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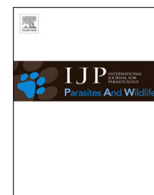

Seroprevalence of *Borrelia burgdorferi* antibodies in white-tailed deer from Texas

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A B S T R A C T

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 sero-reactive 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 longitudinal study to demonstrate fluctuation in sero-reactivity of white-tailed deer to *B. burgdorferi* sensu stricto antigens in southern United States. 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.
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1. Introduction

Lyme Disease (LD) is a multisystem infectious bacterial disease caused by *Borrelia burgdorferi* and is 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. In addition, recent studies project that LD is more prevalent than previously expected with over 300,000 infected individuals annually, and therefore significantly under-reported (Kuehn, 2013).

To date, most studies investigating LD prevalence in the US have focused on the endemic northeastern and midwestern states (Lane and Burgdorfer, 1986; Gill et al., 1994; Ostfeld and Keesing, 2000; Pepin et al., 2012) with few studies carried out in nonendemic southern US. Nevertheless, in recent years several studies on LD in the Texas-Mexico transboundary southern US region have emerged (Illoldi-Rangel et al., 2012; Clark et al., 2014; Feria-Arroyo et al., 2014; Rudenko et al., 2014; Szonyi et al., 2015; Mitchell et al., 2016). In addition, a number of reports about the isolation of *B. burgdorferi* spirochetes from humans, as well as from *I. scapularis* ticks removed from animals in Texas were published during the 1980'a and 1990's (Burgdorfer and Keirans, 1983; Rawlings, 1987; Rawlings et al., 1987; Piesman and Sinsky, 1988; Teltow et al., 1991; Rawlings and Teltow, 1994).

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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 the recently discovered *B. mayonii* (genospecies number 19) (Ryffel et al., 1999; Stanek and Reiter, 2011; Dolan et al., 2016). 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*, *I. affinis* and *I. uriae* (Olsen et al., 1995; 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, birds, reptiles, and also larger animals such as white-tailed deer (WTD) (Frank et al., 1998; Ostfeld et al., 2006). Adult stages of *Ixodes* prefer to feed on large mammals, especially WTD, *Odocoileus virginianus*.

The literature has emphasized the importance of WTD as hosts for *Ixodes*. WTD not only facilitate mating by the adult stages of *Ixodes*, but also serve as a source of blood meal for female *Ixodes* egg production (Main et al., 1981; Wilson et al., 1985). Nevertheless, WTD is not considered a reservoir for *B. burgdorferi* (Ostfeld et al., 2006). It is also worthy to note that WTD do not show symptoms of diseases when infected female *Ixodes* feed on them. On the other hand, due to their role in maintaining tick populations, studies in the endemic areas of northeastern and midwestern US have shown 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 (Wilson et al., 1985; Kilpatrick et al., 2014). However, some studies showed that elimination of WTD does not remove the risk of LD in an area (Ostfeld and Keesing, 2000) due to the complexity of the ecology of this disease. In addition, data regarding the ecology of LD in nonendemic southern US is very limited.

Serological tests 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) (Main et al., 1981; Magnarelli et al., 1984; Brownstein et al., 2003). The application of serologic surveillance in WTD has been used to establish geographic locations where *B. burgdorferi* circulates (Magnarelli et al., 1984, 1986; Lane and Burgdorfer, 1986; Gill et al., 1994; 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 detection of *I. scapularis* ticks infected with *B. burgdorferi* in Texas (Feria-Arroyo et al., 2014), and the growing population of WTD nationwide (Rawinski and Square, 2008; McShea, 2012; Raizman et al., 2013), 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 to 6.5 years of age were sampled during the Texas hunting season from 14 counties of the state. About 56.9% of the sampled WTD 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 provided by Dr. Alice Blue-McLendon at the Texas A&M University Winnie Carter Wildlife Center, served as negative controls. These were collected from 2003 to 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 detection of the pathogen

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 0.1 M 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 450 nm. Samples were considered sero-reactive when the optical density 450 nm (OD) values were three standard deviations (SD) above the mean for the negative controls (OD = 0.662). All samples were tested in triplicates.

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 (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

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