SEROPREVALENCE OF ANTI-*BORRELIA BURGDORFERI* ANTIBODIES IN WHITE-TAILED DEER (*ODOCOILEUS VIRGINIANUS*) FROM TEXAS

A Thesis

by

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ABSTRACT

Lyme Disease is caused by the bacterial pathogen *Borrelia burgdorferi*, and is transmitted by the tick-vector *Ixodes scapularis*. It is the most prevalent arthropod-borne disease in the United States. To determine the seroprevalence of *B. burgdorferi* antibodies in white-tailed deer (*Odocoileus virginianus*) from Texas, we analyzed serum samples (n=1493) collected during the 2001-2015 hunting seasons, using indirect ELISA. Samples with higher sero-reactivity (0.803 and above) than the negative control group (0.662) were further tested using a more specific standardized western immunoblot assay to rule out false positives. Using ELISA, 4.7% of the samples were sero-reactive against *B. burgdorferi*, and these originated in two eco-regions in Texas (Edwards Plateau and South Texas Plains). However, only 0.5% of the total samples were positive by standardized western immunoblot assay. Additionally, both ELISA and standardized western immunoblot assay results correlated with an increased incidence in human Lyme Disease cases reported in Texas. This is the first study to demonstrate a seroprevalence of anti-*B. burgdorferi* antibodies in Texas white-tailed deer. Future ecological and geographical studies are needed to assess the environmental factors governing the prevalence of Lyme Disease in non-endemic areas of the southern United States.

DEDICATION

This work is dedicated to the glory of Almighty God, the omnipotent and omniscient.

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NOMENCLATURE

ELISA	Enzyme-linked Immunosorbent Assay
CDC	Centers for Disease Control and Prevention
LD	Lyme Disease
MB	Marblot Immunoblot Assay
WTD	White-Tailed Deer

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1. INTRODUCTION

Lyme Disease (LD) is caused by the bacterial pathogen *Borrelia burgdorferi* and transmitted by the tick vector *Ixodes scapularis.* This disease is considered the most prevalent arthropod-borne disease in the United States (US). In recent years, there has been an increase in the number of human LD cases confirmed by the Centers for Disease Control and Prevention (CDC, 2015) across its geographic distribution, and more prevalent than expected with over 300,000 infected individuals estimated annually (Kuehn, 2013). In 1975, a significant number of juvenile arthritis cases were identified in the towns of Lyme and Old Lyme, Connecticut, US. Further investigations revealed that these children were not suffering from arthritis, but rather, an infectious clinical disease (Steere et al., 1977). The bacterium responsible for this disease was thereafter identified by the medical entomologist Dr. Willy Burgdorfer in 1981 (Burgdorfer et al., 1982), and later named *B. burgdorferi* in his honor.

To date, most studies investigating LD prevalence in the US have focused on the endemic northeastern and midwestern states (Gill et al., 1994; Lane and Burgdorfer, 1986; Ostfeld and Keesing, 2000; Pepin et al., 2012) with few studies carried out in nonendemic southern US. Several recent studies focused on LD in the Texas-Mexico transboundary southern US region (Clark et al., 2014; Feria-Arroyo et al., 2014; Rudenko et al., 2014; Szonyi et al., 2015). In addition, there are several studies reporting *B. burgdorferi* spirochetes isolation from humans, as well as from *I. scapularis* ticks removed from animals in Texas (Burgdorfer and Keirans, 1983; Piesman and Sinsky,

1988; Rawlings, 1987; Rawlings and Teltow, 1994; Rawlings et al., 1987; Teltow et al., 1991).

LD is a multisystem infectious bacterial disease caused by several of the 18 genospecies in the *B. burgdorferi* sensu lato complex. In North America, *B. burgdorferi* sensu stricto causes LD while *B. afzelii* and *B. garinii* are considered the cause of most European cases. Other *Borrelia* genospecies possibly associated with human clinical cases are *B. valaisiana*, *B. bissettii*, *B. americana*, and *B. mayonii* (Ryffel et al., 1999; Stanek and Reiter, 2011; Pritt et al., 2016). Natural vertebrate hosts of *B. burgdorferi* include white-footed mice, shrews, raccoons, squirrels and birds etc., and these can serve as sources of infection when parasitized by infected ticks (Anderson, 1989).

