THE FREQUENCY AND OCCURRENCE OF LYME DISEASE IN TEXAS

An Undergraduate Research Scholars Thesis

by

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ABSTRACT

The Frequency and Occurrence of Lyme Disease in Texas. (May 2013)

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This study examines the frequency and spatial occurrence of Lyme Disease (LD) within the state of Texas. According to the CDC, LD is the most prevalent arthropod borne disease in the US with 33,097 cases reported last year 2011. In 2009 the case definition of LD was revised and it differentiates in between confirmed and probable cases for this disease. Taking this into account, Texas is the only state in the US in which the ratio of probable versus confirmed cases is repetitively as high as 2:1. This ratio can be attributed to many different causes, from doctors' disregard for the disease and not testing for it, to the presence of genetically distinct *Borrelia* species and/or *Ixodes scapularis* tick vectors in Southern US. Thus it is important to develop a firm understanding of the distribution of tick vectors in Texas. An important tool hampering the study of LD has been the lack of a valid and reliable LD diagnosis protocol for veterinary purposes. Dr. Esteve-Gassent's laboratory here at Texas A&M has generated data regarding canine LD and ticks containing *B. burgdorferi* sensu stricto. The data for the canine LD comprised of western blots, ELISA tests and IFA diagnostics on a collection of over 800 animals. Ticks were collected during the same timeframe (Fall 2011 through Sumer 2012) and

analyzed with PCR for various genetic markers to determine the presence of *B. burgdorferi*. This work has already determined cutoff values for the ELISA test that determine which animal samples are true positives and which are true negatives. This work has shown that the standard IFA testing is not a reliable methodology for diagnostics in Veterinary Medicine. We aim to generate a series of maps displaying the distribution of canine LD cases, positive ticks and human cases, and determine the level of correlation of these distributions. The goal of this project is to determine whether or not canine LD can be used as an indicator of areas of high risk for human LD.

DEDICATION

To Sean, thanks for putting up with me and my weirdness.

ACKNOWLEDGEMENTS

I would like to take this opportunity to acknowledge the many people who made this project possible. Dr. Sandy Rodgers from Texas Veterinary Medicine Diagnostic Laboratory (TVMDL) for their help collecting dog samples from different counties in the state of Texas necessary to carry out this study, the AgriLife-TVMDL seed grant funding the project entitled "Improving diagnostic methods for Lyme disease, and epidemiology of human and animal infections in TX", that will provide the financial support to conduct the experiments outlined in this thesis. I would also like to thank The Texas A&M Honors Program for their support through the Undergraduate Fellowship program and giving me the opportunity to partake in such an excellent program. Special thanks to Dr. May Bogges for her valuable help and knowledge during the statistical analysis of the data generated in this thesis. I would also like to give a very special thanks to all the organizations that helped us with the collection of ticks (Texas parks and wildlife, the Brazos Animal Shelter, the TVMA and hunters that provided ticks). To my advisor, Dr. Esteve-Gassent

NOMENCLATURE

LD	Lyme disease
CDC	Centers for disease control and prevention
LB	Lyme borreliosis
EM	Erythema migrans
IFA	Immunofluorescent-antibody assay
ELISA	Enzyme linked immuno sorbed assay
WB	Western blot
PCR	Polymerase chain reaction
FIPS Codes	Federal information processing standards codes

CHAPTER I

INTRODUCTION

According to the CDC, Lyme Disease (LD), a bacterial infection transmitted by ticks, is the most prevalent arthropod borne disease in the United States (31, 33, 36). Since 2002, there has been a gradual increase of cases and recently in 2011 the CDC reported 33,097 cases of LD[1] with an incidence rate set at 7.8/100,000 people[2]. Texas alone reported 28 confirmed and 46 probable cases. In 2008 the case definition of LD was revised and nowadays the CDC differentiates in between confirmed and probable cases for this disease[3]. Taking this into account, since 2008 Texas is the only state in the US in which the ratio of probable versus confirmed cases is repetitively as high as 2:1. This high ratio of probable versus confirmed cases can be attributed to many different causes from doctors' blatant disregard for the disease to the presence of genetically distinct *Borrelia* species and/or *Ixodes scapularis* tick vectors in Southern United States. Thus, it is important to develop a firm understanding of the distribution of tick vectors in Texas and highlight the prevalence of canine LD cases. An important tool hampering the study of LD has been the lack of a consistent LD diagnosis protocol for veterinary purposes among different diagnostic laboratories. In addition, this is a none-reportable disease in veterinary medicine, contrary to what happens in human medicine. This study examines the frequency and spatial occurrence of canine LD within the state of Texas as well as the occurrence of the tick vector in the same area of study. Generating LD distribution maps of the different strains of *B.*

[1] http://www.cdc.gov/lyme/stats/chartstables/reportedcases_statelocality.html
[2] http://www.cdc.gov/lyme/stats/chartstables/incidencebystate.html
[3] http://wwwn.cdc.gov/NNDSS/script/casedef.aspx?CondYrID=752&DatePub=1/1/2011%2012:00:00%20AM

burgdoferi circulating in the Southern United States as well as the distribution of *I. scapularis* in the same area, will help us understanding the human risk for disease in an area that has been poorly studied since the disease was first described. In addition, mapping the canine and human LD cases will help to determine whether dogs can be proposed as sentinels for LD in Texas.

