

DISTRIBUTION OF ANTIBODIES REACTIVE TO *BORRELIA LONESTARI* AND
BORRELIA BURGDORFERI IN WHITE-TAILED DEER (*ODOCOILEUS VIRGINIANUS*)
POPULATIONS IN THE EASTERN UNITED STATES

by

JESSICA HELEN MURDOCK

(Under the Direction of Michael J. Yabsley)

ABSTRACT

Little is understood about a Lyme-like syndrome, Southern Tick-Associated Rash Illness (STARI), which occurs in the southeastern United States. *Borrelia lonestari*, a possible agent of STARI, is transmitted by the lone star tick (*Amblyomma americanum*) and naturally infects white-tailed deer (WTD, *Odocoileus virginianus*). I tested 714 WTD from 20 eastern states for antibodies reactive to *B. lonestari* using an indirect immunofluorescent antibody test, 107 (15.0%) were seropositive. Significantly more southeastern deer (17.5%) were positive compared to northeastern deer (9.2%). Using a SNAP® test, 71 (9.9%) were positive for *Borrelia burgdorferi* and significantly more northeastern deer (23.9%) were positive compared with southeastern deer (3.8%). My data demonstrate that WTD are exposed to both *Borrelia* species, but antibody prevalence for the two species differs regionally and distributions correlate with the presence of *I. scapularis* and *A. americanum* ticks. Age and gender do not affect prevalence.

INDEX WORDS: Lyme, STARI, *Borrelia burgdorferi*, *Borrelia lonestari*, White-tailed deer, *Odocoileus virginianus*

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STATES

by

JESSICA HELEN MURDOCK
B.S., Kent State University, 2003

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JESSICA HELEN MURDOCK

Major Professor: Michael J. Yabsley

Committee: David Stallknecht
Robert Warren

Electronic Version Approved:

Maureen Grasso
Dean of the Graduate School
The University of Georgia
August 2008

DEDICATION

I would like to dedicate this manuscript to my parents, James and Jenny Murdock, and to Scot Groghan, whom without their inspiration and support I would not be half the person that I am today.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Introduction

Tick-borne pathogens, such as *Borrelia burgdorferi*, are important causes of morbidity and mortality for humans and some domestic animals. Due to the recent expansion of many tick species, the distribution of these diseases is spreading to new geographic areas. By improving surveillance techniques, we can develop better methods of monitoring and controlling their spread. There were 23,305 documented cases of Lyme disease in the United States in 2005 alone (Centers for Disease Control and Prevention (CDC) 2007), emphasizing the impact of this zoonotic disease. Typical symptoms of Lyme disease include headache, fever, sore muscles and joints, and the characteristic erythema migrans rash. If left untreated, Lyme disease can lead to Bell's palsy (facial paralysis), chronic arthritis, neurologic problems, and heart problems, including pericarditis (Steere et al. 1977, Davidson and Nettles 1997, Steere 2004).

Although many studies have investigated the natural history of Lyme disease in the northeastern and northern Midwestern U.S., little is understood about a similar syndrome, called Southern Tick-Associated Rash Illness (STARI), which occurs in the southeastern and south-central United States. STARI has been diagnosed numerous times following the bite from a lone star tick (*Amblyomma americanum*) (Campbell et al. 1995, Masters et al. 1998, James et al., 2001). The clinical signs of STARI (erythema migrans rash, headache, fever, heart problems, and muscle and joint stiffness) so closely mimic the symptoms of Lyme disease that it may be misdiagnosed as Lyme disease (James et al. 2001), making it hard to assess the epidemiology of

Lyme and Lyme-like disease in the South. The absence of *B. burgdorferi* from STARI patients in the South suggests that another agent is responsible for these symptoms (James et al. 2001, Bacon et al. 2003, Wormser 2005). The spirochete, *B. lonestari*, has been detected in a single case of STARI, but was not detected in a series of cases in a later study (James et al. 2001, Bacon et al. 2003, Wormser et al. 2005).

B. lonestari has been detected in numerous wild lone star ticks collected throughout the southeastern United States (Burkot et al. 2001, James et al. 2001, Bacon et al. 2003, Stegall-Faulk et al. 2003, Bacon et al. 2005) and this species has been proven competent to transmit *B. lonestari* to white-tailed deer (WTD, *Odocoileus virginianus*) (Varela-Stokes 2007, Little et al. unpublished). A previous study has shown that wild WTD are naturally infected with *B. lonestari* (Moore et al. 2003), and experimentally, WTD are susceptible to *B. lonestari* infection. However, experimental inoculations resulted in a short-term infection with antibodies detectable for only a few weeks after circulating organisms were cleared from the blood (Moyer et al. 2006, Varela-Stokes 2007). Further, some WTD that were exposed either by needle inoculation or tick transmission did not seroconvert, or produced low antibody titers (Moyer et al. 2006, Varela-Stokes 2007). Although not considered competent reservoirs for *B. burgdorferi* (Telford et al. 1988, Luttrell et al. 1994), WTD seroconvert after experimental infection and can have high antibody prevalence rates in northern states, indicating frequent exposure to *B. burgdorferi* (Magnarelli et al. 1986, Gill et al. 1994, Luttrell et al. 1994, Magnarelli et al. 1995, Gallivan et al. 1998, Magnarelli et al. 1999).

Because WTD are suspected natural reservoirs of *B. lonestari*, I aimed to determine the distribution of antibodies reactive to *B. lonestari* in WTD populations throughout the eastern United States. Although experimental infections suggest short-term detection of antibodies, I

hypothesized that antibodies to *B. lonestari* would be detected in wild WTD because of frequent exposure to ticks and the agent. I also tested WTD for antibodies to *B. burgdorferi* since these pathogens overlap in some eastern regions. Further, because antibodies reactive to *B. burgdorferi* could cross react with the *B. lonestari* antigen used in the serologic test, this would address problems with test specificity. Because *B. lonestari* is transmitted by *A. americanum*, I hypothesized that antibodies reactive to *B. lonestari* would be detected in WTD populations with known exposure to *E. chaffeensis* (as reported in Yabsley et al. 2003), and that antibodies to *B. burgdorferi* would be detected predominantly in the northeastern and Midwestern states where Lyme disease is endemic.

Tick-borne Disease Transmission

In order to understand tick-borne diseases it is imperative to understand the various factors involved in the transmission process, which includes tick and vertebrate hosts as reservoirs. Environmental factors affecting tick survivability and availability of non-competent reservoir hosts that can be used by ticks as a blood meal also must be considered. A reservoir host is a host upon which the agent is dependent for survival, that allows a pathogen to replicate, and can subsequently transmit the pathogen to appropriate vectors. The pathogen is transmitted when a non-infected tick feeds on a reservoir host and picks up the pathogen. If the tick is an appropriate vector, the pathogen will replicate, and when the tick then feeds upon another animal, the pathogen will be transmitted during feeding. In the case of *B. burgdorferi*, the spirochetes replicate and persist in the tick gut until the tick's next bloodmeal, during which the spirochetes migrate to the salivary glands to be transmitted in the saliva.

Hard ticks feed once during each of their three life stages; larval, nymphal, and adult. Depending on the life stage and tick species, different primary hosts are utilized by the ticks. For

example, larval and nymphal blacklegged ticks (*Ixodes scapularis* and *Ixodes pacificus*) primarily feed on rodents, such as the white-footed mouse (*Peromyscus leucopus*) in the case of *I. scapularis*. However, adult blacklegged ticks prefer feeding on white-tailed deer (WTD, *Odocoileus virginianus*) (Levine et al. 1985, Oliver 1996, Patrican 1997). In contrast, all three life stages of the lone star tick (LST) prefer feeding on WTD, although larvae and nymphs will feed on other medium-sized mammals and birds (e.g. turkeys). Following feeding, the ticks drop from the host, molt to the next stage, and remain in the environment until they attach to another host. If a tick picks up a pathogen during one of these feeding stages, it is maintained to the next stage through transstadial transmission.

