Distribution of Antibodies Reactive to *Borrelia lonestari* and *Borrelia burgdorferi* in White-Tailed Deer (*Odocoileus virginianus*) Populations in the Eastern United States

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Abstract

Southern tick-associated rash illness is a Lyme-like syndrome that occurs in the southern states. *Borrelia lonestari*, which has been suggested as a possible causative agent of southern tick-associated rash illness, naturally infects white-tailed deer (WTD; *Odocoileus virginianus*) and is transmitted by the lone star tick (*Amblyomma americanum*). To better understand the prevalence and distribution of *Borrelia* exposure among WTD, we tested WTD from 21 eastern states for antibodies reactive to *B. lonestari* using an indirect immunofluorescent antibody assay and *Borrelia burgdorferi* using the IDEXX SNAP[®] 4Dx[®] test. A total of 107/714 (15%) had antibodies reactive to *B. lonestari*, and prevalence of antibodies was higher in deer from southern states (17.5%) than in deer from northern states (9.2%). Using the SNAP 4DX test, we found that 73/723 (10%) were positive for *B. 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 *Ixodes scapularis* and *A. americanum* ticks.

Key Words: *Borrelia*—Lyme disease—Tick(s)—Vector-borne—Zoonosis.

Introduction

TICK-BORNE PATHOGENS, such as *Borrelia burgdorferi*, are important causes of morbidity and mortality of humans and some domestic animals. Due to the recent geographic expansion of some tick species, the distribution of these diseases is spreading to new 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 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, 2004).

Although many studies have investigated the natural history of Lyme disease in the northeastern and northern Midwestern United States, little is understood about Lyme disease in the southern United States. A Lyme-like disease syndrome, called southern tick-associated rash illness (STARI), occurs throughout the southeastern and south-central United States. STARI has been diagnosed numerous times after the bite from a lone star tick (*Amblyomma americanum*) (Campbell et al. 1995, Masters et al. 1998, James et al. 2001). The clinical signs

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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* detection from numerous STARI patients in the South suggests that another agent is responsible for producing this condition (James et al. 2001, Bacon et al. 2003, Wormser et al. 2005, Philipp et al. 2006). The spirochete *Borrelia lonestari* has been associated with a single case of STARI (James et al. 2001), but was not detected in a series of cases in a later study (Wormser et al. 2005).

Lone star ticks naturally infected with *B. lonestari* have been collected throughout the southeastern United States (Burkot et al. 2001, James et al. 2001, Bacon et al. 2003, 2005, Stegall-Faulk et al. 2003, Stromdahl et al. 2003), 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* by PCR (Moore et al. 2003), and experimentally, WTD are susceptible to *B. lonestari* infection (Moyer et al. 2006). However, some WTD that were experimentally exposed, either by needle inoculation or by tick transmission, developed an infection characterized by a short detectable spirochetemia and 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 (Luttrell et al. 1994). Naturally exposed deer can have high antibody prevalence rates in northern states, indicating frequent exposure to *B. burgdorferi* (Magnarelli et al. 1986, 1995, 1999, Gill et al. 1994, Gallivan et al. 1998); however, some of these surveys utilized assays that can cross-react with related organisms.

Because WTD are suspected natural reservoirs of *B. lonestari*, the objective of the current study was to determine the distribution of antibodies reactive to *B. lonestari* in WTD populations from various locations in the eastern United States. Although experimental infections of deer suggest that antibody titers rapidly decrease after a single exposure, we hypothesized that antibodies to *B. lonestari* would be detected in wild WTD, predominantly due to frequent reexposure of deer to ticks harboring the agent that would boost the immune response to *B. lonestari*. We also tested WTD for antibodies to *B. burgdorferi* because these pathogens overlap in some eastern regions and because antibodies reactive to *B. burgdorferi* could cross-react with the *B. lonestari* antigen used in our IFA assay. Because *B. lonestari* is transmitted by lone star ticks, the same tick species that transmits *Ehrlichia chaffeensis*, 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 highly endemic.

Materials and Methods

Sample collection

Most serum samples used in this project were collected from a serum bank comprised of random hunter-killed WTD samples taken between 1994 and 2006 for various projects

performed by the Southeastern Cooperative Wildlife Disease Study (SCWDS), College of Veterinary Medicine, University of Georgia, Athens, Georgia. Samples were chosen from the serum bank based on the availability of samples. When possible, the most recent samples from a county and counties that had the highest number of samples available for testing were selected for inclusion. To increase the geographic scope of the study, additional serum samples from WTD were collected in Indiana, Minnesota, Pennsylvania, and Vermont.

