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SURVEY OF Rickettsia spp. IN TICKS IN NACOGDOCHES COUNTY, TEXAS

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By

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SURVEY OF *Rickettsia* spp. IN TICKS IN NACOGDOCHES COUNTY, TEXAS

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ABSTRACT

Rickettsia parkeri is an obligate intracellular pathogenic bacterium that is commonly transmitted by the Gulf Coast tick, *Amblyomma maculatum*. *Rickettsia parkeri* is the causative agent of *Rickettsia parkeri* rickettsiosis, which is a disease characterized by nonspecific symptomology. Significant effort by numerous research groups focuses on determining the geographic distribution of potential vectors of this pathogen. The purpose of this study was to study *A. maculatum* populations in Nacogdoches County, Texas, for the presence of *Rickettsia parkeri.* Over a two-year period, 49 ticks were collected in Nacogdoches county and taxonomically identified. The DNA was extracted using DNeasy Blood and Tissue Kit, and the UltraClean Microbial DNA Isolation Kit. The genomic contents of the tick were subjected to PCR amplification to identify *Rickettsia* genus bacteria. Any ticks testing positive for *Rickettsia* spp. were subjected to PCR to test for *R. parkeri* and *R. rickettsii*. Ultimately, 26% of ticks collected were positive for a rickettsia bacteria with 6% positive for *Rickettsia parkeri.*

PREFACE

In 2012-2013, a study was performed in Nacogdoches County to identify Gulf Coast ticks that were carriers for *Rickettsia parkeri*. In that study, 35 ticks were collected and tested for *Rickettsia* spp. Only the Gulf Coast ticks, *Amblyomma maculatum*, were tested for *Rickettsia parkeri.* So, the current study is an extension of research published in 2015. I set out to identify *Rickettsia parkeri* in all of the ticks in the county not solely *Amblyomma maculatum.* In addition, the current research including testing for *Rickettsia rickettsii*, the causative agent for Rocky Mountain Spotted Fever.

ACKNOWLEDGMENTS

First, I would like to thank Dr. Sarah Canterberry without whom I never would have gotten this far. Not only did I learn how to be a good scientist but so much more. With her untimely passing, this thesis represents some of the last work from her lab and my sincere hope is that she is proud of it and all the author accomplished and continue to accomplish because of her.

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INTRODUCTION

In 1937, rickettsiologist Dr. Ralph R. Parker described a rickettsia bacterium that infects Gulf Coast ticks, *Amblyomma maculatum*[1]. *Rickettsia parkeri* was named in his honor and was correlated with causing disease in human in 2004[2]. *Rickettsia parkeri* is now recognized as the etiological agent of three rickettsial diseases: (1) Tidewater spotted fever, (2) *R. parkeri* rickettsiosis, and (3) American boutonneuse fever. Now *R. parkeri* rickettsiosis[2,3] is the most commonly used name for the infection caused by *R. parkeri.* *Rickettsia parkeri* rickettsiosis is a febrile illness with nonspecific symptomology often resulting in misdiagnosis which in some cases results in death[3,4]. An eschar, a necrotic lesion at the site of the tick bite, is one of the unique symptoms of *R. parkeri* rickettsiosis[3,4].

Since discovering that *R. parkeri* is pathogenic to humans, research has focused on determining the distribution of *R. parkeri*, its potential hosts, and pathogenesis. The primary vector of *R. parkeri* in North America is the tick species, *Amblyomma maculatum*[4,5]. *Amblyomma maculatum* is an ixodid tick distributed throughout much of the Southeastern and south-central United States[6]. It is currently unknown as to the percentage of *A. maculatum* that carry *R. parkeri.*

Ixodid Ticks and *Amblyomma maculatum*

The study of ticks as a vector for disease is an important aspect of public health. Although mosquitoes infect more people per year, ticks are carriers for a greater variety of pathogenic organisms including viruses, bacteria, fungi, protozoa, and even some parasitic nematodes[7]. Annually, ticks cost billions of dollars from the cost of treatment, population management, and loss of economic product[7]. Ticks are arachnid, and members of the subclass Acari (mites and ticks)[7]. Ticks are subdivided into Argasidae (soft) ticks, Ixodidae (hard) ticks, and one species of Nuttalliellidae ticks[7]. The Ixodidae are referred to as hard ticks. Hard ticks are characterized by a sclerotized dorsal scutal plate[7]. The Ixodidae family is divided into the Prostriata and the Metastriata. The Prostriata is made up of only one genus, *Ixodes*. While the Metastriata make up the remaining 11 genera of Family Ixodidae[7]. The *Amblyomma* genus is one of the 11 genera within the Metastriata[7].

Ticks are obligate blood sucking arthropods found across much of the globe. *Amblyomma maculatum* has a recorded range covering the Eastern seaboard of the United States into the southeast United States[6]. Their historical range in the United States covers from Maryland southward into Texas. The tick geographical range continues to spread to inland states as far north as Missouri and Iowa and as far west as Oklahoma and the Big Bend region of Texas[4,6] (See Figure 1). The range of *A. maculatum* continues south from Texas into central and northern South America[8]. The extensive range of *A. maculatum*

makes identifying the populations of ticks that serve as a vector for rickettsial bacteria challenging.

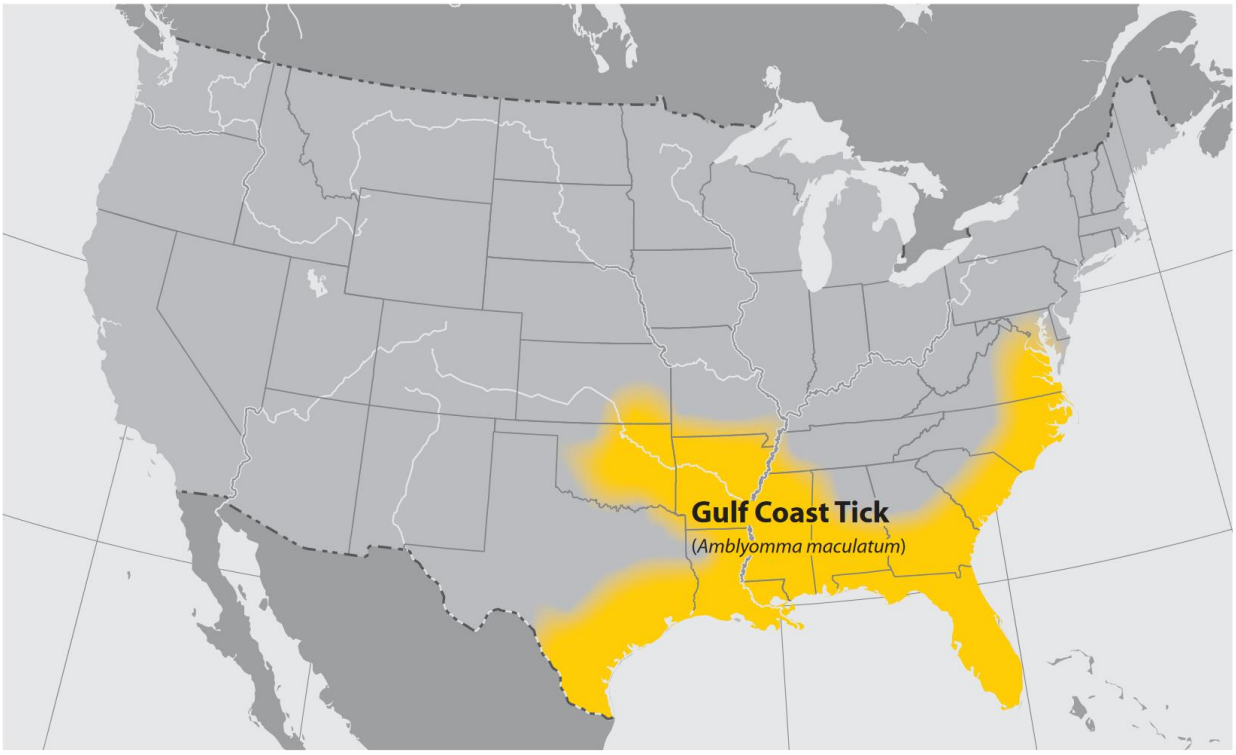

Figure 1: Geographic Distribution of Gulf Coast ticks in North America
The yellow shading represents the historic and current distribution of the Gulf Coast Tick in the United States. The map does not show locations of *Rickettsia parkeri* distribution only the distribution of the Gulf Coast tick. Adapted from the CDC at https://www.cdc.gov/ticks/maps/gulf_coast_tick.pdf

