DISTRIBUTION OF *BORRELIA BURGDORFERI*, THE CAUSATIVE AGENT OF LYME DISEASE IN TICKS ACROSS TEXAS

An Undergraduate Research Scholars Thesis

by

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ABSTRACT

Distribution of *Borrelia burgdorferi*, the causative agent of Lyme disease in ticks across Texas.

(May 2013)

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The goal of this study is to determine where the Lyme disease (LD) causative agent is prevalent in Texas. According to the CDC, LD is the most prevalent arthropod borne disease in the US with 33,097 cases reported last year. In 2009 the case definition of LD was revised and nowadays the CDC differentiates in between confirmed and probable cases for this disease. Taking this into account, since 2009 Texas is the only state in the US in which the ratio of probable versus confirmed cases is repetitively 2:1. This can be attributed to many different causes, from doctors' disregard for the disease and not testing for it or to the presence of genetically distinct *Borrelia spp.* and/or *Ixodes scapularis* vectors in Southern U.S. LD is transmitted by the bite of an infected Ixodes ticks. There are approximately 18 recognized genospecies of Borrelia that are present in ticks and make up the *B. burgdorferi* sensu lato complex. Only one of them has been shown to cause disease in humans in the U.S., *B. burgdorferi* sensu stricto, while *B. garinii* and *B. afzelii* have been proven to cause Lyme borreliosis in Europe. In addition *B. spielmani*, *B. bissettii*, *B. valsiana* and *B. lusitane* are currently under study in Europe to determine their implication in Lyme borreliosis (54). We have

collected ticks, the vectors of LD, from 23 counties in Texas and tested them for the presence of the bacteria pathogen, *Borrelia burgdorferi* sensu lato, by PCR utilizing different genetic markers (7, 27, 56, 58, 60) in order to determine what *B. burgdorferi* strains are circulating in Texas and how they are distributed across the state.

DEDICATION

I lovingly dedicate this thesis to my family who has supported me every step of the way. Special thanks go to my parents, Darby and Michael Jolly, and Ben and Shelly Brown. I would also like to thank my many grandparents for all of their encouragement and support as well.

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NOMENCLATURE

LD	Lyme disease
CDC	Center for Disease Control and Prevention
EM	Erythema migrans
PCR	Polymerase chain reaction
OspA	Outer surface protein A
OspB	Outer surface protein B
OspC	Outer surface protein C
IGR	Intergenic region 16SrRNA-23SrRNA
IGS	Intergenic spacer 23SrRNA-5SrRNA
FlaB	Flagelar gene
Bbss	*Borrelia burgdorferi* sensu stricto
Bbsl	*Borrelia burgdorferi* sensu lato

CHAPTER I

INTRODUCTION

Lyme disease (LD), or Lyme borreliosis, is the most commonly reported arthropod-borne disease in the United States (12). It is caused by the spirochetal bacterial pathogen *Borrelia burgdorferi* that is transmitted to mammalian hosts by the *Ixodes spp*. ticks (55). According to the Centers for Disease Control and Prevention (CDC), there has been a gradual increase of LD cases since 2002. Most recently, there were a total of 33,097 reported cases in 2011 with an incidence rate of 7.8 per 100,000 people (12). In 2009 the case definition of LD was revised and nowadays the CDC differentiates between confirmed and probable cases for this disease. Taking this into account, since 2009 Texas is the only state in the U.S. in which the ratio of probable versus confirmed cases is repetitively 2:1. This can be attributed to many different causes, from doctors' disregard for the disease and not testing for it or to the presence of genetically distinct *Borrelia* species and/or *Ixodes scapularis* vectors in Southern U.S. In addition, the maintenance of the enzootic cycle for this pathogen might be different in the South compared to the well established models described in the Northeast and Midwest U.S.

LD is a multisystemic disease, which can be characterized by three different stages, the first of which is a localized infection. Erythema migrans (EM) is the most common symptom in LD patients and is identifiable by a target-shaped rash and accompanied by flu-like symptoms. This occurs after an incubation period of 3-32 days. The rash is the only way to detect LD without a diagnostic test. Nevertheless, only 70% of all the reported LD cases develop EM at the site of tick bite, and most of the patients cannot recall whether or not a rash was present at the time of

infection (53, 56, 58). The second stage of LD includes the dissemination of *Borrelia burgdorferi* within days to weeks after disease onset. This stage is known as early disseminated LD. This can include multiple secondary EM sites and complications with the involvement of the neurological and cardiac systems (56). The third stage, the persistent infection, also known as chronic LD, occurs after several weeks of disseminated infection and may persist for several years. The pathogen continues to spread to the joints, nervous system, and cardiac tissue. Depending on the species of Borrelia, the frequency of the dissemination to the different sites varies. For example, *Borrelia burgdorferi* in North America is mainly arthritogenic, while European strains cause neuroborreliosis more frequently. Lyme arthritis is asymmetrical, occurs in large joints (i.e. elbows, knees, and ankle), and is recurrent for several years. In approximately 60% of the untreated patients, intermittent attacks of arthritis begin to occur months after the onset of illness, especially in the knees (56, 58).

