and the ticks are then gathered off the researcher. In this method the researcher is the collection apparatus and the researchers clothing should be checked regularly just as any other collection device should be (Sonenshine 1993). This method can also be standardized by distance or time. It is also considered more appropriate for sampling in shrub vegetation (Falco and Fish 1989, Schulze et al. 1997). It has also been stated that this method may be more appropriate for sampling adults of species that quest higher in vegetation as opposed to larvae often found in leaf litter and vegetation closer to the ground. This may also be the best method for determining human risk of contracting tick-borne disease as this method looks at the number of ticks encountered by a human instead of just a piece of material (Ginsberg and Ewing 1989).

2.2.2.4 Carbon Dioxide Tick Traps

Carbon dioxide (CO_2) tick traps will usually have a cooler or container with holes on each side near the bottom. This container holds dry ice in the center of a platform. The holes allow the CO_2 to escape as the dry ice sublimates. On the outside edges of the platform there will be tape, sticky side up, or some type of sticky material that the ticks get stuck on as they try to get close to the source of the CO_2 (Wilson et al. 1972, Kinzer et al. 1990, Sonenshine 1993). These traps work because different tick species have been shown to have CO_2 receptors or like the *A. americanum* have been shown to be attracted by the CO_2 that is exhaled when humans and animals breathe (Wilson et al. 1972, Kinzer et al. 1990, Steullet and Guerin 1992). The CO_2 tick traps are widely used devices that can reduce sampling personnel requirements but do require the ability to easily obtain dry ice, have a way to transport the traps to the research site and to store the traps when not in use (Sonenshine 1993, Schulze et al. 1997).

CO_2 traps are more affective for certain species such as *A. americanum* that search out hosts (Ginsberg and Ewing 1989, Petry et al. 2010). These traps can also be placed in areas of high or low vegetations and do not have the same issues with thick vegetation or thorns that flagging and dragging apparatuses do (Gray 1985, Ginsberg and Ewing 1989). One issue with this method is that the area over which a single trap is effective can be impacted by tick species, tick life stage and the environment. Because of this, it is not possible to use this method to calculate tick density (Kensinger and Allan 2011). If the research project is specifically looking for a species that is not attracted by the CO_2 or if the goal of the project is to determine tick densities, then this will not be an appropriate method.

2.2.2.5 Sampling from Hosts

This collection procedure involves the trapping of animals and removing any ticks that may be currently feeding on them. This can be done by catching random animal species or in some cases depending on the research purposes specific species will be trapped. This can be an effective method as using preferred hosts can allow for collection of specific tick species even in low density areas (Ginsberg and Ewing 1989). It has also been identified as a method that is expensive and can require a lot of personnel hours to accomplish, but may be the least impacted by weather (Schulze et al. 1997). This method can be an issue for tick-borne disease research projects for two reasons. First, is that the ticks gathered were not actively questing and therefore not currently considered a risk to humans. Second, is that if the tick was actively feeding it could have picked up microorganisms from the blood of its host. Testing for pathogens in these ticks can lead to confusion in our understanding of tick, pathogen, and host associations. There is no way for us to tell if the tick was carrying the pathogen when it was

searching for its blood meal, if it was in the blood meal itself or if the pathogen would have been there when the tick went questing for its next blood meal (Kahl et al. 2002).

2.2.3 Tick-Borne Disease Research in Denton County, Texas

The research project for Denton County Texas was designed to look at what ticks were present and determine if they were carrying any species of *Rickettsia* bacteria in an area that does experience incidence of disease, but not high incidence, and therefore has not been a focus of tick research. The goal of the study was not only to look at disease risk in the area but to help fill in some gaps in the overall knowledge of ticks and tick-borne disease by collecting data in a low disease incidence area. The collected data could provide information on what species of *Rickettsia* might be present in that area and in the future the data could be used to analyze how environmental characteristics vary from high disease incident areas.

2.3 Methods

2.3.1 Study Areas

The study areas chosen were part of the Lake Lewisville Environmental Learning area (LLELA) and Clear Creak Natural Heritage Center (CCNHC) both located in Denton County, Texas. The first-year sampling was conducted in LLELA with CCNHC being added the second year.

2.3.2 Drag Sampling Method

A 1 m^2 piece of white corduroy cloth was attached at one end to a piece of PVC pipe that had a length of cord running through the pipe with the ends tied together. The cord was long enough to allow the entire cloth to lay on the surface during dragging. This cloth was dragged

over the leaf litter and low vegetation. The cloth was checked every 10 m for ticks (Falco and Fish 1992, Estrada-Pena et al. 2013).

2.3.3 Tick Trap Method

CO_2 traps were created using the basic design from Kinzer et al. 1990 and Sonenshine 1993. The base of the traps was a piece of thin board approximately 2ft by 2ft square. Duct tape was placed along the outside of the board sticky side up. A small square Styrofoam container was placed in the middle of the board with holes about 1 inch up from the bottom of the middle of each side. When the traps were placed approximately 1 pound of dry ice was placed in the Styrofoam container. The traps were left in their location for between 4 to 5 hours before they were inspected for ticks.

2.3.4 Tick Sampling and Documentation

Tick sampling the first year was performed from May 6th through September 15th, 2013. Each environmental area sampled within the LLELA study area was divided up into 100m transects and dragging was performed twice a week. Drag sampling only was done the first year. Tick sampling the second year was done from May 14th through July 9th, 2014 and was performed in LLELA and CCNHC. Tick traps were mostly used with dragging being performed periodically for comparison purposes. Any ticks captured were placed in 70% ethanol until further examination could be performed (Williamson et al. 2010). All ticks were examined visually to determine developmental stage and, for adult ticks, their sex and species. Pictures of each tick were taken using ZEISS Axio Zoom. V16 Fluorescence Stereo Zoom Microscope since DNA testing would require destruction of each tick.

2.3.5 Method Performance

Initially, the drag sampling was the method selected for tick collection, as it is a commonly performed method and an initial goal was to perform comparisons with previous research which had been conducted using this method. Drag sampling does sample questing ticks and from that standpoint would be appropriate for a study on ticks and tick-borne disease. I chose multiple 100 m transects in areas of LLELA and was actively dragging these sites from May 6, 2013 to September 15, 2013. During this period, a total of 8 ticks were gathered. It became apparent that either there were extremely few ticks in this area or the method I had selected was not appropriate. At this point it was necessary for me to reanalyze my approach or risk not being able to obtain enough data to continue the project.

After determining my initial research plan would not produce enough ticks, I reassessed the available options and used the previous year's tick sampling experience as well as the information discussed earlier in this paper to choose CO_2 traps as my second method. As a comparison, I continued to do periodic drags through areas where I had successfully gathered ticks using CO_2 traps. This was done to ensure that the difference in tick quantities caught was due to method and not due to variation in tick population from the previous year. Five ticks were caught by the dragging method during the second season of sampling. I also expanded my research area to include the CCNHC in case the LELLA research area simply had an extremely low tick density.

2.4 Results

Six ticks were caught by dragging between June and September 2013 and five between April and June 2014. The 114 ticks caught by trapping were caught between May and July of

2014. All but one nymph tick caught was of the *Amblyomma* genus. The nymph ticks within the *Amblyomma* genus were not able to be confirmed as *A. americanum* or *A. maculatum,* however, it is likely that these were mostly if not all *A. americanum* ticks because few *A. maculatum* adults were caught. Both *I. scapularis* were caught in May of 2014. The one caught by dragging was in LLELA and was an adult male, the one caught in CCNHC was by trapping and was a nymph.

Table 2.1: Number of ticks caught by each method.