The lifecycle of *Amblyomma maculatum* is typical by having a 3-host life cycle like a majority of the Ixodid tick species[7] (See Figure 2). At least 71 species of birds and mammals have been identified as potential hosts for this ectoparasite[6]. Larval and nymph stages utilize rodents and birds as primary hosts[6]. Adult *A. maculatum* utilize (1) white-tailed deer (2) cattle, (3) horses and (4) swine as hosts[6]. Interestingly, coyotes and domestic sheep are the only recorded host for all three stages of *A. maculatum*[6].

Ticks take blood meals from their host at each stage of ectoparasitism[7]. After the blood meal is taken, the tick drops off and initiates the interstadial period. The interstadial period is the period between life stages when a molt occurs. The female lays the eggs, which hatch to yield the larvae. The larvae then find a host, feed, and drop off. The larvae then molt and transform into the nymph, which feeds again, then drops off and molts into an adult. The eight-legged adult feeds and mates, and the gravid female drops off the host, lays her eggs on a low laying plant, and then dies. Ixodid ticks are homogonadotrophic, reproducing only once in their lifetimes[7].

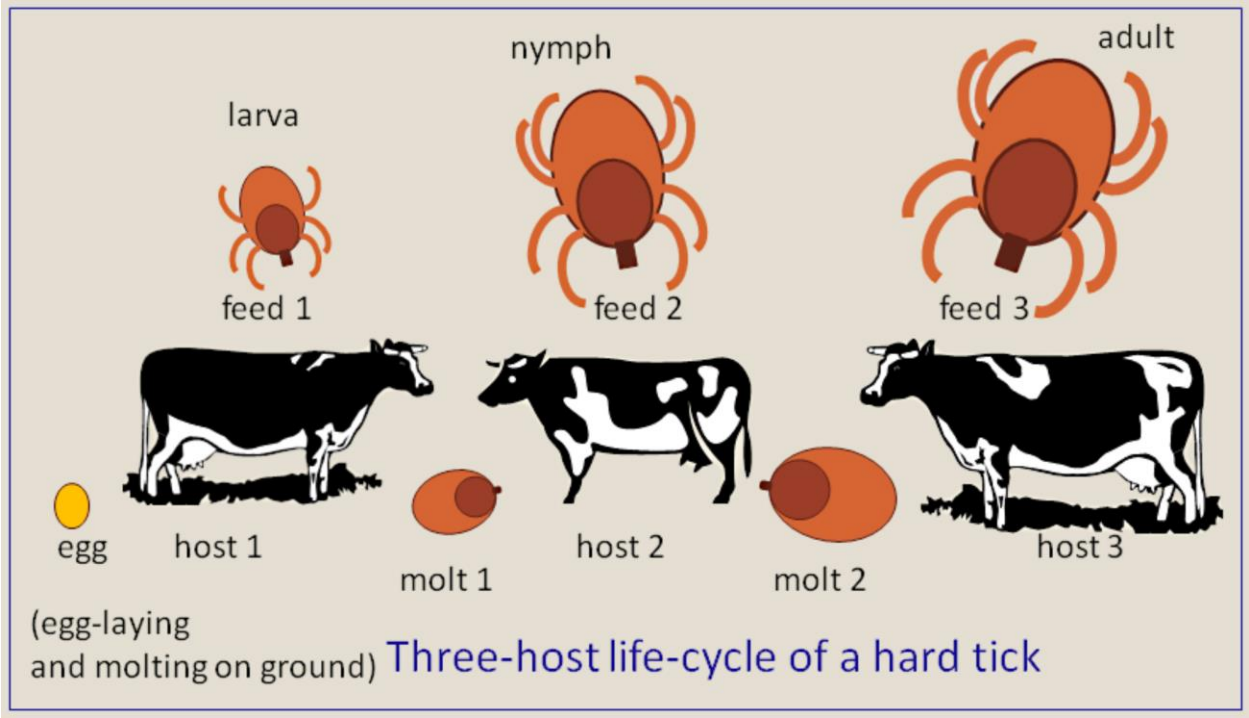

Figure 2: Three Host Life-Cycle
Female ticks lay eggs on ground. Larva feed on a first host, drops off, and molts. Nymphs feed on another host, drops off, and molts. Adults feed on a host and mate, drop off. The male dies and the females lay their eggs before dying.
Adapted from: Alan R Walker [CC BY-SA 3.0 (https://creativecommons.org/licenses/by-sa/3.0)], from Wikimedia Commons

Rickettsia parkeri Rickettsiosis

Rickettsia bacteria are gram-negative, obligate intracellular bacteria. They belong to the α-subdivision of the Proteobacteria[9]. There are four bacterial clades of *Rickettsia*. *Rickettsii parkeri* is a member of the rickettsia spotted fever group (SFGR)[10]. *Rickettsia parkeri* rickettsiosis is characterized by fever, mild headache, muscle pain, and an eschar[9]. In some patients, a reddish-pink macular rash develops in the peripheral extremities and moves into the core of the body with development of the disease[3,4,9]. *Rickettsia parkeri* rickettsiosis is often misdiagnosed as a viral illness because of its nonspecific symptoms (e.g. fever, rash, and headache). Table 1 adapted from Dantas-Torres shows some potential diseases that are often falsely diagnosed as the symptoms are similar to *R. parkeri* rickettsiosis. As the treatment for rickettsiosis is doxycycline, a viral misdiagnosis can be fatal[11,12]. In fact, multiple cases have been reported in which misdiagnosis of a rickettsial disease resulted in death of the patient[13,14].

Table 1: Diseases and Conditions to Consider with *Rickettsia parkeri* Rickettsiosis. Each of the listed diseases could be considered with a diagnosis of RMSF or *R. parkeri* rickettsiosis. The nonspecific symptomology makes diagnosis of these diseases difficult. Adapted from Dantas-Torres (2007).

Typhus	Ehrlichiosis
Other rickettsial diseases	Immune complex vasculitis
Thrombotic thrombocytopenia purpura	Meningococcemia
Enterovirus infection	Typhoid fever
Leptospirosis	Dengue
Infectious mononucleosis	Bacterial sepsis
Gastroenteritis or acute abdomen	Bronchitis or pneumonia

Rickettsia parkeri rickettsiosis is also commonly misdiagnosed as Rocky Mountain Spotted Fever (RMSF)[3,12]. Rocky Mountain Spotted Fever is caused by another member of the spotted fever group bacteria, *R. rickettsii*. The common symptomology between the diseases is confusing and requires novel diagnostics. Current diagnostics rely on antibody detection. While sensitive and specific to rickettsial organisms, the antibodies cross react between *R. parkeri* and *R. rickettsii* [15]. Paddock et al. (2008) reviewed approximately 6000 "lab confirmed" RMSF diagnoses reported to the CDC from 1981-2005[3]. Paddock found that only 305 (5.3%) cases identified *R. rickettsii* correctly as the etiological agent of disease. In fact, ~95% of the approximately 6000 US cases confirmed as RMSF did not differentiate between the autochthonous rickettsial species of the United States[3]. This is likely due to *Rickettsia rickettsii* being carried in a low percentage of ticks (<1%) even in areas where a diagnosis of RMSF has occurred[16,17]. This is compared to a higher percentage of ticks that carry *R. parkeri*[7].