LD manifests in three clinical stages, including acute, subacute and chronic clinical stages. The acute clinical stage, which manifests as a characteristic circular reddened skin lesion called *Erythema migrans* (EM), is usually accompanied by headache, fever, arthralgia, myalgia and lymphadenitis. The subacute clinical stage occurs about few weeks after the acute stage, and includes cardiac and neurologic involvement. The chronic clinical stage involves sporadic arthritic and nervous complications (Steere et al., 1983; Steere et al., 1986).

Antibiotics such as doxycycline and amoxicillin have proved effective in treating the acute and chronic stages of LD (Adelson et al., 2004). Currently, there is no LD vaccine for human use, the only formulation available is approved for veterinary use, and labeled for dogs only (Embers and Narasimhan, 2013). Therefore, one of the

preventive methods of LD in humans is the use of insect repellents, light-color clothing when outdoors, and prompt checking of attached ticks after outdoor activities.

In the US, the ticks responsible for the transmission of this pathogen are *I. scapularis* and *I. pacificus*. Other *Ixodes* species known to participate in the enzootic cycle of this bacterial pathogen in the US are *I. dentatus* and *I. affinis* (Brownstein et al., 2003).

Ixodes are three-host ticks with a lifecycle spanning two to four years during which they undergo four developmental stages including egg, larva, nymph and adult. The larval and nymphal stages of these ticks feed on a wide host range including small mammals such as the white-footed mouse (*Peromyscus leucopus;* a natural reservoir of *B. burgdorferi*), chipmunks and squirrels, and also birds and reptiles (Frank et al., 1998; Ostfeld et al., 2006). Adult stages of *Ixodes* prefer to feed on large mammals, such as the white-tailed deer (WTD), *Odocoileus virginianus*. Other stages of *I. scapularis* especially nymphs are also known to feed on WTD (Ostfeld et al., 2006).

In the northeastern and midwestern areas of the US where LD has been extensively studied, the risk of LD is considered greater for humans in summer when their activities occur in tick-infested habitats (Pepin et al., 2012), and when nymphs are more numerous. In contrast, adult ticks are active in fall, but because human outdoor activities decrease in this climatic season, infection is less likely (Ostfeld et al., 2006).

The literature has emphasized the importance of WTD as hosts for *Ixodes*. The adult ticks feed on WTD and mate, which maintain their populations (Main et al., 1981; Wilson et al., 1985). Studies in the endemic areas of northeastern and midwestern US

have showed that WTD densities and *Ixodes* abundance correlated positively with human cases of LD. A lower density of WTD and number of ticks in these areas is correlated with lower numbers of reported cases of LD in humans (Daniels et al., 1993; Kilpatrick et al., 2014; Wilson et al., 1985). However, other studies have reported that the removal of WTD in certain geographic areas did not necessarily eliminate the circulation of *I. scapularis* (Perkins et al., 2006). To date, very limited information is known about LD ecology in the southern US.

Serological tests including ELISA, immunofluorescence and immunoblot assay, have revealed the presence of antibodies to *B. burgdorferi* in various animal species. These include WTD as well as other wild mammals (white-footed mouse, raccoon), and domestic animals (dog, cat, horse, cattle) in northeastern and midwestern regions of the US (Brownstein et al., 2003; Magnarelli et al., 1984; Main et al., 1981). The application of serologic surveillance in WTD has been used to establish geographic locations where *B. burgdorferi* circulates (Gill et al., 1993; Gill et al., 1994; Lane et al., 1986; Magnarelli et al., 1984; Magnarelli et al., 1986; Martinez et al., 1999).

With little being known about LD ecology in the southern US (Esteve-Gassent et al., 2015; Szonyi et al., 2015), the expansion of *Ixodes* tick population in the US (Eisen et al., 2016), the detection of *I. scapularis* ticks infected with *B. burgdorferi* in Texas (Feria-Arroyo et al., 2014), and the growing population of WTD nationwide (McShea, 2012; Raizman et al., 2013; Rawinski and Square, 2008), the objective of the current study was to determine the sero-reactivity of Texas WTD to *B. burgdorferi* during a 15-year longitudinal study (2001-2015).