Vectors, hosts and pathogens

There are four main hard tick vectors of Lyme disease which are *Ixodes scapularis, Ixodes pacificus, Ixodes ricinus,* and *Ixodes persulcatus*. In North America, *I. pacificus,* the Western black-legged tick, is the primary vector in western United States and *I. scapularis,* the black-legged tick, is the vector in northeastern and midwestern United States and even extend into Mexico (26) (Dr. Esteve-Gasssent and collaborators, manuscript in preparation). *I. ricinus* primarily live in Europe and *I. persulcatus* are in Asia (55). Spirochetes have been isolated from certain non-Ixodes ticks such as the lone star tick, *Amblyomma americanum,* and the American dog tick, *Dermacentor variabilis,* suggesting that these ticks also may play some role in Lyme epidemiology (41). The different species of ticks have different vector competencies, as in, how

well they are able to spread the bacteria. There have been sporadic cases of Lyme disease that have been transmitted by *D. variabilis* and *A. americanum,* however, they are not efficient vectors compared to *Ixodes spp. (32, 40).* One study performed in Alabama showed *I. scapularis* have much higher infection rates (83%) than *A. americanum* (5%) and *D. variabilis* (8%), and thus, this species is considered the primary vector in the Northern Hemisphere (41).

Ixodes ticks have a three-stage life cycle which includes a larval stage, nymphal stage, and adult stage. The tick has one blood meal during each of these stages then drops off to molt to the next stage, which takes several months (Fig. 1). The life cycle of a tick can vary between 2 to 6 years depending on different environmental factors such as climate, host availability, etc (39, 55). Larva hatch from eggs laid by the female and emerge with 6 legs. They are not important vectors of LD because transmission of *B. burgdorferi* does not occur trans-ovarially. Transmission occurs trans-stadially, which is only passed on after feeding on an infected host. Larvae feed on small to medium mammals and birds. After the larva feeds and drops to the ground, it molts into an 8-legged nymph. The nymphal stage is most closely associated with the transmission of LD because they are harder to see and there are higher numbers of them (1). Nymphs feed on small mammals as well as on some larger mammals such as deer. They are active from early summer to early autumn. In the adult stage, the ticks mainly feed on larger mammals including deer and humans, and they are most active from autumn through winter, until early spring (55). Humans, as well as companion animals (dogs and horses), are considered accidental hosts and are a dead end for the transmission of *B. burgdorferi.* On the other hand, the white footed mouse (*Peromyscus leucopus*) is the most important reservoir in the US for *B. burgdorferi* (1). In addition, deer are important for maintaining tick populations because they provide the perfect

environment to feed sufficient numbers of adult ticks, and will allow the mating of male and females, necessary to generate the next generation of ticks. However, they are not competent reservoirs for the disease agent (55). A typical habitat for the transmission of LD includes wooded areas with decaying vegetation on the ground in order to maintain humidity for the survival of ticks and have a sufficient amount of vertebrate hosts. Moreover, recent studies have shown that the level of biodiversity will also affect the maintenance of the enzootic cycle as well as the risk of disease transmission to humans (17, 28, 33, 35-37, 51).

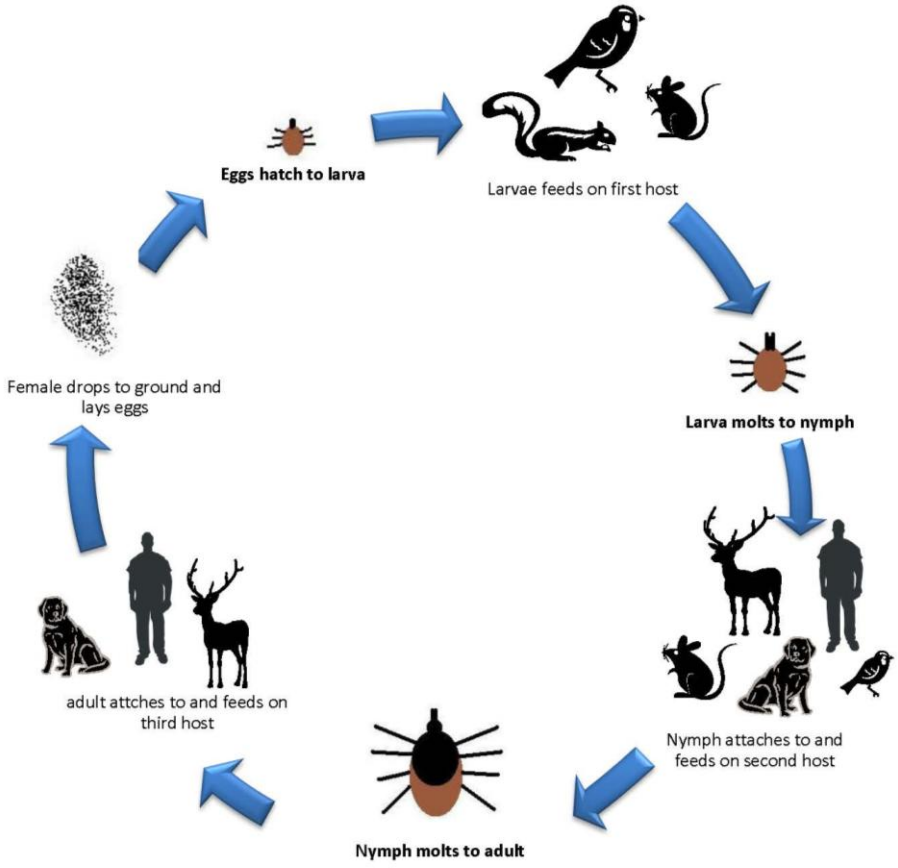

Figure 1. Life cycle of *Ixodes* ticks (56, 57)