Two mechanisms have evolved for maintaining *Rickettsia* spp. in the population of ticks: (1) transovarial and (2) transstadial transmission. Transovarial transmission occurs from an infected female to her offspring[9,15]. Once the female has acquired a rickettsial infection, the bacteria infect and multiply in all of her organs. As the bacteria increases the ovaries become infected and infect the developing oocytes in her ovaries[15]. When the oocytes are fertilized, and laid, the newborn ticks can become infected by a rickettsial bacteria. The rate of transovarial transmission may be as high as 65-85% in *A maculatum*[5], but varies from species to species.

The second method of rickettsia bacterial maintenance is transstadial transmission. Transstadial transmission is the transmission of a rickettsia bacteria from one life stage (stadia) to another within a single tick[9]. This process maintains the infection throughout a tick's life and increases the likelihood of another ticks being infected by cofeeding. The rate of transstadial transmission of *R. parkeri* in infected ticks was 100%[5]. This evidence indicates that *A. maculatum* will maintain the *R. parkeri* infection throughout the entire life of the tick.

Rickettsia rickettsii has a much lower prevalence (>1%) in tick populations compared to *R. parkeri* (5-60%). Some research suggests that *Rickettsia rickettsii* is only maintained transovarially in ticks for a few generations before the tick is killed[11]. The pathogenicity of the bacteria is the likely reason *R. rickettsii* is not maintained at a higher percentage[11]. Rickettsial studies in other species have shown that competition between different rickettsia favors the maintenance of a single nonpathogenic rickettsial infection[9,18]. This begs the question: is *R. parkeri* pathogenic to ticks? According to Wright et al. (2015), *R. parkeri* caused no loss of fitness in the populations of *A. maculatum* that were studied[5]; however, the populations were only studied for three generations as to not create loss of fitness from inbreeding.

JUSTIFICATION

The Gulf Coast tick has an extensive range across the United States. Much of this region is densely populated resulting in an increased likelihood of human contact with ticks carrying *R. parkeri*. Data presented herein document the population of *A. maculatum* in Nacogdoches that serve as vectors for *R. parkeri*. Understanding of the prevalence of rickettsial bacteria in the local tick population will allow physicians to be more aware of *R. parkeri*, which will lead to a better diagnosis potential cases of *R. parkeri* rickettsiosis.

A previous study was done in the area showing 60% of collected ticks were positive for *Rickettsia* spp.[19]. The previous study only tested *Amblyomma maculatum* ticks for *R. parkeri.* The current study identified all the tick species collected as well as assayed for *Rickettsia parkeri.* Data presented herein is an extension of the previous research which was to include *R. rickettsii* in addition to *R. parkeri.* This was the first-time to the author's knowledge that *R. rickettsii* was tested for in Nacogdoches county. An important aspect of this study is the need for more information to track the prevalence of *Rickettsia* spp. within tick populations.

MATERIALS AND METHODS

Objective 1. Collection of Ticks

Ticks were collected via "dragging" in various locations across Nacogdoches County during the height of adult tick season (May through August 2017). A primary location for collection was Alazan Bayou Wildlife Management Area. Dragging involves uses white cotton cloth approximately 1meter in length behind the researcher[20,21]. The researcher drags the cloth behind them and actively walks through the wooded area or forest. After dragging for approximately 25-30 meters, the cotton cloth was then examined for any ticks. All ticks were stored in a 15-mL twist top conical tube containing a 90% isopropyl alcohol solution and placed in a 4°C refrigerator for further testing.

A request was made of local veterinary clinics and animal shelters around the Nacogdoches area to collect ticks from animals in the clinics. This resulted in a few ticks (n=6) being collected. Additional ticks were donated by individual animal owners in the area. All ticks were stored in a 15-mL twist top conical tube containing a 90% isopropyl alcohol solution and placed in a 4°C refrigerator for further testing.

Objective 2. Morphological Identification of Ticks

A visual identification was performed based upon morphological characteristics[22]. A dichotomous key was used to identify the ticks to the species level. Briefly, ticks were

removed from the alcohol solution and placed onto a weighting boat under a dissecting scope and examined.

The first step in the key was to determine the structure of the anal groove. The tick was identified as genus *Ixodes* if the anal groove extended anteriorly around the anus. If the anal groove did not extend anteriorly around the anus, the tick was identified as one of the other genera within Family Ixodidae. The basis capituli was examined for all non-*Ixodes* ticks, which is the mouth portion of the tick. The next step was to determine the size and shape of the palpi compared to basis capituli. The palpi were compared to the basis capituli to determine if the palpus was longer or about as broad as the basis capituli. Specimens determined to have longer palpi and in which the second palpal segment was longer than it was broad were classified as members of genus *Amblyomma*. While those specimens whose palpi were about as long as the basis capituli and second palpal segment was about as long as they were broad were determined to be members of genus *Dermacentor*. Final identification of *Dermacentor variabilis* was determined by verification of a spiracular plate with small goblets. The two members of genus *Amblyomma* of interest to this study, *A. maculatum* and *A. americanum*, were verified by inspection of the first coxa. If the internal spur of the first coxa was short or insignificant then the species was identified as *Amblyomma maculatum*. If the internal spur was about half as long as the external spur, then the specimen was identified as *Amblyomma americanum*.

Objective 3. Identification of *Rickettsia* spp. bacteria

DNA Extraction

The tick DNA was extracted using two protocols. DNA from tick specimens numbered 1-46 was extracted using the DNeasy Blood and Tissue Isolation Kit (QIAGEN, Valencia, CA, USA) following manufacturer's instructions with modifications for the isolation of DNA from tick specimens. Briefly, the process involved cutting the tick into pieces followed by digestion and lysis utilizing Proteinase K overnight. The next day the lysate was run through a column and the DNA was eluted from the column after a series of washing steps.

DNA from ticks numbered 47-51 was extracted using UltraClean Microbial DNA Isolation Kit (MoBio Laboratories, Inc., Carlsbad, CA, USA). The secondary protocol involved freezing the remaining ticks in liquid nitrogen and using a mortar and pestle to crush the tick and placing the pulverized remains into a column with microbeads and shaking the column for 10 minutes. The resulting solution was then loaded into the column and the DNA was eluted from the column after a series of wash steps as per the manufacturer's protocol.

After all of the genomic DNA was collected from the ticks, the DNA concentration was measured. From this measurement, PCR stocks were made so the concentration was approximately 100 μg per mL. If the original concentration was below

this concentration, a PCR stock was not made from that specimen. Approximately 10 ng of template was added to each reaction for all subsequent PCR reactions.

