Borrelia burgdorferi Not Detected in Widespread *Ixodes scapularis* (Acari: Ixodidae) Collected from White-Tailed Deer in Tennessee

Author(s): M. E. Rosen, S. A. Hamer, R. R. Gerhardt, C. J. Jones, L. I. Muller, M. C. Scott, and G. J. Hickling
Source: Journal of Medical Entomology, 49(6):1473-1480. 2012.
Published By: Entomological Society of America
URL: http://www.bioone.org/doi/full/10.1603/ME11255

Borrelia burgdorferi Not Detected in Widespread *Ixodes scapularis* (Acari: Ixodidae) Collected From White-Tailed Deer in Tennessee

M. E. ROSEN,[1,2] S. A. HAMER,[3,4] R. R. GERHARDT,[5] C. J. JONES,[5,6] L. I. MULLER,[1] M. C. SCOTT,[1] AND G. J. HICKLING[1]

J. Med. Entomol. 49(6): 1473–1480 (2012); DOI: http://dx.doi.org/10.1603/ME11255

ABSTRACT Lyme disease (LD), caused by the bacterium *Borrelia burgdorferi* and transmitted in the eastern United States by blacklegged ticks, *Ixodes scapularis* Say, is classified as nonendemic in Tennessee and surrounding states in the Southeast. Low incidence of LD in these states has been attributed, in part, to vector ticks being scarce or absent; however, tick survey data for many counties are incomplete or out of date. To improve our knowledge of the distribution, abundance, and *Borrelia* spp. prevalence of *I. scapularis*, we collected ticks from 1,018 hunter-harvested white-tailed deer (*Odocoileus virginianus* (Zimmerman)) from 71 of 95 Tennessee counties in fall 2007 and 2008. In total, 160 deer (15.7%) from 35 counties were infested with adult *I. scapularis*; 30 of these counties were new distributional records for this tick. The mean number of *I. scapularis* collected per infested deer was 5.4 ± 0.6 SE. Of the 883 *I. scapularis* we removed from deer, none were positive for *B. burgdorferi* and one tested positive for *B. miyamotoi*. Deer are not reservoir hosts for *B. burgdorferi*; nevertheless, past surveys in northern LD-endemic states have readily detected *B. burgdoreferi* in ticks collected from deer. We conclude that *I. scapularis* is far more widespread in Tennessee than previously reported. The absence of detectable *B. burgdorferi* infection among these ticks suggests that the LD risk posed by *I. scapularis* in the surveyed areas of Tennessee is much lower than in LD-endemic areas of the Northeast and upper Midwest.

KEY WORDS Lyme disease, *Ixodes scapularis*, *Borrelia burgdorferi*, *Odocoileus virginianus*, Tennessee

Lyme disease (LD), caused by the bacterium *Borrelia burgdorferi*, is the most common vector-borne disease of humans in the United States, with over 20,000 new cases reported each year (Bacon 2008). LD is endemic in parts of the Midwest and Northeast, but is classified as nonendemic in the Southeast (Centers for Disease Control and Prevention [CDC] 2008). For the period 2000–2009, Tennessee (population 5.6 million; 2000 Census) reported an average of only 19.8 LD cases annually to the CDC (CDC 2012), with most of these reports recorded by the Tennessee Department of Health as 'probable' rather than 'confirmed' (A. Moncayo, personal communication).

The blacklegged tick (*Ixodes scapularis* Say) is the vector of *B. burgdorferi* to humans in the eastern United States (Piesman 2002). Before this study, *I. scapularis* had been reported from ten Tennessee counties based on opportunistic tick collections (Fig. 1; Dennis et al. 1998, Durden and Kollars 1992). More recent efforts to map the eastern United States distribution of *I. scapularis* (Diuk–Wasser et al. 2010) did not survey sites in Tennessee, so the distribution of *I. scapularis* throughout the state has been uncertain.

The primary host for adult *I. scapularis* is the white-tailed deer (Wilson et al. 1990). Systematic collection of ticks from hunter-harvested deer can provide useful data on the regional distribution and abundance of this tick (e.g., Cortinas and Kitron 2006, Keefe et al. 2009). *I. scapularis* removed from deer also can be tested for the presence of *B. burgdorferi* (Magnarelli et al. 1986). *Borrelia* spp. prevalence estimated in this way tends to underestimate the prevalence seen in questing ticks sampled from the same areas (e.g., Telford et al. 1998) because complement-mediated immune responses of the white-tailed deer progressively lyse spirochetes in feeding ticks (Kurtenbach et al. 1998). Nevertheless, this approach has proven useful for determining the geographic extent of *Borrelia* spp. in *I. scapularis* populations (e.g., Magnarelli et al. 1986, 1995; Gill et al. 1993; Keefe et al. 2009).

Reported cases of several tick-borne diseases (including spotted fever group rickettsioses and human ehrlichioses infection) are on the rise in Tennessee

[1] Center for Wildlife Health, University of Tennessee Institute of Agriculture, Knoxville, TN 37996.
[2] Corresponding author: Wildlife Disease Laboratory, Michigan Department of Natural Resources, 4125 Beaumont Road, Lansing, MI 48910 (e-mail: rosenm@michigan.gov).
[3] Department of Fisheries and Wildlife, Michigan State University, East Lansing, MI 48824.
[4] Current Affiliation: Veterinary Integrative Bioscience Department, Texas A&M University, College Station, TX 77802.
[5] Department of Entomology and Plant Pathology, University of Tennessee Institute of Agriculture, Knoxville, TN 37996.
[6] Deceased.