Lyme Disease Causative Agent

Bacteria in the *B. burgdorferi* sensu lato (in the broad sense, encompassing multiple strains) complex are corkscrew-shaped spirochetes that are about 0.2-0.5 µm wide, and 20-30 µm long (Pal and Fikrig 2003), and are covered by a lipo-protein outer surface membrane (Coleman et al. 1986). These bacteria can form non-motile cysts when deprived of serum (Alban et al. 2000) in order to survive nutrient deficiencies. The *B. burgdorferi* sensu lato complex has been subdivided into several genospecies, three of which are found in North America: *B. burgdorferi* sensu stricto (in the strict sense- referring to a particular strain), *Borrelia andersonii*, and *Borrelia bissettii* (Postic et al. 1998). Various genomic sequences have been used to distinguish these species (Lin 2003). Of these three genospecies found in North America, *B. burgdorferi* sensu stricto is the only one known to cause Lyme disease in humans (Strle et al. 1997, Postic et al. 1998, Clark 2004). In Europe, two additional members of the complex, *B. afzelli* and *B. garinii*, cause Lyme disease in humans (Nadelman and Wormser 1998, Richter et

al. 2006). Therefore, the use of the term *B. burgdorferi* in this paper shall mean the specific genospecies *B. burgdorferi* sensu stricto, rather than the complex of *B. burgdorferi* sensu lato.

Lyme Disease Vector

B. burgdorferi has been detected in many *Ixodes* species, including three species that will feed on humans: *Ixodes scapularis* (Burgdorfer et al. 1982, Anderson et al. 1985, Magnarelli et al. 1986, Clark 2004), *I. pacificus* (Lane et al. 1991, Eisen et al. 2004), and *I. affinis* (Anderson et al. 1985, Magnarelli et al. 1986, Clark 2004). It has also been detected in other ticks in the family *Ixodidae*, such as the winter tick (*Dermacentor albipictus*) (Magnarelli et al. 1986), the American dog tick (*Dermacentor variabilis*) (Anderson et al. 1985, Magnarelli et al. 1986), and the LST (Magnarelli et al. 1986, Clark 2004). However, attempts to transmit *B. burgdorferi* by *Dermacentor* and *Amblyomma* have failed (Piesman and Sinsky 1988, Mather and Mather 1990, Mukolwe et al. 1992, Oliver et al. 1993). Additional enzootic cycles may occur between *I. minor* and the eastern woodrat (*Neotoma floridana*) and numerous bird species, as well as between *I. denatus* and the eastern cottontail rabbit (*Sylvilagus floridanus*); however these tick species rarely bite humans (Oliver 1996). Only two of these tick species frequently infest WTD, *I. scapularis* and *A. americanum*.

I. scapularis has a large distribution, and is commonly found from southern Canada and coastal Maine south through the Mid-Atlantic and northern Midwestern states, including parts of Minnesota, Wisconsin, Illinois, and Indiana. The distribution extends across the southeastern United States, although the distribution appears to be more focal, and west to parts of Oklahoma, Texas, Missouri, and Kansas (Stafford 2007). Adults are active in the fall and into the winter, depending on the location, and have a second activity peak in the spring (Ostfeld et al. 1996,

Stafford 2007). Nymphs are active in the late spring to early summer, while larvae are active in the mid to late summer (Ostfeld et al. 1996, Stafford 2007).

A. americanum is the predominate tick in the southeastern United States and ranges from Texas to Florida, north through Iowa and Illinois through to Pennsylvania. Recently the range has expanded along the east coast into New Jersey, Rhode Island, and New York (Stafford 2007), and into northern Indiana (www.bsu.edu/physiology/article). Similar to *I. scapularis*, *A. americanum* peaks in activity at varying times depending on the life stage. Adults become active in early spring, nymphs become active in late spring, and larvae become active in the early summer (Stafford 2007). Since the distribution of these ticks overlap, there is potential for pathogens carried by these two species to also overlap.

Wildlife Reservoirs of Lyme Disease

Reservoir host competence for Lyme disease depends on the ability of the causative agent, *B. burgdorferi*, to infect and replicate in the host and subsequently be passed on to feeding ticks. Because the tick vectors of this pathogen feed on a wide variety of hosts, reservoir competence is extremely important; ticks feeding on non-competent hosts will not become infected with *B. burgdorferi*, thereby decreasing the amount of infected ticks in the environment. Since *I. scapularis* has a wide distribution, the density and host preferences vary depending on the region. Immature stages preferentially feed on rodents, including white-footed mice in the northern states, but can be found on a wider variety of species in the South, including reptiles (Apperson et al. 1993, Levin et al. 1996, Durden et al. 2002, Clark et al. 2005). If southern lizard species are shown to have borreliacidal properties similar to those in western areas (Wright et al. 1998, Lane and Quistad 1998), this may explain why Lyme disease is seen less frequently in the South.

In the eastern United States, numerous host species are susceptible to *B. burgdorferi* infection and may serve as competent reservoirs, including the white-footed mouse (Anderson et al. 1985, Anderson and Norris 2006), cotton mouse (*Peromyscus gossypinus*), cotton rat (*Sigmodon hispidus*) (Lane et al. 1991, Barbour and Fish 1993, Oliver et al. 1993, Oliver et al. 2005, Oliver et al. 2003), eastern chipmunk (*Tamias striatus*) (Anderson et al. 1985), northern short-tailed shrews (*Blarina brevicauda*) (Brisson et al. 2008), gray squirrels (detected in Europe) (*Sciurus carolinensis*) (Craine et al. 1997), eastern woodrat (Oliver et al. 2003), meadow voles (*Microtus pennsylvanicus*) (Markowski et al. 1998), American robins (Richter et al. 2000, Ginsberg et al. 2005), and many migratory birds (Weisbrod and Johnson 1989, Ginsberg et al. 2005), although competence varies by species. Although WTD are important hosts for adult *I. scapularis*, they are not competent reservoirs for *B. burgdorferi* (Patrican 1997). However, they do seroconvert after exposure, both experimentally (Luttrell et al. 1994) and in the wild (Magnarelli et al. 1986, Anderson et al. 1987, Magnarelli et al. 1991). To date, detection of antibodies to *B. burgdorferi* in WTD has been restricted to a limited number of studies in the northern United States (Anderson et al. 1987, Gill et al. 1993, Magnarelli et al. 1999), and a few in the southern U.S. (Magnarelli et al. 1986, Mahnke et al. 1993). Furthermore, deer are typically parasitized by adult *I. scapularis*, therefore, do not often transmit the pathogen to naïve ticks that would subsequently feed on other hosts.

In the southeastern United States, lizards are common hosts for immature *I. scapularis* ticks (Apperson et al. 1993). The role of reptiles in the natural history is controversial and numerous studies have provided contradictory evidence. For example, reservoir competence studies on herpetofauna have shown that some, but not all, reptiles contain *B. burgdorferi* bacteriolytic properties in their serum (Kuo et al. 2000, Ullmann et al. 2003). Experimentally, the

southeastern five-lined skink (*Eumeces inexpectatus*) can serve as a reservoir host for Lyme disease for at least five weeks (Levin et al. 1996); however, serum from the eastern fence lizard (*Sceloporus undulatus*) has some bacteriocidal properties (Grigery et al. 2005). In contrast, the western fence lizard (*Sceloporus occidentalis*) and southern alligator lizard (*Elgaria multicaerinata*) are incompetent hosts for the spirochetes (Kuo et al. 2000), and the western fence lizard shows complete borreliacidal activity (Ullmann et al. 2003). *B. burgdorferi* has been detected by polymerase chain reaction (PCR) in broad-headed skinks (*Eumeces laticeps*), brown anoles (*Anolis sagrei*), eastern fence lizards, eastern glass lizards (*Ophisaurus ventralis*), Florida scrub lizards (*Sceloporus woodi*), green anoles (*Anolis carolinensis*), ground skinks (*Scincella lateralis*), Mediterranean geckos (*Hemidactylus turcicus*), and six-lined racerunners (*Cnemidophorus sexlineatus*) (Clark et al. 2005, Swanson and Norris 2007); however, *B. burgdorferi* was not isolated in culture from any of these naturally infected reptiles. Furthermore, the five-lined skink (*Eumeces fasciatus*) is an incompetent host for larval *I. scapularis* ticks (Giery and Ostfeld 2007), and has been demonstrated as a dilution host in models (Giery and Ostfeld 2007). Since some, but not all, reptile species contain bacteriolytic properties, the presence of these reptiles may serve to reduce tick infection rates (Kuo et al. 2000), thereby decreasing cases of Lyme disease.