Tick Species	Drag	Trap	Total
Amblyomma (nymph)	7	63	70
A. americanum	1	42	43
A. maculatum	0	4	4
D. variabilis	2	4	4
I. scapularis	1	1	2
Total	11	114	125

2.5 Discussion

Many factors should be considered when choosing an appropriate tick sampling method. After performing the first year of sampling during my project, I found that selecting the sampling method based on literature and desired research goals alone and not taking into consideration tick density expectations, tick species in the study areas and any environmental issues interfering with the sampling method did lead to unexpected issues. Except for a couple of fortunate drags and one drag resulting in catching many larval ticks which were not included in the study I found that dragging in this low tick density environment was not able to produce enough data to be useful. Adding the tick traps the second year resulted in a large increase in

the overall number of ticks collected. The frequency of drag sampling the second year was dramatically decreased but periodic drag sampling was performed to help show that the low amount of ticks collected the previous year was more likely related to the sampling method not being appropriate than there simply being lower tick abundance in the first year compared to the second year. The addition of the CO_2 trapping method allowed me to continue my project although it did require sacrificing the ability to look at tick densities.

The fact that the tick density in my study area is much lower than the densities seen in locations in the eastern United States, that I had initially hoped to make comparisons with, may be the primary reason that the dragging method did not produce as many ticks as I had hoped. There were additional factors, that when taken into consideration along with low tick density, led to me making modifications to my sampling method. First have being that the primary tick sampled in my research site was the *A. americanum* which is known to be attracted to CO_2 traps. An additional complication with multiple areas within my initial study area was the presence of plants from the *Smilax* genus with thorns that would often be present in the dragging areas. The thorns on these plants would tangle up in the dragging apparatus. Effort had to be made to avoid these plants which did impact the placing of different drag transects as well as cause damage to multiple drag apparatuses. After multiple encounters with these plants, I was enlightened to the necessity that research areas be visited and examined prior to committing to a method. It may even be appropriate to perform a test drive prior to making the final decision on which method would be best suited to the research goals while still working within the environment.

Facing the fact that my project was not working out as planned, I had to re-evaluate why

and how I had selected the sampling method. While the drag sampling method suited the research goals for the project, it did not match well with the tick density, certain environments within the initial study area and there was another method that had been shown to work better for the primary tick species *A. americanum*. While the drag sampling method could have allowed me to look at tick densities and make comparisons with other studies using that method, it could only do these things if it also allowed me to gather a sufficient sample size. If I had conducted an appropriate evaluation prior to the start of sampling I would have likely determined that this commonly used method would not work for my project. It would have also saved me valuable research hours and likely provided a larger sample for analysis.

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CHAPTER 3

DETECTION OF *Rickettsia* spp. IN TWO RECREATIONAL AREAS IN DENTON COUNTY, TEXAS

3.1 Abstract

There is a lack of information on areas that may present risk of exposure to ticks in places not endemic for tick-borne diseases. Lack of known exposure sites in areas where there are documented cases of tick-borne disease, but they are considered uncommon, can deter physicians from looking at tick-borne disease as a potential diagnosis. Physicians may only take these diseases into consideration if the patient indicates they have been bitten by a tick or have recently visited an area at high-risk for these diseases. If these diseases are not diagnosed early on, the length of time taken to diagnose a tick-borne illness can cause additional complications for an individual who has been infected (Eisen and Eisen 2007, Williamson et al. 2010, Hatcher et al. 2018). In the case of Rocky Mountain spotted fever (RMSF), which is caused by *Rickettsia rickettsii*, late diagnosis and treatment can be linked to higher risk of mortality (Dantas-Torres 2007, Regan et al. 2015, Hatcher et al. 2018). In this paper, two study areas in Denton County Texas, an area not considered to have endemic tick-borne diseases, are surveyed to determine what tick species visitors may be exposed to and if these ticks were carrying species of *Rickettsia*.

3.2 Introduction

Starting in October of 2004, the Texas Department of State Health Services has had the Tick-Borne Disease Research Laboratory at the University of North Texas Health Science Center test ticks removed from humans for infection with the bacteria *Borrelia, Ehrlichia* and *Rickettsia* (Williamson et al. 2010, Mitchell et al. 2016). The presence of a process to test ticks for disease

does show that there is concern about tick-borne illnesses in the state of Texas. However, tick-borne diseases such as RMSF, Lyme disease, and human monocytotropic ehrlichiosis are not considered endemic in Texas. Because of the lower incidence of these diseases in Texas, there has not been the same level of tick survey data collected in this state as in states where these diseases are highly prevalent (Williamson et al. 2010, Mitchell et al. 2016, Hatcher et al. 2018). Although not prevalent, there are occurrences of these diseases and they do currently present a risk to residents. Individuals who become ill with these and other tick-borne diseases in Texas may be at additional risk as doctors in the state may not initially consider tick-borne diseases during diagnosis of patients. Delays in accurate diagnosis can lead to delays in receiving appropriate treatment (Williamson et al. 2010, Hatcher et al. 2018). Collection of data in areas where these diseases are not prevalent could play a part in increasing our knowledge of the ecology of these emerging infectious diseases (Williamson et al. 2010).

In this study ticks collected in two areas in Denton County, Texas were tested for the presence of *Rickettsia* spp. In the United States there are four *Rickettsia* species that are well-documented as causing human disease. These known disease-causing species are *R. rickettsii*, *Rickettsia parkeri*, *Rickettsia felis*, and *Rickettsia akari*. Of these *R. rickettsii* and *R. parkeri* are tick-borne diseases while *R. felis* is flea-borne and *R. akari* is transmitted by mites (Parola et al. 2005, Shapiro et al. 2010, M. Biggs et al. 2016).

R. rickettsii is the etiological agent of RMSF was first clinically described in 1899 and it continues to be a cause of mortality and severe outcomes in the United States (Parola et al. 2005, M. Biggs et al. 2016). RMSF symptoms start five-to-seven days after the tick bite and include fever, headache, nausea and vomiting with lesions appearing on various parts of the

body. It is also possible that there can be respiratory issues, neurologic issues and circulatory failure (Bratton and Corey 2005).

The primary tick vector associated with *R. rickettsii* in the United States is *Dermacentor variabilis*. The *D. variabilis* species range goes from central North American over to the Atlantic Coast, up through southern Canada and down to the Gulf Coast of Mexico. There are also populations along the West Coast in California and southwestern Oregon (Parola et al. 2005, Berrada et al. 2011, Stromdahl et al. 2011, Minigan et al. 2018). Although *D. variabilis* is considered the primary vector for *R. rickettsii* Documentation has shown low incidence of it in *D. variabilis* even in areas of fatal outbreaks. In some cases where this has been documented other SFGR such as *Rickettsia amblyommatis* or *Rickettsia montanensis* were identified instead. The low prevalence in *D. variabilis* is considered interesting and leaves open the possibility of some human cases being attributed to less pathogenic rickettsiae. However, even with the low prevalence found in *D. variabilis,* severe and deadly human cases of RMSF continue to be reported in this ticks geographic range (Stromdahl et al. 2011).

D. variabilis is considered the primary tick vector, but there are other ticks that have been confirmed as vectors or as having the potential to be a vector. *Dermacentor andersoni* is a known vector in the western United States and there have been documented occurrences of *Rhipicephalus sanguineus* being a vector in Arizona and Mexico (M. Biggs et al. 2016, Tinoco-Gracia et al. 2018). *A. americanum* is a tick known to frequently bite humans with a species range in the United States that overlaps with reported RMSF cases (Levin et al. 2017). It has historical evidence implicating it as a vector, as well as experimental evidence showing that it is capable of being a competent vector. Although the evidence exists to support that it can play

an epidemiologically important role, there have not been many *A. americanum* ticks collected that have tested positive for *R. rickettsii.* It is believed that it may only play an infrequent role as a RMSF vector in North America (Childs and Paddock 2003, Parola et al. 2005, Berrada et al. 2011, Breitschwerdt et al. 2011, M. Biggs et al. 2016, Levin et al. 2017).