There are approximately 18 recognized genospecies of Borrelia that are present in ticks and constitute the *B. burgdorferi* sensu lato complex. Only *B. burgdorferi* sensu stricto has been shown to cause disease in humans in the US, while *B. garinii* and *B. afzelii* have been proven to cause Lyme borreliosis in Europe (Table 1). Lyme borrelia belong to the eubacterial phylum and are corkscrew-shaped spirochetes. It is a non-typical, gram negative bacteria that does not express the lipopolysaccharide in the outer membrane but instead it encodes for a significant amount of lipoproteins that anchor to the outer membrane through the lipid moiety (47). Moreover, members of the *Borrelia burgdorferi* sensu lato complex depend on the host for most of its nutrition requirements (56).

Borrelia genospecies are transmitted in the tick saliva, possibly through the regurgitation of gut material (62). It is distributed in the midgut of ticks, so once the tick has a meal, *B. burgdorferi* disseminates into the hemolymph in its way to the salivary glands in order to inoculate the host (10). It causes infection to the host by migration through tissues, adhesion to host cells, and evasion of the hosts' immune defenses (13). *B. burgdorferi* has a unique and very fragmented genome with one linear chromosome and up to 21 plasmids (12 linear plasmids, and 9 circular plasmids) (47, 49). These spirochetes have antigenic surface lipoproteins, of which there are three main outer surface proteins (*Osp*): *Osp*A, *Osp*B, and *Osp*C (4, 56). These proteins are good genetic markers when testing for the presence of the bacteria and can be used in diagnosis of LD. When transmission occurs there is a phenotypic switch in these proteins. *Osp*A has been associated to a protein in the tick mid gut (TROSPA) and therefore it is down-regulated (25, 38) during the blood meal, while *Osp*C that binds to a tick saliva protein (16, 42) is up-regulated at

the same time (4, 18). On the other hand, the Lyme borrelias have not been found to produce toxins (55).

Table 1. Distribution of *Borrelia* and their vectors (34, 48, 54)

Species	Main Vector	Species	Main Vector	Species	Main Vector
North America		**Europe**		**Asia**	
B. americana	*Ixodes pacificus*	*B. afzelii*	*I. ricinus*	*B. afzelii*	*I. ricinus*
	I. minor		*I. persulcatus*		*I. persulcatus*
B. andersonii	*I. dentatus*		*I. hexagonus*		*I. hexagonus*
B. bissettii	*I. pacificus*	*B. bissettii*	unknown	*B. garinii*	*I. ricinus*
	I. spinipalpis	*B. burgdorferi*	*I. ricinus*		*I. persulcatus*
	I. affinis		*I. hexagonus*		*I. uriae*
B. burgdorferi	*I. ricinus*		*I. scapularis*	*B. japonica*	*I. ovatus*
	I. hexagonus		*I. pacificus*	*B. sinica*	*I. ovatus*
	I. scapularis		*I. affinis*	*B. tanukii*	*I. tanuki*
	I. pacificus		*I. minor*	*B. turdi*	*I. turdus*
	I. affinis		*I. spinipalpis*	*B. valaisiana*	*I. turdus*
	I. minor		*I. muris*		*I. ricinus*
	I. spinipalpis	*B. garinii*	*I. ricinus*		*I. columnae*
	I. muris		*I. persulcatus*	*B. yangtze*	*I. granulatus*
B. californiensis	Unknown		*I. uriae*		*I. nipponensis*
B. carolinensis	Unknown	*B. lusitaniae*	*I. ricinus*		
B. kurtenbachii	*I. scapularis?*	*B. spielmanii*	*I. ricinus*		

**Borrelia* species highlighted in blue are considered pathogenic

PCR Testing

Polymerase chain reaction (PCR) tests have been found to be an accurate and reliable source of testing in early Lyme disease patients and to identify *B. burgdorferi* from infected ticks (5, 50). A few of the most targeted genes include *flaB*, *recA*, *p66*, *ospA*, and several other rRNA genes such as the 16SrRNA and the intergenic region (IGR) 16SrRNA-23SrRNA and the internegic spacer (IGS) 23SrRNA-5SrRNA (5, 34, 50, 52). In previous studies, the genetic markers: *flaB*

(flagelar gene), *IGR*, *ospA*, *p66*, and *ospC* have been reported as to being optimal to identify *Borrelia burgdorferi* sensu lato complex genospecies, as well as of great value when doing population genetic studies of the Borrelia genospecies identified (7, 9, 34). Therefore, we decided to utilize the same markers previously used in the Northeast, Midwest, and Western US as well as in Europe, in order to simplify the analysis performed as well as to be consistent with the literature. Sensitivities can vary depending on the site of the sample extraction. In our study we have decided to extract DNA from individual ticks instead of pooling internal organs such as salivary glands or midguts, so we can determine infection load at the individual level rather than the organ studied.