The tick life cycle

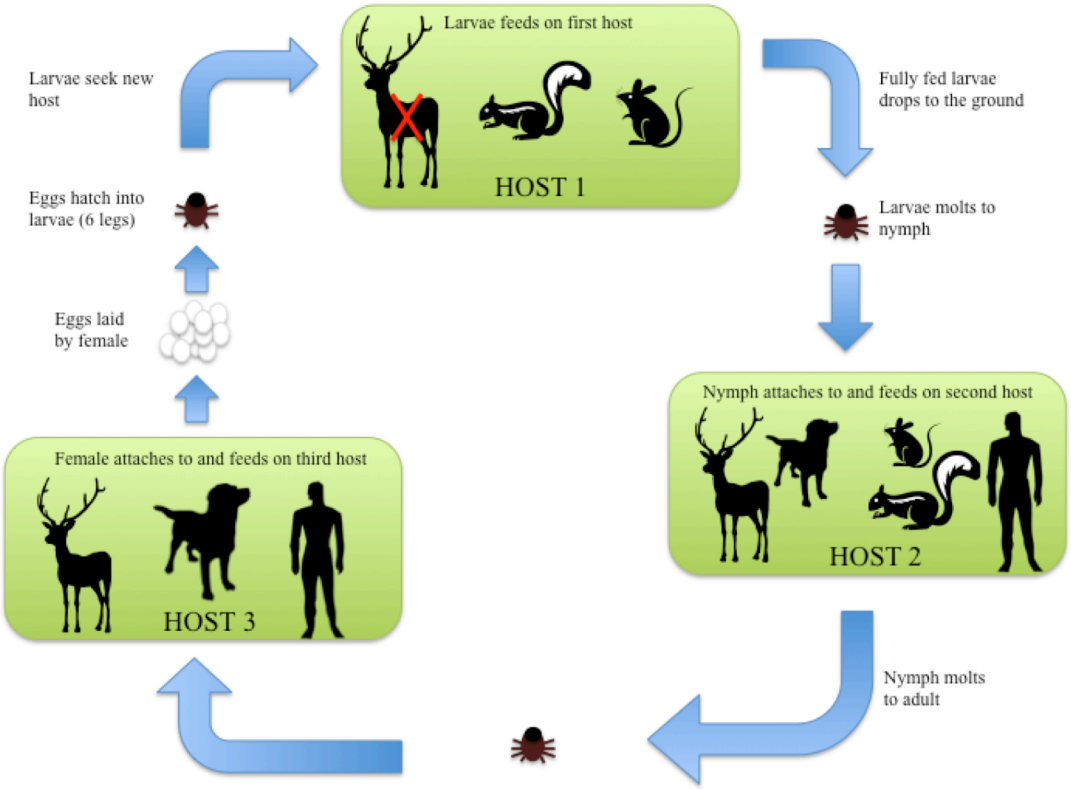

FIG 1. Tick Life Cycle. Infectious cycle of the European *Borrelia burgdorferi* sensu lato genospecies *B. burgdorferi* sensu lato is the only pathogenic genospecies present in the US and Europe, both rodents and birds are reservoirs. A red cross indicates a non-reservoir host. (Adapted from "Lyme borreliosis" Stanek G.

Hard ticks have a life cycle consisting of three primary stages- a larval stage, a nymphal stage, and an adult stage (2). The tick feeds on a host in between each major stage in their life cycle

before dropping off the host and molting into the next stage. Each stage takes several months to complete before the tick can move on and grow. The time between each stage varies and is dependent on a number of environmental factors such as surrounding temperature, the amount of rainfall, and the availability of host prey to feed on. Ticks start of their life cycle as hatched larvae with six legs. They proceed to feed on their first host before dropping off and molting into a nymph with eight legs. The larvae is the first stage in which a tick can be infected with the bacterial spirochete *B. burgdorferi (34).* Until then, the tick cannot become a vector of LD because it has not fed on any host, and *B. burgdorferi* does not transmit transovarial. LD is transmitted through the blood meal (20, 23, 26). Since larvae have not had the chance to feed yet, they cannot transmit the disease. Consequently, nymphs are the first stage at which *Ixodes* ticks can transmit the infection. At the adult stage, the ticks grow in size and feed on larger prey-primarily deer. While adult ticks possess the ability to transmit LD, they are not as dangerous as nymphs because nymphs are smaller in size and thus, harder to spot.

Lyme disease stages and symptoms

LD is caused by the bacterial spirochetes *Borrelia burgdorferi* senso lato and is transmitted by the tick vector *Ixodes scapularis* in northeastern North America, *Ixodes pacificus* in midwestern North America, *Ixodes ricinus* and *Ixodes persulcatus* in Europe, and *Ixodes ovatus* in Asia (23, 32, 33). There are approximately 18 recognized genospecies of Borrelia that are present in ticks and conform to what is known as the *B. burgdorferi* sensu lato complex. Only *B. burgdorferi* sensu stricto has been proven to cause disease in humans within the United States, while *B. garinii* and *B. afzelii* have been proven to cause LB in Europe. In addition, *B. spielmani*, *B.*

bissettii, *B. valsiana* and *B. lusitane* are currently being studied to confirm their implication in Lyme borreliosis (23, 32, 33).

LD, or also called Lyme borreliosis (LB), is a multisystemic disease that can be characterized into three stages (33). The first stage of LD, the early-localized stage, features the most common symptom, erythema migrans (EM) present in 70% of the reported cases (as per CDC recent published statistics based on reported cases during the last 9 years[4]). This stage usually occurs within a month of infection and presents flu-like symptoms along with an expanding rash, called erythema migrans, which usually stems from the site of the tick bite. The second stage of LD, or the early-disseminated stage, exhibits signs of dissemination with multiple EM sites, pain and stiffness in joints and muscles, fever and other flu-like symptoms, and complications with neurologic and cardiac systems. The third stage of LD, the late-disseminated stage or Chronic Lyme Disease, is comprised of neurological involvement (14% of reported cases) [5], further cardiac problems (1% of reported cases) [6], numbness in the extremities, and chronic arthritis (30% of reported cases) [7].

Testing methods

There are effective treatments for LD if caught early, but the ability to diagnose LD is so inconsistent that it hinders many patients from being able to receive treatment within an adequate time span (33). There are multiple techniques that can be used to test for LD such as an enzyme linked immuno sorbed assay (ELISA), an immunofluorescent-antibody assay (IFA), a PCR using

[4] http://www.cdc.gov/lyme/stats/chartstables/casesbysymptom.html
[5] http://www.cdc.gov/lyme/stats/chartstables/casesbysymptom.html
[6] http://www.cdc.gov/lyme/stats/chartstables/casesbysymptom.html
[7] http://www.cdc.gov/lyme/stats/chartstables/casesbysymptom.html

genetic markers, and the Western Blot (immunoblot). While all of these tests have their strengths and their weaknesses, PCR and ELISA testing leaves less room for error. The IFA test has a problem with blurring the lines between "true" positives and negatives, and the WB test leaves room for human error when reading test results (1). Consequently, in human medicine, the diagnostic of LD in the absence of EM is based in a two-tier system consistent of a first ELISA test followed by an immunoblot assay[8]. When a patient has an ELISA test positive for the diseases, it is confirmed by running the immunoblot assay in which specific *B. burgdorferi* proteins will be tested for its reactivity with the serum sample. CDC has established some guidelines for the purpose of surveillance that allows physicians to determine whether the patients are confirmed or probable cases for Lyme diseases helping the reporting system for this disease. On the other hand, since LD is not reportable in veterinary medicine, a wide array of veterinary LD test are available in the market, which hampers the ability of getting an estimate of the annual LD cases in a particular companion animal species.

On the other hand, ticks can also be tested for the presence of *B. burgdorferi*. 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 (4, 29). 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 (4, 24, 29, 30). 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 to do population genetic studies of the

[8] http://www.cdc.gov/lyme/diagnosistesting/LabTest/TwoStep/index.html

Borrelia genospecies identified (6, 7, 24). Therefore, we decided to utilize the same markers previously used in the northeast, Midwest and Western US 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 location studied.

Hypothesis

The goal of this project is to determine whether or not canine LD can be used as an indicator of areas of high risk for human LD. In order to obtain this goal we generate LD distribution maps for Texas that will help us in understanding the strains of *B. burgdorferi* circulating within the Southern United States as well as their circulation in Central and Eastern Texas where *I. scapularis* is present. Data generated at Dr. Esteve-Gassent's laboratory will be entered into an Excel and statistical analysis was carried out using Stata. Three maps (canine, tick, and human) will be created in Stata displaying the density of cases in each county for the period Fall 2011 through Spring 2012. Geo-statistical regression analysis will be used to compare cases and location and determine the strength of the evidence in the data of a relationship between canine, tick and human LD.

CHAPTER II

METHODS

The following descriptions explain how analysis of the dog and tick samples were prepared and executed.