Southern Tick-Associated Rash Illness (STARI)

In the mid-1980's, physicians in the Southeast and south-central states described an erythema migrans rash following the bite of a LST (Campbell et al. 1995). For years this was diagnosed as Lyme disease, despite the fact that patients lacked laboratory evidence of *B. burgdorferi* infection (Campbell et al. 1995). Although *B. burgdorferi* has been detected in LST by PCR (Magnarelli et al. 1986, Clark 2004), several trials have proved that LST are not vector

competent; therefore, another pathogen was suspected as the causative agent of these Lyme-like symptoms (Piesman and Sinsky 1988, Mukolwe et al. 1992, Sanders and Oliver 1995, Kirkland et al. 1997).

Another spirochete, *B. lonestari*, has been detected in LST across the eastern United States, including the states of Alabama, Arkansas, Delaware, Georgia, Indiana, Kansas, Kentucky, Maryland, Missouri, Mississippi, New Jersey, New York, North Carolina, South Carolina, Tennessee, Texas, and Virginia (Burkot et al. 2001, Bacon et al. 2003, Stegall-Faulk et al. 2003, Stromdahl et al. 2003, Varela et al. 2004, Steiner, personal communication). Subsequently, this organism was detected by PCR in a patient with an erythema migrans rash and a history of a lone star tick bite who recently traveled to North Carolina and Maryland (James 2001). Portions of the *B. lonestari* genome have been sequenced (Lin et al. 2003), and the sequence is distinct from *B. burgdorferi*. Because of these findings, *B. lonestari* has been suggested as a putative agent of this newly recognized disease, now referred to as Masters' disease, or Southern Tick Associated Rash Illness (STARI).

Lone star ticks prefer feeding on WTD during all three mobile stages, and have been found on WTD in high relative abundance when compared to other common hosts (Kollars et al. 2000), giving them ample opportunity to pick up this organism. Because LST are very common in the South and aggressively feed on humans (Felz and Durden 1996), exposure to this pathogen could occur relatively often.

Since there is a known relationship between WTD, LST, and transmission of at least two pathogens (*Ehrlichia chaffeensis* and *E. ewingii*) associated with LST (Yabsley et al. 2002, Yabsley et al. 2003), it is conceivable that WTD may be reservoirs for *B. lonestari* from LST vectors. Experimental and field studies support this hypothesis. Wild LST have been shown to

transmit *B. lonestari* to naïve fawns (Varela-Stokes 2007, Little et al. unpublished data), proving vector competence of the LST and susceptibility of WTD. White-tailed deer were also susceptible to a culture isolate of *B. lonestari* (Moyer et al. 2006). Natural infections of WTD have been detected in Arkansas, Georgia, North Carolina, and South Carolina (Moore et al. 2003). Experimentally, *B. lonestari* failed to infect mice, dogs, and Holstein cattle (Moyer et al. 2006), and does not survive exposure to eastern fence lizard or Swiss-Webster mouse (*Mus musculus*) sera in vitro (Grigery et al. 2005). Because of the presence of *B. lonestari* in the eastern U.S., its role as a human pathogen should be investigated.

Immune Response

Since WTD develop a detectable antibody response during experimental infection with *B. burgdorferi* (Gill et al. 1993, Mahnke et al. 1993, Luttrell et al. 1994), serologic tests can detect whether or not an animal has been exposed to this pathogen. *B. burgdorferi* has several outer surface proteins (Osp, designated as OspA-F), which are variably expressed in tick vs. vertebrate hosts which may contribute to transmission, survival, or virulence (Gilmore and Piesman 2000, Schwan and Piesman 2000, Pal et al. 2000). For example, OspA and -B are up-regulated while the spirochete is in the arthropod vector, and down-regulated during transmission to the host, while OspC is up-regulated during transmission to the host (Barthold et al. 1995, Montgomery 1996, de Silva 1996, Schwan and Piesman 2000, Pal and Fikrig 2003, Neelakanta et al. 2007).

OspA is present on the spirochetes in larval ticks as soon as 24h after the larvae have been placed on infected rodents (de Silva et al. 1996), and is believed to serve a colonization function in the tick gut (Pal et al. 2000). Reservoir hosts generally do not show a strong immune response to OspA because this protein is down-regulated by the spirochete during transmission from vector to host (Gill et al. 1993, Montgomery et al. 1996). Since OspA antibodies decrease

the perpetuation of *B. burgdorferi* in larval ticks (de Silva et al. 1996), the production of antibodies to OspA may also account for the difference in reservoir potential of different animals (de Silva et al. 1996). Additionally, the 31 kilodalton (kDa) OspA and the 34 kDa OspB proteins are specific to *B. burgdorferi* (Mitchell et al. 1994).

As the spirochetes move from the vector to the host during feeding, they begin to clear OspA and -B and replace them with OspC to survive in the host (Schwan et al. 1995). This change in Osp expression may be cued by tick feeding and temperature changes between the tick midgut and host (Schwan et al. 1995). Because OspC can be detected in the tick and the host, it is thought to play a role in vector to host transmission (Gilmore and Piesman 2000, Schwan and Piesman 2000). In the host, OspC produces an early, strong, immune response (Montgomery et al. 1996). Although the 22kDa OspC protein is specific to *B. burgdorferi sensu lato* (Mitchell et al. 1994), it is not specific to *B. burgdorferi sensu stricto* (Du et al. 2007).

Like Osps, VlsEs are variable surface lipoproteins found on *B. burgdorferi*, but which are capable of antigenic variation (Embers et al. 2007). These molecules contain invariable regions (IRs) which do not engage in antigenic variation, and which are more conserved among *B. burgdorferi* genospecies (Liang et al. 1999). C6 is the portion of the VlsE that is the most conserved, and can be used to detect antibodies to *B. burgdorferi*.

Immunoglobulins (Ig) are antibodies that respond to foreign objects in the body, such as Osps of *Borrelia*. IgM and IgG antibodies are often detected in response to proteins of *B. burgdorferi*. The production of IgG or IgM depends on the duration of the infection (Du et al. 2007). For example, IgM antibodies are usually not detected until 3 weeks after infection, and can persist for months to years (Craft et al. 1986), and IgG antibodies are not detected until 4-6 weeks following infection, and can persist for years (Craft et al. 1986). The earliest detectable

IgG response is usually to OspC, and there is a high IgG response to OspA and –B when there is severe and prolonged arthritis (Akin et al. 1999).

However, WTD do not always show a strong immune response to *B. lonestari*, and when they do seroconvert the immune response appears to taper after a short period of time. For example, WTD experimentally inoculated with *B. lonestari* only weakly seroconvert, showing low titers (1:64) 28 days post-inoculation (Moyer et al. 2006). Additionally, deer infected through wild tick transmission did not always seroconvert, and those that did seroconvert remained seropositive for only 14 days (Varela-Stokes 2007).