Identification of *Rickettsia* spp. Bacteria

DNA from all ticks was subjected to PCR amplification of the rickettsial 17 kDa antigen gene. The primers utilized were R17K128F2 and R17K128R[19,23] with sequences displayed in Table 2. PCR cycle included an activation cycle of 3 minutes at 95°C followed by melting at 95°C for 30 seconds, annealing at 54°C for 30 seconds and elongation at 72°C for 30 seconds. The cycle was repeated 35 times, ending with a terminal extension of 72°C for 10 minutes. All PCR reactions were run without light capture on a C1000 Thermal Cycler CFX96 Touch Real-time PCR Detection System (BioRad, Des Plaines, IL USA). The PCR products visualized via a 2% agarose gel electrophoresis. The gel was visualized using the Typhoon FLA 9500 (GE Healthcare Bio-Sciences AB Uppsala, Sweden). The presence of a 111 bp product indicated the presence of a rickettsial species in the tick specimen. Each PCR was run in duplicate to verify results.

Identification of *Rickettsia parkeri* bacteria

Specimens that tested positive for a rickettsia species were tested for *R. parkeri* by PCR amplification. The primers for this test were designed to amplify the *ompB* gene which is an outer membrane protein that is expressed in *R. parkeri*[19,23]. Primers Rpa129F and Rpa224R (Table 3) were used for the reaction. PCR cycle included an activation

cycle of 3 minutes at 95°C followed by melting at 95°C for 30 seconds, annealing at 55°C for 30 seconds and elongation at 68°C for 30 seconds. The cycle was repeated for 34 times, ending with a terminal extension of 72°C for 10 minutes. All PCR reactions were run without light capture on a C1000 Thermal Cycler CFX96 Touch Real-time PCR Detection System (BioRad, Des Plaines, IL USA). The PCR products visualized via a 2% agarose gel electrophoresis. The gel was visualized using the Typhoon FLA 9500 (GE Healthcare Bio-Sciences AB, Uppsala, Sweden). The presence of a 96 bp band indicated a positive result for *R. parkeri.*

Optimization of *Rickettsia parkeri* Primers

The papers containing the primer sequence used to detect *R. parkeri* did not contain the specifications for the PCR reaction. The first *R. parkeri* reaction was run with a 52°C annealing temperature and did not yield any positive results. A temperature gradient PCR was performed on the *R. parkeri* reaction without any positive result. Another temperature gradient PCR was performed using the original stock DNA, with T_m ranging from 50–70°C. All positive results were obtained using 55°C annealing temperature with 5% DMSO added to the master mix.

Identification of *Rickettsia rickettsii*

All specimens testing positive for a rickettsial bacteria were subjected to PCR testing for *Rickettsia rickettsii.* Primers RRi6_F and RRi6_R were used to identify a gene encoding hypothetical protein A1G_04230 (GenBank accession no. ABV76353)[24]. PCR cycle included an activation cycle of 8 minutes at 98°C, followed by melting at 95°C for 30

seconds, annealing at 60.0°C for 30 seconds and elongation at 68°C for 30 seconds. The cycle was repeated for 30 cycles following the protocol in Kato et. al 2013. All PCR reactions were run without light capture on a C1000 Thermal Cycler CFX96 Touch Real-time PCR Detection System (BioRad, Des Plaines, IL, USA). The PCR products visualized via a 2.5% agarose gel electrophoresis. The gel was visualized using the Typhoon FLA 9500 (GE Healthcare Bio-Sciences AB, Uppsala, Sweden). The PCR products for *R. rickettsia* were visualized on 2.5% agarose gel with a 100 bp ladder for size comparison using the Typhoon FLA 9500 (GE Healthcare Bio-Sciences AB, Uppsala, Sweden). A positive result was indicated by the presence of a band of 153 bp.

Table 2: Primers used in the PCR reactions. The primer sequence for each reaction is given. Additionally, the target and product size of the amplification reaction are given as well. [a] and [b] adapted from Wright et al. 2011. [c] adapted from Kato et al. 2013.

Template	Primer Sequence	Product Size	Bacterial Target
R17K128F2[a]	5'GGGCGGTATGAAYAAACAAG3'	111 bp	*Rickettsia* 17 kDa
R17K128R[a]	5'CCTACACCTACTCCVACAAG3'	111 bp	*Rickettsia* 17 kDa
Rpa129F[b]	5'CAAATGTTGCAGTTCCTCTAAATG3'	96 bp	*R. parkeri ompB*
Rpa224R[b]	5'AAAACAAACCGTTAAAACTACCG3'	96 bp	*R. parkeri ompB*
RRi6_F[c]	5'AAATCAACGGAAGAGCAAAAC3'	153 bp	hypothetical protein A1G_04230
RRi6_R[c]	5' CCC TCC ACT ACC TGC ATC AT3'	153 bp	hypothetical protein A1G_04230

RESULTS

Tick Collection and Identification

Over the two-year collection period, 49 ticks were collected, from which three species were identified (Table 2). The three species identified were two members of genus *Amblyomma*, *A. maculatum* and *A. americanum*, the Gulf Coast tick and the Lone Star tick, respectively. Additionally, one member of genus *Dermacentor*, *D. variabilis*, the American Dog tick was identified. Of the 49 ticks, 30 of the ticks were identified as *A. maculatum*, 7 were identified as *A. americanum*, and 10 ticks were identified as *D. variabilis*. One tick was only identified to genus *Amblyomma* as it was too engorged to determine further morphologically (Figure 3).

Identification of *Rickettsia* spp. bacteria

Of the 48 tick specimens collected, only 13 specimens tested positive for a rickettsial bacteria species (approximately 26%). Specifically, specimens 17, 18, 24, 26, 27, 28, 30, 32, 36, 39, 50, 51, 52 tested positive for a rickettsia bacteria. These specimens represent all three tick species collected. Table 4 shows each tick species and the results of PCR testing.

The results of PCR testing were inconclusive for *R. parkeri*. The results for *R. parkeri* were inconclusive because the results were not reproducible. Tick specimens 32,

51, and 52 tested positive for *R. parkeri* only once. After the PCR was optimized some of the template DNA from the tick specimens ran out before the result was reproduced. Only *A. maculatum* and *D. variabilis* ticks tested positive for *R. parkeri* in this experiment. All ticks tested negative for *R. rickettsii.* Ultimately, 26% of the 48 ticks tested positive for *Rickettsia* spp. Approximately 6% of the 48 ticks tested positive for *Rickettsia parkeri* (Figure 4).

Table 3: Data for each tick collected in the study. Many of the ticks were donated to the research so the location and date collected are unknown.