Preparation of data sets

Data was first generated in Dr. Esteve-Gassent's laboratory. After receiving the test samples and results from the experiments, data was input into various Excel sheets. During this study we worked with three data bases: Human Lyme disease cases from 2000 till 2012, Canine Lyme disease cases from October 2011 till October 2012 and ticks collected from multiple locations in Texas from March 2011 till September 2012. Each database contained information regarding: location from which samples were acquired, animal species and/or tick species, sex, tests ran (Immuno fluorescence assay (IFA), Immunosorbed assay (ELISA) and immunoblot or Western blot (WB) assay for dogs; PCR test with different markers for tick samples) and result of each one of the tests ran per sample. Once databases were established for each test subject (canine/animal hosts, tick, and human), all entries marked "positive" or "yes" and "negative" or "no" were substituted for their binary equivalents- 1 for yes/positive, 0 for no/negative. The data was then loaded into STATA version11® (STATA, Inc. College Station), where the content was screened for any inconsistencies, ie: Yes, yes, YES, etc. Corrections for the inconsistencies were made by inputting coded instructions into STATA that forced each varying input issue to conform to one form of input. For example, if a sample tested positive for LD under the IFA test, possible results could be recorded in a variety of ways (Yes, YES, yes, POS, Pos, pos, Positive,

POSITIVE, positive, etc). In order to run a functioning program that can analyze these results, corrections must be made. All of the possible result recordings are streamlined into either 1 for a positive result or 0 for a negative result by inputting code into STATA that forces all possible positive options to be replaced by 1 and all possible negative results to be replaced by 0. Once all of these steps have been taken, analysis can begin on the actual data itself.

TABLE 1. Summary of databases used in this study

Data Set	Years	Total Samples	Tests Run	Positive Cases (%)	Geographical Region
Human	2000-2012	1212	*	100**	TX
Canine	2011-2012	890	ELISA, WB, IFA	27.7	TX
Ticks	2011-2012	681	PCR	24.7(14.7)***	TX

* Data from the Texas Department of Safety Health Services
** All LD diagnosed cases
*** First number denotes ticks positive for *B. burgdoferi* senso lato, the second number in parenthesis denotes ticks positive for *B. burgdoferi* senso stricto.

Generation of tables

Using the databases generated from Excel and STATA, tables can be generated to make it easy to keep a running record of test results. These tables help the analysis of data in a variety of ways: keeping a general count of select data, comparison of several sets of data, and generating cut-offs in testing values. In Dr. Esteve-Gassent's laboratory, all three uses have been utilized. General counts are taken so that comparisons are simple to make and easy to see. Several tables have been generated to help generalize test results: % Positive IFA vs. % Positive WB, % Positive WB vs. % Positive ELISA, etc. The generated tables have also been helpful when used in conjunction with STATA to reanalyze cut-offs for each band in the Western Blot test. Each test subject was tested for LD using the Western Blot test, and the results were recorded in a

database. The results recorded each band in the test and whether or not each subject was positive or negative in showing each of those bands. After recording the results, the database was refined it into a table with binary input. The table was then run through a program in STATA that compared the occurrence of each band with the diagnosis of each animal to see the relevance of each band, ie: was a certain band always positive, regardless of the diagnosis? or was certain bands only prevalent when the diagnosis was negative/positive? After comparing each bands' occurrence to the diagnosis, the program identified which bands were relevant and which ones were useless in diagnosing LD. Therefore, this analysis allowed our team to clearly differentiate in between positive and negative canine LD cases, so comparison with human reported cases and presence of positive ticks was done.

Generation of graphs

Databases were also utilized to generate visual aides that were used to define our results. Each database was run through STATA to generate a graph that would display the monthly seasonality of Lyme Disease within Texas. This graph was then examined for similarities and differences with the nation's reported seasonality to see if Texas adhered by the same pattern of outbreaks or whether it opposed or mirrored it. Graphs were also generated to determine the correct cut-off levels for ELISA and IFA tests by running the databases through several programs that were created in STATA. The graphs compared the results and measured the accuracy rating of each test to determine proper cut off values.

Maps

After running the databases through programs that generated tables and graphs, the databases were run through a program that generated maps for geo-statistical analysis. A map modeling the density of human cases in each county in Texas from Fall 2011 through Spring 2012 was generated using the prepared database and a program run through STATA. To generate the map and the county lines, the zip codes in each county had to be converted to FIPS codes that could be read in STATA. FIPS codes are a standardized set of codes that help computer programs uniformly determine geographic regions. Unlike zip codes, FIPS codes only have one code per county, making it easier to program a map with county borders. In order to do this, a program had to be generated and executed so that the conversion would be consistent and quick. Once the map was generated, geo-statistical regression analysis was then used to compare cases and location to determine the strength of the evidence in the data of a relationship between canine, tick and human LD.

Spatial analysis

Spatial analysis of the data was conducted by using Arc GIS 10. By using spatial analysis for this data will help study locational attributes of the presence of Lyme in Texas. The locational attributes of spatial data are formally expressed by means of the geometric features of points, lines or areal units (polygons) in a plane, or, less frequently, on a surface. This spatial referencing of observations is also the salient feature of a Geographic Information System (GIS), which makes it a natural tool to aid in the analysis of spatial data. Conventionally, geographic information systems had four basic functions to perform on spatial data: input, storage, analysis and output (3, 12-16, 22). Anselin and Getis (3) further divided analysis function of a GIS was

into four components, consisting of selection (sampling of data from the data base), manipulation (partitioning, aggregation, overlay, buffering, and interpolation), exploration and confirmation. This study comprised of mapping ticks positive for Borrellia, dogs positive for Lyme and reported Lyme cases among humans at county and zip code level. Mapping at county level will provide the information related to hot spots for presence of Lyme in Texas and mapping at zip code level gives an idea about clustering of the cases in a county around a particular region. Further, mapping the positive cases with average precipitation and ecology of the state would define the conditions necessary for survival of infected ticks in Texas. As prevalence of Lyme in Texas remains questionable, visual representation of the same can help break the myth about same along with, will give an idea about the problem areas in this State to other fellow researchers as well.

CHAPTER III

RESULTS

Canine samples and sero-prevalence of Lyme disease in Texas

Analysis of western blot tests- irrelevant bands

Once the rest of the data for the dogs was compiled, tests were generated through a program in STATA that analyzed the amount of positive and negative cases and how many bands were accounted for in each of those cases. The program counted the amount of times each band occurred and compared it to whether or not the case it correlated to displayed a positive or negative diagnosis. Some bands occurred continuously regardless of the diagnosis deeming them irrelevant because they did not help in differentiating the different cases.