2. MATERIALS AND METHODS

2.1. White-Tailed Deer Serum Sample Collection

From October 2001 to February 2015, a total of 1493 male and female WTD ranging from 0.5-6.5 years of age were sampled during the Texas hunting season from 14 counties of the state. About 56.9% of the WTD population sampled were adults (two years and older), 23% were yearlings (one to two years old), and 20.1% fawns (less than one year of age). The counties from which samples were collected included Bee, Bell, Brazos, Gonzales, Guadalupe, Hamilton, Karnes, Kerr, Medina, Real, Travis, Uvalde, Webb, and Williamson (Fig. 1).

All blood samples were collected, centrifuged, sera separated and stored in a -20°C freezer until used. Dr. J. Morrill from the University of Texas Medical Branch (UTMB) (Galveston, Texas), and the Orion Research and Management Services, Inc. Belton, Texas provided samples. Additional WTD serum samples, which were obtained at the Texas A&M University Winnie Carter Wildlife Center, served as negative controls. These were collected from 2003-2013 from pen-raised WTD with no known exposure to ticks or *B. burgdorferi*. These animals received ivermectin injections (for its acaricidal properties) triple the recommended dose annually in the fall, and repeated every 10-14 days. In addition, a second group of negative controls were obtained in 2015 from WTD on deer ranches that implemented tick control measures, and where *Ixodes* is less prevalent.

2.2. Serological Analyses

Indirect ELISA was used to detect antibodies to *B. burgdorferi* in the sera of WTD following previously described protocols (Small et al., 2014), and modified for WTD. This modification used *B. burgdorferi* B31 strain A3 grown in (Barbour-Stoenner-Kelly II) BSK-II medium (pH 7.6), and supplemented with 1% inactivated rabbit serum, at 32°C and 1% CO_2. ELISA plates were blocked with 3% bovine serum albumin (BSA) to reduce nonspecific reactivity. The primary antibody dilution used was 1:200 (WTD serum samples) and 1:2000 was used for the secondary antibody dilution (Horseradish peroxidase-conjugated Rabbit anti-deer Immunoglobin G, Rockland Immunochemicals, Inc., Limerick, PA, USA). Both primary and secondary dilutions were carried out in 0.1M phosphate-buffered saline (pH 7.4) with 0.1% Tween 20. The substrate used for the enzyme included both o-phenylene diamine dihydrochloride (OPD) (Thermo Fisher Scientific, Life Technologies, Carlsbad CA, USA) and hydrogen peroxide. Optical density values were read at 450nm. Samples were considered sero-reactive when the optical density 450nm (OD) values were three standard deviations (SD) above the mean for the negative controls (OD = 0.662).

Commercially developed standardized western immunoblot assays for the analyses of WTD sera are not available. Therefore, the samples with a high sero-reactivity (high optical density above the cut off value) when compared to the negative controls, were tested further with a standardized western immunoblot assay. This assay was used to determine the specificity of the immune reaction to *B. burgdorferi* specific antigens, and to rule out false positives. The standardized western immunoblot assay

used in this study was modified using previous studies (Gill et al., 1994). *B. burgdorferi* B31 strain A3 was the test antigen used. This modification used *B. burgdorferi* pure cell lysates, which were separated in 12% SDS-PAGE gels following standardized electrophoresis protocols at 100 volts for 90 minutes (Maruskova et al., 2008). After *Borrelia* proteins were separated, gels were transferred to nitrocellulose membranes (GE HealthCare) using the RTA transfer blot kit (Bio-Rad Laboratories, Inc. Hercules, CA, USA) following manufacturer's recommendations. The membranes were blocked using 1% nonfat skimmed milk in Tris Buffer Saline (TBS) containing 0.2% Tween 20. Primary antibody (WTD serum samples) was utilized at 1:1500 dilution and incubated overnight at 4°C, while secondary antibody (Peroxidase conjugated Rabbit anti-deer IgG, Rockland Immunochemicals, Inc., Limerick, PA, USA) dilution at 1:5000 was incubated for one hour at room temperature. All blots were visualized using Chemiluminescence (Bio-Rad Chemiluminescence and Colorimetric detection kit, Bio-Rad Laboratories, Inc., Hercules, CA, USA) and imaged using a ChemiDoc ™ Touch (Bio-Rad Laboratories, Inc., Hercules, CA, USA). All blots were imaged every 10 seconds for a maximum of 10 minutes. Samples with five or more bands (excluding cross-reactive bands that were also present on the immunoblots of the negative control samples) were considered positive, partly in line with the LD diagnostic standards established by the CDC, while samples with four or less bands were considered negative.