Hypothesis

This study is based on the hypothesis that **there are Lyme disease infected ticks in Texas** and they have been historically under detected, which makes this disease a significant Public Health concern. Therefore, it is our goal to collect environmental samples of ticks in different parts of Texas and test them for the presence of *Borrelia burgdorferi* using PCR amplification of different genetic markers (7, 27, 60) to understand the strains of *B. burgdorferi* circulating in Southern US as well as their distribution in Central and East Texas where *I. scapularis* is present. Texas has optimal environmental factors present in these areas including the main vector, *I. scapularis*, the wooded regions, climate, seasons, and primary hosts associated with the transmission of *B. burgdorferi*. In addition, the latest studies addressing this issue in Texas date from mid and late 1990's, at which time the detection of *B. burgdorferi* in ticks collected in different areas of the state was done by means of basic culture and microscopic (immunofluorescence) techniques (2, 6, 11, 14, 15, 22, 24, 43-45, 59). Nowadays, there is a wide

array of highly sensitive and specific molecular techniques that can be used to better detect this bacterial pathogen in environmental samples. In particular we will be using PCR followed by sequencing of the positive samples to confirm the presence of *B. burgdorferi* sensu lato complex genospecies in Texas.

CHAPTER II

MATERIALS AND METHODS

Collection and Identification

A total of 573 tick samples were sent to our facilities from all over Texas between February 2011 and July 2012. Most of the samples were sent from veterinary clinics or the Texas Parks and Wildlife management areas. Ticks were found on a variety of animals as well as questing on the vegetation. Thirty-five questing tick samples were actively collected using flags and CO_2 traps. Ticks were stored in 70% ethanol until DNA extraction was performed. Before processing, each tick received an identification code, logged in the laboratory database, and subsequently identified using several hard tick identification keys (3, 23, 29-31, 61). Location (address, zip code, county and GPC coordinates), tick species, sex, and life cycle stages were all noted for each sample as well as host species and tag number (when appropriate) in which they were feeding on.

DNA Extraction and PCR

Total DNA was isolated from the tick samples by the use the commercially available kit "High pure PCR template preparation kit" (Roche Diagnositcs Corp., Indianapolis, IN). After the DNA was extracted from each individual tick, PCR amplification was performed using AccuStart™ PCR SuperMix (Quanta Biosciences, Inc., Gaithersburg, MD). An initial PCR amplification was carried out using primers targeting the flagellin gene (f*laB*) as a screening for all *B. burgdorferi* sensu lato species. Negative water controls were included in all PCR procedures to monitor for contamination. As a positive control, DNA isolated form *B. burgdorferi* B31 strain MSK-5 was

used in each reaction. Amplifications were separated in 2% agarose gel in TAE (Tris-Acetate-EDTA buffer) for 40 minutes at 90 volts. The positive samples were then amplified using four more primer sets including the intergenic region 16SrRNA-23SrRNA (IGR) (7, 8), *p66, ospC*, and *ospA* (27). IGR, p66, and ospC reactions were nested PCR amplifications. Primers and protocols used in this section of the proposal are described below (Table 2). All primers were designed and successfully used by different authors in previous studies of *B. burgdorferi* distribution in the US (7, 9, 27). All final amplicons were separated in 1% agarose gel in TAE buffer for 40 minutes and 90 volts.

Sequencing

All samples with positive amplification, regardless of the primer used, were sent for sequencing to Eton Biosciences Inc. (San Diego, CA). Samples were cleaned utilizing the Wizard® SV Gel and PCR clean up kit (Promega, Madison, WI) following manufacturer's recommendations. The *flaB* amplification was used as screening and positive specimens for this marker were sent for sequencing to verify the presence of a *B. burgdorferi* sensu lato genospecies. When *B. burgdorferi* was confirmed, the other molecular targets were used in different PCR reactions. Positive amplicons were sent for sequencing to verify the presence of *B. burgdorferi* sensu stricto, following the same protocol as the one used for the *flaB* amplification. All sequences obtained have been submitted for population genetic study to understand the distribution of the different *B. burgdorferi* detected in the state of Texas. All sequences were analyzed using MacVector vs. 12.6 (MacVector, Inc.).