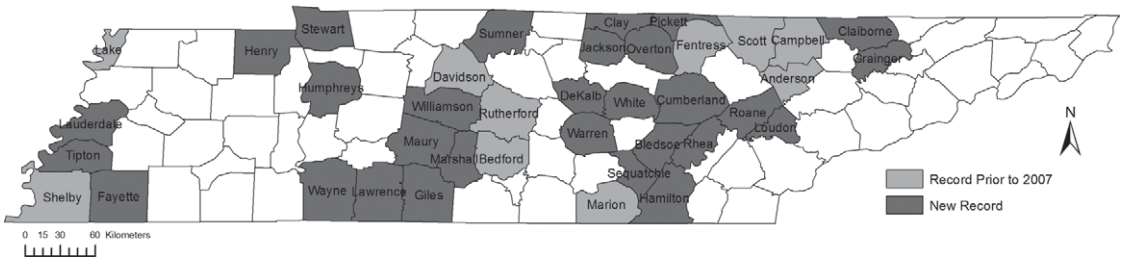

Fig. 1. Current distribution of *I. scapularis* by Tennessee county. Prior published reports listed *I. scapularis* as present in only Anderson, Bedford, Campbell, Fentress, Davidson, Lake, Marion, Rutherford, Scott, and Shelby Counties (Dennis et al. 1998, Durden and Kollars 1992).

(Tennessee Department of Health 2009). This pattern, evident throughout the Southeast, has been linked to increasing abundance and geographic range of several tick species and their hosts (particularly deer; Paddock and Yabsley 2007). Whether LD is similarly on the rise in the Southeast remains contentious. To date, there have been no published reports of *B. burgdorferi* being detected in *I. scapularis* in Tennessee. Our goal in this study was to examine blacklegged ticks collected from hunter-harvested deer in Tennessee to clarify their current distribution, relative abundance, and LD-pathogen status.

Materials and Methods

Sample Collection. Ticks were removed from hunter-harvested deer at 47 Tennessee Wildlife Resource Agency (TWRA) check stations in November of 2007 and 2008 to quantify the presence of *I. scapularis* in the counties from which the deer were harvested. "County" was used as the geographic unit for this study because tick distributions have previously been reported and mapped by county (e.g., Dennis et al. 1998), and because tick presence in some other states is known to correlate with LD incidence reported at the county level (e.g., Kitron and Kazmierczak 1997).

Check stations were selected to maximize coverage across the state. Before searching for ticks, volunteers and TWRA employees watched a training video explaining the purpose of the research and the sampling protocol. They were instructed to search for ticks on each deer's head and neck to just below the scapula, on both sides of the animal. All ticks from one deer were placed in a vial with 70% ethanol. Data on county-of-harvest, sex, and age (determined by TWRA biologists) were recorded for each deer.

All ticks were brought to the University of Tennessee's Center for Wildlife Health (CWH) laboratory and identified to species using dichotomous keys (Sonenshine 1979). Voucher specimens were submitted to the U.S. National Tick Collection in Statesboro, GA. Body length (base of the basis capitulum to the tip of the abdomen) and scutum width (at the widest point) of intact ticks were measured (nearest 0.01 mm), and an engorgement index (EI) was calculated as the ratio of body length to scutum width (after Falco et al. 1996).

***Borrelia* spp. Assay.** Total DNA was extracted from each tick using a DNeasy Blood and Tissue Kit (Qiagen Inc., Valencia, CA). A polymerase chain reaction (PCR) amplifying the mitochondrial 16S rRNA gene (Black and Piesman 1994) was run on all samples to verify DNA extraction. If this amplification was not successful the original extraction product was diluted 1:10 and rerun for both *Borrelia* spp. and mitochondrial DNA. This dilution step was included because blood from the tick's meal can inhibit PCR (Schwartz et al. 1997). All samples were then tested at the CWH laboratory using a *Borrelia* genus-specific nested PCR targeting the 16S-23S rRNA intergenic spacer (IGS) locus (Bunikis et al. 2004a). These IGS primers will detect both *B. burdorferi* senso stricto and *B. burdorferi* senso lato (Bunikis et al. 2004a). Water served as the negative control and DNA from *I. scapularis* ticks infected with strain B31 *B. burgdorferi* (Centers for Disease Control and Prevention, Fort Collins, CO) served as the positive control. PCR amplicons were visualized by gel electrophoresis. Positive samples were extracted from the gel, purified using a Gel DNA Recovery Kit (Zymoclean, Orange, CA) according to manufacturer's instructions, and submitted for sequencing at the University of Tennessee's Core Sequencing Facility. Sequences were compared with those published in GenBank (http://blast.ncbi.nlm.nih.gov/Blast.cgi) to facilitate species and strain identification.

For independent verification of sample-infection status, a quantitative PCR targeting the 16S rRNA gene (Tsao et al. 2004) was run at Michigan State University's Insect Microbiology Laboratory on the DNA from all *I. scapularis* collected in 2007.

Data Analysis. There was minimal overlap of check-station locations in the 2nd year, so 2007 and 2008 data were pooled for analysis and no attempt was made to investigate change between years. The proportion and percent of deer infested with *I. scapularis* was visualized on a Tennessee county map (ESRI, Redlands, CA). Differences in the proportion of deer infested with *I. scapularis* among TWRA management regions were assessed using a Chi-square test of association. Differences among these regions in the mean number of adult *I. scapularis* detected on infested deer were assessed using a Kruskal–Wallis nonparametric analysis of variance (ANOVA). Tick engorgement may

Table 1. Numbers and percentages of hunter-harvested deer infested with ticks in each Tennessee Wildlife Management Agency (TWRA) region in Nov. 2007 and Nov. 2008 (years pooled)