2.3. Statistical Analysis

The statistical analysis used to determine the cut-off value for the indirect ELISA was STATA 12.0 statistical software (STATA 2011, StataCorp LP, College Station, Texas 77845 USA). The cut-off value (Fig. 2) was calculated as three standard deviations plus the average of negative controls. The graphs were plotted using Prism 6.0.

3. RESULTS

Serum samples of 1493 WTD were collected and evaluated for sero-reactivity against *B. burgdorferi*. Of these samples, 1384 were categorized as the study samples while 109 were the negative controls. The indirect ELISA results were read at the optical density of 450nm (OD) and ranged from 0-1.395 for the test samples, and 0-0.662 for the negative control samples. The cut off value (0.803) used to detect the highly sero-reactive samples was calculated by adding the average of the negative controls to three times their standard deviation. Therefore, test samples above 0.803 were considered sero-reactive and thereafter further analyzed with standardized western immunoblot assay (Fig. 2).

A total of 65/1384 (4.7%) WTD had a high sero-reactivity to *B. burgdorferi* by indirect ELISA and 7/1384 (0.5%) by standardized western immunoblot assay (Table 1). Control samples (109) were utilized to evaluate cross-reactivity of WTD negative serum to *B. burgdorferi* antigens. In this experiment we observed that a number of bands (45kDa, 50kDa, 70kDa) appeared cross-reactive because they were also found in the negative control samples. Consequently, those three bands were removed from analyses of the test samples, and not considered when marking the highly sero-reactive WTD samples (Table 2). The samples marked as highly sero-reactive were those that had five or more reactive bands by the standardized western immunoblot assay (Fig. 3B). Consequently, from the sero-reactive group, 10.8% (7 out of 65) of the highly sero-reactive samples were positive by standardized western immunoblot assay, which

represented approximately 0.5% (7 out of 1384) of the total samples studied. The gender distribution of the sero-reactive samples by standardized western immunoblot assay was as follows; 42.9% (3/7) were males (two fawns and a yearling) while 57.1% (4/7) were adult females. There is no apparent age or sex trend observed with respect to the positive samples. In addition, the positive samples by standardized western immunoblot assay were further tested using the standardized Marblot western blot assay (Fig. 3B). This assay is designed for qualitative *in vitro* detection of human immunoglobulin G (IgG) antibody to individual proteins of *B. burgdorferi* (B31) in human serum (http://www.trinitybiotech.com). As shown in Fig. 3, four of the seven sero-reactive WTD samples also provided a positive result using the commercially available Marblot test. The remaining three samples provided a very weak immunoreactivity to the different *Borrelia* antigens presented in this test.

Overall, the counties with the highest prevalence of sero-reactive samples were Travis and Williamson, located in central region of Texas (Fig. 1). Interestingly, those sero-reactive WTD samples were detected in years 2002 (6 samples) and 2009 (1 sample), which correlate with the years in which Texas reported to CDC their highest numbers of human LD (Fig. 4). In addition, since WTD are not competent reservoirs of *B. burgdorferi*, even a low seroprevalence may be suggestive of the activities of *Ixodes* in these areas. Moreover, when looking at the density of WTD in the counties with the sero-reactive samples, we observed that they are located in the eco-regions with the highest WTD densities in the state of Texas. Furthermore, those densities were above state average, with a continuous increase since 2007 (Fig. 5).

4. DISCUSSION

The current study was designed to evaluate the sero-reactivity of WTD to LD pathogen (*B. burgdorferi*) in central and south Texas, and we showed a sero-reactivity of only 4.7% (65/1384) by indirect ELISA and 0.5% (7/1384) by standardized western immunoblot assay, with distribution across different eco-regions of Texas, in particular, the central region of the state (Texas Parks and Wildlife Department, 2015; https://tpwd.texas.gov/education/hunter-education/online-course/wildlife-conservation/texas-ecoregions). Additionally, the years of high WTD sero-reactivity correlate with the years during which there was a peak of reported LD cases in Texas (Fig. 4).