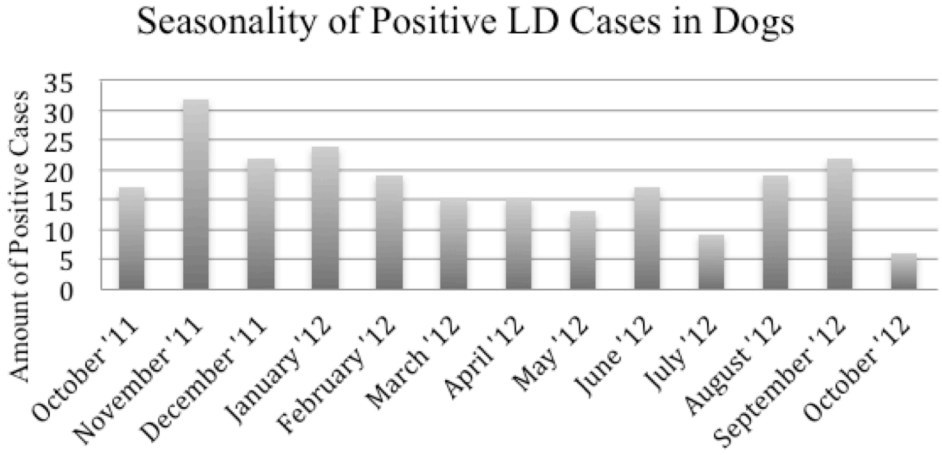

FIG 2. Chart displaying the seasonality of LD cases from 2011-2012 for dogs in Texas.

Seasonality

A total of 925 canine samples were studied, and they distributed in 95 of the 254 couties in

Texas. The positive canine cases mapped in 69 counties, while samples from 26 counties were negative. A graph was generated that counted the amount of positive LD cases in dogs and sorted them into the months that they occurred. This graph made it easier to visualize the seasonality of LD in dogs, and made it easier to identify key peak and rest times for LD in Texas.

TABLE 2. Table showing the different types of tick species received in lab along side the corresponding amounts of IFA positive cases.

Tick Species	Positive Cases
Amblyomma americanum	20/109 (18.35%)
Amblyomma cajennense	19/39 (48.72%)
Amblyomma maculatum	0/1 (0%)
Amblyomma inornatum	2/2 (100%)
Dermacentor occidentalis	0/2 (0%)
Dermacentor albipictus	49/225 (21.78%)
Dermacentor variabilis	4/30 (13.33%)
Rhipicephalus sanguineus	20/137 (14.60%)
Ixodes scapularis	73/145 (50.34%)

Spatial distribution

In addition to the chart, a map was also generated using a GIS computer protocol. The protocol mapped positive LD dog cases in Texas by distinguishing the different counties that the positive cases from the data originated from. The map displays both the amount of positive cases found from our data spanning the past year and the amount of precipitation that each county received.

Ticks

Numerical analysis

The first thing that was analyzed in regards to the tick data was the different type of ticks and the amount of each tick we received. A table was generated to easily compare the amount of each

type of tick received that tested positive of Borrellia.

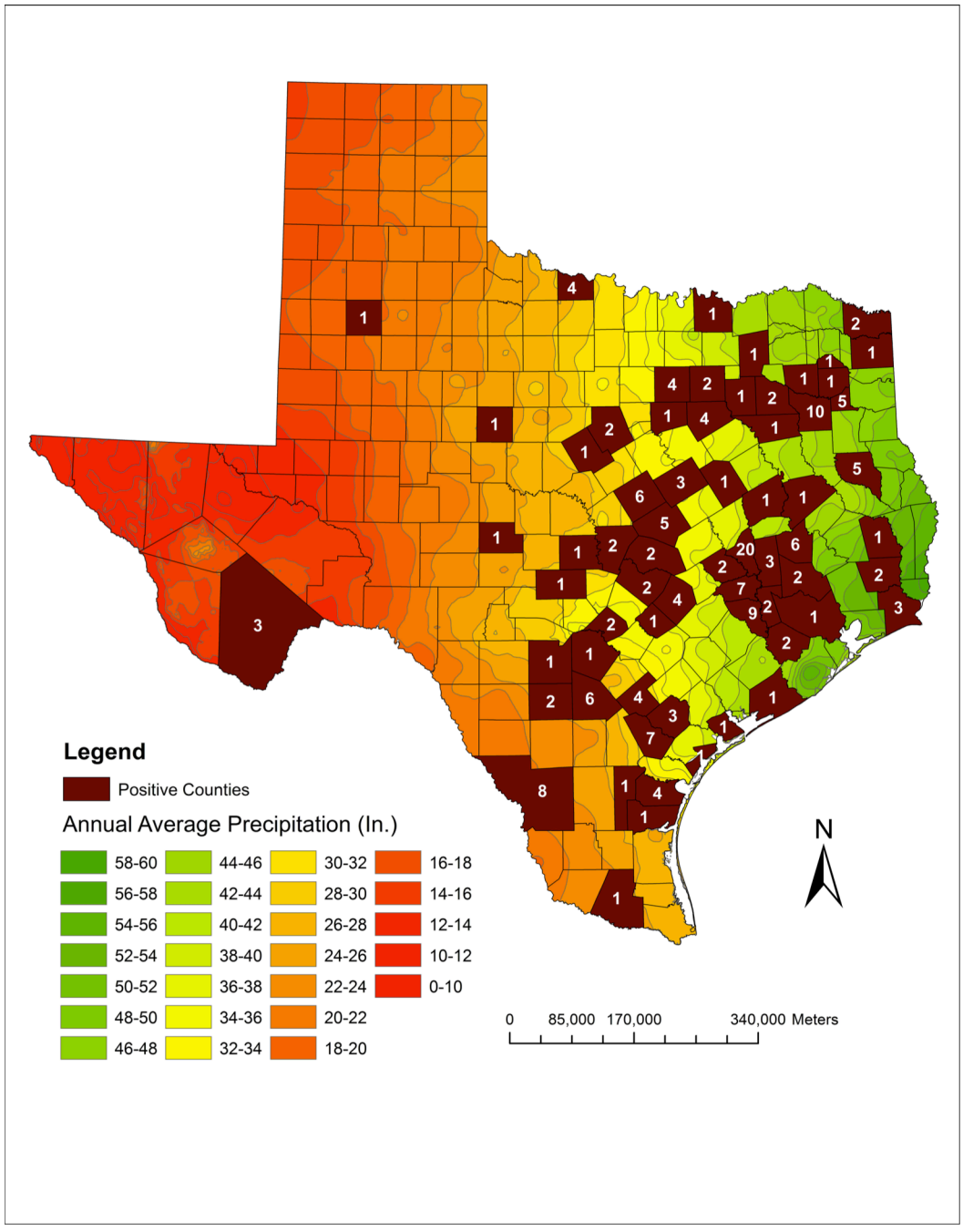

FIG 3. Map displaying the amount of positive LD cases in dogs in Texas compared to the amount of average rainfall.

Table 3. Running list of counties from which dog samples were obtained.