TWRA region	No. of deer surveyed	All tick species		*I. scapularis*		*D. albipictus*		*A. americanum*	
		Infested deer	%	Infested deer	%	Infested deer	%	Infested deer	%
West Tennessee	177	63	35.6	13	7.3	50	28.2	9	5.1
Middle Tennessee	81	49	60.5	36	44.4	22	27.2	13	16.0
Cumberland Plateau	214	154	72.0	70	32.7	110	51.4	11	5.1
East Tennessee	371	41	11.1	14	3.8	30	8.1	3	0.8
Oak Ridge WMA	175	157	89.7	27	15.4	156	89.1	12	6.9
Total	1,018	464	45.6	160	15.7	368	36.1	48	4.7

Region boundaries are shown in Figure 2; Oak Ridge Wildlife Management Area (OR WMA) spans two TWRA regions and is reported separately.

decrease the likelihood of detecting *Borrelia* spp. through lysis of spirochetes and inhibition of PCR, so, we used a chi-test of association to determine whether DNA extraction/PCR outcome was influenced by the EI of the tick.

Results

Tick Infestation. In total, 1,018 deer from 71 Tennessee counties were inspected; ticks of any species were collected from 464 (45.6%) of these deer. In total, 4,237 ticks of three species were collected from deer, with the most common being *Dermacentor albipictus* (Packard) ($n = 3{,}296$) and least common being *Amblyomma americanum* (L.) ($n = 70$; Table 1). The prevalence of tick infestation in deer was highest in the Cumberland Plateau TWRA Management region and lowest in East Tennessee region (Fig. 2, Table 1; $\chi^2 = 391.2$, 4 df, $P < 0.0001$).

Adult *I. scapularis* ($n = 871$) were collected from 15.7% of deer inspected. Deer were most frequently infested with *I. scapularis* in Middle Tennessee (44.4% of deer), and least frequently in East Tennessee (3.8%; Table 1); these differences in *I. scapularis* infestation among regions were statistically significant ($\chi^2 = 147.9$; 4 df; $P < 0.0001$).

I. scapularis were found on deer harvested from 35 of the 71 counties from which deer were sampled, with infestation prevalence ranging from 2–100% (Fig. 2). *I. scapularis* was not detected in some well-sampled counties (e.g., most counties in East Tennessee), but was found in some sparsely sampled counties (e.g., in some Middle Tennessee and Cumberland Plateau counties; Fig. 2).

The mean number of *I. scapularis* removed per infested deer ranged from <2 per deer in East Tennessee to 11 in Middle Tennessee (Table 2); this regional variation in burden was significant (Kruskal–Wallis nonparametric ANOVA; H = 42.4; 4 df; $P < 0.0001$).

***Borrelia* spp. Infection.** PCR was performed on 883 *I. scapularis* (502 females, 381 males; this sample comprised the 871 *I. scapularis* listed in Table 2 plus an additional 12 *I. scapularis* for which no county location was recorded). Of the 833 ticks that were sufficiently intact for an engorgement index to be calculated, 40 (4.8%) required 1:10 dilution of the original extract to produce PCR amplication. There was no indication that a tick's EI (low = <2; high ≥5 EI) influenced whether this dilution step was required ($\chi^2 = 0.80$; 2 df; $P = 0.37$). All males and 124 females (i.e., 58% of total ticks tested) had an engorgement index of <3.

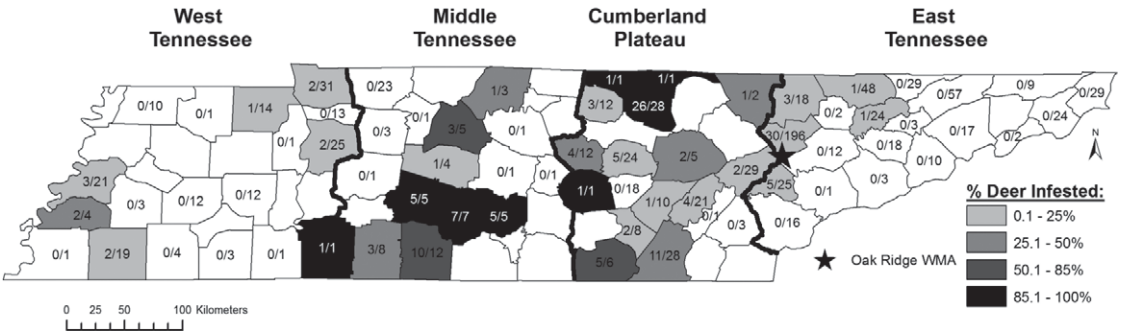

Fig. 2. Deer sampling effort and *Ixodes scapularis* infestation by county (thin lines) and Tennessee Wildlife Resource Agency regions (thick lines). The numerator in each county is the number of deer found infested with *I. scapularis*, the denominator is the number of deer checked. The map is based on 1,018 deer harvested by hunters in November 2007 and November 2008 (years pooled; median number of deer per checked county = 8, range 1–196). There were nine deer where only regional location was assigned that are not mapped here: West Tennessee: two deer (one with *I. scapularis*); Cumberland Plateau: four deer (one with *I. scapularis*); East Tennessee: three deer (one with *I. scapularis*). For the purposes of this map, Oak Ridge WMA is considered part of Anderson County.