Table 1. Primer sequence and amplification programs utilized in this study

Primer	Location	Sequence	Program	References
FlaB:			***FlaB:***	
Bbsl-F	475-496	5'-AACACACCAGCATCACTTTCAGG-3'	94C^{o} for 30 sec	
Bbsl-R	685-709	5'GAGAATTAACTCCGCCTTGAGAAGG-3'	94C^{o} for 30 sec 56C^{o} for 30 sec 74C^{o} for 1 min 4C^{o} o/n	(7-9, 27)
IGR:			**IGR-Nest-1:**	
rrs-rrlA-F	2296-2319	5'-GGTATTTAAGGTATGTTTAGTGAG-3'	94^{o}C for 30 sec	
rrs-rrlA-R	3334-3313	5'-GGATCATAGCTCAGGTGGTTAG-3'	35 times: 94^{o}C for 30 sec, 56^{o}C for 30 sec, 74^{o}C for 1 min 4^{o}C o/n	
rrs-rrlA-Fn	2323-2343	5'-GGTGAAGTCGTAACAAGGTAG-3'	**IGR-Nest-2:**	(7, 8)
rrs-rrlA-Rn	3305-3284	5'-GTCTGATAAACCTGAGGTCGGA-3'	94^{o}C for 30 sec 40 times: 94^{o}C for 30 sec, 60^{o}C for 30 sec, 74^{o}C for 1 min 4^{o}C o/n	
p66-F	1211-1233	5'-GATTTTTCTATATTTGGACACAT-3'	***p66*-Nest-1**:	
p66-R	1966-1943	5'-TGTAAATCTTATTAGTTTTTCAAG-3'	94^{o}C for 30 sec 35 times: 94^{o}C for 30 sec, 50^{o}C for 30 sec, 74^{o}C for 1 min 4^{o}C o/n	
p66-Fn	1252-1275	5'-CAAAAAAGAAACACCCTCAGATCC-3'	***p66*-Nest-2**:	(7)
p66-Rn	1935-1907	5'-CCTGTTTTTAAATAAATTTTTGTAGCATC-3'	94^{o}C for 30 sec 40 times: 94^{o}C for 30 sec, 50^{o}C for 30 sec, 74^{o}C for 1 min 4^{o}C o/n	
OspC-F	306-328	5'-ATGAAAAAGAATACATTAAGTGC-3'	**OspC-Nest-1:**	
OspC-R	963-933	5'-ATTAATCTTATAATATTGATTTTAATTAAGG-3'	94C^{o} for 30 sec 94C^{o} for 30 sec 52C^{o} for 30 sec 74C^{o} for 30 sec 4C^{o} o/n	
OspC-Fn	331-359	5'-TATTAATGACTTTATTTTTATTTATATCT-3'	**OspC-Nest-2:**	(7, 8)
OspC-Rn	948-924	5'- TTGATTTTAATTAAGGTTTTTTTGG-3'	94C^{o} for 30 sec 94C^{o} for 30 sec 52C^{o} for 30 sec 74C^{o} for 30 sec 4C^{o} o/n	
OspA-F	160-178	5'-TATTTATTGGGAATAGGTC-3'	**OspA:**	
OspA-R	1049-1033	5'-GACTCAGCACCTTTTTG-3'	94C^{o} for 30 sec 94C^{o} for 30 sec 51C^{o} for 60 sec 72C^{o} for 2 min 4C^{o} o/n	(7)

CHAPTER III

RESULTS

After sampling from 23 of the 254 counties in Texas, we found ticks positive for Borrelia in 12 counties. A total of 569 ticks were collected and 86 were infected with *Borrelia burgdorferi* sensu stricto (*Bbss*) which were positive for the two genetic markers *fla*B and IGR (Table 3). The infected ticks were collected from several different hosts including dogs, white-tailed deer, cats, gamebock, and javalina, or questing on vegetation. In addition to the 86 positive *Bbss* there were three other strains of borrelia found including 63 *B. burgdorferi* sensu lato (*Bbsl*), 1 *Borrelia americana,* and 3 *Borrelia andersonii*. These other strains of borrelia were *fla*B positive and IGR negative. The *flaB* amplicon was sequenced and the blast analysis was used to confirm species.

Table 3. Infected tick species results

Species	Total	Bb sensu stricto	% infected
Amblyomma americanum	75	16	21%
Amblyomma cajennense	39	11	28%
Amblyomma inornatum	2	2	100%
Dermacentor albipictus	225	18	8%
Dermacentor variabilis	25	1	4%
Ixodes scapularis	**72**	**30**	**42%**
Rhipicephalus sanguineus	132	8	6%
Total	569	86	15%

**Borrelia burgdorferi sensu stricto: fla*B(positive) IGR (positive)*

The 86 *Bbss* samples were further analyzed using the other three markers: *ospC*, *p66*, and *ospA*. We also tested the 63 samples in the *Bbsl* complex with the same three markers to determine the genetic variability in each one of the groups (Figure 2). In this figure we were representing the percent of samples amplifying each marker (*p66*, *ospC* and o*spA*) in both groups, the *Bbss* and

Bbsl detected strains. Interestingly, not all samples amplify all genetic markers, being *p66* the one mostly detected in both groups of Borrelias. On the other hand, *ospA* was the genetic marker less detected in the samples analyzed. Consequently, these results suggest the presence of a great genetic variability in the *Borrelia burgdorferi* sensu stricto strains detected in Texas.