	ELISA positive		
County	**Percent**	**Count**	**Total**
Aransas	100% (100-100)	1	1
Caldwell	100% (100-100)	1	1
Camp	100% (100-100)	1	1
Goliad	100% (100-100)	3	3
Grayson	100% (100-100)	1	1
Hardin	100% (100-100)	2	2
Johnson	100% (100-100)	1	1
Kleberg	100% (100-100)	1	1
Llano	100% (100-100)	1	1
Menard	100% (100-100)	1	1
Somervell	100% (100-100)	1	1
Tyler	100% (100-100)	1	1
Upshur	100% (100-100)	1	1
Wood	100% (100-100)	1	1
Brewster	75% (0-100)	3	4
Burleson	67% (0-100)	2	3
Comal	67% (0-100)	2	3
Erath	67% (0-100)	2	3
Hidalgo	67% (0-100)	2	3
Travis	67% (0-100)	2	3
Waller	67% (0-100)	2	3
Williamson	67% (0-100)	2	3
Atascosa	60% (23-97)	6	10
Bexar	50% (0-100)	1	2
Frio	50% (0-100)	2	4
Matagorda	50% (0-100)	1	2
Medina	50% (0-100)	1	2
Ellis	44% (4-85)	4	9
Karnes	44% (4-85)	4	9
Grimes	43% (0-92)	3	7
Walker	43% (13-73)	6	14
Washington	41% (15-67)	7	17
Nacogdoches	38% (8-69)	5	13
Smith	38% (18-59)	10	26
Webb	36% (15-58)	8	22
Austin	35% (15-54)	9	26
Bee	33% (11-55)	7	21
Burnet	33% (0-88)	2	6
Comanche	33% (0-100)	1	3
Gillespie	33% (0-100)	1	3
Houston	33% (0-100)	1	3
Jefferson	33% (0-72)	3	9
Jim Wells	33% (0-100)	1	3
Leon	33% (0-100)	1	3
Montgomery	33% (0-88)	2	6
Navarro	33% (0-100)	1	3
Taylor	33% (0-100)	1	3
Coryell	32% (9-55)	6	19

Table 3. Continuation

Van Zandt	**29% (0-74)**	**2**	**7**
Brazos	27% (17-37)	20	74
Bell	26% (5-48)	5	19
Gregg	26% (5-48)	5	19
Cass	25% (0-100)	1	4
Kaufman	25% (0-100)	1	4
Limestone	25% (0-100)	1	4
Tarrant	25% (1-49)	4	16
Nueces	24% (1-46)	4	17
Fort Bend	22% (0-56)	2	9
Bowie	20% (0-50)	2	10
Calhoun	20% (0-76)	1	5
Lubbock	20% (0-76)	1	5
Dallas	18% (0-45)	2	11
Hunt	14% (0-49)	1	7
Wichita	14% (0-28)	4	28
Mclennan	14% (0-29)	3	22
Bastrop	13% (0-26)	4	30
Henderson	11% (0-37)	1	9
Harris	7% (0-21)	1	15
Anderson	0% (0-0)	0	1
Angelina	0% (0-0)	0	3
Brazoria	0% (0-0)	0	2
Callahan	0% (0-0)	0	1
Colorado	0% (0-0)	0	1
Freestone	0% (0-0)	0	4
Gonzales	0% (0-0)	0	2
Harrison	0% (0-0)	0	1
Hays	0% (0-0)	0	4
Jasper	0% (0-0)	0	1
Kerr	0% (0-0)	0	2
Lamar	0% (0-0)	0	2
Madison	0% (0-0)	0	3
Milam	0% (0-0)	0	1
Montague	0% (0-0)	0	1
Panola	0% (0-0)	0	1
Pecos	0% (0-0)	0	1
Polk	0% (0-0)	0	1
San Patricio	0% (0-0)	0	2
Starr	0% (0-0)	0	1
Trinity	0% (0-0)	0	3
Victoria	0% (0-0)	0	2
Wharton	0% (0-0)	0	1
Fayette	.% (.-.)	.	0
Jackson	.% (.-.)	.	0
Nolan	.% (.-.)	.	0

Seasonality

Likewise with the tick samples, a graph was generated to help visualize the seasonality of LD in ticks in Texas. The graph counted the amount of tick samples that tested positive for Borrellia that were collected each month. We generated one graph for those samples that were designated as *B. burgdorferi* sensu lato (Bbsl) positive (positive for *flaB* genetic marker), and the same graph for those that were designated as *B. burgdorferi* sensu stricto (Bbss) positive ticks (*flaB* and IGR positive). These results are shown in figures 4 and 5 where the peak times for ticks showing infection with *B. burgdorferi* was mostly from September through April.

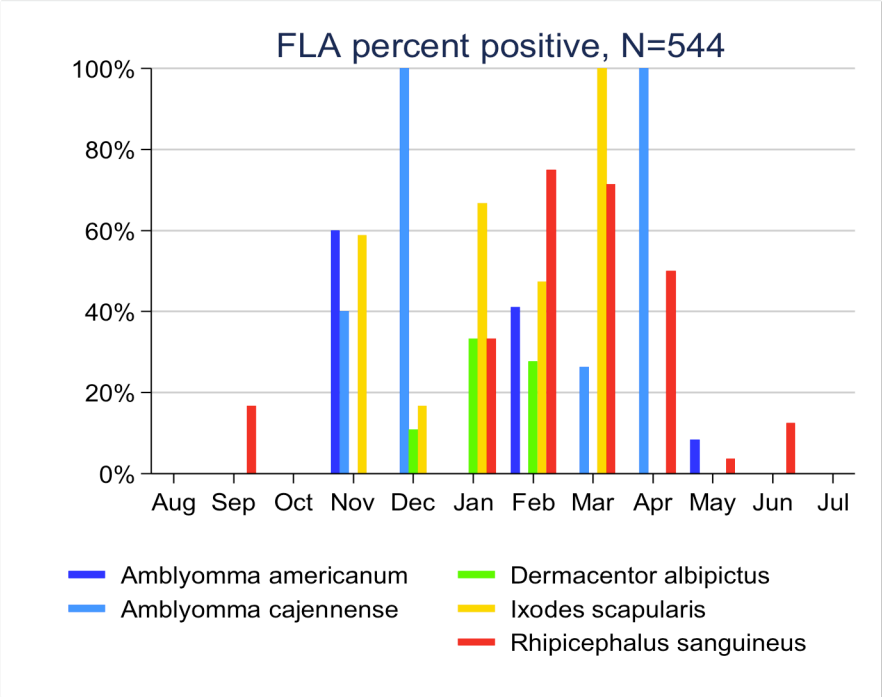

FIG 4. Tick Seasonality Chart. Percentage of total tick cases from 2011-2012 that tested positive for *B. burgdoferi* using *fla* amplification.

Moreover, *I. scapularis*, the competent vector for the transmission of Lyme disease is present from November through March, and no ticks of this species were collected during the summer. Figure 5 shows the fact that the Bbss infected ticks are distributed in time from the months of

November through March; this is a narrower window of presence than the one observed for Bbsl infected ticks. This shows us that dogs can possibly be used as sentinels for LD in Texas because the ticks feed on them during their peak months and then die off, and after the ticks feed and go through their low point in their seasonality, the dog cases peak.