Specimen Number	Species	Location Collected	Collection Method	Collection Date
1	*Amblyomma maculatum*	Unknown		Unknown
2	*Amblyomma maculatum*	Alazan Bayou WMA	Dragging	7/19/17
3	*Amblyomma maculatum*	Alazan Bayou WMA	Dragging	7/19/17
4	*Amblyomma maculatum*	Alazan Bayou WMA	Dragging	7/17/17
5	*Amblyomma maculatum*	Alazan Bayou WMA	Dragging	7/17/17
6	*Amblyomma maculatum*	Alazan Bayou WMA	Dragging	7/17/17
7	*Amblyomma maculatum*	Alazan Bayou WMA	Dragging	7/17/17
8	*Amblyomma maculatum*	Alazan Bayou WMA	Dragging	7/17/17
9	*Amblyomma maculatum*	Alazan Bayou WMA	Dragging	7/17/17
10	*Amblyomma maculatum*	Alazan Bayou WMA	Dragging	7/17/17
11	*Amblyomma maculatum*	Alazan Bayou WMA	Dragging	7/17/17
14	*Dermacentor variabilis*	Alazan Bayou WMA	Dragging	7/17/17
15	*Amblyomma maculatum*	unknown-donated	Donated	2017
16	*Amblyomma americanum*	unknown-donated	Donated	2017
17	*Amblyomma americanum*	unknown-donated	Donated	2017
18	*Amblyomma americanum*	unknown-donated	Donated	2017
19	*Dermacentor variabilis*	unknown-donated	Donated	2017
20	*Dermacentor variabilis*	unknown-donated	Donated	2017
21	*Amblyomma maculatum*	unknown-donated	Donated	2017
22	*Amblyomma maculatum*	unknown-donated	Donated	2017
23	*Amblyomma maculatum*	unknown-donated	Donated	2017
24	*Amblyomma americanum*	unknown-donated	Donated	2017
25	*Dermacentor variabilis*	unknown-donated	Donated	2017
26	*Amblyomma maculatum*	unknown-donated	Donated	2017
27	*Amblyomma americanum*	unknown-donated	Donated	2017
28	*Amblyomma maculatum*	unknown-donated	Donated	2017
29	*Amblyomma americanum*	unknown-donated	Donated	2017
30	*Amblyomma maculatum*	unknown-donated	Donated	2017
31	*Amblyomma americanum*	unknown-donated	Donated	2017
32	*Amblyomma maculatum*	unknown-donated	Donated	2017
33	*Dermacentor variabilis*	unknown-donated	Donated	2017
34	*Amblyomma maculatum*	unknown-donated	Donated	2017
35	*Dermacentor variabilis*	unknown-donated	Donated	2017
36	*Dermacentor variabilis*	unknown-donated	Donated	2017
37	*Amblyomma americanum*	unknown-donated	Donated	2017
38	*Amblyomma maculatum*	unknown-donated	Donated	2017
39	*Amblyomma maculatum*	unknown-donated	Donated	2017
40	*Amblyomma maculatum*	unknown-donated	Donated	2017
41	*Amblyomma maculatum*	unknown-donated	Donated	2017
42	*Amblyomma maculatum*	unknown-donated	Donated	2017
43	*Amblyomma maculatum*	unknown-donated	Donated	2017
45	*Amblyomma maculatum*	Unknown	Donated	2017

46	*Dermacentor variabilis*	Unknown	Donated	2017
47	*Amblyomma maculatum*	Chireno	Donated-Sterling Vet Clinic	2017
48	*Dermacentor variabilis*	Chireno	Donated-Sterling Vet Clinic	2017
49	*Amblyommma* sp.	Chireno	Donated-Sterling Vet Clinic	2017
50	*Amblyomma maculatum*	Nacogdoches	Donate-Southside Animal Clinic	09-2017
51	*Dermacentor variabilis*	Nacogdoches	Donated-Southside Animal Clinic	09-2017
52	*Amblyomma maculatum*	Nacogdoches	Donated-Southside Animal clinic	09-2017

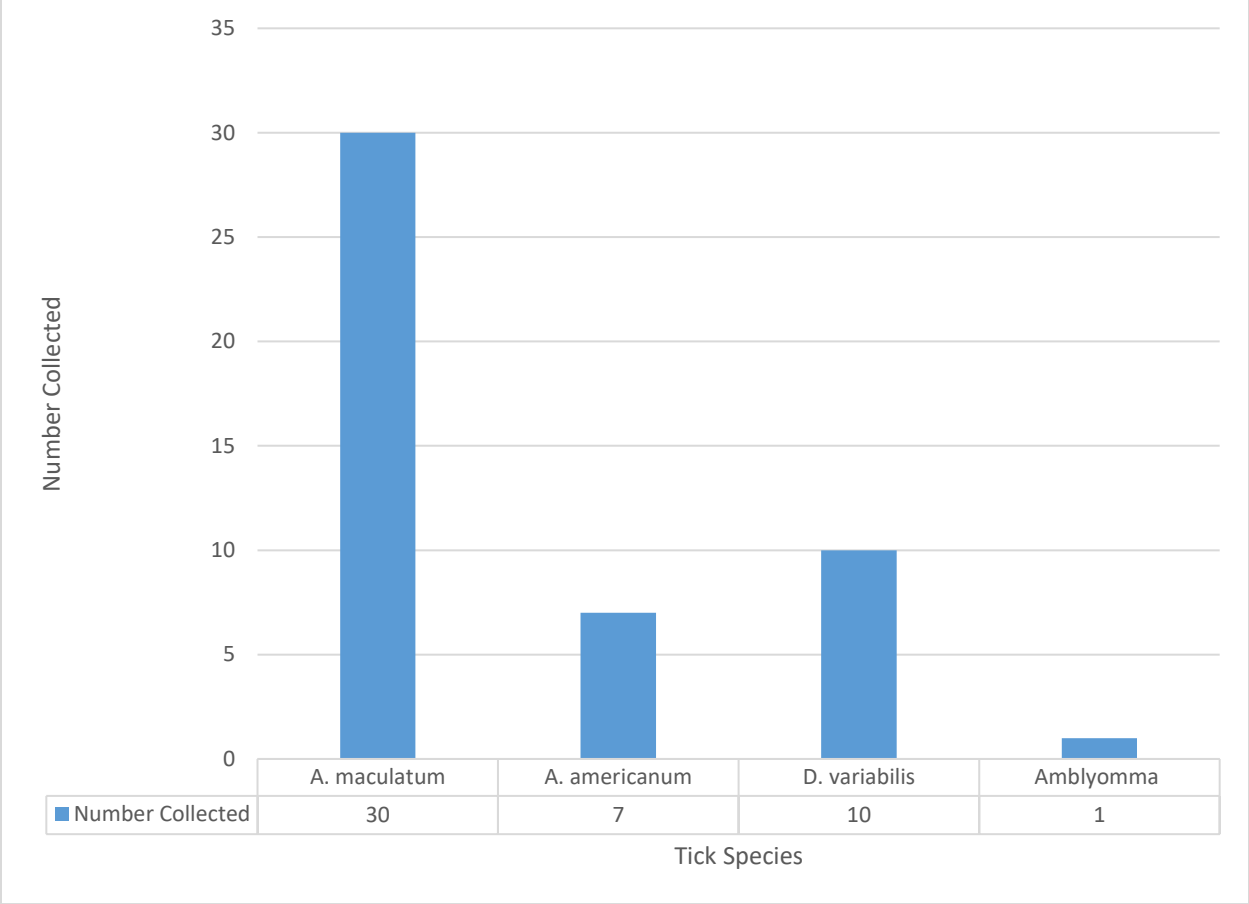

Figure 3: The number of each tick species collected during the study. The last column represents the tick that could only be identified to genus.