B. burgdorferi ***sensu stricto***
*fla*B and IGR positive
(86 samples)

Marker	Samples (+)	% positive
ospC	51	56%
p66	73	80%
ospA	47	52%

B. burgdorferi ***sensu lato***
*fla*B positive IGR negative
(63 samples)

Marker	Samples (+)	% positive
ospC	32	56%
p66	43	75%
ospA	27	47%

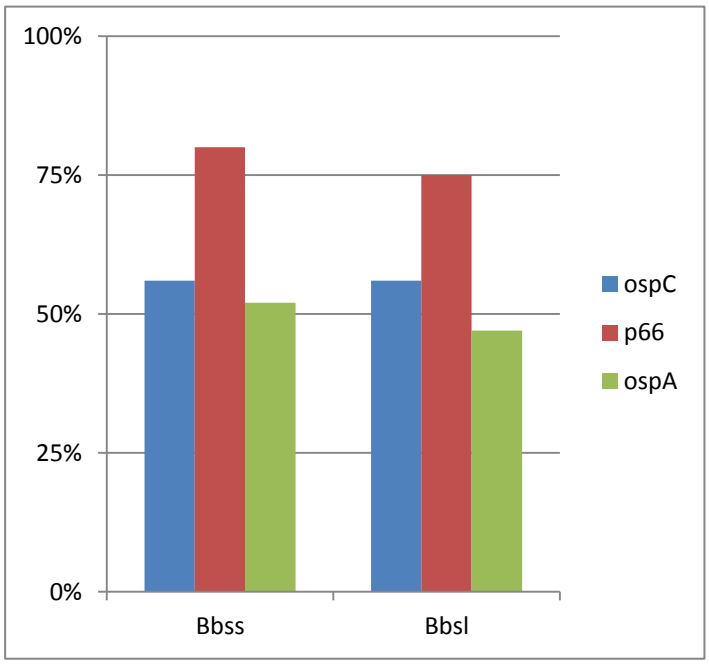

Figure 2. Positive markers for two strains of Borrelia

The total positive samples were mapped by county and compared with the annual amounts of precipitation (Figure 3). The majority of our positive *I. scapularis* samples were found in East Texas due to the greater amounts of rainfall and preferable habitat for tick populations. Nevertheless, some infected *I. scapularis* ticks were found in West Texas, in a region we consider as the borderline for the distribution of this tick species. In addition, other tick species were also found positive for *B. burgdorferi* in this study. They have not been reported as competent vector for the transmission of *B. burgdorferi*, but we can use them as bio-reporters. In

this sense, when detecting *Bbss* in this tick species we are acquiring information regarding the potential distribution of the infectious agent in Texas.

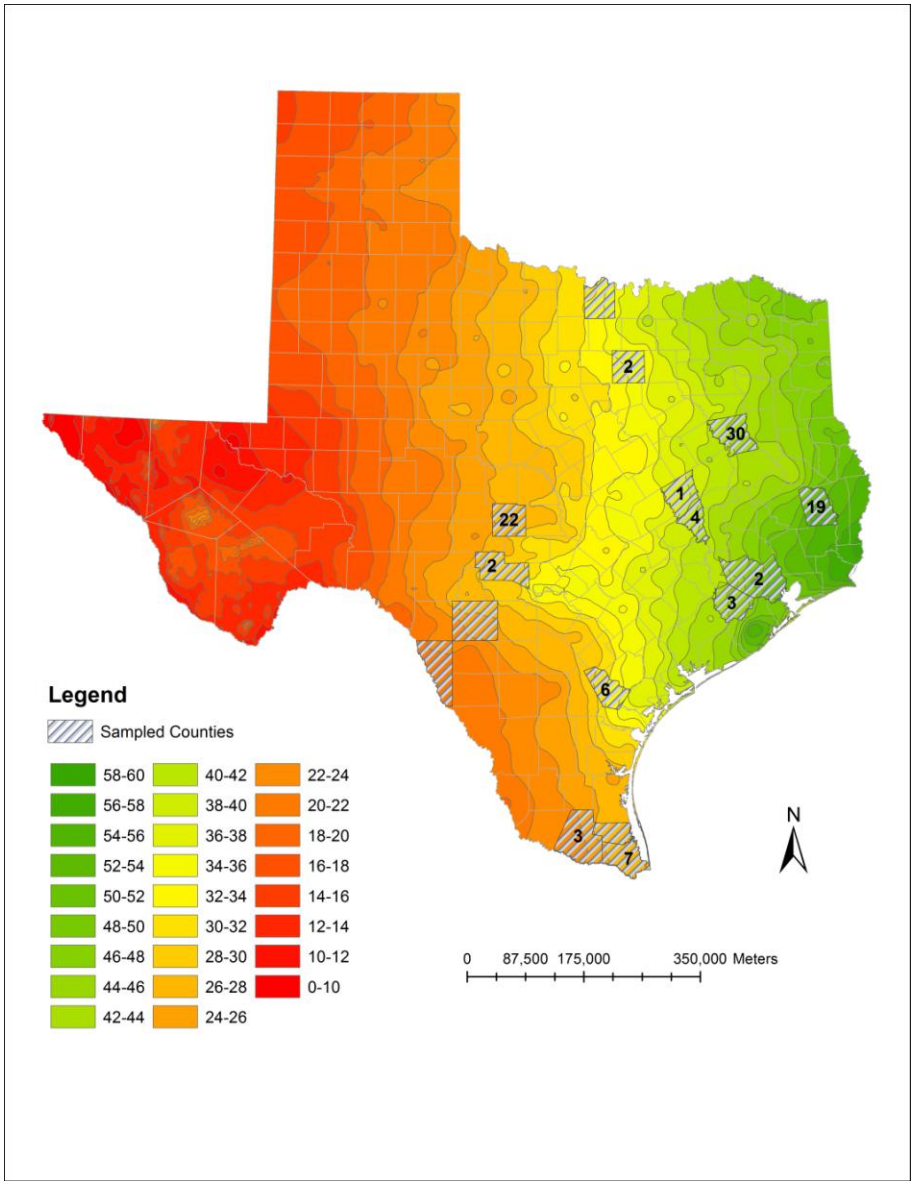

Figure 3. Number of positive samples corresponding to the average precipitation in Texas