R. parkeri was first isolated in 1939 by R. R. Parker an entomologist and rickettsiologist. The tick it came from was an *Amblyomma maculatum* tick from Liberty County, Texas. R. R. Parker also determined that *R. parkeri* did cause a mild febrile disease in guinea pigs when they were inoculated with it (Goddard 2003, Paddock et al. 2004, Parola et al. 2005). It was not reported in the literature as a human disease-causing agent until 2004. The 2004 article discussed a case that occurred in August of 2002 where a 40-year-old male presented with mild febrile illness with multiple eschars and a maculopapular rash. DNA samples from this patient were found to be identical to existing GenBank sequences for *R. parkeri* (Paddock et al. 2004). The tick causing the first identified human infection with *R. parkeri* was not identified but the *A. maculatum* tick is considered the primary vector with most reported cases having been linked to transmission from this tick (Paddock et al. 2004, Eremeeva and Dasch 2015, Herrick et al. 2016). The range of the *A. maculatum* tick in the United States is along the Gulf of Mexico and in the states along the Eastern Seaboard. It's range also extends inland primarily into Oklahoma and Kansas (Sumner et al. 2007, Herrick et al. 2016).

3.3 Methods

3.3.1 Study Areas

Ticks were collected from two areas used for education and recreation in Denton County, Texas. Lewisville Lake Environmental Learning Area (LLELA) which is located on the

south end of Lake Lewisville and Clear Creek Natural Heritage Center (CCNHC) which is located within Lake Lewisville's upper floodplain. The CCNHC study site was added the second year of collection to help increase the total number of ticks collected.

3.3.2 Tick Collection

The first collection period was from May 6, 2013 to September 15, 2013 with the second tick collection period being between April 24, 2014 and July 9, 2014. The second year of tick collection was ended early after the primary areas used for collection at CCNHC were highly impacted after flooding. Because this study is concerned with the risk of tick-borne disease to humans, it is most appropriate to sample ticks that are questing. Questing ticks that are infected pose a current risk to humans. Ticks that are sampled off of hosts may have been infected by their current host, and while they could pose a risk to human during their next life stage, they would not have been a risk during the sampled life stage (Ginsberg and Ewing 1989).

Two methods were used to gather ticks. The first method used, dragging, was performed through the entire collection period but resulted in a low number of collected ticks. The second method was CO_2 tick traps, was added during the second year after it was determined that tick dragging would result in collection of an insufficient number of ticks. All ticks collected were labeled with information on the location, the date, and the method used to collect them. Ticks were placed in 70% ethanol for later identification and testing.

3.3.2.1 Drag Sampling Method

1m^2 piece of white flannel cloth was attached at one end to a wooden dowel and

weighted at the other end with small lead weights. This flannel cloth was drug over the leaf litter and low vegetation in order to capture ticks seeking hosts. The cloth was checked every 20 m for ticks (Falco and Fish 1992, Ostfeld et al. 1995, Allan et al. 2003). When ticks were captured, they were placed in a vial containing 70% ethanol to preserve them for later identification and DNA testing (Ostfeld et al. 1995, Allan et al. 2003).

3.3.2.2 Carbon Dioxide Traps

The tick traps used were based on those used by Kinzer et al. 1990 and Sonenshine 1993. The traps consisted of a piece of tempered hardboard approximately 2 ft by 2 ft square. Duct tape was secured sticky side up along the outer edge of the hardboard with a small Styrofoam cooler in placed in the center. The Styrofoam cooler had one hole on each side close to the bottom. Approximately 1 lb. of dry ice was placed in the cooler during each trapping session. The coolers were dropped off in the morning between 8-10 AM and were picked up approximately 4 to 5 hours later. Any ticks gathered were placed in 70% ethanol for later identification and testing.

3.3.3 Identification of Adult Tick Species and Gender

Because all ticks would have to be destroyed in order to test for any bacteria they may have been carrying, pictures were taken of the front and back of each tick using a ZEISS Axio Zoom V16 Fluorescence Stereo Zoom Microscope. This provided a permanent visual record for each tick sampled and allowed DNA extraction to occur independently of tick identification. Two keys were primarily used for identification. The first key was from the book *Pictorial Keys Arthropods, Reptiles, Birds, and Mammals of Public Health Significance.* The second key was

from an article titled *Pictorial Key to the Adults of Hard Ticks, Family Ixodidae (Ixodida: Ixodoidea), East of the Mississippi River*. Although the article references ticks east of the Mississippi River the ticks expected in my research area were included in the key (Keirans and Litwak 1989, Centers for Disease Control and Prevention (U.S.) 2000). An additional resource used was an online interactive key inspired by the key I used created by Keirans and Litwa (Bischof).

3.3.4 Tick Testing for Presence of *Rickettsia* spp.

Initial plans were to test for the presence of *Rickettsia, Ehrlichia* and *Borrelia* spp. However, the expectation in Texas would be to find *Borrelia lonestari* which is no longer thought to be a human pathogen (Stromdahl et al. 2018). For this reason, *Borrelia* spp. were removed from the study. During testing no presumptive positives were found for *Ehrlichia* spp. when using the Ehr DSB 330F and Ehr DSB 728R primer set for the DSB gene (Doyle et al. 2005).

3.3.4.1 DNA Extraction

The E.*Z.N.A.® Mollusc DNA isolation Kit* (Omega Bio-tek, Inc., Norcross, GA, USA) was used for DNA extraction. For this procedure, adult ticks were laterally bisected with half of the tick being used for DNA extraction and the other half stored in 70% ethanol at –80°C. For nymphal ticks, the entire tick needed to be used to ensure enough DNA could be extracted. After performing the DNA extraction, each sample was checked on a NanoDrop® 2000C Spectrophotometer (ThermoScientific) to confirm the nucleic acid purity was acceptable for downstream applications (ThermoScientific). The normally acceptable ratio of absorbance at 260 nm and 280 nm is approximately 1.8.

3.3.4.2 Polymerase Chain Reaction (PCR)

Following DNA extraction, PCR was performed on all samples using primers directed to the *Rickettsia* spp. *rompA* gene listed in Table 3.1. Agarose gel electrophoresis was used to identify presumptive-positive *rompA* amplicons and these were later sequenced.

Table 3.1: Nucleotide sequences of primers used for PCR of tick samples for *Rickettsia*

Primer name	Gene	Primer sequence, 5′ → 3′	Specificity	Reference
Rr.190 70P	*rompA*	ATGGCGAATATTTCTCCAAAA	Genus	(Regnery et al. 1991)
Rr.190 602N	*rompA*	AGTGCAGCATTCGCTCCCCCT	Genus	(Regnery et al. 1991)

3.3.4.3 Sequencing of *rompA* Amplicons

After quantification of each sample using a NanoDrop® 2000 spectrophotometer (ThermoScientific), each sample was diluted with molecular grade water to approximately 10 ng/µl. This diluted sample was then used for quantification of dsDNA on a Qubit ® 2.0 Fluorimeter using the Qubit® dsDNA HS assay kit.

The Nextera XT DNA kit was then used to fragment and tag samples, preparing them for sequencing in the MiSeq ® next-generation sequencer. The detailed instructions can be located in the *Nextera XT DNA Sample Preparation Guide* (Illumina 2012). In this procedure, a transposase randomly cuts the DNA creating double-stranded breaks with staggered ends where an adapter sequence is attached. The adapter sequences are used in a limited cycle PCR to amplify the DNA fragment and add index sequences at both ends. Following this, the indexed samples were purified using the Agencourt AMPure® XP magnetic beads to remove unincorporated dNTPs, salts, primer dimers, primers and other contaminants (Beckman-Coulter). This is also size selection step that is used to remove short indexed fragments. The

results of the process were fragments of an average size of 250 bp plus the additional indexed adapter sequences.