Laboratory Assays

Diagnosis of Lyme disease in humans from endemic areas is assisted by the common finding of an erythema migrans rash associated with fever and exposure to ticks. However, these clinical signs which assist in detection are not present in wildlife (Lindenmayer et al. 1990). To conduct surveillance for borrelial pathogens in wildlife, we must rely on diagnostic assays such as bacterial culture, immunohistochemistry, PCR, and serological tests, such as enzyme-linked immunosorbent assay (ELISA), immunofluorescent antibody assay (IFA), SNAP tests, or Western Blot (WB). Serologic assays, which detect antibodies to pathogens to determine if the animal has been exposed to that pathogen, are often used because of their ease of use, and are beneficial in surveillance programs to monitor the spread of disease (Gill et al. 1993, Gill et al. 1994).

The Centers for Disease Control and Prevention (CDC) recommend using a two-test approach for serologic tests to detect antibodies to Lyme disease, noting that these tests may result in false negatives during early infection (CDC 1995). This two-step approach includes using an ELISA or IFA as a first step in detection, and following up positive cases with a WB for

confirmation (CDC 1995). This approach is specific enough to ensure a correct positive result. Additionally, if the WB was found to be negative, this approach would determine a false positive in the original ELISA or IFA, and negative ELISA or IFA tests would not need further testing (CDC 1995). The CDC also recommends that early infections be tested using both IgG and IgM tests, since late stage infections have a strong IgG response, and IgM alone could show false positive results for active disease after 1 month post infection (CDC 1995).

Several ELISA formats have been developed for *B. burgdorferi*. Loebermann et al. (2006) showed that IgM ELISAs detected the presence of antibodies to *B. burgdorferi* before immunoblotting tests. ELISAs also had higher sensitivity, and similar specificity when compared to IFAs (Magnarelli et al. 2004); however, ELISAs which used 41kDa flagellar antigen showed problematic specificity, and gave false positive results (Mitchell et al. 1994).

When compared to ELISAs, P39 recombinant ELISAs, and WB, IgM IFAs were found to be more accurate in detecting antibodies to *B. burgdorferi* (Mitchell et al. 1994). IgM IFAs were also found to be more sensitive than IgG- IgM fluorescence ELISA, P39 ELISA, and IgG WB (Mitchell et al. 1994). Since IFAs do not detect antibodies to specific Osps, but rather detect antibodies to all Osps, IFAs are generally very sensitive, and cross-reaction can occur. Furthermore, the subjectivity of IFAs creates problems in accuracy, since general background may be misinterpreted as a positive result, and this subjectivity is alleviated with experience (Mitchell et al. 1994). Therefore, the novice may have more false positive and false negative results because of their lack of experience with reading IFA slides.

Western blots have a sensitivity as high as 83% (Dressler et al. 1993) and a specificity as high as 100% (Dressler et al. 1993). WB are more laborious, expensive, and require more serum

than ELISA (Karlsson et al. 1989, Magnarelli et al. 2004), and are also dependant on *B. burgdorferi* strains (Du et al. 2007).

Commercially available SNAP® 4Dx® test kits provide 96% sensitivity and 100% specificity to *B. burgdorferi* (IDEXX, package insert), which serves as the best current method for accurately detecting this bacterium. Use of synthetic C6 peptide has been shown to provide high accuracy with high specificity and sensitivity to *B. burgdorferi* (Smismans et al. 2006). Since serologic cross-reactivity among *Borrelia* spp. can occur with the IFA test, the SNAP® 4Dx® test can be employed to identify antibodies specific to *B. burgdorferi*.

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CHAPTER 2

DISTRIBUTION OF ANTIBODIES REACTIVE TO *BORRELIA LONESTARI* AND *BORRELIA BURGENDORFERI* IN WHITE-TAILED DEER (*ODOCOILEUS* *VIRGINIANUS*) POPULATIONS IN THE EASTERN UNITED STATES¹

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ABSTRACT

Southern Tick-Associated Rash Illness (STARI) is a Lyme-like syndrome that occurs in the southern states. *Borrelia lonestari*, which naturally infects white-tailed deer (WTD, *Odocoileus virginianus*), has been suggested as the cause of STARI, and is transmitted by the lone star tick (*Amblyomma americanum*). We tested 714 WTD from 20 eastern states for antibodies reactive to *B. lonestari* using an indirect immunofluorescent antibody test (IFA), of which 107 (15.0%) were positive. Antibody prevalence was higher in southern deer (17.5%) than northern deer (9.2%). Using the SNAP® 4DX® test, 71 (9.9%) were positive for *Borrelia burgdorferi* and significantly more northern deer (23.9%) were positive compared with southern deer (3.8%). Our data demonstrate that WTD are exposed to both *Borrelia* species, but antibody prevalence for exposure to the two species differs regionally and distributions correlate with the presence of *I. scapularis* and *A. americanum* ticks.

INTRODUCTION

Tick-borne pathogens, such as *Borrelia burgdorferi*, are important causes of morbidity and mortality for humans and some domestic animals. Due to the recent expansion of many tick species, the distribution of these diseases is spreading to new geographic areas. By improving surveillance techniques, we can develop better methods of monitoring and controlling their spread. There were 23,305 documented cases of Lyme disease in the United States in 2005 alone (Centers for Disease Control and Prevention (CDC) 2007), emphasizing the impact of this zoonotic disease. Typical symptoms of Lyme disease include headache, fever, sore muscles and joints, and the characteristic erythema migrans rash. If left untreated, Lyme disease can lead to Bell's palsy (facial paralysis), chronic arthritis, neurologic problems, and heart problems, including pericarditis (Steere et al. 1977, Steere et al. 2004).

Although many studies have investigated the natural history of Lyme disease in the northeastern and northern Midwestern U.S., little is understood about a similar syndrome, called Southern Tick-Associated Rash Illness (STARI), which occurs in the southeastern and south-central United States. STARI has been diagnosed numerous times following the bite from a lone star tick (*Amblyomma americanum*) (Campbell et al. 1995, Masters et al. 1998, James et al., 2001). The clinical signs of STARI (erythema migrans rash, headache, fever, heart problems, and muscle and joint stiffness) so closely resemble the symptoms of Lyme disease that it may be misdiagnosed clinically (James et al. 2001), making it difficult to assess the epidemiology of Lyme and Lyme-like disease in the South. The absence of *B. burgdorferi* from STARI patients in the South suggests that another agent is responsible for producing the infection that results in these symptoms (James et al. 2001, Bacon et al. 2003, Wormser et al. 2005). The spirochete *B. lonestari* has been detected in a single case of STARI, but was not detected in a series of cases in a later study (James et al. 2001, Bacon et al. 2003, Wormser et al. 2005).

B. lonestari has been detected in numerous wild lone star ticks collected throughout the southeastern United States (Burkot et al. 2001, James et al. 2001, Bacon et al. 2003, Stegall-Faulk et al. 2003, Bacon et al. 2005) and lone star ticks have been proven competent to transmit *B. lonestari* to white-tailed deer (WTD, *Odocoileus virginianus*) (Varela-Stokes 2007). A previous study has shown that wild WTD are naturally infected with *B. lonestari* (Moore et al. 2003), and experimentally, WTD are susceptible to *B. lonestari* infection. However, some WTD that were experimentally exposed either by needle inoculation or tick transmission resulted in short-term infections characterized by an absent, weak, or short duration antibody response (Moyer et al. 2006, Varela-Stokes 2007). Although not considered competent reservoirs for *B. burgdorferi* (Telford et al. 1988, Luttrell et al. 1994), WTD seroconvert after experimental

infection and can have high antibody prevalence rates in northern states, indicating frequent exposure to *B. burgdorferi* (Magnarelli et al. 1986, Gill et al. 1994, Luttrell et al. 1994, Magnarelli et al. 1995, Gallivan et al. 1998, Magnarelli et al. 1999).