CHAPTER IV

CONCLUSION

Based on these results, it can be concluded that **there are infected *Ixodes scapularis* ticks in Texas**. The data shows that 15% of the ticks collected were infected with *Borrelia burgdorferi* sensu stricto, the infectious strain of Borrelia in the United States. Of these infected ticks, 47% were in *I. scapularis*. Moreover, 6% of all the collected ticks infected with *B. burgdorferi* were *I. scapularis*. Based on previous studies, *Ixodes scapularis* is the only tick species that transmits *B. burgdorferi* to humans and companion animals. However, the presence of other infected tick species was important in order to determine where this bacterium is kept in the enzootic cycle. This opens many questions for future research. For instance, are other tick species involved in transmitting pathogenic Borrelia to other mammalian host, including humans and companion animals? Are these other species involved in maintaining *B. burgdorferi* in its enzootic cycle? How is *B. burgdorferi* maintained in its enzootic cycle in areas where *I. scapularis* is not found? Are other tick species competent vectors for this bacterium? Does Texas have a slightly different strain of *B. burgdorferi* circulating?

There is a strong trend in the seasonality of the infected ticks in the fall and winter (Fig. 4,5). Previous papers have shown that the ticks most active in late spring and the summer in northeastern regions. However, the difference in Texas can be attributed to the harsh summer, which causes the ticks to be less active during this season. Texas also has mild winters leading to higher activity during this time as seen in Figure 4. The data supports the fact that the tick activity is more consistent throughout the year, fading slightly during the summers. This data

also shows a correlation with the hunting season in Texas and therefore presents a public health concern.

Most of our samples came from South and East Texas. As shown in Figure 3, the majority of the positive samples were found in areas with higher precipitation, which is in East Texas. This correlates with the understood behavior of Ixodes ticks and environmental preferences. They tend to inhabit areas with higher relative humidity, vegetation, and the wild life that occurs in East Texas. We are continuing to collect tick samples from these areas, but we also want to find out how far west we can go and still find positive samples. This study will allow understanding the real distribution of *I. scapularis* as well as that of pathogenic *B. burgdorferi*.

Most studies look at the presence of questing *Ixodes scapularis* nymphs, since they have been correlated with higher densities of LD human cases. However, in Texas the presence of *I. scapularis* nymphs, and specifically infected nymphs, has not been an easy task (19-21). Consequently, these studies have concluded that Southern U.S. has low risk for infection with Lyme disease. Nevertheless, new cases of Lyme disease are being diagnosed and reported every year in this region of the country. Thus, we still do not understand the transmission cycle from the environment to humans and companion animals. In this first screening study of the state of Texas, we found questing adults *I. scapularis* ticks that were infected with *B. burgdorferi*. This finding suggests the fact that questing nymphs from this species are present in Texas. We are currently working on finding out where and when the nymphs and larvae are questing or feeding on small and medium animals. This effort will help understanding the distribution and real risk of LD in not only Texas but in Southern US. In our current efforts to find *I. scapularis* immature stages, we have continued collecting ticks from a series of location across the state of Texas, and

we are now finding *Ixodes scapularis* larvae and nymph for further analysis. Most of the ticks collected come from wild animals, such a white tail deer and javalina as well as small rodents that are normal wildlife of the region of study. However, some ticks were collected in gated properties and off of exotic animal species such as scimitar-horned oryx. In addition, some of the ticks were also infected with *B. burgdorferi*. This leads to questions such as what is the role of this exotic species in the maintenance of the enzootic cycle of *B. burgdorferi* in our study area?

Further studies need to be done on other tick species capable of infecting intermediate mammalian hosts, such as rodents and other medium size mammals that are responsible for the maintenance of *B. burgdorferi* in its enzootic cycle, and look further into the ecology of this disease. *Amblyomma americanum,* and *Dermacentor variabilis* have been found to acquire, maintain, and transmit *B. burgdorferi,* however, they have very low rates of infection (41). This is a potential explanation for the maintenance of this pathogen in areas where *Ixodes scapularis* ticks are not present or at times of the year in which they are not active and still humans and dogs are getting infected.

Figure 2 shows a discrepancy in the detection of the other 3 genetic markers *osp*A, *osp*C, and p66 in either *Bbss* (*flaB* and IGR positive) or *Bbsl* (*flaB* positive and IGR negative). The genetic marker *p66* was detected 80% positive in the tick samples. The variability found in the detection of the different genetic markers, suggests the presence of significant genetic diversity in *Borrelia burgdorferi* strains detected in our region of study. It also shows that there is more genetic diversity found in *Bbsl* since the detection of the different genetic markers was even more variable than the *Bbss*. Other members of the laboratory are conducting population genetic

studies with the sequences we have obtained in this study for each one of the markers and compared with the strains found in other areas of the country. This study will determine whether we have similar or different Borrelia strains in Southern U.S. and whether or not this can correlate with the ticks in which Borrelia was detected.