Table 2. Numbers of *I. scapularis* (mean ± SE) collected from infested deer brought by hunters to Tennessee check-stations in Nov. 2007 and Nov. 2008 (years pooled, noninfested deer excluded)

TWRA region	Infested deer	Female *I. scapularis*	Male *I. scapularis*	Total Adult *I. scapularis*
West Tennessee	13	13 (1.0 ± 0.2)	15 (1.2 ± 0.4)	28 (2.2 ± 0.5)
Middle Tennessee	36	211 (5.9 ± 0.9)	176 (4.9 ± 0.8)	387 (10.8 ± 1.6)
Cumberland Plateau	70	217 (3.1 ± 0.4)	154 (2.2 ± 0.4)	371 (5.3 ± 0.8)
East Tennessee	14	18 (1.3 ± 0.3)	8 (0.6 ± 0.2)	26 (1.9 ± 0.4)
Oak Ridge WMA	27	33 (1.2 ± 0.3)	26 (1.0 ± 0.2)	59 (2.2 ± 0.4)
Total	160	492 (3.1 ± 0.3)	379 (2.4 ± 0.3)	871 (5.4 ± 0.6)

Oak Ridge Wildlife Management Area (WMA) spans two TWRA regions and is reported separately. No immature *I. scapularis* were found on the sampled deer.

B. burgdorferi was not detected in any of the *I. scapularis* tested (i.e., 883 at full strength, plus 40 retested at 1:10 dilution) using the *Borrelia* species IGS PCR. Furthermore, no *B. burgdorferi* was detected in the subset of 431 samples retested using a *B. burgdorferi*-specific quantitative PCR at the independent Michigan State University laboratory. Using IGS PCR, one adult female produced a band at ≈500 bp (rather than the ≈1,000 bp expected for *B. burgdorferi*). This template was sequenced at the IGS locus, and matched *B. miyamotoi* with 100% sequence homology (NCBI accession: AY363706).

Discussion

I. scapularis is far more widely distributed in Tennessee than has been reported previously (Fig. 1). Our mapping effort, while based on the greatest geographic coverage and largest sample sizes to date in this state, did not sample all counties and so remains an underestimate of the state-wide distribution of this tick. Earlier distribution maps (Dennis et al. 1998, Durden and Kollars 1992) were derived from sparse, passively collected, nonstandardized data, so the expanded distribution shown in Fig. 1 reflects, in part, our improved surveillance effort. Nevertheless, we consider it likely that *I. scapularis* distribution has expanded in recent years in response to changes in land use, increasing deer numbers, and perhaps climate change. For example, one of us (R.G.) has surveyed for ticks in Cumberland County since the early 1990s, and has never collected *I. scapularis.* In the process of this study (2008), we collected *I. scapularis* from deer harvested close to R.G.'s research area. In addition, in the spring and fall of 2009, Harmon et al. (2011) obtained the first collections of questing *I. scapularis* from vegetation in that same area. Similarly, a study of ticks on white-tailed deer from Steward County found only *A. americanum* on 97 deer checked in 1985 and 1986 (Bloemer et al. 1988), whereas in 2008 we found 2 of 31 deer from that county to be harboring *I. scapularis.*

We identified significant variation in abundance of *I. scapularis* in different parts of Tennessee. This is a strong indication that *I. scapularis* distribution is heterogeneous across the state at regional and county scales. Nationally, it has been shown that there are significant associations of *I. scapularis* with certain habitat, landform, and climate patterns (e.g., Brownstein et al. 2005, Diuk–Wasser et al. 2010). One useful source of information on habitat variation in Tennessee is the ecoregion classification created by the U.S. Environmental Protection Agency to aid state agencies in management, research, and monitoring of ecosystems and ecosystem components. These ecoregions are based on the abiotic and biotic factors that influence ecosystem characters—including geology, physiography, vegetation, climate soils, land use, wildlife, and hydrology (Griffith et al. 2009). A comparison of our deer infestation prevalence data with a map of Tennessee's ecoregions indicates that *I. scapularis* is most abundant on deer from the low elevation Interior Plains ecoregion of Middle Tennessee. The hills and plains in this ecoregion are composed of a diverse mixture of sandstone, siltstone, and shale associated with oak-hickory (*Quercus* spp. and *Carya* spp.) forests with some areas of bluestem (*Schizachyrium scoparium* and *Andropogon* spp.) prairie and cedar glades (Griffith et al. 2009). Conversely, *I. scapularis* were least abundant in the Blue Ridge Mountains ecoregion, and the eastern parts of the Ridge and Valley ecoregion, suggesting higher-elevations habitats are unfavorable for this tick (as has been reported elsewhere; Jouda et al. 2004). *I. scapularis* was also largely absent from the Southeastern Plains ecoregion, where agricultural land use dominates. Future in-depth analysis of *I. scapularis*' ecological associations in Tennessee could help guide studies of *B. burgdorferi* and other associated pathogens such as *Anaplasma phagocytophilum* and *Babesia* spp.

On average, 15.7% of the harvested Tennessee deer were infested with *I. scapularis* in the fall. This is a low level of infestation relative to most other published surveys of hunter-harvested deer, in both endemic and nonendemic areas (e.g., Amerasinghe et al. 1993; Cortinas and Kitron 2006; Gill et al. 1993; Keefe at al. 2009; Kitron et al. 1992; Loken et al. 1985; Magnarelli et al. 1986, 1995; Riehle and Paskewitz 1996). In these cited studies, deer infestation averaged 57% in five LD-endemic states and 35% in three nonendemic states.

We collected an average of 5.4 *I. scapularis* per infested deer from across the state. This was similar to burdens reported both from endemic states and from other nonendemic states (these averaged 5.5 and 6.2 ticks per deer, respectively; Amerasinghe et al. 1993;

Cortinas and Kitron 2006; Gill et al. 1993; Keefe et al. 2009; Kitron et al. 1992; Magnarelli et al. 1986, 1995; Riehle and Paskewitz 1996). Lack of variation in the intensity of infestation may reflect low search efficiency given the time pressure when examining deer for ticks at busy check stations. It may also be that low-density tick populations are aggregated, so that deer that encounter ticks tend to pick up several at a time, whereas deer in less favorable habitats encounter no ticks at all.