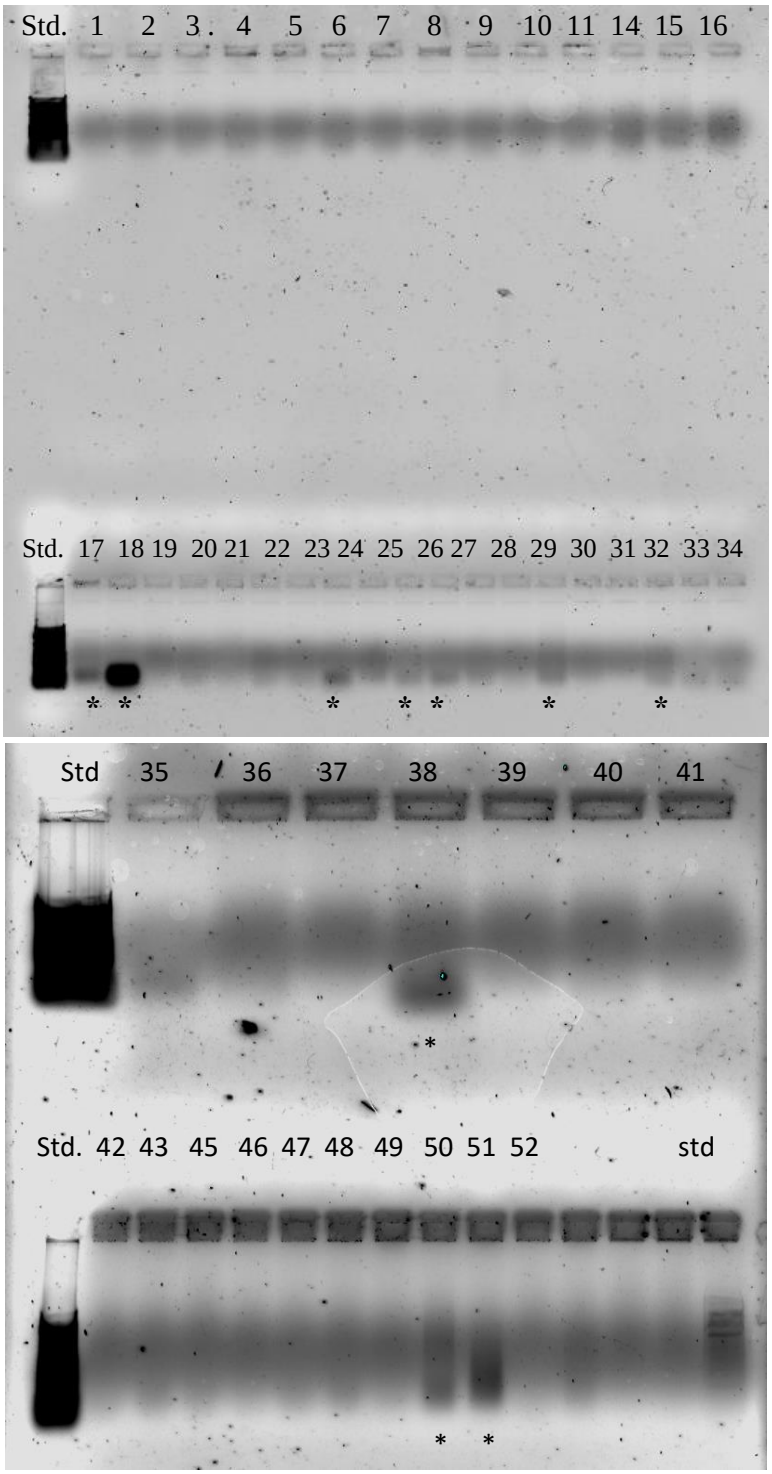

Figure 4: PCR gels for *Rickettsia* spp. (First Run)
Gels showing results for *Rickettsia* spp. A positive result shows a 111 bp band. Specimens are numbered from left to right starting at the top left in the first image.
* indicates a positive result.

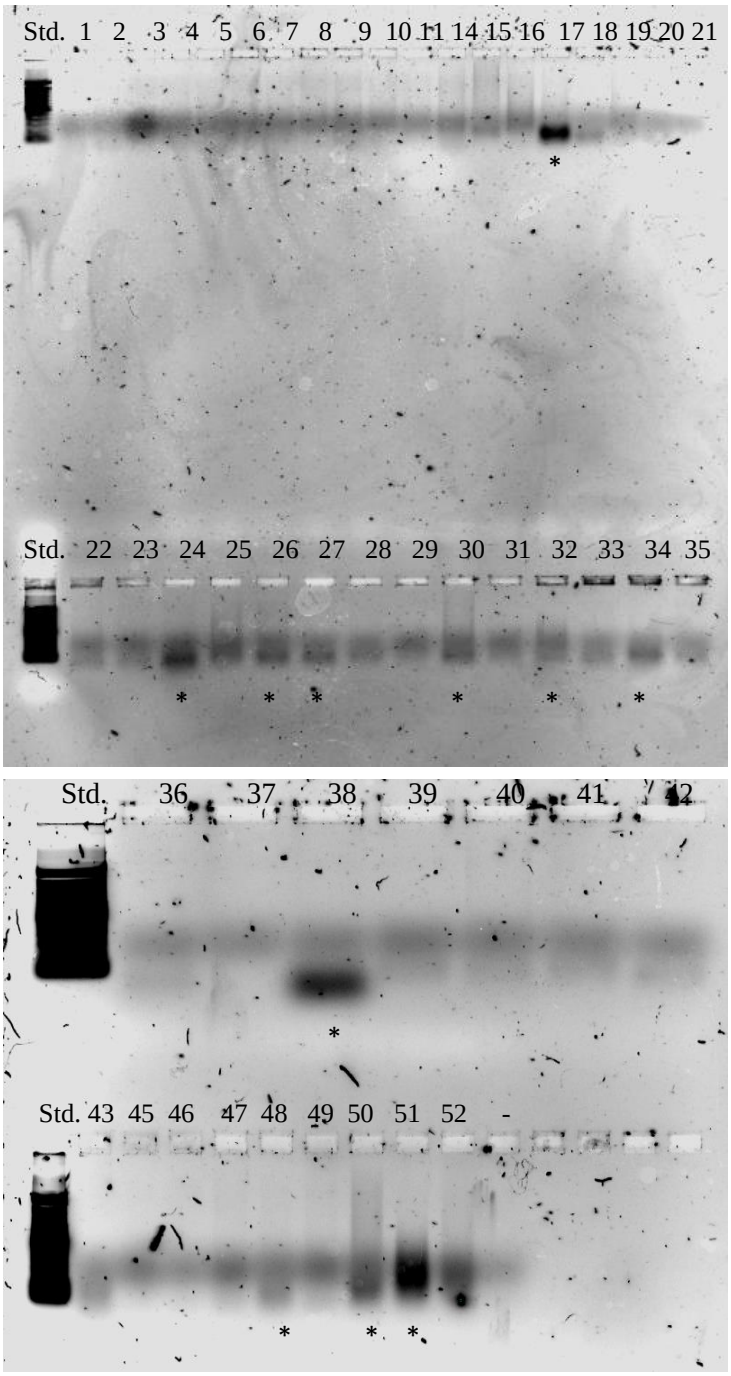

Figure 5: PCR Reactions for *Rickettsia* spp. (Second Run)
Gels showing results for *Rickettsia* spp. A positive result shows a 111 bp band. Specimens are numbered from left to right starting at the top left in the first image.
* indicates a positive result.

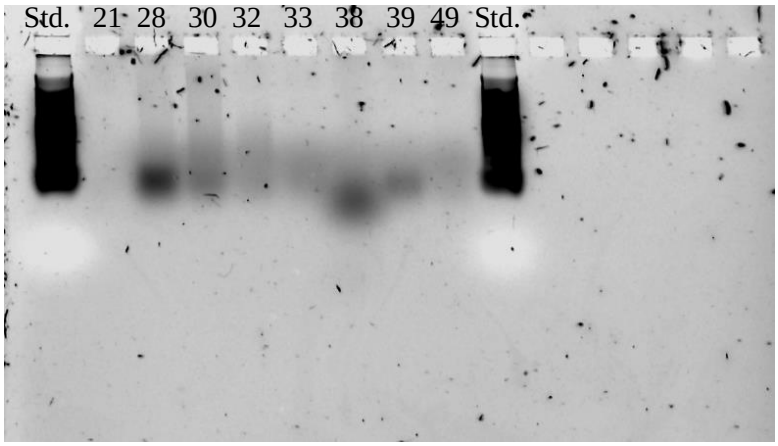

Figure 6: PCR Gels for *Rickettsia* spp.
Any specimen positive in at least on PCR was rerun for verification. Those specimens are shown here.