Next, a library normalization was performed to ensure balanced library representation when the samples are combined to create the pooled samples. The completed pooled library is made up of single stranded DNA. 600 µl of this pooled library was loaded into the MiSeq® reagent cartridge to be sequenced.

3.3.4.4 Sequence Alignment and Identification of *Rickettsia* Species

Unipro Ugene version 1.31.1 software was used to align sequences. The BWA-MEM mapping tool, from withing Unipro Ugene, was used to perform each alignment. The BWA-MEM algorithm is part of the Burrows-Wheeler Aligner (Li and Durbin 2010, Li 2013). A consensus sequence was created and within Unipro Ugene was used to query the NCBI Blast database (Okonechnikov et al. 2012).

3.4 Results

The number of each tick species caught in each study location is listed in Table 3.2. The *Amblyomma americanum* tick species was gathered far more than any other species. With the number of *A. americanum* ticks gathered being much higher than any other species of adult tick, it is highly likely that a vast majority, if not all, of the *Amblyomma* nymphs were also *A. americanum*. However, the nymphal ticks were not identified beyond the *Amblyomma* genus.

Table 3.2: Tick species caught in each study location

Tick Species	Total	LLELA	CCNHC
Amblyomma (nymph)	68	10	58
Amblyomma americanum	42	8	34

Tick Species	Total	LLELA	CCNHC
Amblyomma maculatum	4	3	1
Dermacentor variabilis	4	2	3
Ixodes scapularis	2	1	1

Eleven presumptive positives for *Rickettsia* were found. There were seven *A. americanum* nymphs found to be positive with two positives being from adult *A. americanum* ticks, one from a *D. variabilis* tick, and one from an *Ixodes scapularis* tick. All but one of these had strong matches to *Rickettsia amblyommatis.* The one that did not was from the *I. scapularis* tick, and it showed multiple matches to different *Rickettsia* spp. with many listed as *Ixodes* endosymbionts. As shown in Table 3.3, most of the ticks were collected from CCNHC with nine of the presumptive positives being collected there and only two of the nymphs being collected from LLELA.

Table 3.3: Study site where ticks positive for *Rickettsia* were caught

Tick Species	LLELA	CCNHC
Amblyomma (nymph)	2	5
Amblyomma americanum	0	2
Dermacentor variabilis	0	1
Ixodes scapularis	0	1

3.5 Discussion

Although there were no ticks carrying *Rickettsia rickettsii*, the causative agent of RMSF, there were 11 ticks found to be carrying *R. amblyommatis* between the two study areas*. R. amblyommatis* was originally isolated in 1973 from an *A. americanum* tick and was designated strain WB-8-2^{T} but never formally named. Previously in scientific literature, it was referred to as

'Candidatus Rickettsia amblyommii' before being called *R. amblyommatis* (Karpathy et al. 2016). It is commonly seen in *A. americanum* ticks with noted frequency of 40-70%. It has also been found in other *Amblyomma* spp. as well as being previously detected in *D. variabilis* (Fritzen et al. 2011, Parola et al. 2013, Karpathy et al. 2016, Santibanez et al. 2018) Initially, it was not thought to be pathogenic, but more recent evidence has indicated that this organism can trigger strong immune response in humans (Apperson et al. 2008, Karpathy et al. 2016). A report in 1993 implicated *R. amblyommatis* as a possible cause of infection among military personnel (Moncayo et al. 2010). In Tennessee, it was found that patients with specific reactivities to it often suffered headache, fever and myalgia, with thrombocytopenia, anemia, rash and eschar occurring in <50% of suspected cases (Delisle et al. 2016). A North Carolina patient developed a rash at the bite site of an *A. americanum* tick determined to be carrying *R. amblyommatis* (Billeter et al. 2007).

Beyond the possibility that *R. amblyommatis* does cause a mild illness there have been studies and discussions on how the presence of *R. amblyommatis* might impact *R. Rickettsii* both in ticks and humans (Karpathy et al. 2016). One study found that *A. americanum* larvae infected with both showed a decrease in *R. rickettsii* being maintained in the tick into the nymph stage when compared with those ticks infected with *R. Rickettsii* only. However, the acquisition of *R. rickettsii* was not impacted by existing *R. amblyommatis* infection in nymphs or adults with the vector competence determined to be not significantly impacted by the presence of *R. amblyommatis* (Levin et al. 2018). Additionally, concerns have been raised that some reported cases of RMSF may not have been caused by *R. Rickettsii*, but by *R. amblyommatis* or other SFGR. This issue is present because different species of SFGR have been found to be

serologically cross-reactive. Due to this the use of the *R. rickettsii* antigen for serologic testing can miss identify incidents of SFGR caused but other species (Apperson et al. 2008, Moncayo et al. 2010, Vaughn et al. 2014, Delisle et al. 2016)

The identification of multiple ticks, primarily *A. americanum* ticks, carrying *R. amblyommatis* can be useful information for those trying to increase understanding of tick-borne disease risk in Texas. *R. amblyommatis* was believed to be nonpathogenic or a symbiont. However, there has been increasing documentation indicating that *R. amblyommatis* may be causing illness among those infected or even leading to incorrectly reported cases of RMS. Its presence may also be impacting *R. rickettsii* and acting as an interference in the environments where they co-exist. *R. amblyommatis* is commonly found in *A. americanum* ticks which are known to be aggressive when it comes to biting humans. With it frequently being found in this tick known to frequently bite humans, further investigation is warranted to increase our understanding of it and how it is playing a role in SFGR in the state of Texas.

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CHAPTER 4

CREATION OF SPECIES DISTRIBUTION MAPS OF THE LONE START TICK (ACARI: IXODIDAE) AND *Rickettsia amblyommatis* (RICKETTSIALES: RICKETTSIACEAE) INFECTED TICKS FOR ANALYSIS OF SPOTTED FEVER GROUP RICKETTSIOSIS IN TEXAS

4.1 Abstract

Understanding the distributions of ticks and the diseases they carry, in relation to the cases of human disease they cause, can be a very complex and difficult task. The distributions of both ticks and infectious agents can vary in different ways based on the environment. A specific challenge faced when trying to further understand these dynamics is the type and availability of data. Often when dealing with humans and disease the data available will not be at a point level, but often provided within specific political boundaries. In this study Texas zip code level data on *Amblyomma americanum* ticks with and without *Rickettsia amblyommatis* were used to create maps of potential species distributions. These species distributions are analyzed to determine if any differences exist between the two and to compare them with a map of rates of spotted fever group rickettsiosis with the goal of providing further understanding of the relationship between this tick, this disease, and how they might play a part in spotted fever group rickettsiosis (SFGR) cases in Texas.

4.2 Introduction

R. amblyommatis (previously called *Rickettsia amblyommii* or *Candidatus* Rickettsia amblyommii) was believed to be a symbiont in certain tick species, but is currently implicated as a possible human infectious agent that may cause a mild form of illness (Apperson et al. 2008, Gleim et al. 2016, Hardstone Yoshimizu and Billeter 2018). It has also been suggested that

in the presence of *R. amblyommii*, it may be more difficult for adult female ticks to pass on *Rickettsia rickettsii*, the etiological agent of Rocky Mountain spotted fever (RMSF), to offspring (Blanton et al. 2014, Rivas et al. 2015, Levin et al. 2018). In guinea pigs *R. amblyommii* has been shown to impart an immune response which lessens the severity of later infection with *R. rickettsii* (Blanton et al. 2014, Rivas et al. 2015). With the possibility that *R. amblyommatis* may play a role in causing disease, decreasing risk of contracting RMSF by helping limit *R. rickettsii*, or decreasing the severity of RMSF for those who do contract it, there is importance in furthering our understanding of this organism. Having a better understanding of the distribution of *R. amblyommatis*, the distribution of the aggressive human biting tick *A. americanum* that is commonly known to carry it, and how they both relate to locations of human incidence of SFGR may lead to a better understanding of the roles being played by each (Hardstone Yoshimizu and Billeter 2018, Santibanez et al. 2018, Pascoe et al. 2019).