Because WTD are suspected natural reservoirs of *B. lonestari*, we planned to determine the distribution of antibodies reactive to *B. lonestari* in WTD populations throughout the eastern United States. Although experimental infections suggest only short-term detection of antibodies occurs, we hypothesized that antibodies to *B. lonestari* would be detected in wild WTD because of frequent re-exposure to ticks harboring the agent. We also tested WTD for antibodies to *B. burgdorferi* since these pathogens overlap in some eastern regions. Further, because antibodies reactive to *B. burgdorferi* could cross react with the *B. lonestari* antigen used in the serologic test, this would address problems with test specificity. Because *B. lonestari* is transmitted by lone star ticks, we hypothesized that antibodies reactive to *B. lonestari* would be detected in WTD populations with known exposure to *E. chaffeensis* (as reported in Yabsley et al. 2003), and that antibodies to *B. burgdorferi* would be detected predominantly in the northeastern and Midwestern states where Lyme disease is endemic.

METHODS

Sample Collections

The majority of blood and serum samples used in this project were collected from hunter-killed WTD between 1994-2006 for various projects performed by the Southeastern Cooperative Wildlife Disease Study (SCWDS), College of Veterinary Medicine, University of Georgia, Athens, Georgia. All samples were stored at -20°C until testing. Additional serum samples from hunter-killed WTD were collected by collaborators in Indiana, Minnesota, and Pennsylvania.

Whole-blood samples collected from the cavity of hunter-killed WTD, or from post-mortem jugular venipuncture, were placed into 50ml tubes (Corning, Lowell, MA). Blood was allowed to clot and then samples were centrifuged at 3000 rpm for 8 minutes. Serum was placed into storage microtubes (Starstedt Ag & Co., Nümbrecht, Germany) and stored in a -20°C freezer until serological testing.

IFA Serology

An indirect immunofluorescent assay (IFA) using *B. lonestari* as an antigen and serum at a 1:64 dilution was used to detect anti-*Borrelia* antibodies in samples as previously described (Moyer et al. 2006). Positive samples were determined by the presence of green fluorescing spirochetes while negative samples lacked any detectable fluorescence. Indeterminate samples were retested and if they were again characterized as indeterminate, the sample was classified as negative. Positive control serum samples were collected previously from pen-raised WTD fawns that were hyperimmunized with *B. burgdorferi* antigens (Mahnke et al. 1993) which cross-reacted with *B. lonestari* antigens. New control sera were collected from fawns raised in isolation that have consistently been negative for antibodies to *Borrelia* and other tick-borne pathogens (*Ehrlichia* and *Anaplasma*).

SNAP® 4Dx® assay Serology

To detect *B. burgdorferi*-specific antibodies, samples were tested using the SNAP® 4Dx® test (IDEXX Laboratories, Inc., Westbrook, Maine) following the manufacture's instructions. This test has been used to detect *B. burgdorferi* antibodies in dogs (Duncan et al. 2004, Carlos et al. 2007), cats (Levy et al. 2003), horses (IDEXX unpublished data), and rabbits (Yabsley unpublished data). The ability of this assay to detect anti-*B. burgdorferi* antibodies in WTD was confirmed using serum from experimentally infected WTD (Luttrell et al. 1994). Sera

from WTD experimentally infected with *B. lonestari* (Moyer et al. 2006) were negative when tested with the SNAP® 4Dx® test.

Data Analyses

The southeastern region included the states of Alabama, Arkansas, Florida, Georgia, Kentucky, Louisiana, Missouri, Mississippi, North Carolina, South Carolina, Tennessee, Texas, and Virginia. The northern region included the states of Indiana, Kansas, Maryland, Minnesota, New Jersey, Pennsylvania, and West Virginia. To facilitate graphic presentation, data for each population were categorized by county or parish; if one or more WTD with antibodies reactive to *Borrelia* was detected, that county or parish was classified as seropositive. The mean seropositive population seroprevalence was calculated for populations with at least one positive WTD. Using the mean seroprevalence from IFA seropositive populations (35.1%) and the mean seroprevalence from SNAP seropositive populations (41.0%), post-hoc analyses indicated that testing 5-7 WTD per population would result in detecting at least one seropositive animal with 85% to 95% confidence (Thrusfield 1995). Only those counties/parishes with ≥ 5 samples were included for assigning seronegative results.

Chi-square analysis ($p=0.05$) was used to determine if differences in seroprevalence existed between age classes and gender. Ages were divided into the following age classes: 0-0.75 years, 0.76-1.5 years, 1.6-2.5 years, 2.6-3.5 years, 3.6-4.5 years, and 4.6+ years.

We also determined if there was an association between seropositive county status and presence of ticks. Data documenting the presence of *A. americanum* was collected during previous studies and routine deer necropsies conducted by SCWDS (unpublished data). Because the majority of these routine deer necropsies were conducted in the summer and early fall, *I. scapularis* was rarely detected on WTD populations; therefore, we used a distribution map

created by Dennis et al. (1998) to compare with seropositive populations. This map indicated which counties had *I. scapularis* ticks reported and which did not. For both tick species it is important to note that the lack of reporting tick presence does not indicate that the tick is not present in those areas.

RESULTS

Regional serology

A total of 714 WTD serum samples from 136 counties (average of 5.25 samples/county, range 1-21) in 20 states were tested (Table 2.1, Fig. 2.1). Antibodies reactive with *B. lonestari* were detected in 107 (15.0%) WTD (Table 2.1). The mean seroprevalence in populations with at least one positive WTD was 35.1% (SD= 27.6%, range= 5-100%). A significant difference was seen for mean population seroprevalence between southern and northern regions (52.2% and 32.6%, respectively, $\chi^2= 4.72$, df=1, p=0.03). The presence of *B. lonestari* antibodies was associated with the presence of *E. chaffeensis* antibodies (36 of 49 counties, 73.5%) (Yabsley et al. 2003).

Using the SNAP® 4Dx® assay, antibodies to *B. burgdorferi* were detected in 71 of 714 (9.9%) WTD (Table 2.1). The mean seroprevalence in seropositive populations was 41.0% (SD= 30.2%, range= 5-100%). Significantly more seropositive populations were detected in the north (47.8%) compared with the south (16.7%) (Fig. 2.1) ($\chi^2=14.92$, df=1, p<0.01). Only 41 of the 71 samples that were SNAP positive for *B. burgdorferi* also had antibodies reactive to *B. lonestari* by IFA testing. Eight of these 41 (19.5%) samples were from southern states while 33 (80.5%) were from northern states.

Age and Gender Effects on Prevalence

Of the 507 WTD for which age data were available (mean 2.7 yrs), 106 (20.9%) had antibodies reactive to *B. lonestari* by IFA (mean age 2.7 yrs) and 30 (5.9%) were seropositive for *B. burgdorferi* by SNAP (mean age 2.9 yrs) (Fig. 2.2). No age differences were detected with *B. burgdorferi* ($\chi^2=8.48$, $df=5$, $p=0.132$) or *B. lonestari* antibodies ($\chi^2=7.71$, $df=5$, $p=0.0173$). Gender data were available for 521 WTD, of which 217 were male and 304 were female. There was no difference in seroprevalence between gender for *B. lonestari* (16.1% in males vs 14.8% in females, $\chi^2= 0.171$, $df=1$, $p=0.68$) or *B. burgdorferi* (7.4% in males vs 5.6% in females, $\chi^2= 0.677$, $df= 1$, $p=0.41$).

Tick Presence Effects on Prevalence

Most (19/24; 79.2%) of the *B. lonestari* seropositive populations that had corresponding tick data contained WTD that were infested with *A. americanum*. Likewise, most (26/37; 70.3%) of the *B. burgdorferi* seropositive populations that had corresponding tick data contained WTD that were infested with *I. scapularis*.