We evaluated 1493 WTD serum samples (1384 test samples and 109 negative control samples), obtained across 14 counties in Texas. The test samples were obtained from several locations across central and south Texas, including areas where there have recently been a high number of reported LD cases in the past decade (TickChek, 2015; https://www.tickchek.com/stats/state/texas/lyme). The negative control samples were obtained from pen-raised WTD at the Texas A&M University Winnie Carter Wildlife Center, with no known exposure to *B. burgdorferi*; deer ranches where ectoparasites and acarid control measures were implemented; and areas where there was a lower possibility of *Ixodes* tick survival. Due to the fact that WTD are free roaming animals, the objective was to obtain a large pool of negative samples, including those with known lack of tick exposure, as well as those that could have been exposed to ticks but not to

the bacterial pathogen, to account for a more realistic background reactivity of WTD serum samples in the different serological tests performed.

Upon further analysis of the 65 samples that were positive by ELISA with standardized western Immunoblot assay, samples with five or more bands were considered highly sero-reactive, while samples with four or less bands were marked negative. There is a lack of standardized tests for WTD and any information on their reactivity to specific *B. burgdorferi* antigens. Therefore, the decision of high sero-reactivity was partly based on the LD diagnostic standards of the CDC and the immunoblot results obtained in this study (Fig. 3). Some of the reactive bands seen with the samples positive by standardized western immunoblot assay (31kDa, 34kDa, and 41kDa) were consistent with the bands seen on western immunoblots of samples from humans with LD (Craft et al., 1986; Gill et al., 1993; Grodzicki and Steere, 1988). In the study conducted by Gill et al., (1993), the mean number of bands in their immunoblot assay for WTD samples collected from established *I. scapularis* areas, and positive by ELISA were 8.2. In contrast, our data showed the mean number of bands in the immunoblot study to be 5.6. A possible explanation for this lower number might be the nonendemicity of our study areas, in addition to the fact that these samples were not tested with antigens from a local *B. burgdorferi* isolate, due to the lack of it. Also, Gill et al., (1993) reported that the *Borrelia* antigen at 19.5kDa reacted with 94% of their ELISA-positive samples. In contrast, none of our ELISA positive samples reacted with *Borrelia* antigen at 19.5kDa. In the current study, the 45kDa, 50kDa and 70kDa *Borrelia* antigens may be nonspecific as these were found in most of the WTD serum

samples negative by ELISA, as well as the negative control samples (Fig. 5). Furthermore, only one of the seven positive samples showed a response to one of the two major outer surface proteins, OspB (Table 2). However, animals immunized with killed LD spirochetes have been shown to respond to the outer surface proteins OspA and OspB (Gill et al., 1993). Previous studies have also reported a lack of response to the outer surface proteins of *B. burgdorferi* in humans infected in the early stage of LD (Craft et al., 1986; Grodzicki and Steere, 1988; Guy, 1993). Therefore we could hypothesize that the sero-reactive animals had a recent exposure to *B. burgdorferi*, because our sampling period (fall and winter months in Texas) correlates with the activity of *I. scapularis* adult stage.

Three of the seven sero-reactive WTD samples did not give a positive result when the Marbot test was used, but these samples showed a number of reactive bands in the developed in-house immunoblot test, and high ELISA readings. This discrepancy could be due to the fact that other *Borrelia* species such as *B. lonestari*, the causative agent of Southern Tick Associated Rash Illness (STARI), could also be present in the state of Texas. In this respect, *B. lonestari* is known to be transmitted by *Amblyomma americanum* (Lone Star tick), which also feeds on WTD, and co-infections could be present.

Even though there was neither a positive control for our study, nor a previous study in Texas to compare our WTD data, we evaluated the high sero-reactivity based on a comparison with our negative controls. These WTD negative controls were obtained from three sources; 1) pen-raised and kept in captivity at the Texas A&M University

Winnie Carter Wildlife Center, with no known exposure to ticks and *B. burgdorferi*; 2) ranches where ectoparasite and acaricide control measures were implemented, and 3) areas where there is a lower distribution of *Ixodes* tick geographically. Taken together, it is therefore likely that, the generally low sero-reactivity recorded in this serological analysis of WTD in Texas, may be an indication of a low incidence of LD in this non-endemic southern region of the US.