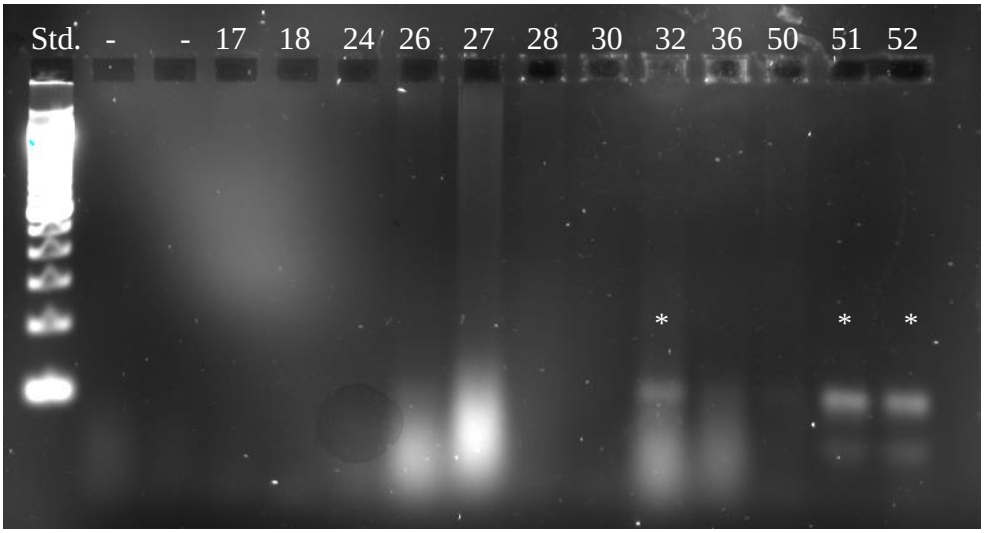

Figure 7: PCR Gels for *Rickettsia parkeri.*
Gels showing results for *Rickettsia* spp. A positive result shows a 96 bp band. Specimens are numbered from left to right starting at the top left in the first image.
* indicates a positive result.

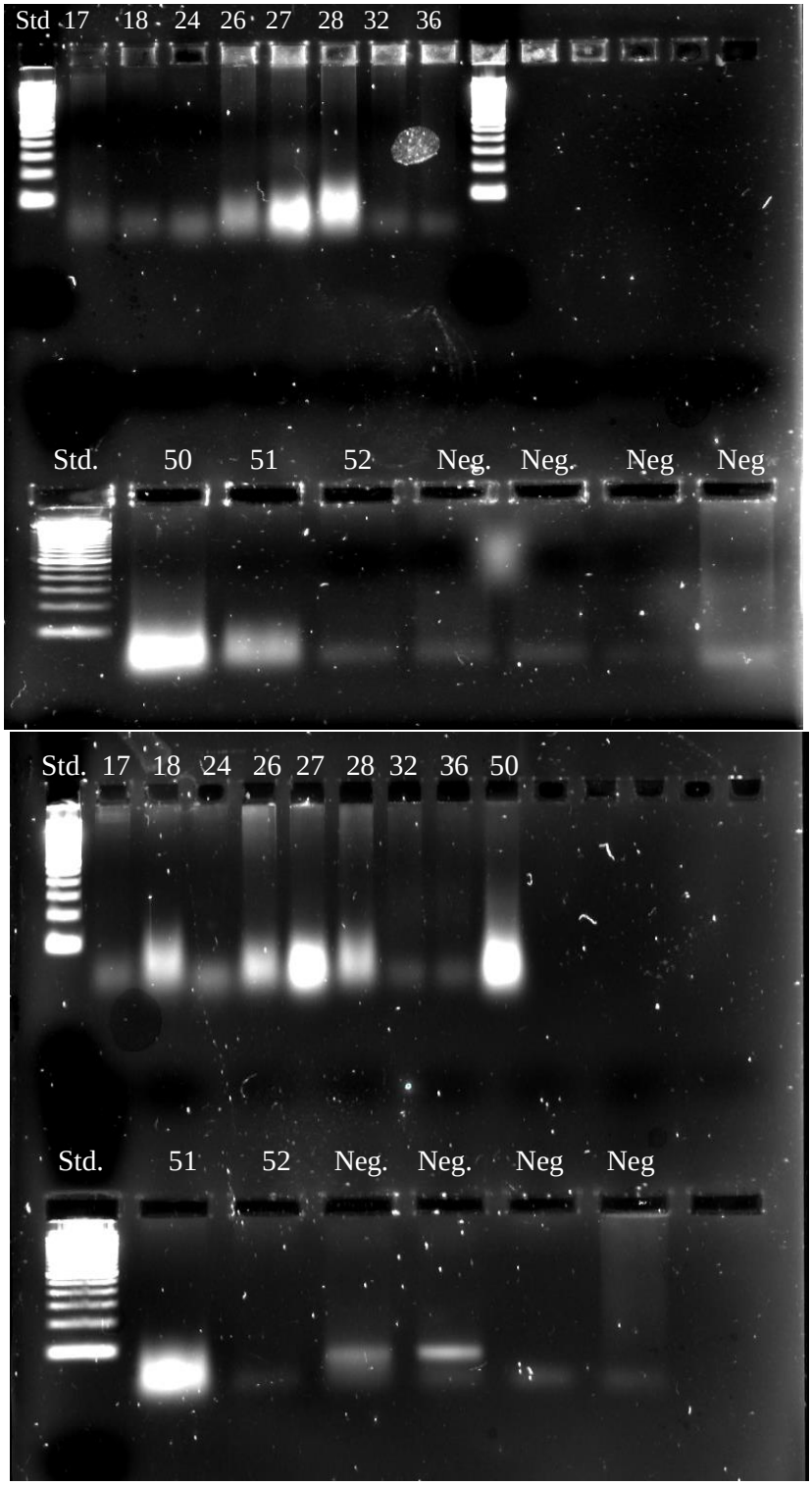

Figure 8: PCR Reaction for *Rickettsia rickettsii*
Gels showing results for *Rickettsia* spp. A positive result shows a 153 bp band. Specimens are numbered from left to right starting at the top left in the first image. -No positive results for this test.

Table 4: PCR results for the specimens that tested positive for a rickettsial species. Results for *R. parkeri* were inconclusive. The result for each *R. parkeri* test was not repeatable.

Specimen No.	Species	*Rickettsia* spp.	*R. parkeri*	*R. rickettsii*
17	*A. americanum*	+	-	-
18	*A. americanum*	+	-	-
24	*A. americanum*	+	-	-
26	*A. maculatum*	+	-	-
27	*A. americanum*	+	-	-
28	*A. maculatum*	+	-	-
30	*A. maculatum*	+	-	-
32	*A. maculatum*	+	+	-
36	*D. variabilis*	+	-	-
50	*A. maculatum*	+	-	-
51	*D. variabilis*	+	+	-
52	*A. maculatum*	+	+	-