D. albipictus was common on deer throughout Tennessee, and in other Midwestern states (e.g., Cortinas and Kitron 2006). This is a one-host tick; after the larvae attach to a host, all subsequent life stages are completed on that same host. Consequently, it has no ecological capacity to transmit pathogens to another host species, including humans. *A. americanum* is a very abundant tick in Tennessee, but was not commonly found on deer in our fall surveys; this is not surprising as its life stages are mostly inactive in November (Bloemer et al. 1988, Marsland 1997, Kollars et al. 2000, Goddard 2007). *A. americanum* is an incompetent vector for *B. burgdorferi* as its saliva lyses the spirochetes (Piesman and Happ 1997, Ledin et al. 2005).

We know of no previous reports of *B. burgdorferi* from *I. scapularis* in Tennessee, and we did not detect this spirochete in the 883 ticks tested in this study. This is in marked contrast to similar deer-check surveys undertaken in LD-endemic states, all of which detected *Borrelia*-positive ticks (Amerasinghe et al. 1993; Gill et al. 1993; Magnarelli et al. 1986, 1995). The average *Borrelia* spp. prevalence in these surveys in other states (14%) was lower than is typically seen in questing adult ticks in similar endemic areas (20–80%; Qiu et al. 2002, Piesman et al. 1999), which is to be expected, given complement-mediated lysis of *B. burgdorferi* in ticks feeding on deer (Telford et al. 1988). Nevertheless, *B. burgdorferi* is readily detected by PCR among male ticks (that do not engorge) and in female ticks with low engorgement (EI $\leq$3; D. Gaines, personal communication); 58% of the ticks we tested were in one of these two categories.

The few equivalent deer-check surveys undertaken in nonendemic states generally have reported low, but nonzero, prevalence of *Borrelia* spp. (Magnarelli et al. 1986, Kitron et al. 1992, Keefe at al. 2009), except for Goddard et al. (2003) who did not detect *Borrelia* spp. among ticks collected from a small number of deer in Mississippi. To our knowledge, this is the first large (>500) sample of adult *I. scapularis,* tested with species-specific molecular techniques, to report a zero prevalence of *B. burgdorferi.*

There are published reports of *B. burgdorferi* in ticks and hosts from Tennessee. In 2003, a skin biopsy taken from the erythema migrans rash of a human patient in Greene County tested positive for *B. burgdorferi* strain B31 (Haynes et al. 2005); however, the patient's travel history was not provided. Shariat et al. (2007) reported *B. burgdorferi* from 2 of 18 pooled samples of *D. albipictus* removed from white-tailed deer in Cheatham County. Similarly, Jordan et al. (2009) reported the pathogen from 14% of wild turkey and 17% of migratory waterfowl blood samples collected from a site in middle Tennessee and reported further detections from these species in ten surrounding counties. *A. americanum* was the most common tick collected in that study; no *I. scapularis* was observed. However, both Shariat et al. (2007) and Jordan et al. (2009) used a hybridization technique that did not provide sequence confirmation of *B. burgdorferi* presence. A similar survey 1 yr later detected high levels of *B. miyamotoi,* but zero prevalence of *B. burgdorferi,* in wild turkeys from similar habitats in eastern and central Tennessee (Scott et al. 2010).

Sequence-confirmed *B. burgdorferi* has been reported from one red wolf on the Tennessee/North Carolina border of Great Smoky Mountains National Park (Penrose et al. 2000). Other wolves in the same vicinity tested positive for *B. burgdorferi* antibodies but were PCR negative for the organism. ELISA-testing of canine serum (using LymeCHEK, San Diego, CA) of 159 dogs from Cumberland and Knox Counties in 1996 suggested that 14.5% had been exposed to *B. burgdorferi* (Marsland 1997). However, the specificity of the test is uncertain and the vaccination and travel histories of these dogs were incomplete. More recently, highly specific SNAP 3Dx and 4Dx testing for the *B. burgdorferi* C_6 peptide in 18,891 pet dogs in Tennessee produced 47 positive samples (a 0.02% prevalence), with the travel histories of the positive dogs again unknown. In comparison, SNAP testing of dogs in known LD-endemic areas typically produces seroprevalences >1.0% among dogs (Bowman et al. 2009). SNAP 4Dx tests of blood samples from 20 deer from three west Tennessee counties in 2001 identified one deer as positive for *B. burgdorferi,* and another three as positive for an unknown *Borrelia spp.* (Murdock et al. 2009). We conclude that, although *B. burgdorferi* may be present in Tennessee, its prevalence in wildlife hosts and human-biting ticks appears to be exceedingly low relative to LD-endemic states.

One *I. scapularis* from our collection tested positive for *B. miyamotoi.* Finding a *Borrelia* spp. other than *B. burgdorferi* in our tick sample emphasizes the importance of using species-specific pathogen tests. *B. miyamotoi* is closely related to the relapsing fever group of *Borrelia,* a separate group from the LD *Borrelia* (Bunikis et al. 2004b). In 2006, *B. miyamotoi* was detected in 15 of 36 (42%) adult *I. scapularis* collected opportunistically from white-tailed deer at three TWRA East Tennessee check stations (G. H., unpublished data). The prevalence of this *Borrelia* species in Tennessee ticks thus appears to be variable in both time and space, which warrants further study.

Implications for Human-Disease Risk. The reported LD-case rate in Tennessee is very low compared with endemic areas of the northeastern United States, and it has commonly been argued that this was because *I. scapularis* is absent from most of the state. Our findings indicate, however, that *I. scapularis* is widespread in Tennessee, albeit at low abundance and without detectable infection with the LD pathogen. This latter finding is noteworthy, as there have been

few published reports of well-established *I. scapularis* tick populations without concurrent *B. burgdorferi* infection. We know of only one such publication to date: Maggi et al. (2010) identified a population of *I. scapularis* in coastal North Carolina where none of 258 adult *I. scapularis* tested harbored *B. burdorferi*, although 63.2% of sympatric *I. affinis* were infected with *Borrelia* spp.