Table 2.1. Prevalence of antibodies reactive with *Borrelia lonestari* and *Borrelia burgdorferi* among white-tailed deer (*Odocoileus virginianus*) from 136 counties in 20 states.

State	Year(s)	No. of counties tested	Number IFA positive and SNAP negative/ no. tested (%)	Number SNAP positive/ no. tested (%)
Alabama	2000-2001	5	3/22 (14)	0/22 (0)
Arkansas	2000-2007	7	11/38 (29)	2/38 (5)
Florida	1992-2007	9	11/62 (18)	1/62 (2)
Georgia	1997-2007	10	7/60 (12)	2/60 (3)
Indiana	2006-2007	14	4/60 (7)	11/60 (18)
Kansas	1998-2002	5	2/23 (9)	0/23 (0)
Kentucky	2000-2001	4	3/20 (15)	0/20 (0)
Louisiana	1994-2007	14	13/76 (17)	2/76 (3)
Maryland	1999-2002	5	9/24 (38)	9/24 (38)
Minnesota	2004	7	0/24 (0)	11/24 (46)
Missouri	1994-2002	7	5/38 (13)	1/38 (3)
Mississippi	2002	4	5/18 (28)	0/18 (0)
North Carolina	2000-2002	6	12/24 (50)	0/24 (0)
New Jersey	2006	4	1/33 (3)	17/33 (52)
Pennsylvania	2006	3	0/12 (0)	4/12 (33)
South Carolina	2001-2002	4	5/24 (21)	0/24 (0)
Tennessee	2001	3	3/20 (15)	1/20 (5)
Texas	1992-2002	9	3/45 (7)	0/45 (0)
Virginia	2000-2002	8	6/49 (12)	10/49 (20)
West Virginia	1999-2001	8	4/42 (10)	0/42 (0)
Total		136	107/714 (15)	71/714 (10)

IFA, indirect fluorescent antibody

FIG 2.1. Distribution of antibodies reactive to *Borrelia lonestari* and *Borrelia burgdorferi* (determined by indirect fluorescent antibody and SNAP® 4Dx® assays, respectively) among white-tailed deer (*Odocoileus virginianus*). Counties shaded dark red were determined to be positive for *B. burgdorferi* because deer were SNAP and IFA positive, counties shaded pink were classified as positive for *B. burgdorferi* because deer were SNAP positive and IFA negative. Counties shaded dark blue were classified as positive for *B. lonestari* because deer were only positive for antibodies reactive to *B. lonestari* antigen (and were all SNAP negative). Counties shaded light blue were negative for both *Borrelia* species by both assays (and had a minimum of 5 deer/population tested).

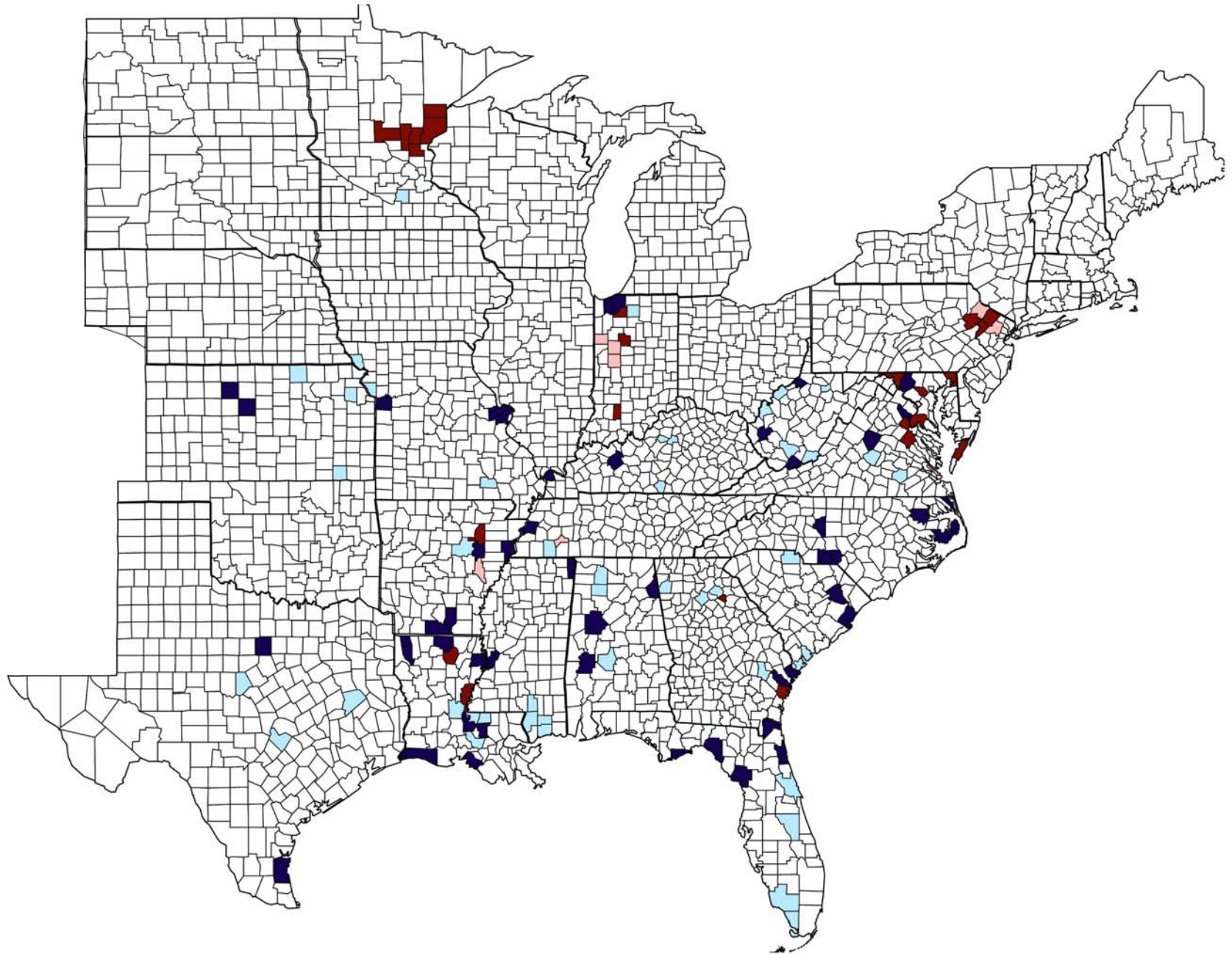
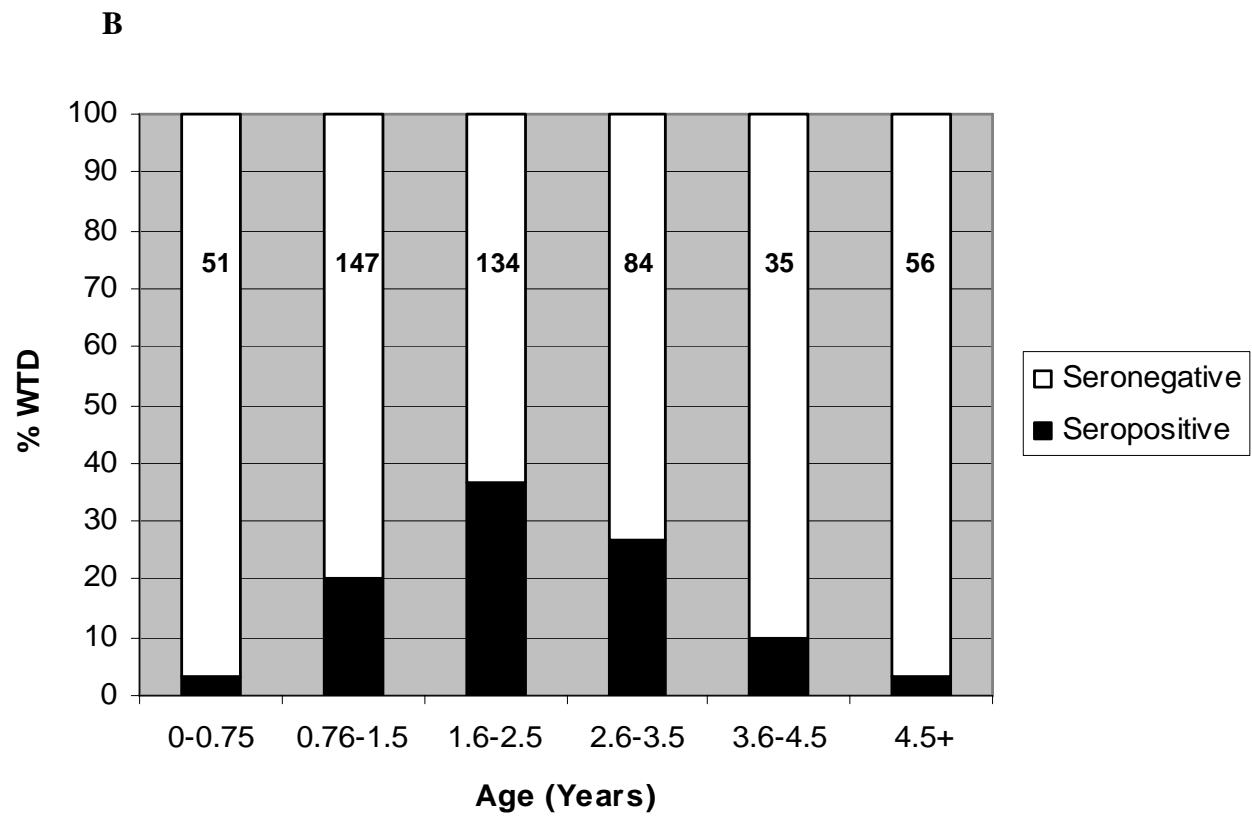
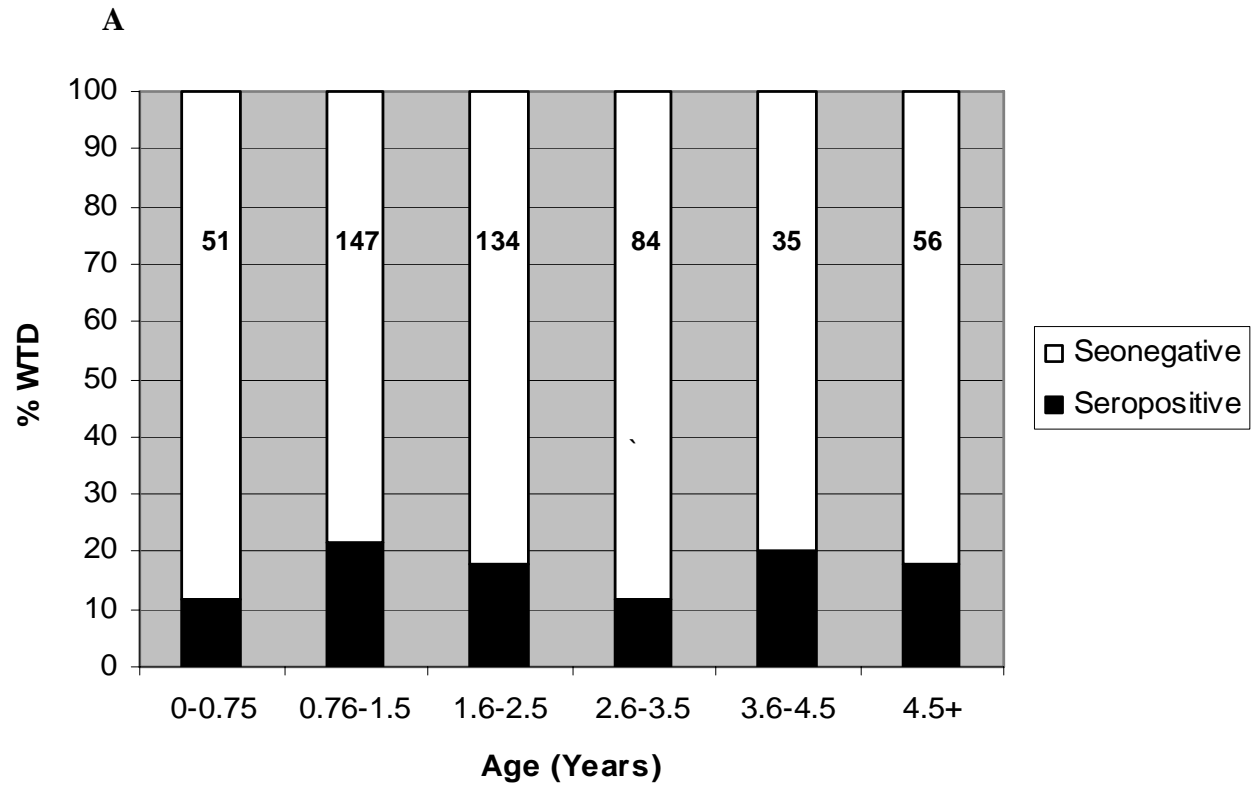