In reviewing the literature, no data was found on the correlation of a high population density of WTD with LD incidence in Texas, but there have been reports of a positive correlation between WTD population and LD cases in endemic regions of the US (Kilpatrick et al., 2014; Wilson et al., 1985). Nonetheless, there has been an increase in population density of WTD from 14,000 to 3.8 million across Texas over the past decade (Alan, 2013). In the current study, the highly sero-reactive WTD were reported in one of the Texas eco-regions in which WTD population density has significantly increased (Alan, 2013) over the past decade (Fig. 5). Therefore, we hypothesize that the increased populations of WTD may be a contributing factor to the increased LD cases in these two counties.

From our data, the overall seroprevalence for *B. burgdorferi* in WTD in Texas is low, but two counties in the central part of the state in which we recorded higher levels of antibodies correlate with the years during which a peak of human LD cases occurred. Additionally, we have demonstrated, for the first time, the distribution of anti-*B. burgdorferi* antibodies in WTD in Texas. Future research is aimed at Geographical Information System (GIS) mapping methods and spatio-temporal analyses to evaluate

land use changes over the period of time during which these serum samples were obtained, in order to understand the link between WTD sero-reactivity to *B. burgdorferi*, land use changes in Texas, the observed increase in WTD populations in the central region of the state, and their impact on LD risk in non-endemic areas.

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APPENDIX 1

Figures

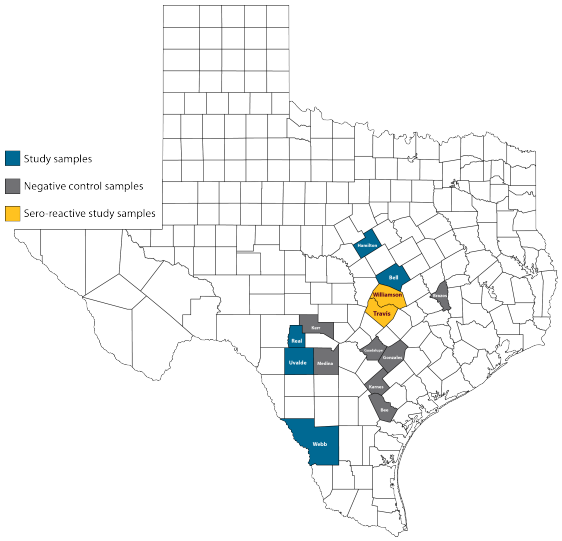

Fig. 1. Texas map showing 14 counties in which white-tailed deer (WTD) were sampled for anti-*Borrelia burgdorferi* antibodies from 2001-2015. Counties in blue are where samples, which were negative by ELISA and standardized western immunoblot assay were obtained. Counties in gray are where negative control samples were obtained. Counties in yellow are where samples sero-reactive by standardized western immunoblot assay (Travis and Williamson counties) were obtained.

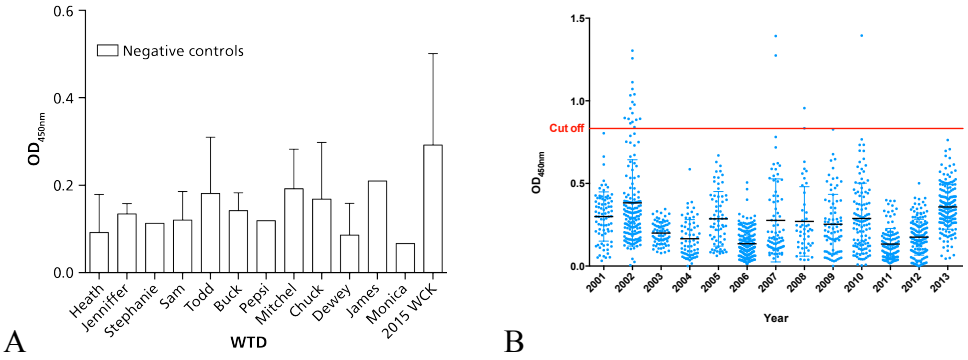

Fig. 2. Optical density values of the white-tailed deer (WTD) serum samples analyzed with indirect ELISA for anti-*Borrelia burgdorferi*. (A.) ELISA data from 109 WTD serum samples used as negative controls, collected from 2003-2015. (B.) ELISA data from 1384 WTD serum samples collected from 2001-2013. The overall range of values is 0-1.395 and 0-0.662 for the study samples and negative controls, respectively. The red line denotes the cut off value (0.803) used in this study (average optical density 450nm, OD, of negative controls plus three times their standard deviation). The samples above this line were analyzed with standardized western immunoblot assay to validate the ELISA results and to rule out false positives.