B. miyamotoi has recently been identified as a public health concern in Russia, where it has been associated with influenza-like illness, relapsing fever, and erythema migrans (Platonov et al. 2011). Russian strains of *B. miyamotoi* differ slightly from those in North America, and the eco-epidemiological settings differ also, so the zoonotic potential of *B. miyamotoi* in the southeastern United States remains uncertain.

At a national level, one approach taken to provide the public with information and advice regarding Lyme disease has been the creation of national LD risk maps (CDC 1999) and, more recently, a distribution map of *Borrelia*-infected nymphal ticks (that are considered the key life-stage for transmission to humans; Diuk–Wasser et al. 2012). As a consequence of random allocation of sampling effort in the latter study, almost no survey work was undertaken in Tennessee. Therefore, our study provides the most detailed information on *I. scapularis* distribution, relative abundance, and LD pathogen prevalence presently available for Tennessee. We conclude that *I. scapularis* is far more widespread in Tennessee than previously reported; however, the absence of detectable *B. burgdorferi* infection among these ticks indicates that the LD risk they pose in the areas of Tennessee surveyed is presently far lower than is the case in the LD-endemic areas of the Northeast and upper Midwest.

Acknowledgments

We thank the student volunteers who collected ticks: J. Harmon, E. Baker, R. Dutkowski, H. Edwards, N. Hiles, R. Johnson, A. Ohde, J. Pregel, D. Rice, L. Shankle, A. Wimbush, and the Student Chapters of the Wildlife Society at University of Tennessee Knoxville, University of Tennessee Martin and Tennessee Technological University. We thank J. Evans and his many check-station colleagues at TWRA. D. Paulsen assisted with tick identifications. J. Piesman and G. Dietrich at the Centers for Disease Control and Prevention provided *Borrelia*-positive controls. J. Sidge assisted with the diagnostic assays at Michigan State University. We gratefully thank Jean Tsao for her never-ending hours of guidance. This study was funded by the University of Tennessee Institute for Agriculture. G.J.H. received support from the NIMBioS, the National Institute of Mathematical and Biological Synthesis.

References Cited

Amerasinghe, F. P., N. L. Breisch, K. Neidhardt, B. Pagac, and T. W. Scott. 1993. Increasing density and *Borrelia burgdorferi* infection of deer-infesting *Ixodes dammini* (Acari: Ixodidae) in Maryland. J. Med. Entomol. 30: 858–864.

Bacon, R. M., K. J. Kugeler, K. S. Griffith, and P. S. Mead. 2008. Surveillance for Lyme disease—United States, 1992–2006. MMWR 56: 1–9.

Black, W. C., and J. Piesman. 1994. Phylogeny of hard- and soft-tick taxa (Acari: Ixodida) based on mitochondrial 16s rDNA sequences. Proc. Nat. Acad. Sci. U.S.A. 91: 10034–10038.

Bloemer, S. R., R. H. Zimmerman, and K. Fairbanks. 1988. Abundance, attachment sites, and density estimators of lone star ticks (Acari: Ixodidae) infesting white-tailed deer. J. Med. Entomol. 25: 295–300.

Bowman, D., S. E. Little, L. Lorentzen, J. Shields, M. P. Sullivan, and E. P. Carlin. 2009. Prevalence and geographic distribution of *Dirofilaria immitis, Borrelia burgdorferi, Ehrlichia canis,* and *Anaplasma phagocytophilum* in dogs in the United States: results of a national clinic-based serologic survey. Vet. Parasitol. 160: 138–148.

Brownstein, J. S., D. K. Skelly, T. R. Holford, and D. Fish. 2005. Forest fragmentation predicts local scale heterogeneity of Lyme disease risk. Oecologia 146: 469–475.

Bunikis, J., U. Garpmo, J. Tsao, J. Berglund, D. Fish, and A. G. Barbour. 2004a. Sequence typing reveals extensive strain diversity of the Lyme borreliosis agents *Borrelia burgdorferi* in North America and *Borrelia afzelii* in Europe. Microbiol.-Sgm. 150: 1741–1755.

Bunikis, J., J. Tsao, U. Garpmo, J. Berglund, D. Fish, and A. G. Barbour. 2004b. Typing of *Borrelia* relapsing fever group strains. Emerg. Infect. Dis. 10: 1661–1664.

(CDC) Centers for Disease Control and Prevention. 1999. Appendix methods used for creating a national Lyme disease risk map. MMWR 48: 21–24.

(CDC) Centers for Disease Control and Prevention. 2008. Surveillance of Lyme disease—United States, 1992–2006. MMWR 57: 1–9.

(CDC) Centers for Disease Control and Prevention. 2012. Reported Lyme disease cases by state, 2000–2010. CDC, Atlanta, GA. (http://www.cdc.gov/lyme/stats/index.html).

Cortinas, M. R., and U. Kitron. 2006. County-level surveillance of white-tailed deer infestation by *Ixodes scapularis* and *Dermacentor albipictus* (Acari: Ixodidae) along the Illinois River. J. Med. Entomol. 43: 810–819.

Dennis, D. T., T. S. Nekomoto, J. C. Victor, W. S. Paul, and J. Piesman. 1998. Reported distribution of *Ixodes scapularis* and in *Ixodes pacificus* (Acari : Ixodidae) in the United States. J. Med. Entomol. 35: 629–638.