The last study trying to detect *B. burgdorferi* in Texas was published in 1994 and utilized conventional immune-staining techniques as well as culture of Borrelia from collected ticks (46). It was found that none of the Ixodes ticks were infected, despite previous studies in the Northeast that had found anywhere between 12-100% rate of infection. Now that we have proof that there are *B. burgdorferi* infected *Ixodes scapularis* ticks in Texas, we can continue to isolate *B. burgdorferi* strains from different locations in the state so as to understand genetic differences of the strains in Southern U.S. compared to those in Northern U.S. We can also determine whether or not there is genetic variation that could explain differences in disease onset, immune response in humans or presence of different competent vectors in Southern U.S. that differ from what has been described in Northern U.S.

Taken together, our studies showed that *B. burgdorferi* infected ticks are widely distributed in Texas, mostly in Eastern and Central Texas but also in South Texas. Furthermore, *Ixodes scapularis*, the competitive vector for the transmission of Lyme disease was also present in the same locations and showed a 42% infection rate. This finding is similar to those described for other regions of the country with higher LD incidence in humans and companion animals. Furthermore, questing infected ticks or infected ticks feeding on medium to large mammalian hosts were mostly detected during the fall and winter months, which coincide with the hunting

season for white tail deer. Consequently, we suggest that contrary to what has been observed in other parts of the country, the risk for Lyme disease infection in Texas will increase during the fall and winter months, reducing significantly during the summer months. Further studies to validate this observation are currently in progress. If confirmed, this will significantly impact the detection and diagnostics of Lyme disease in Southern U.S.

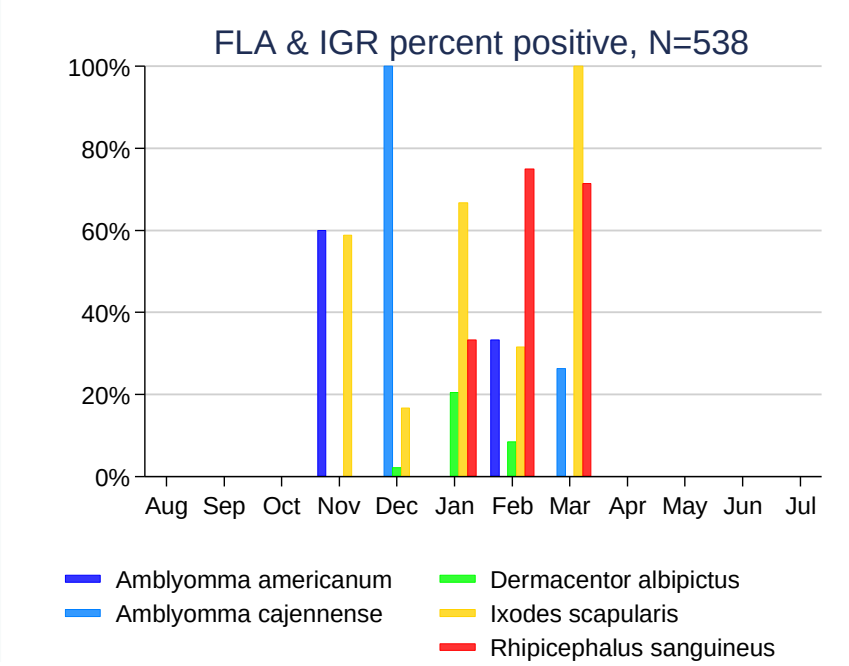

Figure 4. Seasonality of *Bb* sensu stricto infected tick species in Texas

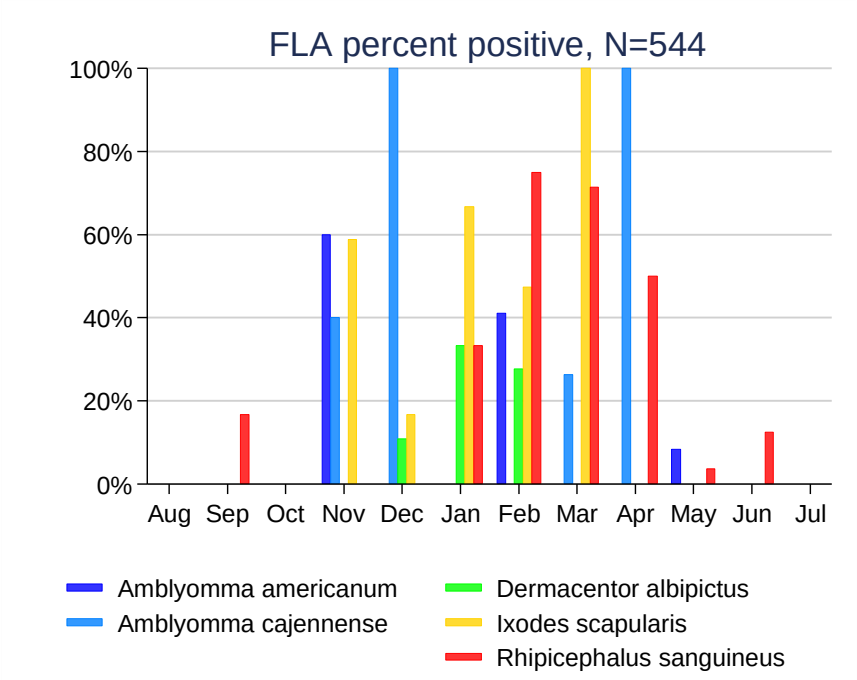

Figure 5. Seasonality of all Borrelia positive tick species in Texas

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