Whole-blood samples collected from the thoracic and/or abdominal cavity of hunter-killed WTD, or from postmortem jugular venipuncture, were placed into 50 mL tubes (Corning, Lowell, MA). Blood was allowed to clot at room temperature for 10–30 min and then stored at 4°C until centrifugation at 3000 rpm for 8 min within 2–12 h of collection. Serum was placed into storage microtubes (Starstedt Ag, Nümbrecht, Germany) and stored in a –20°C freezer until serological testing.

Serologic testing

An indirect immunofluorescent antibody 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 bright green fluorescing spirochetes, while negative samples lacked any detectable fluorescence. Indeterminate samples (samples showing light fluorescence) were retested, and if they were again characterized as indeterminate, the sample was classified as negative.

To detect *B. burgdorferi*-specific antibodies, samples were tested using the SNAP® 4Dx® test (IDEXX Laboratories, Westbrook, ME) following the manufacturer's instructions. The SNAP platform assay has been used to detect *B. burgdorferi* antibodies in dogs (Duncan et al. 2004, Carlos et al. 2007, Yabsley et al. 2008), cats (Levy et al. 2003), horses (Chandrashekar et al. 2008, Johnson et al. 2008), and rabbits (Yabsley unpublished data).

Serology controls

Positive control sera for IFA assays were collected from pen-raised WTD fawns that were hyperimmunized with *B. burgdorferi* antigens (Mahnke et al. 1993). Our testing showed that these sera cross-reacted with *B. lonestari* antigens. Negative control sera for both the IFA and SNAP assays were collected from 3-week-old fawns raised in isolation that have consistently been negative for antibodies to *Borrelia* and other tick-borne pathogens (*Ehrlichia* and *Anaplasma* spp.). The ability of the SNAP 4Dx test to detect anti-*B. burgdorferi* antibodies in WTD was confirmed using serum from experimentally infected WTD (Luttrell et al. 1994). To ensure specificity of the SNAP 4Dx test for *B. burgdorferi*, sera from WTD experimentally infected with *B. lonestari* (Moyer et al. 2006) were tested with the SNAP 4Dx test and found to be negative (data not shown).

Data analyses

For analysis, states were divided into two regions: a southern region including Alabama, Arkansas, Florida, Georgia, Kentucky, Louisiana, Missouri, Mississippi, North Carolina, South Carolina, Tennessee, Texas, and Virginia, and a northern

TABLE 1. PREVALENCE OF ANTIBODIES REACTIVE WITH *BORRELIA LONESTARI* AND *BORRELIA BURGDORFERI* AMONG WHITE-TAILED DEER (*ODOCOILEUS VIRGINIANUS*) FROM 137 COUNTIES IN 21 STATES

State	Collection year(s)	No. of counties tested	No. IFA positive and SNAP negative/no. tested (%)	No. SNAP® 4Dx® 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)
Mississippi	2002	4	5/18 (28)	0/18 (0)
Missouri	1994–2002	7	5/38 (13)	1/38 (3)
New Jersey	2006	4	1/33 (3)	17/33 (52)
North Carolina	2000–2002	6	12/24 (50)	0/24 (0)
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)
Vermont	2008	9	NT	2/9 (22)
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)	73/723 (10)

IFA, indirect immunofluorescent antibody assay; NT, these samples were not tested by IFA.

region including Indiana, Kansas, Maryland, Minnesota, New Jersey, Pennsylvania, Vermont, and West Virginia. To facilitate graphic presentation, serologic data 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 seroprevalence for a population was calculated for populations with at least one positive WTD. Using the mean seroprevalence from IFA-positive populations (35.1%) and the mean seroprevalence from SNAP-positive populations (41.0%), we performed the *post hoc* analyses, and the analyses indicated that testing 5–7 WTD per population would result in detecting at least one seropositive animal with 85–95% confidence (Thrusfield 2005). Therefore, only those counties/parishes with ≥ 5 samples were included for assigning negative results.

Chi-square analysis ($p=0.05$) was used to determine if differences in seroprevalence existed between age classes and sexes. Deer 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 determined if there was an association between seropositive county status and the presence of ticks. Data documenting the presence of *A. americanum* was collected during previous studies and routine deer necropsies conducted by SCWDS (Lockhart et al. 1996, Yabsley et al. 2003). Because the majority of these necropsies were conducted in the summer and early fall, *Ixodes scapularis* was rarely detected on WTD populations; therefore, we used an *I. scapularis* distribution map created by Dennis et al. (1998) to compare to seropositive populations. For both tick species it is important to note that the lack of reporting tick presence does not indicate a lack of tick presence in those areas.

Results

Serologic testing

A total of 714 WTD serum samples from 136 counties (average of 5.25 samples/county or parish, range 1–21) in 21 states were tested by IFA (Table 1; Fig. 1). Antibodies reactive with *B. lonestari* were detected in 107 (15%) WTD. The mean seroprevalence in all of the populations with at least one positive WTD was 35.1% (SD = 27.6%, range = 5–100%). There was a significant difference in the overall seroprevalence between southern and northern regions (52.2% and 32.6%, respectively; $\chi^2 = 4.72$, $p = 0.03$). The presence of antibodies reactive with *B. lonestari* was associated with the presence of antibodies reactive with *E. chaffeensis* (36 of 49 [73.5%] counties were seropositive for both agents) (*E. chaffeensis* data derived from Yabsley et al. 2003).