Diuk–Wasser, M. A., A. G. Hoen, P. Cislo, R. Brinkerhoff, S. A. Hamer, M. Rowland, R. Cortinas, G. Vourc'h, F. Melton, G. J. Hickling, J. I. Tsao, J. Bunikis, A. G. Barbour, U. Kitron, J. Piesman, and D. Fish. 2012. Human risk of infection with *Borrelia burgdorferi,* the Lyme disease agent, in eastern United States. Am. Soc. Trop. Med. Hyg. 86: 320–327.

Diuk–Wasser, M. A., G. Vourc'h, P. Cislo, A. G. Hoen, F. Melton, S. A. Hamer, M. Rowland, R. Cortinas, G. J. Hickling, J. I. Tsao, A. G. Barbour, U. Kitron, J. Piesman, and D. Fish. 2010. Field and climate-based model for predicting the density of host-seeking nymphal *Ixodes scapularis,* an important vector of tick borne disease agents in the eastern United States. Global Ecol. Biogeogr. 19: 504–514.

Durden, L. A., and T. M. Kollars. 1992. An annotated list of the ticks (Acari: Ixodidea) of Tennessee, with records of four exotic species of the United States. Bull. Soc. Vector Ecol. 17: 125–131.

Falco, R. C., D. Fish, and J. Piesman. 1996. Duration of tick bites in a Lyme disease-endemic area. Am. J. Epidemiol. 143: 187–192.

Gill, J. S., R. C. Johnson, M. K. Sinclair, and A. R. Weisbrod. 1993. Prevalence of the Lyme-disease spirochete, *Borrelia burgdorferi,* in deer ticks (*Ixodes dammini*) collected

from white-tailed deer (*Odocoileus virginianus*) in Saint Croix State Park, Minnesota. J. Wildl. Dis. 29: 64–72.

Goddard, J. 2007. Seasonal activity of *Amblyomma* spp. in Mississippi. J. Vector Ecol. 32: 157–158.

Goddard, J., J. W. Sumner, W. L. Nicholson, C. D. Paddock, J. Shen, and J. Piesman. 2003. Survey of ticks collected in Mississippi for *Rickettsia, Ehrlichia,* and *Borrelia* species. J. Vector Ecol. 28: 184–189.

Griffith, G. E., J. M. Omernick, and M. McGinley. 2009. Ecoregions of Tennessee (EPA). *In* J. Cutler (ed.), Encyclopedia of Earth. Environmental Information Coalition, National Council for Science and the Environment, Washington, DC. (http://www.eoearth.org/article/Ecoregions_of_Tennessee_(EPA)).

Harmon, J. R., G. J. Hickling, M. C. Scott, and C. J. Jones. 2011. Evaluation of 4-poster acaricide applicators to manage tick populations associated with disease risk in a Tennessee retirement community. J. Vector Ecol. 36: 404–410.

Haynes, J. M., P. P. Lee, R. L. Seipelt, and S. M. Wright. 2005. Detection of *Borrelia burgdorferi* sequences in a biopsy from a Tennessee patient. J. Tennessee Acad. Sci. 80: 57–59.

Jordan, B. E., K. R. Onks, S. W. Hamilton, S. E. Hayslette, and S. M. Wright. 2009. Detection of *Borrelia burgdorferi* and *Borrelia lonestari* in birds in Tennessee. J. Med. Entomol. 46: 131–138.

Jouda, F., J. L. Perret, and L. Gern. 2004. *Ixodes ricinus* density, and distribution and prevalence of *Borrelia burgdorferi* sensu lato infection along an altitudinal gradient. J. Med. Entomol. 41: 162–169.

Keefe, L. M., M. H. Moro, J. Vinasco, C. Hill, C. C. Wu, and E. A. Raizman. 2009. The use of harvested white tailed deer (*Odocoileus virginianus*) and geographic information system (GIS) methods to characterize distribution and locate spatial clusters of *Borrelia burgdorferi* and its vector *Ixodes scapularis* in Indiana. Vector-Borne Zoon. Dis. 9: 671–680.

Kitron, U., and J. J. Kazmierczak. 1997. Spatial analysis of the distribution of Lyme disease in Wisconsin. Am. J. Epidemiol. 145: 558–566.

Kitron, U., C. J. Jones, J. K. Bouseman, J. A. Nelson, and D. L. Baumgartner. 1992. Spatial-analysis of the distribution of *Ixodes dammini* (Acari: Ixodidae) on white-tailed deer in Ogle County, Illinois. J. Med. Entomol. 29: 259–266.

Kollars, T. M., J. H. Oliver, L. A. Durden, and P. G. Kollars. 2000. Host associations and seasonal activity of *Amblyomma americanum* (Acari : ixodidae) in Missouri. J. Parasitol. 86: 1156–1159.

Kurtenbach, K., H. S. Sewell, N. H. Ogden, S. E. Randolph, and P. A. Nuttall. 1998. Serum complement sensitivity as a key factor in Lyme disease ecology. Infect. Immun. 66: 1248–1251.

Ledin, K. E., N. S. Zeidner, J.M.C. Ribeiro, B. J. Biggerstaff, M. C. Dolan, G. Dietrich, L. Vredevoe, and J. Piesman. 2005. Borreliacidal activity of saliva of the tick *Amblyomma americanum.* Med. Vet. Entomol. 19: 90–95.

Loken, K. I., C. C. Wu, R. C. Johnson, and R. F. Bey. 1985. Isolation of the Lyme disease spirochete from mammals in Minnesota. Proc. Soc. Exp. Biol. Med. 179: 300–302.

Maggi, R. G., S. Reichelt, M. Toliver, and B. Engber. 2010. Borrelia species in *Ixodes affinis* and *Ixodes scapularis* ticks collected from the coastal plain of North Carolina. Ticks Tick-Borne Dis. 1: 168–171.