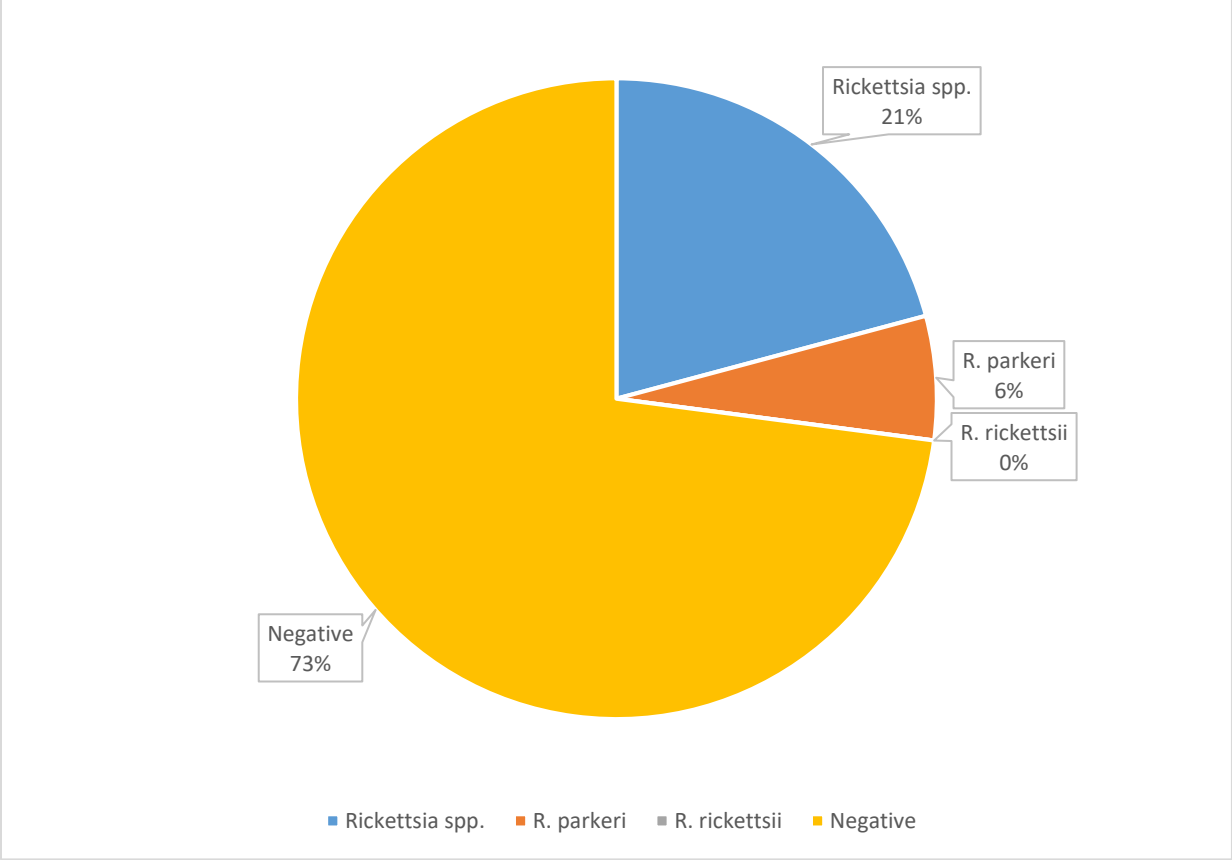

Figure 9: The percentage of ticks testing positive for each PCR test.
Ticks testing positive for *R. parkeri* are not included in the percentage for *Rickettsia* spp.

DISCUSSION

The results of the experiment fall within the range of expected prevalence (5-60%)[9] of carriage rate for *R. parkeri.* The data does support observations that *R. parkeri* is carried in a greater percentage in ticks than *R. rickettsii*.

The primary vector for *Rickettsia parkeri* is *Amblyomma maculatum. Dermacentor variabilis* can carry *R. parkeri* (0.3-2.3%)[4]; however, *D. variabilis* is the primary vector of *R. rickettsii*[9,11,25]. *Amblyomma americanum* can become infected with *R. parkeri* in small amounts (0.4-1.0%)[4,9,26]. Both tick species can become infected from a rickettsemic vertebrate host or co-feeding alongside an infected *A. maculatum* tick[26,27].

Ticks 51 and 52 could be an example of this phenomenon occurring. Both ticks tested positive for *R. parkeri.* Ticks 51 and 52 were received from Southside Animal Clinic in Nacogdoches. Both ticks were removed from the same dog before being donated to the researcher. an argument could be made for a co-feeding infection to have occurred. Tick specimen 51 was identified as an *A. maculatum* tick while specimen 52 was identified as *D. variabilis*. The low occurrence of *R. parkeri* in *D. variabilis* ticks supports the idea that specimen 52 was infected from tick 51 during the process, although it cannot be proven that one tick infected the other.

An issue with the survey was the low sample size of ticks collected, and the low number of locations from which ticks were collected. The low sample size limits the ability of the percentages to be extrapolated to the entire Nacogdoches county, which was a goal of the research. The author encountered difficulties with collecting ticks including limited public land to drag, as well as particularly wet summer that limited days available to drag as well as an apparent absence of ticks. The author noticed a pattern wherein ticks would be absent for a few days immediately following a rainstorm. This absence caused drags to be unsuccessful in collecting ticks. Another limitation is the low number of drag sites. The primary collection site for ticks was Alazan Wildlife Refuge. A lack of information exists for the rest of the county because many areas of the county were not surveyed accurately.

For this study, a pictorial key was used to morphologically identify ticks. The key was specifically designed for east of the Mississippi River although this study was performed west of the Mississippi River. For this reason, the key is not the most ideal; however, it worked for these purposes because the only ticks not included in the key are not present in this region of the state. One such tick is the Cayenne tick, *Amblyomma cajennense,* which does not inhabit the East Texas region[28].

For this survey, although the ticks were identified as 1-52 only 49 ticks were actually collected. This is due to a numbering error while separating the ticks into individual tubes. As the ticks were collected, they were moved from 15mL twist top tubes

to smaller 1.5 mL snap top tubes and numbered individually. During the process of numbering and separating the ticks, three tubes were numbered but did not receive a tick. The author did not discover the error had occurred until after all of the ticks had be numbered. The numbers skipped were 12,13, and 44.

Future work from this study would be to increase overall tick collection as well as the number of species collected. In this survey, no *Ixodes* ticks were collected. A more thorough survey would include this genus of ticks as well as the *Amblyomma* and *Dermacentor* genera. Another extension of the work would include more pathogenic and non-pathogenic *Rickettsia* species as well. For example, another rickettsial bacteria, *R. amblyommii*, is most commonly found in *A. americanum*[11]. This bacterial species could explain the discrepancy between the number of ticks testing positive for a rickettsial bacteria and the number of ticks testing positive for *R. parkeri* and *R. rickettsii*[29] in this study and could help to create a more accurate picture of *Rickettsia* species that are endemic to Nacogdoches County.

This survey identified three tick species common to the East Texas area using only morphological identification. Although visual identification is the simplest, molecular identification of ticks using PCR would be a more precise determination of the species of ticks. The author experienced multiple ticks that were either missing morphological structures. The mouth pieces can sometime be removed and left in the parasitized animal or obscured by host tissues. If the tick is engorged, it may be difficult

to identify the species. When a tick becomes engorged the body structures alter to accommodate the large amount of blood. These changes creating difficulty and imprecision in the identification process. Molecular identification using PCR could confirm the identity of the tick and support the morphological identification.

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VITA

After graduating from Livingston High School in Livingston, Texas, Nathaniel Blakley began his college career at Stephen F. Austin State University as a vocal education major in the Fall of 2009. After a year and a half as a music major, Nathaniel changed his major to Biology, with an emphasis in cellular and molecular biology. After switching majors Nathaniel graduated from Stephen F. Austin State University in August 2013. Upon graduating he taught high school science at Corrigan-Camden high school and Cleveland High school. After teaching for two years, Nathaniel returned to Stephen F. Austin for a semester doing post-baccalaureate work in biology and chemistry. Nathaniel enrolled in the Masters of Science in Biology program in Spring 2016. He received the Master's degree in Spring 2018.

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