Using the SNAP 4Dx assay, we detected antibodies to *B. burgdorferi* in 73 of 723 (10.1%) WTD (Table 1). The mean seroprevalence in seropositive populations was 41% (SD = 30.2%, range = 5–100%). Significantly more seropositive populations were detected in the north (47.8%) compared with the south (16.7%) (Fig. 1) ($\chi^2 = 14.92$, $p < 0.01$). Only 41 of the 73 samples that tested 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 sex effects on prevalence

Of the 507 WTD for which age data were available, 106 (20.9%) had antibodies reactive to *B. lonestari* by IFA (mean age, 2.7 years) and 30 (5.9%) were seropositive for

FIG. 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 labeled with ● were determined to be positive for *B. burgdorferi* because deer were SNAP and IFA positive. Counties labeled with ○ were classified as positive for *B. burgdorferi* because deer were SNAP positive and IFA negative. Counties labeled with ▲ were classified as positive for *B. lonestari* because deer were positive only for antibodies reactive to *B. lonestari* antigen (and were all SNAP negative). Counties labeled with △ were negative for both *Borrelia* species by both assays (and had a minimum of five deer/population tested).

B. burgdorferi by SNAP (mean age, 2.9 years) (Fig. 2). No significant age differences were detected with antibodies reactive to *B. lonestari* ($\chi^2 = 7.71$, $p = 0.0173$), but significantly more deer in the 0.76–1.5, 1.6–2.5, and 2.6–3.5 age groups were seropositive for *B. burgdorferi* compared with younger and older deer groups ($p \leq 0.0001$). Sex data were available for 521 WTD, of which 217 were male and 304 were female. There was no difference in seroprevalence between sexes for *B. lonestari* (16.1% in males vs. 14.8% in females, $\chi^2 = 0.171$, $p = 0.68$) or *B. burgdorferi* (7.4% in males vs. 5.6% in females, $\chi^2 = 0.677$, $p = 0.41$).

Tick presence–agent associations

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 (27/38 [71.1%]) of the *B. burgdorferi* seropositive populations that had

corresponding tick data contained WTD that were infested with *I. scapularis*.

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 *B. burgdorferi* as well as other *Borrelia* spp. and that the distribution of this exposure differs regionally. However, there are areas where overlap in distribution does occur. Although we believe that the majority of deer with antibodies reactive with *B. lonestari* were exposed to *B. lonestari*, we cannot rule out that another related organism caused cross-reactive reactions. No differences were noted between seroprevalence and age and sex for antibodies reactive with *B. lonestari*, which correspond with data from *E. chaffeensis* and *Anaplasma phagocytophilum* in WTD (Yabsley et al. 2003,

FIG. 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.

Dugan et al. 2006). Finally, the presence of *I. scapularis* and *A. americanum* was 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 the respective agents (Burgdorfer et al. 1982, Magnarelli et al. 1986, Kirkland et al. 1997, James et al. 2001).

The distribution of antibodies reactive with *B. lonestari* among WTD corresponds with what little is known about the natural history of this organism. The majority of the *B. lonestari* reactive samples came from counties with known established populations of *A. americanum*, the only known competent vector for *B. lonestari*. Also, the presence of *B. lonestari* reactive samples was associated with the presence of *E. chaffeensis*. Although antibodies reactive to *B. lonestari* were most commonly detected in the southeastern United States, several northern counties (in Indiana, Maryland, New Jersey, and West Virginia) had deer with antibodies reactive to *B. lonestari* that were SNAP 4Dx negative. These populations

are all within the range of *A. americanum*, which has considerably expanded into northern states in recent years (Paddock and Yabsley 2007, Ball State University 2008). Antibodies reactive to *B. lonestari* were detected in a limited number of counties outside of the known distribution of *A. americanum* (western Kansas and West Virginia), but this species may be present in areas not previously documented. Distribution studies in these areas may be warranted to determine whether and to what extent *A. americanum* has expanded into these regions. The remaining *B. lonestari* seropositive northern deer from Minnesota and Pennsylvania were also positive for *B. burgdorferi*, indicating that these seroreactors were likely caused by cross-reaction with *B. burgdorferi*. WTD naturally infected with *B. lonestari* also have been detected in five populations in the Southeast (Moore et al. 2003); in the current study, antibodies to *B. lonestari* were detected in three of these four populations for which serum was available for testing. Further, *B. lonestari* has been detected in *A. americanum* from many of the southeastern and mid-Atlantic states, including Alabama, Arkansas, Delaware, Florida, Georgia, Kentucky, Maryland, New Jersey, North Carolina, South Carolina, Tennessee, and Virginia (Burkot et al. 2001, Stegall-Faulk et al. 2003, Stromdahl et al. 2003, Clark 2004, Varela et al. 2004, Taft et al. 2005, Mixson et al. 2006, Schulze et al. 2006).