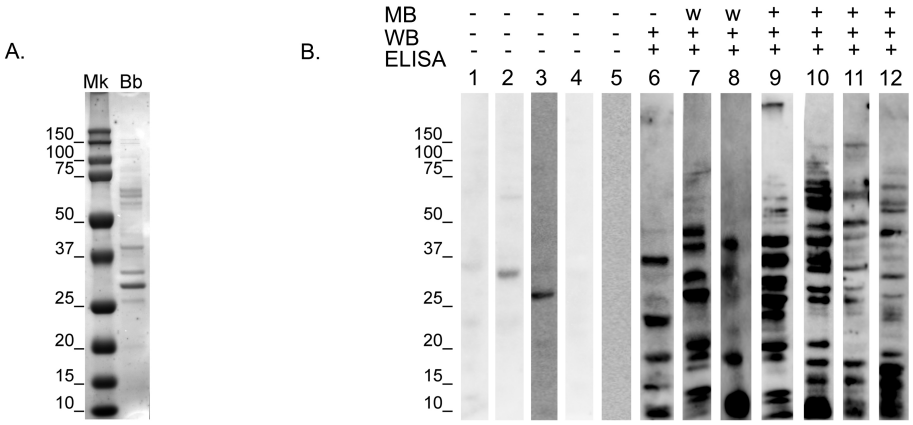

Fig. 3. Assays used to demonstrate reactivity to *Borrelia burgdorferi* antigens in white-tailed deer serum samples. (A) The molecular weight marker (Mk) showing estimated molecular weights of *Borrelia* antigens (Bb). (B) From left to right, negative control samples (1-5) and samples highly sero-reactive (6-12). The three immunoassays used were ELISA, standardized western Immunoblot and Marblot assays. This figure shows that negative control samples were negative for the three immunoassays. Sample 6 was positive by both ELISA and WB but negative by MB. Samples 7 and 8 were positive by both ELISA and WB but weakly reactive by MB. Samples 9 to 12 were positive by the three immunoassays.
MB: Marblot standardized western immunoblot assay. WB: Laboratory standardized western immunoblot assay. ELISA: Enzyme-linked Immunosorbent Assay.

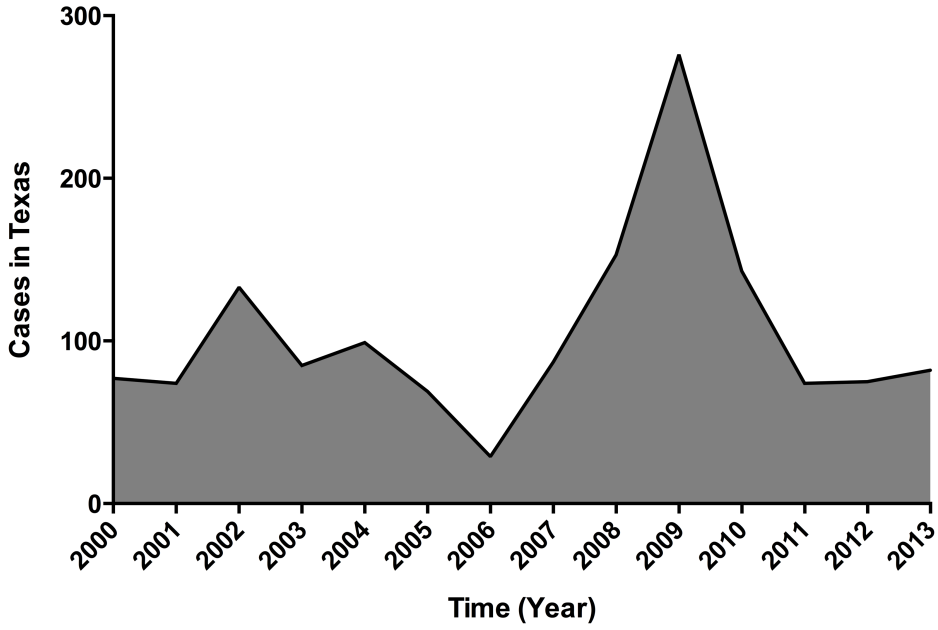

Fig. 4. Confirmed human Lyme Disease cases in Texas from 2000-2013 reported to the CDC (www.cdc.com).