Due to the application in this study, the models created are being referred to as species distribution models (SDM) and are considered representations of potential distributions based on available data. However, documentation cited may refer to either SDM or ecological niche modeling (ENM) as these terms have been used incorrectly or interchangeably. Typically, they both involve the correlation of environmental variables with known species location data (Peterson and Soberón 2012).

The concept of looking at disease spatially is not new. John Snow is one of the first known to map cases of disease. In 1854 he plotted cholera deaths in a district of London which he used to help identify a specific street water pump as a likely source of the cholera epidemic (Koch and Denike 2009, Ruths 2009). More recently, in public health and its application of

disease mapping, disease incidents are often addressed spatially with environment being neglected and looked at as site of exposure and not as a piece of the puzzle contributing to our understanding of disease transmission (Peterson 2014). There have been increases in the use of ecological tools for use in assessing disease risk. This is likely due to the understanding that transmission of disease is related to species being present in an area which is linked to, and not independent of, the environmental conditions present (Peterson 2014).

One individual who has helped further the approach of using species distributions and ecological niche modeling to map areas of disease risk is A. Townsend Peterson. He has advanced this area through both informative journal articles as well as books providing direction on mapping disease risk within an ecological and biogeographical framework. He states his first application of incorporating ecological and biogeographical approaches to disease risk mapping was in 2002 when it was applied to Chagas disease vectors and reservoirs in Mexico and Brazil (Peterson 2014). In one paper species distributions of known Chagas disease vectors and reservoirs were created for Mexico. In this paper Peterson provided the insight that this type of comparison of patterns between vectors and reservoirs in relation to geographic and ecologic space can provide hypotheses for future research projects (Peterson et al. 2002).

Previously, Ostfeld, Glass & Keesing, 2005, divided disease risk maps into the three categories: distributions of either incidence of host disease (often humans), vertebrate reservoirs, or arthropod vectors. All categories do come with their own pros and cons. The category most applicable to this study is distribution maps based on arthropod vectors. A primary referenced limitation for this application is that risk is more closely correlated with

vectors that are infected with the pathogen being studied as opposed to just the general presence of the vector (Ostfeld et al. 2005). Although this is known, many vector distribution maps are created without specific infection information. In many situations it may be that this information is not available. Although these maps can provide information on risk for contact with the vector, these maps cannot provide information on risk of contact with an infected vector.

Although the inclusion of environmental elements is a fairly new approach when mapping factors related to disease risk, there are many papers that have used this type of approach. Agustin Estrada-Peña (1998) studied habitat suitability of *Ixodes scapularis* in the United States and Canada. To create the habit suitability map, a cokriging technique was applied using a dataset of 346 records of *I. scapularis* ticks and temperature and vegetation data from an Advanced Very High Resolution Radiometer (AVHRR) scanning system from the National Oceanographic and Atmosphere Administration (NOAA). This map was created with the knowledge that this type of analysis can be used to provide direction to field work projects with goals of determining actual distribution limits of this tick and also providing information that can be used to make predictions of range changes based on impacts of global change .

An approach seen more frequently is the use of tick data to create tick species distributions. These can have multiple different types of goals. In these projects there is no information on infection. In a paper from Jean-Paul R. Soucy et al., 2018 the Maxent program was used along with passive surveillance data and microclimatic variables to provide an ENM for *I. scapularis* in Ottawa, Ontario, Canada. Their goal was to locate areas of higher habitat suitability where there was increased risk of coming into contact with an *I. scapularis* tick within

the study area. They also wished to support the use of passive surveillance data. Wang et al., 2019, also using Maxent, did a comparison of distributions of three different species within the *Dermacentor* genus. In their study their goal was to provide insights into the ecology of these ticks for use in development of effective tick control.

An approach that is not commonly seen in the literature creates distributions with the use of some type of infection data creating an SDM or ENM representing the distribution of a disease-causing organism. The rarity of this approach is made apparent by the fact that I was only able to locate one example to discuss here. In an article by Mak, Morshed & Henry, 2010, ENMs were created with the Desktop GARP 1.1.6 program. There were three ENMs created. One for the *Ixodes pacificus* tick, one for the *Ixodes angustus* tick, and one for *Borrelia burgdorferi*. The data for *B. burgdorferi* was obtained from positive tests from tick and rodent samples. The project was able to show that the tick distributions created were larger than the actual area of *B. Burgdorferi* distribution which was consistent with previous tick and mouse field data. This article does show that there is a distinct difference in the representation of disease risk between SDMs of known vectors and SDMS created using locations of infected vectors or reservoirs.

There are multiple approaches that are currently available to create distributions of disease agents, their vectors and the environments where they are found. The algorithms used by these different approaches will typically look at patterns between disease cases or vector location and geographic or environmental information (Elith and Graham 2009, Blackburn 2010). Maxent was chosen to create the SDMs for this study as it ranks among highly

performing SDM creation methods and is able to remain robust when working with datasets with location error (Elith et al. 2006, Graham et al. 2008).

4.3 Methods

The methods in this study include the preparation of data for use in Maxent which uses a maximum entropy method to produce predictive ENMs and SDMs (Phillips et al. 2004, Phillips et al. 2006). Preparation included the use of the SDMToolbox (Brown et al. 2017) and ArcMap 10.6.1 (ESRI 2019).

4.3.1 Presence Data

Tick data were provided by the University of North Texas Tick-Borne Disease Research Laboratory (UNTHSC). The ticks in the dataset were ones that had been removed from humans in Texas and submitted for testing between October 2008 and April 2015. The dataset contained species of tick, zip code location for each tick and if the tick had been infected with *Rickettsia*. Although specific coordinates were not available in this dataset, and there is expected error in the created SDMs due to this, it has been shown that SDMs are fairly robust in relation to this issue with Maxent ranking among the most robust to location error of methods tested (Graham et al. 2008).

4.3.2 Preparation of Tick Data

Tick and infected tick distribution models created were for *A. americanum* ticks and for *A. americanum* ticks infected with *R. amblyommatis*. The zip code level data from October 2008 to April 2015 provided by the UNTHSC was reviewed. Cases involving *A. americanum* were separated out and duplicates were removed. Duplicates were considered ticks submitted on

the same day from the same zip code. Frequencies by zip code were determined for all *A. americanum* ticks and separately for only the *A. americanum* ticks that tested positive for the presence of *R. amblyommatis*. These frequencies were each imported into ArcMap 10.6.1 which was used to randomly assign coordinates to each of the ticks, or in the case of *R. amblyommatis* each of the infected ticks. The random coordinates were assigned within the zip code that was attributed to each tick or infected tick.

Presence-only methods can be impacted by sampling bias. Biased or clumped samples often show spatial autocorrelation and can result in overfit models with performance accuracy that is overstated. When working with presence-only data this issue is a serious concern. Correcting for this bias is recommended and can result in improved predictive model quality (F. Dormann et al. 2007, Phillips et al. 2009, Veloz 2009, Kramer-Schadt et al. 2013, Merow et al. 2013, Shcheglovitova and Anderson 2013, Boria et al. 2014, Jarnevich and Young 2015). Multiple steps were taken, which included the initial removal of duplicates and choosing the Maxent settings option to remove duplicate presence records to ensure that there was only one sample record per grid cell. Beyond this, both sets of data were spatially rarefied at a resolution of 10 km using the *Spatially Rarefy Occurrence Data for SDMs* tool available in the SDMtoolbox.