Based on previous studies with *E. chaffeensis*, we expected a higher prevalence of antibodies reactive to *B. lonestari* in WTD populations from the southern states (Dawson et al. 1994, Lockhart et al. 1996, Yabsley et al. 2003, Paddock and Yabsley 2007). However, these data support results of recent experimental studies showing that WTD have a limited serologic reaction after infection with *B. lonestari* (Moyer et al. 2006, Varela-Stokes 2007). Because of this low prevalence, large numbers of WTD need to be tested in individual populations to ensure a proper classification for *B. lonestari* exposure. Interestingly, the prevalence of both *E. chaffeensis* and *B. lonestari* in *A. americanum* was similar (Varela et al. 2004, Mixson et al. 2006, Stromdahl et al. 2008, Yabsley et al. unpublished data). Although WTD show a strong immune response to *E. chaffeensis* (Varela et al. 2003, 2005), WTD experimentally inoculated with *B. lonestari* only develop a low titer, if at all, and for only a short period of time (Moyer et al. 2006, Varela-Stokes 2007). In one experimental inoculation study, antibody titers peaked at 1024 in one of two WTD, and both WTD were seronegative by 6-week postinoculation (Moyer et al. 2006). In another study, only one of three WTD exposed to *B. lonestari* by infected ticks seroconverted with a maximum titer of 128, and this single WTD was seronegative by 5-week postexposure to ticks (Varela-Stokes 2007). Based on these experimental data, it was found that seroprevalence in many southern WTD populations was higher than 30%. It is likely 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 ecology of *B. burgdorferi* in the southern United States is poorly understood. Deer seropositive for *B. burgdorferi* were detected in numerous counties in this study with the majority of *B. burgdorferi* reactive samples being found in the northern states (up to 100% in some counties), which corresponds with areas of high Lyme disease endemicity in dogs and humans

(Duncan et al. 2004, Beall et al. 2008, Bowman et al. 2009). These data confirm several previous studies that have identified antibodies to *B. burgdorferi* in WTD from multiple states in both the northern and southern United States (Magnarelli et al. 1986, 2004, Mahnke et al. 1993, Gill et al. 1994). Also, the prevalence of *B. burgdorferi* detected in deer in the present study was similar to that reported in other studies of deer conducted in similar geographic regions (e.g., southern MN) (Magnarelli et al. 1986, 2004, Wolf et al. 2008). Although less common, seropositive WTD were identified in southern states including Arkansas, Florida, Georgia, Missouri, Tennessee, and Virginia. Although few, if any, autochthonous southern human and canine Lyme disease cases are documented (Felz et al. 1999, Duncan et al. 2004, Bowman et al. 2009), *B. burgdorferi* DNA and/or antibodies have been detected throughout the Southeast in ticks, small mammals, deer, and reptiles (Magnarelli et al. 1992, 2004, Clark 2004). Conversely, in 2005 there was an average of 1414 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). The increased prevalence of *B. burgdorferi* and other *Borrelia* spp. reported in rodents in the South likely reflects the high diversity and density of other competent *Ixodes* species that more often feed on rodents compared with dogs, deer, and humans (Clark et al. 2001, Oliver et al. 2003).

The IFA test is generally a very sensitive assay for detecting anti-*Borrelia* spp. antibodies in many species, although the sensitivity and specificity of this assay on WTD is unknown. 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 coinfecting with *B. lonestari*, because those samples would have likely tested positive using the IFA test had they been coinfecting with *B. lonestari*.

Currently, the causative agent, if any, of STARI is not known. One candidate agent that has been suggested is *B. lonestari* because it was detected in the culture supernatant of a skin biopsy taken from a patient with an attached *A. americanum* (James et al. 2001). Although the cause of STARI is unknown, the condition is commonly diagnosed in the southeastern United States, and many of these patients have tested negative for *B. burgdorferi* (Felz et al. 1999, Wormser et al. 2005, Philipp et al. 2006) and *B. lonestari* (Wormser et al. 2005). Clearly, there is a need for additional research on STARI patients to determine if any particular agent is responsible for this disease.

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 in the mid-Atlantic and upper Midwestern states. These data support the need for enhanced surveillance for these tick species, espe-

cially in regions at the edge of their ranges to detect expansion and increased risk of human disease.

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Disclosure Statement

Ramaswamy Chandrashekar and Tom O'Connor are affiliates of IDEXX Laboratories, Inc. No competing financial interests exist for other authors.

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