FIG 2.2. (A) Prevalence of antibodies with *Borrelia lonestari* in white-tailed deer (*Odocoileus virginianus*) among age classes. (B) Prevalence of *Borrelia burgdorferi* antibodies among age class of white-tailed deer. Number of deer tested in each category is shown in each bar.



DISCUSSION

The overall goal of this project was to determine the exposure rate of WTD to *B. lonestari* and *B. burgdorferi* in the eastern United States. The data presented here demonstrate that WTD are exposed to both *Borrelia* species and the prevalence of the two species differs regionally, although overlap in the distribution does occur. No differences were noted between seroprevalence and age and gender which corresponds with data from *E. chaffeensis* and *A. phagocytophilum* in WTD (Yabsley et al. 2003, Dugan et al. 2006). Finally, the presence of *I. scapularis* and *A. americanum* were correlated with populations positive for *B. burgdorferi* and *B. lonestari*, respectively, which supports numerous studies that indicate that these two ticks are the principal vectors for these two agents (Burgdorfer et al. 1982, Magnarelli et al. 1986, Kirkland et al. 1997, James et al. 2001).

Antibodies reactive to *B. lonestari* were most commonly detected in the southeastern United States; however, *B. lonestari* was present locally in some northern counties. Seroprevalence was highest in several southeastern states such as Louisiana, Alabama, Florida, South Carolina, and North Carolina, and prevalence decreased in more northerly states such as Virginia, Maryland, and Indiana. Although antibodies reactive with *B. lonestari* were detected in Minnesota and Pennsylvania, each individual WTD was also positive for *B. burgdorferi*, indicating that these seroreactors were likely caused by cross-reaction with *B. burgdorferi*. The low prevalence of antibodies reactive to *B. lonestari* in WTD populations in the South where exposure was expected to be highest supports results of recent experimental studies showing that WTD have a limited serologic reaction following infection with *B. lonestari* (Moyer et al. 2006, Varela-Stokes 2007). Because of this limited reactivity, large numbers of WTD would need to be tested in individual populations to ensure a proper classification for *B. lonestari* exposure.