Because the dataset being used contained presence-only data it was necessary to have Maxent create background points (also called pseudo-absences) in areas where presence or absence had not been measured. This information with the presence data and environmental data allows Maxent to predict the probability of presence (Elith et al. 2011, Merow et al. 2013). Controlling where the background points are placed by Maxent through use of a bias file can be

helpful in minimizing issues with sampling bias by aligning the background selection more with any bias that may exist in the presence data. In the SDMtoolbox, a gaussian kernel density bias file that applied a 30 km sample bias distance was created using the remaining samples in each dataset after rarefication.

4.3.3 Preparation of Environmental Data

The standard Worldclim 2.0 variables at a resolution of 10 minutes (~18.4 km) were used for the environmental data (Fick and Hijmans 2017). This resolution was chosen to help account for the spatial error involved in using zip code level data. Initial models were run in Maxent using the jackknife function to assess which variables mattered most for each model (Baldwin 2009, Phillips 2009, Elith et al. 2011). Although it has been stated that there is less need to remove correlated variables in Maxent, it has also been shown that using highly correlated variables can result in difficult to interpret models and in some instances lead to changes in the relationship between the variables and habitat suitability leading to unexpected and illogical results (Elith et al. 2011, Glover-Kapfer 2015). Due to these concerns the *Remove Highly Correlated Variables* tool in the SDMtoolbox was used to analyze the correlation between the standard Wordclim 2.0 variables. Not all variables that mattered in relation to each SDM were able to be used due to correlation. The variables chosen were based on those that mattered for the SDM but that did not have a correlation of > 0.75. The variables chosen for the *A. americanum* ticks were mean diurnal range, minimum temperature of coldest month, mean temperature of driest quarter, precipitation of driest quarter and precipitation of warmest quarter. The variables chosen for the model of the *R. amblyommatis* infected ticks were mean diurnal range, maximum temperature of warmest month, mean temperature of

wettest quarter, mean temperature of driest quarter and precipitation of coldest quarter. The environmental raster files used were all clipped in a square around the state of Texas leaving a buffer of at least 50 km outside the state boundaries (Brown 2017).

4.3.4 Additional Settings Selections in Maxent

Even though considerable research went into determining the default settings in Maxent, it is poor practice to take the "black box" approach of simply running a model with the defaults and not determining what settings are most appropriate for the specific application (Peterson et al. 2011, Merow et al. 2013, Morales et al. 2017). Before final Maxent settings were determined, options were explored, and decisions were made based on available information and the application.

For the sample sizes being used, the features that would be included by default are the linear, quadratic, product and hinge features. The choice was made to remove the product feature as it can lead to models that can be more easily interpreted (Elith et al. 2011). A total of 30 replicates were performed with a replicate type of subsample. Choosing a subsample allowed the test percentage to be specified (Jarnevich and Young 2015, Morales et al. 2017). Random seed was selected, and the random test percentage set at 50%. This percentage was chosen due to the low number of samples in each set. Splitting the dataset in half was determined to be the best option as it allowed the largest amount of sample to be used for both the training and testing data while keeping these independent of each other (Peterson et al. 2011). The maximum number of background points was left at the default value of 10,000. It has been found that best results are attained when using a large amount of background points (Barbet-Massin et al. 2012). Maximum iterations setting was increased from 500 to 5000 to

ensure that the model would reach the convergence threshold (Young et al. 2011, Stohlgren et al. 2015). Setting the maximum iterations to 5000 was a far higher setting than required, but it did go over 500 at times so it was appropriate to increase the setting beyond the default. The threshold rule applied was Minimum Training Presence which is designed to include 100% of the presence points used in model training in the final model. This minimizes omission errors but not commission errors. The omission error was given priority for two reasons. First, the background points often are not characteristic of an environment that lacks the conditions necessary for species presence and because we are looking at infected ticks or tick distributions where disease may potentially be transmitted (Peterson 2014). The regularization multiplier setting can be used to control model complexity. After all other parameters had been determined, the Akaike information criterion was used to determine the regularization multiplier that would allow an appropriate model complexity level for both models (Peterson 2014, Glover-Kapfer 2015). The final regularization multiplier used for *R. amblyommatis* was 2.4 and for *A. americanum* was 1.3.

4.3.5 Spotted Fever Group *Rickettsia* Rate Map

Data on incidents of SFGR in the state of Texas between 2008 and 2013, provided by the Texas Department of State Health Services, was used along with a state of Texas county shapefile for 2010 from a cartographic boundary file downloaded from Topologically Integrated Geographic Encoding and Referencing (U.S. Census Bureau 2019). The Total population by county for 2010 was obtained from American FactFinder (U.S. Census Bureau 2011). Together these were used to create a map of the rate of disease by county for the period of 2008 to 2013.

4.4 Results

Review of the UNTHSC data, prior to adjustments made for use in Maxent, showed that *A. americanum* ticks accounted for over half of the collected ticks identified as being from Texas during the time period of October 2008 to April 2015. The information on what ticks made up this dataset is in Table 4.1. Of the 1,092 sampled ticks, 251 were found to be carrying some species of *Rickettsia*. Of these, 177 were determined to be *R. amblyommatis*. There were 5 *A. maculatum* ticks carrying *R. parkeri* whose locations did fall within the predicted distributions of the *R. amblyommatis* infected ticks and the *A. americanum* ticks. The remaining 69 infected ticks had *Rickettsia* spp. that were either listed as endosymbionts or the species is one that is currently considered avirulent or as having unknown virulence (Labruna et al. 2007, Noriea et al. 2015, Padgett et al. 2016, Allerdice et al. 2019). There were no ticks that tested positive for *R. rickettsii*, the causative agent of RMSF, during the sample period covered.

Table 4.1: Tick composition of the University of North Texas Health Science Center data collected between October 2008 and April 2015.

Tick Species	Frequency	Percent
Amblyomma americanum	594	54.4
Amblyomma cajennense	32	2.9
Amblyomma maculatum	60	5.5
Dermacentor variabilis	167	15.3
Ixodes scapularis	75	6.9
Rhipicephalus sanguineus	143	13.2
Miscellaneous or unidentified	21	1.8
Total	1092	100

The SDMs shown in figure 4.1 show the probability of presence of each species. The closer the probability value is to 1 the more likely the species is present in that area. The *R.*

amblyommatis SDM (Map A) created using only the data on infected *A. americanum* ticks shows the higher presence expectation areas are to the east of the state with upper mid-range probability going down the east side of the bottom tip, with much lower probability covering the center of the state and on to the west.