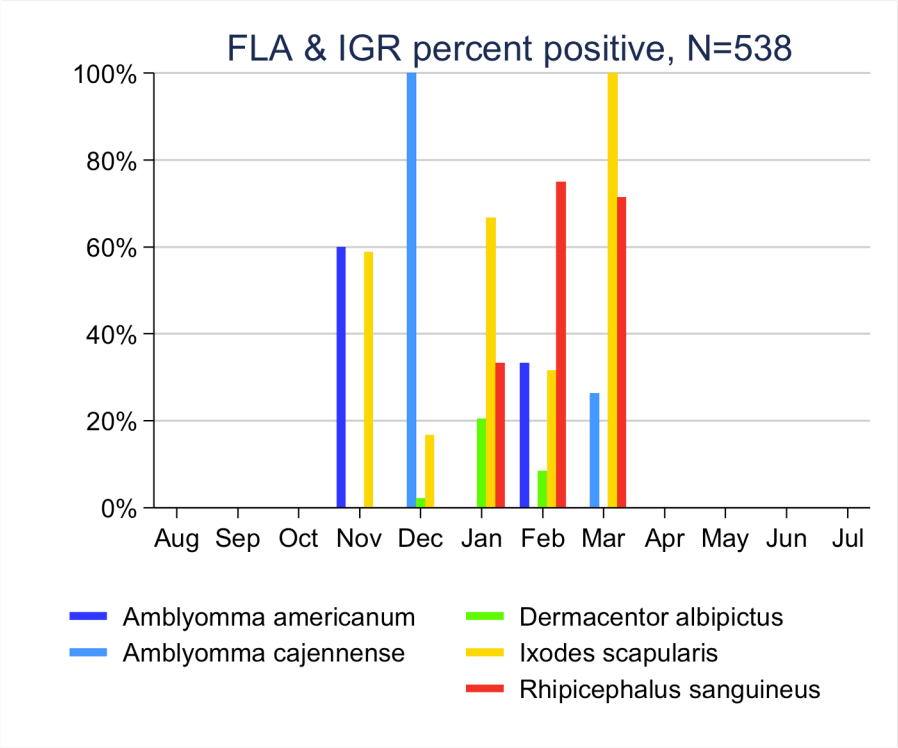

FIG 5. Tick Seasonality Chart. Percentage of total tick cases from 2011-2012 that tested positive for *B. burgdoferi* by using *fla* amplification and then further by IGR amplification.

In addition, when representing each of the genetic marker detected in each month, we observed that most of the genetic variability detected was present in the fall and winter months, which coincides with the lowest maximum and minimum average temperatures.

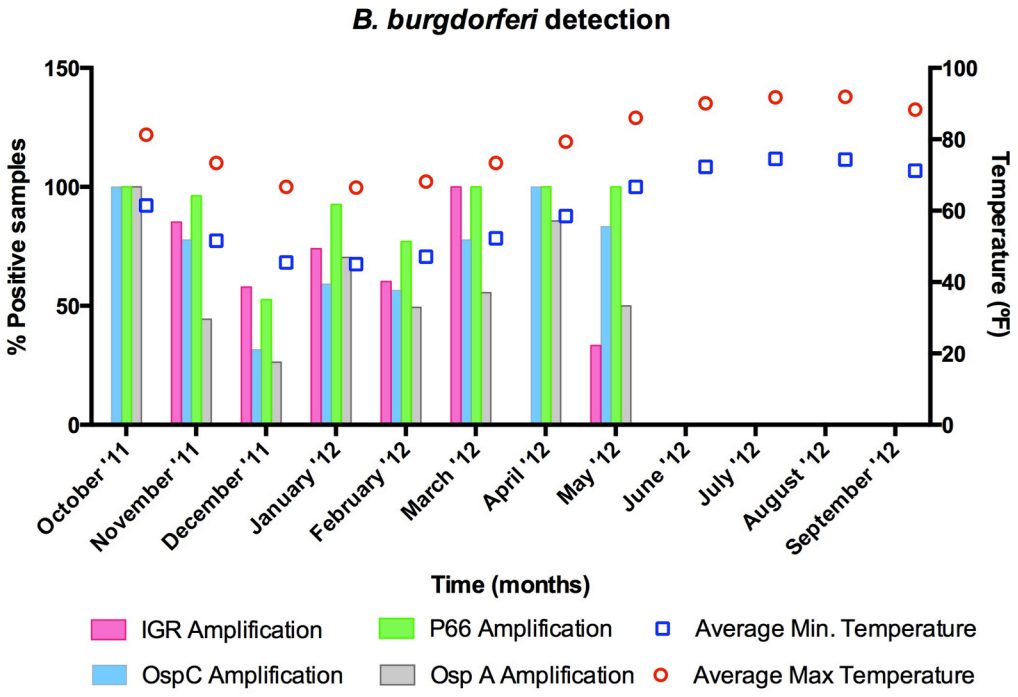

FIG 6. Chart displaying the seasonality of ticks that tested positive for *Borrellia Burgdoferi* in Texas from 2011-2012.

Spatial distribution

Once again, a map was generated using the GIS computer protocol. The protocol mapped positive LD tick cases in Texas by distinguishing the different counties that the positive cases from the data originated from. Also, we have represented the annual average precipitation for the state of Texas so as to observe the correlation of presence of Lyme disease and rainfall. As shown in figure 7, most of the positive ticks were present in East and South Texas. On the other side, Figure 8 shows that the canine Lyme disease cases are distributed across the state of Texas, with most of the cases concentrated in counties with higher rainfall. In addition to this maps, we also generated maps showing the co-localization of canine Lyme disease cases and infected ticks (figure 9) as well as human and canine cases (figure 10). In map depicted in figure 9 we can

observe that there is co-localization of infected ticks with detected canine LD cases at the county level as well as the presence of canine LD around the counties containing infected ticks. Corridors of counties presenting infected animals are also appreciated mostly in East Texas. On the other hand, some counties in West Texas and the Panhandle area also show limited numbers of canine cases, mostly acquired during visits to other parts of the state, since the presence of competent vectors for the transmission of LD have not been described in this ares. This absence of *I. scapularis* can be explain by considering the fact that the relative humidity and average temperatures in this region will not allow the establishment of this tick species.

Finally, figure 9 shows the fact that human and canine LD cases can co-localize in the same county and show a distribution around the foci where we find infected ticks. Unfortunately we do not have LD cases reported from all counties in which we got canine samples and viceversa. Nevertheless, the presence of disease in both animal species and infected ticks in the nearby regions, suggests that dogs can be sentinels for LD in Texas as it is the case in many other states in the country (5, 17, 27, 35).