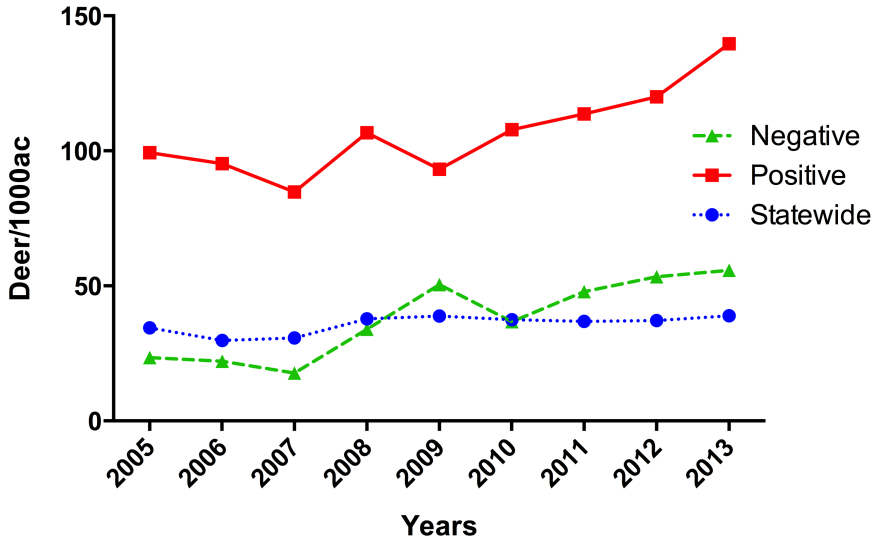

Fig. 5. White-tailed deer (WTD) population density in Texas eco-regions from 2005-2013. This graph is a representation of WTD population density statewide in Texas; in two counties (Travis and Williamson) where sero-reactive samples, and negative control samples for *Borrelia burgdorferi* antibodies were found.
Statewide: WTD population density in Texas.
Positive: WTD population density in Texas eco-region in which the highly sero-reactive samples were found.
Negative: WTD population density in Texas eco-regions where the negative control samples were collected.

APPENDIX 2

Tables

Table 1
Summary of the white-tailed deer serum samples, including year, age, sex and the counties from which they were obtained.

		Fawns		Yearlings		Adults		
Year	**County**	**Does**	**Bucks**	**Does**	**Bucks**	**Does**	**Bucks**	**Total**
2001	Travis	10	10	4	10	32	22	88
2002	Travis	16	23	9	11	78	25	162
2003	Travis	5	8	7	19	20	13	72
2004	Travis	9	8	2	9	11	3	42
2005	Travis, Webb	9	7	3	7	26	16	68
2006	Travis, Bell	20	19	14	22	88	32	195
2007	Uvalde, Travis Webb, Bell	7	5	4	16	34	13	79
2008	Williamson, Travis, Real	6	2	4	4	21	9	46
2009	Travis, Williamson, Hamilton	10	8	4	14	29	17	82
2010	Travis, Williamson	16	14	12	17	38	17	114
2011	Williamson, Travis, Bell	3	8	11	28	39	28	117
2012	Travis, Williamson, Hamilton	9	15	17	23	52	33	149
2013	Travis, Williamson, Hamilton	10	22	8	39	59	32	170
Total by sex (%)		130 (9.4)	149 (10.7)	99 (7.2)	219 (15.8)	527 (38.1)	260 (18.8)	1384 (100%)
Total (%)		279 (20.2)		318 (22.9)		787 (56.9)		

Table 2
White-tailed deer serum samples positive by ELISA and standardized western immunoblot assay, with their respective years and location.

Year	County	No of samples positive by ELISA	No of samples positive by standardized western immunoblot assay
2001	Travis	3	0
2002	Travis	29	6
2005	Travis	3	0
2007	Travis, Uvalde	7	0
2008	Travis	3	0
2009	Travis, Williamson	4	1
2010	Travis	10	0
2013	Travis, Williamson	6	0
Total		65	7

Only seven of the samples highly sero-reactive by ELISA were positive by standardized western immunoblot assay, indicating a potential cross-reactivity with proteins similar to *Borrelia burgdorferi*.