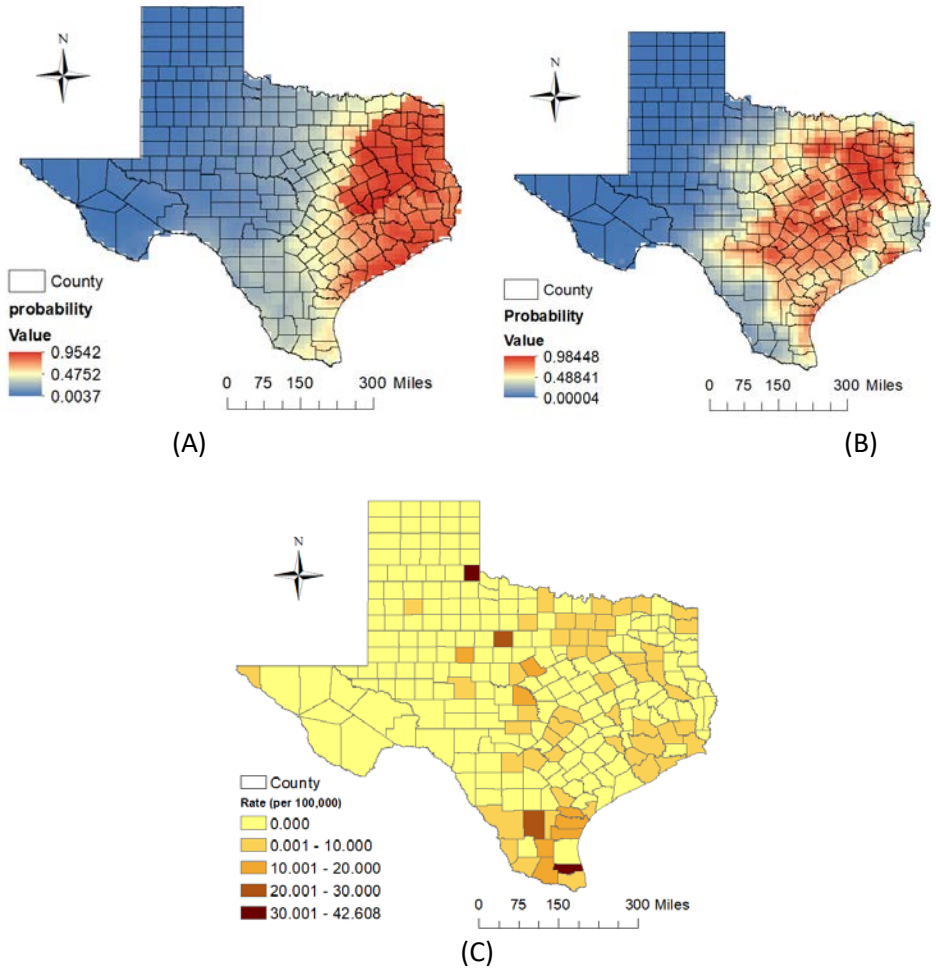

Figure 4.1: Species distribution maps and spotted fever group rickettsia rate map: (A) Species distribution map of *R. amblyommatis* October 2008 and April 2015; (B) Species distribution map of *A. americanum* October 2008 and April 2015; (C) Map of spotted fever group rickettsia rates in Texas between 2008 to 2013.

While the *A. americanum* SDM (Map B) created using infected and uninfected *A. americanum* ticks does not appear to show as much area with as high a probability as the *R. amblyommatis* SDM, but does show a large area of high probability of presence covering most of the eastern

part of the state that goes well into the center of the state, with mid-range and higher probability going down the eastern side of the southern tip of the state.

When reviewing the map of rates of SFGR cases in Texas (Map C) the eastern and central parts of the state have more counties with cases than the western part of the state. The counties with the higher rates appear either in the far south of the state or in the central and north central part of the state. During the period this map represents, from 2008 to 2013, there were only 344 total cases in the state. The counties with the four highest rates on the map, rates above 20.001 per 100,000 population, do not reflect areas where a high number of SFGR cases were reported. These were also counties that had a population of under 25,000. There were only four counties in the state that had the number of cases go into the double digits. With low incidence per county the created rate map will be impacted by the small numbers problem (MacEachren et al. 1998). However, the map does still reflect areas where there have been reported cases of SFGR in Texas. Three of the counties with the highest number of reported cases were in southern Texas along the coast. The highest number of reported cases was in Hidalgo County which had 84. Hidalgo is on the southern coast of Texas immediately west of Cameron County which is the county at the southern tip of the state. The county with the second highest reported cases was Nueces County with 64. Nueces County is also in southern Texas and is the fourth county north of Cameron County. Cameron County itself had the fourth highest with 17. The county with the third highest number of cases, with 29, is Travis County which is in South Central Texas.

4.5 Discussion

The tick dataset being available with only zip code level location information was a

limitation of this study. Not having point level data limited the resolution at which the environmental data could be used. It was also responsible for the choice to not use land use or ecoregion data as there was concern with how that data might work with the built-in error caused by the zip code level data. However, it is not uncommon to have this level of data when working with public health information.

The two species distribution maps do show a definite difference between the predicted possible distribution of the *A. americanum* tick and that of the of *A. americanum* ticks infected with *R. amblyommatis*. This may support using infected ticks to create distributions of areas that may pose a higher risk to humans of contracting a specific tick-borne disease. The SDM of the ticks infected with *R. amblyommatis* may provide useful information for future research into the conditions that are required to support this tick infection. Since the SDMs for the *A. americanum* ticks and the ticks infected with *R. amblyommatis* are different, it is likely that there are environmental characteristics that are necessary for the presence of *R. amblyommatis* that are not present in the entire *A. americanum* distribution. It may be that the area where we see the possible distribution of *R. amblyommatis* has a competent reservoir species or other environmental requirement needed to sustain a population of infected ticks. Species referenced as being possible reservoirs for *R. amblyommatis* include birds, rodents, companion animals, and wildlife (Hardstone Yoshimizu and Billeter 2018). In a study done analyzing *A. americanum* nymph blood meals to identify hosts, the top five identified host included Ruminantia taxa (likely white-tailed deer in that study), Galliformes order, Passeriformes order, Sciurus genus, and Leporidae family (Allan et al. 2010). Species from all of these can be located in Texas. Further research would need to be conducted looking for

overlaps in likely *A. americanum* blood meals with possible reservoir species that also overlap in the area of possible *R. amblyommatis* distribution but are lacking or have lower population in general *A. americanum* distribution. In addition to the possibility of a competent reservoir being present, *A. americanum* is also likely playing a role in the perpetuation of *A. amblyommatis*, given that it can transmit *R. amblyommatis* both transstadially to its next life state and transovarially to its offspring (Levin et al. 2018). It is also possible that this area may be able to support ongoing tick infections in other ticks that can be found in the area (e.g. *A. maculatum, A. cajennense)* that have been shown to carry *R. amblyommatis* (Nieto et al. 2018, Santibanez et al. 2018).

The species distribution maps, when compared with the map of rates of SFGR in Texas, do show that the *A. americanum* tick as well as *R. amblyommatis* may be playing a role in the cases of Spotted fever Rickettsiosis reported in Texas. However, understanding how they are playing a part is difficult to determine based on this information. There are counties with incidence of SFGR that show up within both the tick distribution and *R. amblyommatis* distribution range. To further complicate this analysis the primary vector of RMSF is *D. variabilis.* It has been previously shown that only 0.3% of the state has high probability of suitable habitat for this primary vector. Less than 10% of the state has a 45% or greater probability of suitable habitat. The distribution area with a 7.2% or greater probability of having a suitable habitat for *D. variabilis* overlaps well with the distribution area of *A. americanum* shown here, with the exception that one of the areas that has high probability of suitable habitat for *D. variabilis* is at the southern tip of Texas which may relate to the higher rates shown here in the SFGR rate map (Atkinson et al. 2012). *A.* maculatum also has a range along

the Gulf Coast of Texas which does encompass the area of higher rates seen at the southern tip of the state. The *A. maculatum* tick is a known vector for *R. parkeri,* another SFGR that is known to cause disease. Beyond *D. variabilis*, another tick that has been implicated in outbreaks of RMSF is *R. sanguineus*. This tick also has a wide range which according to the CDC does cover the entire state of Texas (Centers for Disease Control and Prevention 2019). This tick may be responsible for spreading incidents of RMSF or SFGR in areas in the west where other ticks are not expected to be present.

It has also been suggested that some cases in North Carolina reported as RMSF may have been caused by *R. amblyommatis* due to the issue with cross-reaction when performing serologic tests and the high presence of *R. amblyommatis* (Apperson et al. 2008). With only 5 ticks showing positive for *R. parkeri* and no ticks in the dataset used for this study in Texas being positive for *R. rickettsii*, the disease-causing agent of RMSF, it is likely that at least some of these cases were caused by a different spotted fever group rickettsia. So, the possibility does exist that some of these incidences may have been caused by infection with *R. amblyommatis,* though the rate of *R. amblyommatis* seen in *A. americanum* ticks would lead to expectations of higher human infection rates that do not appear to be present in literature reviewed or here in Texas.