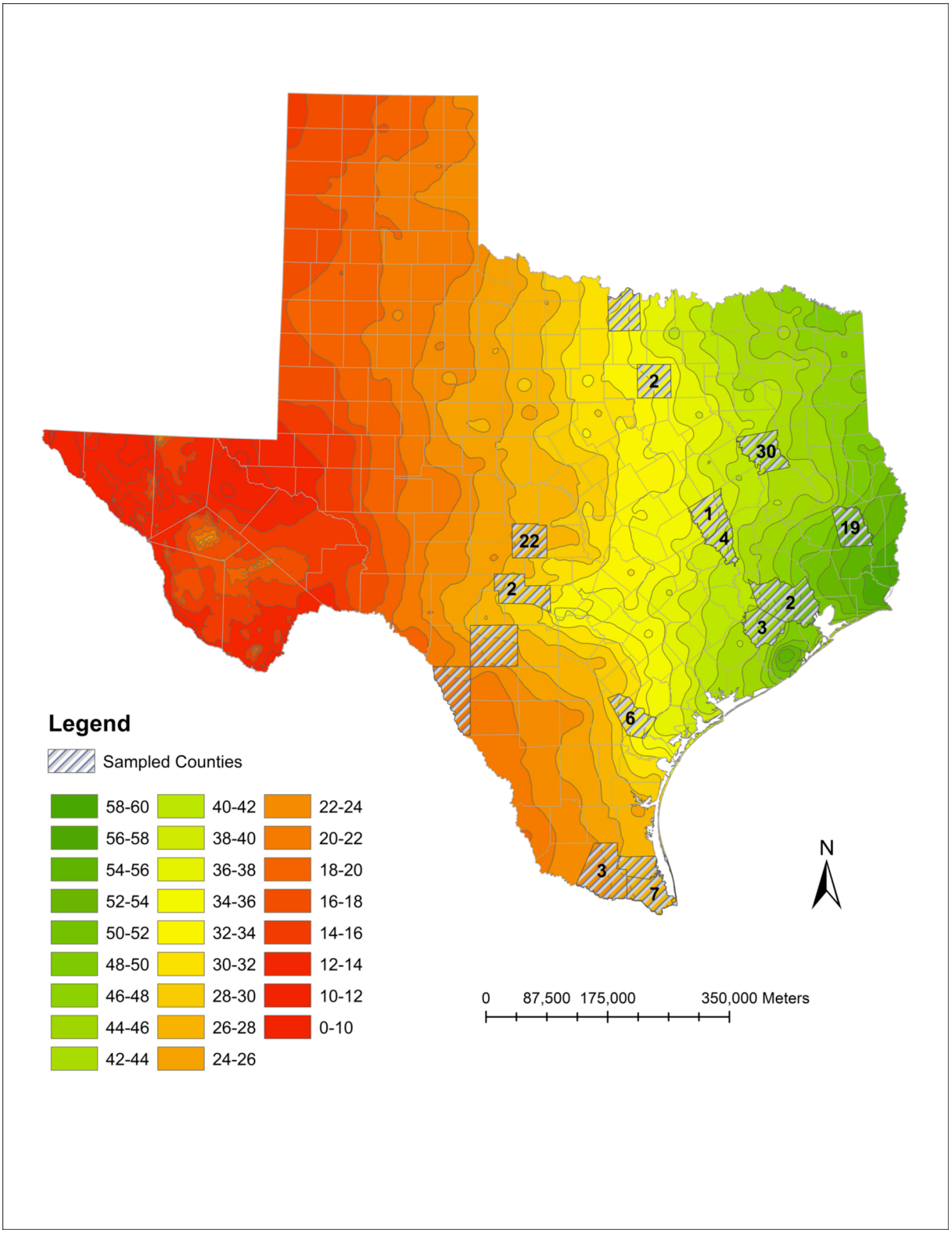

FIG 7. Map displaying the amount of positive *B. burgdorferi* infected ticks in Texas compared to the amount of average rainfall.

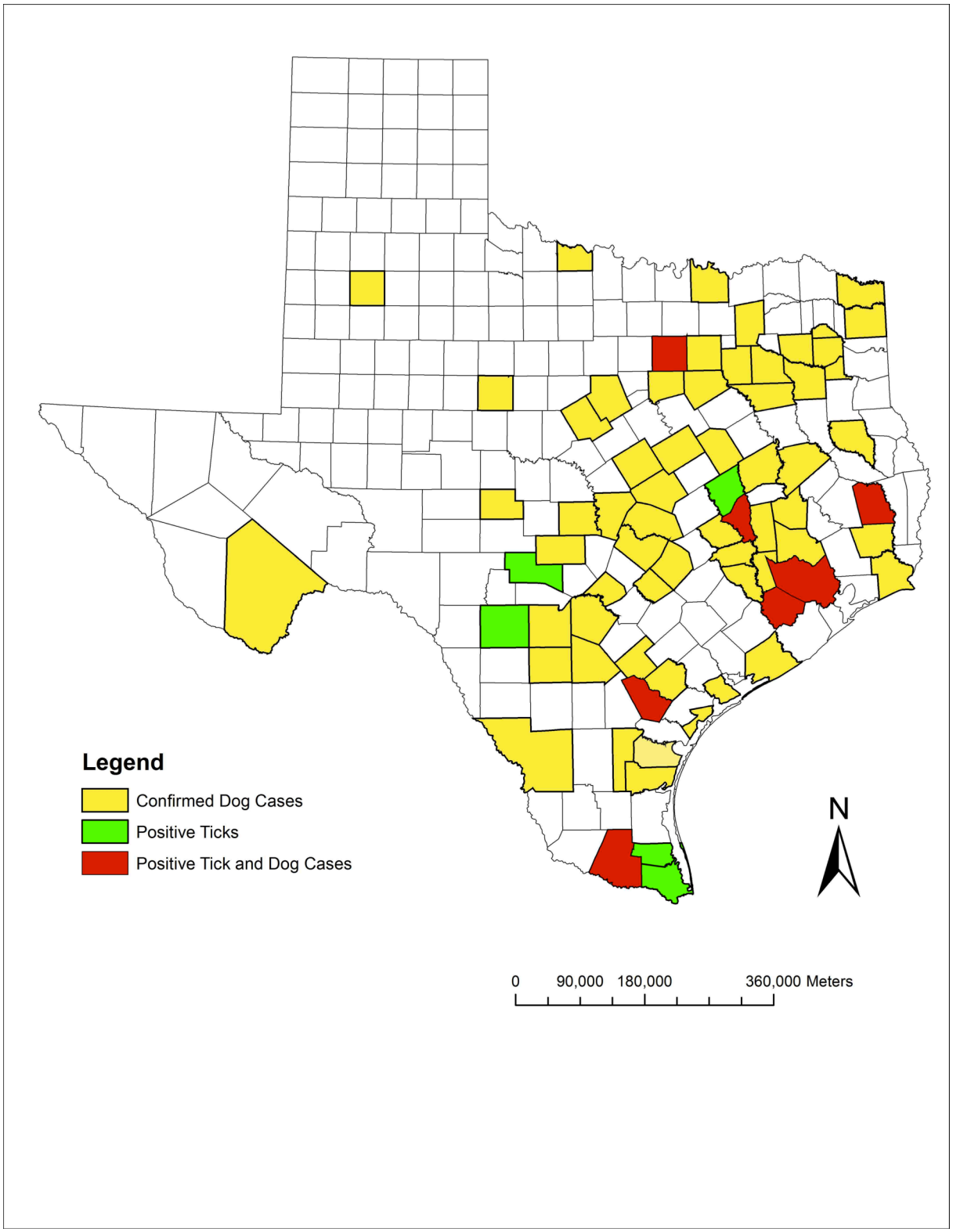

FIG 8. Map displaying the co-localization of positive dog cases and infected ticks from 2011-2012.