The distribution of *B. lonestari* antibodies in WTD corresponds with what is known about the natural history of this microorganism. The majority of the *B. lonestari* reactive samples came from counties with a known established presence of *A. americanum*, the only known competent vector for *B. lonestari*, including the recent detection of established populations in northern Indiana (www.bsu.edu/physiology/article). Naturally infected WTD also have been detected in five populations in the Southeast (Moore et al. 2003), three of which were seropositive for *B. lonestari* in the current study. Furthermore, *B. lonestari* has been detected in *A. americanum* from many of the southeastern and mid-Atlantic states, including Alabama (Burkot et al. 2001), Georgia (Varela et al. 2004, Mixson et al. 2006), Florida (Clark 2004, Mixson et al. 2006), Maryland (Taft et al. 2005), New Jersey (Mixson et al. 2006, Schulze et al. 2006), North Carolina (Mixson et al. 2006), South Carolina (Mixson et al. 2006), and Tennessee (Stegall-Faulk et al. 2003). Antibodies to *B. lonestari* were detected in a limited number of counties outside of the known distribution of *A. americanum* (western Kansas and West Virginia); however, the range of *A. americanum* is rapidly expanding northward and westward (Paddock and Yabsley 2007) and may be present in areas not previously detected. Distribution studies in these areas may be warranted to determine if, and to what extent, *A. americanum* has expanded into these regions.

Although the prevalence of *B. lonestari* antibodies in southeastern WTD populations was much lower than prevalence to *E. chaffeensis*, the presence of *B. lonestari* reactive samples was associated with the presence of *E. chaffeensis*. Interestingly, the prevalence of both *E. chaffeensis* and *B. lonestari* in *A. americanum* were similar in surveys conducted in Georgia (Varela et al. 2004, Mixson et al. 2006, Yabsley et al. unpublished data) and South Carolina (Mixson et al. 2006). Although WTD show a strong immune response to *E. chaffeensis* (Varela

et al. 2003, Varela et al. 2005), WTD experimentally inoculated with *B. lonestari* only seroconvert weakly, if at all, and for only a short period of time (Moyer et al. 2006, Varela-Stokes 2007). In one study, antibody titers peaked at 1,024 in one of two WTD and both WTD were seronegative by six weeks post inoculation (Moyer et al. 2006). In another study, only one of three WTD seroconverted with a maximum titer of 128, and this single WTD was seronegative by five weeks post-exposure to ticks (Varela-Stokes 2007). Based on these experimental data, it was surprising that seroprevalence in many southern WTD populations was higher than 30%. It is possible that wild WTD may maintain antibodies for longer periods of time due to frequent exposure to *B. lonestari*. Because of the overall weak immune response, the seroprevalence detected in this study should be considered a minimum for the exposure rate among wild WTD.

The majority of *B. burgdorferi* reactive samples were found in the northern states, which corresponds with areas of high Lyme disease endemicity in dogs and humans (Duncan et al. 2004, Beall et al. 2008), with only a few deer in the Southeast found to be positive. These data confirm several previous studies that have identified antibodies to *B. burgdorferi* in WTD from multiple states (Magnarelli et al. 1986, Mahnke et al. 1993, Gill et al. 1994). The highest seroprevalences (up to 100%) were detected in Minnesota, New Jersey, Maryland, and Indiana and only limited numbers of positive WTD were detected in Arkansas, Louisiana, and Georgia (only one to three positive WTD per population). Although *B. burgdorferi* is detected in relatively high prevalences in ticks, small mammals, and reptiles in the Southeast (Magnarelli et al. 1992, Clark 2004), there are few human and dog cases (Felz et al. 1999, Duncan et al. 2004). For example, in 2005 there were an average of 1,414 human cases reported from the northern states sampled in this study compared to an average of 38 reported cases per state in the southern

states sampled in this study (Centers for Disease Control and Prevention 2007), and there have not been any confirmed cases of Lyme disease acquired south of Maryland and Virginia (Wormser et al. 2006). The increased prevalence seen in rodents in the South likely reflects the high diversity and density of other competent *Ixodes* species that feed on rodents (Clark et al. 2001, Oliver et al. 2003). Because the detected prevalence of *B. burgdorferi* in WTD from the Southeast was so low, it is probable that we missed deer that were positive, especially due to the low sample sizes of some populations.

The IFA test is generally a very sensitive assay for detecting anti-*Borrelia* spp. antibodies. This study detected a low number of samples that were IFA negative for *Borrelia* spp., but were SNAP positive for *B. burgdorferi*. A possible explanation for this disparity is that not all *B. burgdorferi* samples cross-react with the *B. lonestari* antigen that was used for IFA testing. This would lead to a decreased number of IFA positive samples being detected, especially those having low titers. However, we suspect that these IFA negative, SNAP positive samples (primarily from northern states) were not co-infected with *B. lonestari*, since those samples would have likely tested positive using the IFA test.

Currently the causative agent of STARI is not known, but some researchers have suggested that it might be *B. lonestari* because this organism has been detected in a skin biopsy of a patient with an attached *A. americanum* (James et al. 2001) and human patients in the Southeast demonstrating Lyme-like symptoms test negative for *B. burgdorferi* (Felz et al. 1999, Wormser et al. 2005). The results of this study indicate that antibodies reactive with *B. lonestari* are widespread in WTD populations and the distribution corresponds with the distribution of *A. americanum*. In some areas, the presence of *B. lonestari* overlapped with *B. burgdorferi* which was expected because the range of *A. americanum* has been increasing and currently overlaps

with *I. scapularis* in many states, particularly on the East Coast. These data support the need for enhanced surveillance for both tick species in the known edges of their ranges to detect expansion and increased risk of human disease. Further work is needed to determine the role, if any, of *B. lonestari* as a human pathogen and in general, on the natural history of *Borrelia* species in the southern United States.

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CHAPTER 3

CONCLUSIONS

Lyme disease, caused by the spirochete *Borrelia burgdorferi*, is common in the northern and western United States and is transmitted by the blacklegged ticks (*Ixodes scapularis* and *Ixodes pacificus*). Symptoms may include an erythema migrans rash, fever, fatigue, headache, and can cause chronic sequelae (e.g., heart palpitations, arthritis, etc). Although endemic in rodents and ticks in the southeastern United States, confirmed Lyme cases in humans in this region are rare. However, a Lyme-like disease, coined Southern Tick-Associated Rash Illness (STARI), is commonly seen in the southeast. *Borrelia lonestari*, which naturally infects white-tailed deer (WTD, *Odocoileus virginianus*) and is transmitted by the lone star tick (*Amblyomma americanum*), has been suggested as the causative agent of STARI. Because of the similar symptoms associated with STARI and Lyme disease, studying the epidemiology of Lyme and Lyme-like disease in the South is complicated.

Because WTD are suspected natural reservoirs of *B. lonestari*, the goal of the current research was to determine the distribution of antibodies reactive to *B. lonestari* in WTD populations throughout the eastern United States. A total of 714 WTD from 136 counties in 20 eastern states were tested for antibodies reactive to *B. lonestari* and *B. burgdorferi* in the southeastern United States based on IFA and SNAP® 4Dx® tests, respectively. Using these data, the distribution of WTD populations with antibodies reactive to both *Borrelia* species were mapped at the county level.

Antibodies reactive to *Borrelia lonestari* were detected in 107 (15.0%) WTD by IFA testing. Antibody prevalence was higher in southern deer (17.5%) compared with northern deer (9.2%). Using the SNAP® 4DX® test, 71 (9.9%) deer were positive for *Borrelia burgdorferi* and significantly more northern deer (23.9%) were positive compared with southern deer (3.8%). My data demonstrate that WTD are exposed to both *Borrelia* species, but antibody prevalence for exposure to the two species differs regionally and distributions correlate with the presence of *I. scapularis* and *A. americanum* ticks. Age and gender were not found to affect prevalence for antibodies reactive to either *Borrelia* species.

This study indicates that antibodies reactive with *B. lonestari* are widespread in WTD populations and the distribution corresponds with the distribution of *A. americanum*. The presence of *B. lonestari* overlaps with *B. burgdorferi* in some areas, which is explained by the increasing range of *A. americanum* which currently overlaps with *I. scapularis* in many states. These data support the need for enhanced surveillance for both tick species at the edge of their known ranges to detect expansion and increased risk of human disease. Further work is needed to determine the role, if any, of *B. lonestari* as a human pathogen and on the natural history of *Borrelia* species in the southern United States.