One thing that does appear to be probable is that *R. amblyommatis* may be limiting *R. rickettsii* in this environment in Texas. A study in 2018 looked at vector competence of *A. americanum* ticks for *R. rickettsii* when they were previously infected with *R. amblyommatis.* Data from this study indicates that *R. amblyommatis* may be limiting *R. rickettsii* by causing transovarial interference and decreasing the possibility of *R. rickettsii* being passed on to the

next generation from the mother. Additionally, this study determined that dually infected ticks transmitted *R.* rickettsii to their progeny 30.8% of the time while ticks infected with only *R. rickettsii* transmitted it to 48.1% of the time. In this same study it was also shown that in *A. americanum* ticks previously infected with *R. amblyommatis* and subsequently infected with *R. rickettsii* that there may be a reduction in the proliferation of *R. rickettsii.* Guinea pigs that were subsequently exposed to these dually infected ticks appeared to develop a milder case of infection than if exposed to ticks only infected with *R. rickettsii.* This may be due to the reduction in proliferation or it may be due to the "interference Phenomenon" where to some degree the presence of *R. amblyommatis* may help, to certain extent, protect against the more virulent *R. rickettsii* transmitted from the same tick (Levin et al. 2018). Though there is no research showing this, it may be that this same relationship exists between *R.* amblyommatis and *R.* rickettsii in other tick species that are capable of being vectors for both. Based on the research done with ticks infected both with *R. amblyommatis* and *R. rickettsii* it is possible that the presence of *R. amblyommatis* may be playing a role in the general low numbers of SFGR incidents reported in within its predicted distribution range.

Further research that can be conducted with the SDMs created would be to look at the differences in the environment between areas with higher probability of *R. amblyommatis* presence compared to the distribution of *A. americanum* to determine if there are any variations that can be seen based on landscape, land use, environmental conditions and possibly any known distributions of possible competent reservoirs. This may provide additional information towards understanding the ecology of *R. amblyommatis*. This information along with tick surveys providing point level data would provide necessary information for improved

future models. Further studies on known tick-borne diseases using infected ticks as presence data to create SDMs are needed to further explore the potential of using this approach. It may be possible that this approach has the potential to provide information on areas of increased risk and to possibly further our understanding these diseases.

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CHAPTER 5

SUMMARY AND CONCLUSIONS

This chapter summarizes the findings of this dissertation and provides a discussion of these findings and potential for further research.

5.1 Summary

Tick-borne disease in Texas is considered non-endemic but there is still a risk to its residents with an additional risk of delayed diagnosis and delay in treatment (Williamson et al. 2010). For these reasons understanding ticks and tick-borne disease in the state can provide benefit. Additionally, there may be information obtained that will offer understanding as to why these diseases are less prevalent and to provide a baseline for further analysis if their prevalence changes in the future.

In this dissertation, tick-borne disease in the non-endemic areas of Denton County, Texas and more broadly the state of Texas was explored using skills from multiple disciplines in order to better understand multiple aspects of ticks, tick-borne disease research and tick-borne disease in these areas. Simply understanding the state of tick-borne disease in areas where it is endemic does not provide the full scope of understanding necessary to address this public health issue. It is necessary to see the full picture in order to compare the differences that exist. For this reason, expanded knowledge in non-endemic areas such as the areas used in this dissertation is beneficial not only to those within the non-endemic areas studied but can also provide information that can offer insight into tick species, the disease-causing agents they carry and environments they can be found in.

The research covered started in chapter 2 at the point of tick collection, which is the starting point of many projects in this field. In working through the process of tick collection many pitfalls were discovered that are not well covered in the literature. In this part of the dissertation I was able to impart knowledge on considerations that need to be addressed to those venturing into tick collecting for the first time. All tick collection methods do not work in all locations or applications. Density of ticks in the environment, the species of ticks in the environment, the environment itself, budget, manpower and the goal of the research project the ticks are being collected for can all impact what method or methods are selected. Making decisions prior to knowing information on each of these can lead to either incorrect method selection that will not suit the goals of the project or selecting a method that may not work with the species of tick or the environment that the collecting is taking place in. I feel that the information I have provided in a single document would have been of great benefit and would have saved a lot of time had I had it prior to starting my project. As this field involves those from many disciplines, I believe that not everyone who decides to embark on a tick collection project may have experience in this area or easy access to someone who does.

In Chapter 3 tick identification, DNA extraction and sequencing were used to determine what *Rickettsia* spp. might be present in the ticks sampled in Denton County, Texas. These are sites where tick collection and subsequent testing had not been performed previously. Any information gathered would be new and add information to what we know about ticks and tick-borne disease in these two recreational areas and in Texas. While there were no ticks that tested positive for *R. rickettsii* the bacteria known to cause Rocky Mountain spotted fever (RMSF), there were multiple positives for *R. amblyommatis* a spotted fever group *Rickettsia*

that had previously been considered a symbiont but more recently has been implicated as a possible disease-causing agent (Apperson et al. 2008, Hardstone Yoshimizu and Billeter 2018). It has also been shown to impart immune response in guinea pigs lowering severity of *R. rickettsii* infection and in co-infected *A. americanum* ticks it may result in females being less likely to pass *R*. *rickettsii* on to their offspring (Blanton et al. 2014, Rivas et al. 2015, Levin et al. 2018). Knowing that *R. amblyommatis* is located here is important as it may be playing a role in both causing illness in those visiting these sites as well as possibly limiting the presence of *R. rickettsii* or providing protection against more severe illness from it.

In chapter 4 a larger dataset of ticks was used to look at species distributions of both the *A. americanum* tick and *A. americanum* ticks infected with *R. amblyommatis*. It was determined that there was a difference in species distributions when the entire dataset of *A. americanum* ticks were used compared to when only the *A. Americanum* ticks infected with *R. amblyommatis* were used to create the distribution models. Being able to show this difference supports using infected ticks as presence data for creating species distribution models (SDMs)s. Creating SDMs using infected ticks may show areas of increased risk to humans of contracting specific tick-borne diseases. Both distributions created did overlap with areas where incidence of spotted fever group rickettsiosis were reported which may show that both the *A. americanum* tick and *R. amblyommatis* could be playing a role in this disease in the state of Texas. However, it is not possible to tell if human infection with *R. amblyommatis* might be playing a part in reported SFGR cases. It is also not possible to confirm if *R. amblyommatis* might be limiting the ability of *R. rickettsii* to proliferate in ticks in Texas or even if it might be providing some type of protection against the severe illness that *R. rickettsii* can cause. If *R.*

amblyommatis is providing protection against severe illness this may also be impacting if these less severe cases are reported.

5.2 Future Research

Obtaining a better understanding of ticks and the diseases they carry is a complex and multifaceted task. A multidisciplinary approach will be needed to allow us to do this.

Disease causing organisms are species that have distributions with environmental factors that impact those distributions. Initially, having a better understanding of the distribution of these agents can allow us to warn individuals who may use certain areas of the potential danger and encourage them to take extra precautions. To assist in furthering our knowledge and ability to create accurate tick and tick-borne disease distributions, tick collections in areas where this has not been done needs to take place with follow up testing for infectious agents. However, this is a large undertaking and not likely to occur in most areas. Researchers will need to continue to take advantage of the data available at the spatial resolutions available. Being able to eventually move from species distributions into understanding species niches and what factors play important roles in supporting ongoing tick infections with these disease-causing agents may allow us to better determine ways to stop or limit their proliferation.

Determining useful methods to locate areas of higher risk with available data is always necessary. Often information within areas of human health are not available as point level data but can sometimes be obtained in smaller political boundaries such as at the zip code level. Being able to take advantage of other datasets with similar data as the one used in this

dissertation to create distributions of infected ticks and therefore distributions of the tick-borne disease agents should be further explored.

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