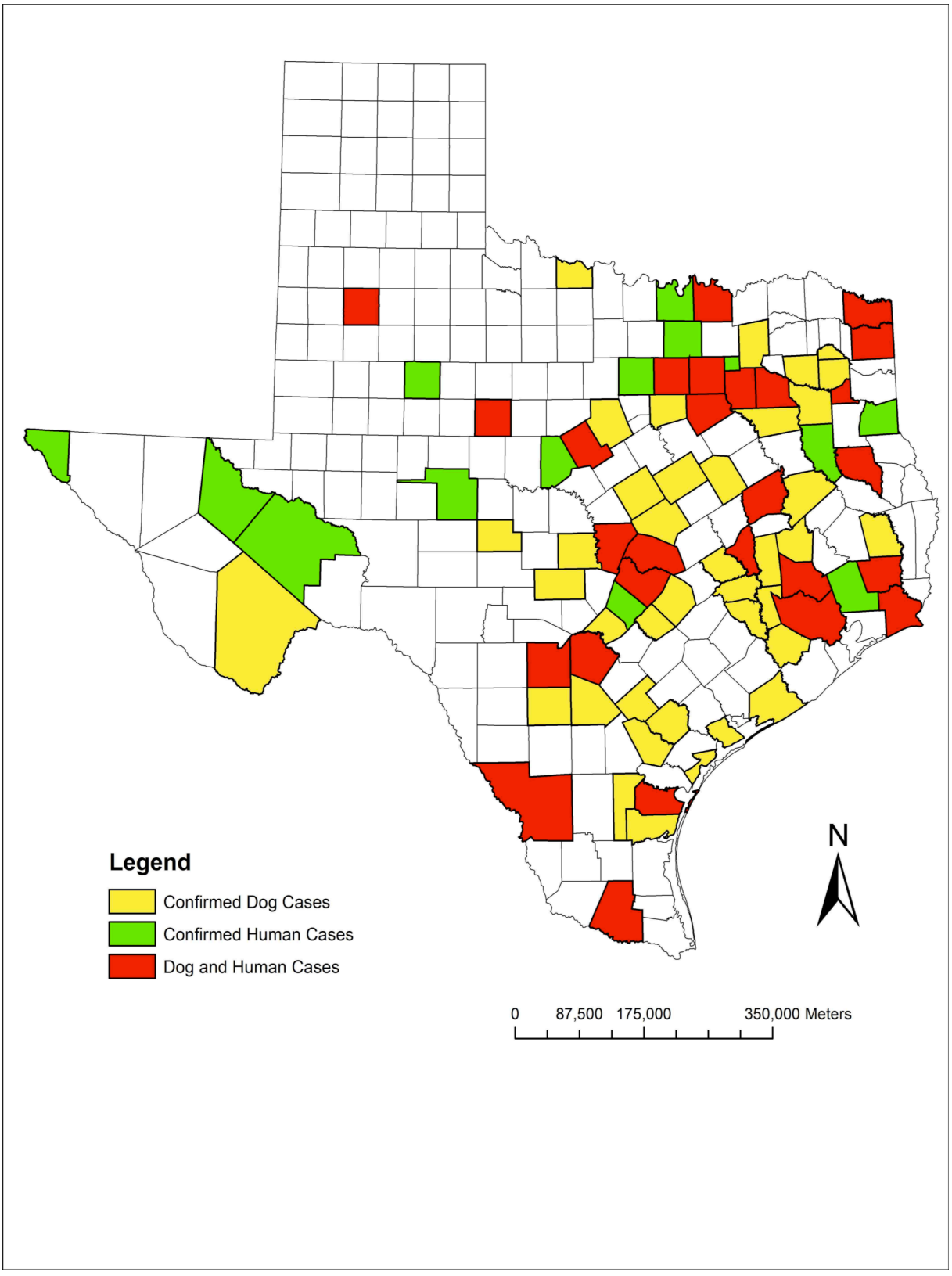

FIG 9. Map displaying the co-localization of positive dog and human cases from 2011-2012.

CHAPTER IV

DISCUSSION

The inquiry of whether or not canines can be identified as sentinels for LD is a question that has not been overlooked. There have been quite a few speculations made by various scientists and veterinarians that suggest that animals that are commonly owned as pets can be utilized as sentinels for LD (11, 18, 21, 25). Looking at our results, both our maps and our graphs suggest that this generalization is a possibility.

Our geographic area of study marks a region of the United States where LD has not been thoroughly studied (8-10, 19, 28). It was interesting to find evidence that the predetermined patterns of acquisition of LD applicable to northeastern and midwestern US did not necessarily apply in Texas. As shown in figure 2,, we have found evidence that the seasonality of positive LD cases in canine tested samples was in fact, inverted from the predetermined seasonality of positive LD cases established in the Northern regions. In particular, most of the canine cases happened during the months of November through March (figure 2). In addition to this, and as shown in figures 4 and 5, we also found that the seasonality of tick samples that tested positive for *B. burgdoferi* was similar when comparing to the seasonality of the dog cases that tested positive for LD. This implies that the presence of ticks that are testing positive for *B. burgdoferi* can mark the time of the year at which mammalian hosts, including humans and companion animals (mostly dogs and horses) are at higher risk to get infected witht this bacterial pathogen. When studing the ticks collected across Texas, we encountered a number of different species from which we could detect the causative agent of Lyme disease by PCR. Among all tick

species, *Ixodes scapularis*, the competent vector for the transmission of Lyme disease and the Ammblyoma ticks showed the highest percent infection (table 3). In addition and as mentioned above, both Bbsl and Bbss positive ticks were detected during the fall and winter months (figures 4 and 5). Moreover, during these months we found high variability in the detection of different molecular markers utilized in this study (Figure 6). Consequently, further population genetics studies are being conducted in our laboratory with the results of the sequencing reactions done to each positive PCR result. With these studies we foresee the determination of potential clusters of distribution of *Borrelia burgdorferi* strains most common in Texas. Similar studies have been done in other areas of the country and have shown the presence of high diversity of Borrelia burgdorferi strains (6).

Furthermore, along with our graphs, the maps that were generated in this study help support the claim of how ticks, dogs, and humans all co-localize within space and time. Our generated maps make it clear that both ticks and dogs and dogs and humans co-localize in the same zip codes, see figures 8 and 9. These maps also further strengthen our argument towards the idea that dogs can be utilized as sentinels for the analysis of LD in the state of Texas, because they show that while infected ticks and dogs live in the same general areas, infected dogs and infected humans also live in close quarters. Figure 9 shows the fact that human and canine LD cases can co-localize in the same county and they distribute around the foci where we found infected ticks. Unfortunately we do not have LD cases reported from all counties in which we got canine samples and viceversa. Nevertheless, the presence of disease in both animal species and infected ticks in the nearby regions, suggests that dogs can be sentinels for LD in Texas as it is the case in many other states in the country (5, 17, 27, 35).

The primary goal of this study was to provide preliminary groundwork that would help establish the concept of using dogs as sentinels for the analysis of LD in the state of Texas. In addition to this, Dr. Esteve's lab is currently working towards furthering the exploration of LD by collecting dog (blood) and tick samples across the state. Taken together, with this study we have determined that the seasonality for the diagnostic of canine LD in Texas is mostly in the winter and fall, rather than the summer months as described by CDC. Moreover, the ticks infected with this bacterial pathogen were also detected during this same time period while no infected ticks were detected during the summer months. In addition, the canine cases and ticks were detected during hunting season in Texas (mostly white tail deer), which suggests that these months are those of higher risk for the acquisicion of Lyme disease. Further studies are being done in Dr. Esteve-Gassent's laboratory in order to better understand the life cycle of the *I. scapularis* ticks, their association with rodents, geographical distribution and prevalence of *B. burgdorferi* infection. All this study will significantly improve our understanding of the real risk for Lyme disease in areas considered to date as of low to none risk for this disease.

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