Magnarelli, L. A., A. Denicola, K. C. Stafford, and J. F. Anderson. 1995. *Borrelia burgdorferi* in an urban environment: white-tailed deer with infected ticks and antibodies. J. Clin. Microbiol. 33: 541–544.

Magnarelli, L. A., J. F. Anderson, C. S. Apperson, D. Fish, R. C. Johnson, and W. A. Chappell. 1986. Spirochetes in ticks and antibodies to *Borrelia burgdorferi* in white-tailed deer from Connecticut, New-York-State, and North-Carolina. J. Wildl. Dis. 22: 178–188.

Marsland, E. J. 1997. Tick control and tick transmitted disease monitoring in eastern Tennessee. MS thesis, University of Tennessee, Knoxville, TN.

Murdock, J. H., M. J. Yabsley, S. E. Little, R. Chandrashekar, T. P. O'Connor, J. N. Caudell, J. E. Huffman, J. A. Langenberg, and S. Hollamby. 2009. Distribution of antibodies reactive to *Borrelia lonestari* and *Borrelia burgdorferi* in white-tailed deer (*Odocoileus virginianus*) populations in the eastern United States. Vector-Borne Zoon. Dis. 9: 729–736.

Paddock, C. D., and M. J. Yabsley. 2007. Ecological havoc, the rise of white-tailed deer, and the emergence of *Amblyomma americanum*-associated zoonoses in the United States. Curr. Top. Microbiol. Immunol. 315: 289–324.

Penrose, R., E. C. Ramsay, J. C. New, and S. A. Kania. 2000. *Borrelia burgdorferi* infection in a Red Wolf. Infect. Dis. Rev. 2: 223–224.

Piesman, J. 2002. Ecology of *Borrelia burgdorferi* sensu lato in North America. *In* J. S. Gray, O. Kahl, R. S. Lane, and G. Stanek (eds.), *Lyme borreliosis.* Biology, Epidemiology, and Control. CAB International, Wallingford, Oxon, United Kingdom.

Piesman, J., K. L. Clark, M. C. Dolan, C. M. Happ, and T. R. Burkot. 1999. Geographic survey of vector ticks (*Ixodes scapularis* and *Ixodes pacificus*) for infection with the Lyme disease spirochete, *Borrelia burgdorferi.* J. Vector Ecol. 24: 91–98.

Piesman, J., and C. M. Happ. 1997. Ability of the Lyme disease spirochete *Borrelia burgdorferi* to infect rodents and three species of human-biting ticks (blacklegged tick, American dog tick, lone star tick) (Acari: Ixodidae). J. Med. Entomol. 34: 451–456.

Platonov, A. E., L. S. Karan, N. M. Kolyasnikova, N. A. Makhneva, M. G. Toporkova, V. V. Maleev, D. Fish, and P. J. Krause. 2011. Humans infected with relapsing fever spirochete *Borrelia miyamotoi,* Russia. Emerg. Infect. Dis. 17: 1816–1823.

Qiu, W. G., D. E. Dykhuizen, M. S. Acosta, and B. J. Luft. 2002. Geographic uniformity of the Lyme disease spirochete (*Borrelia burgdorferi*) and its shared history with tick vector (*Ixodes scapularis*) in the northeastern United States. Genetics 160: 833–849.

Riehle, M., and S. M. Paskewitz. 1996. *Ixodes scapularis* (Acari: Ixodidae): status and changes in prevalence and distribution in Wisconsin between 1981 and 1994 measured by deer surveillance. J. Med. Entomol. 33: 933–938.

Schwartz, I., S. Varde, R. B. Nadelman, G. P. Wormser, and D. Fish. 1997. Inhibition of efficient polymerase chain reaction amplification of *Borrelia burgdorferi* DNA in blood-fed ticks. Am. J. Trop. Med. Hyg. 56: 339–342.

Scott, M. C., M. E. Rosen, S. A. Hamer, E. Baker, H. Edwards, C. Crowder, J. I. Tsao, and G. J. Hickling. 2010. High-prevalence *Borrelia miyamotoi* infection among wild turkeys (*Meleagris gallopavo*) in Tennessee. J. Med. Entomol. 47: 1238–1242.

Shariat, B. M., J. A. Freimund, S. M. Wright, C. S. Murphree, and J. T. Thomas. 2007. *Borrelia* infection rates in winter ticks (*Dermacentor albipictus*) removed from white-tailed deer (*odocoileus virginianus*) in Cheatham County, Tennessee. J. Tennessee Acad. Sci. 82: 57–61.

Sonenshine, D. E. 1979. Insects of Virginia, No. 13. Ticks of Virginia (Acari: Metastigmata). Virginia Polytechnic Institute and State University, Blacksburg. Res. Div. Bull. 139.

Tennessee Department of Health. 2009. The communicable disease interactive data site: interactive statistics. (http://health.state.tn.us/ceds/WebAim/aimhome.htm).

Telford, S. R., T. N. Mather, S. I. Moore, M. L. Wilson, and A. Spielman. 1988. Incompetence of deer as reservoirs of the Lyme disease spirochete. Am. J. Trop. Med. Hyg. 39: 105–109.

Tsao, J. I., J. T. Wootton, J. Bunikis, M. G. Luna, D. Fish, and A. G. Barbour. 2004. An ecological approach to preventing human infection: vaccinating wild mouse reservoirs intervenes in the Lyme disease cycle. Proc. Nat. Acad. Sci. U.S.A. 101: 18159–18164.

Wilson, M. L., T. S. Litwin, T. A. Gavin, M. C. Capkanis, D. C. Maclean, and A. Spielman. 1990. Host-dependent differences in feeding and reproduction of *Ixodes dammini* (Acari: Ixodidae). J. Med. Entomol. 27: 945–954.

Received 17 November 2011; accepted 8 August 2012.