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Investigating the Maintenance of the Lyme Disease Pathogen, *Borrelia burgdorferi*, and its Vector, *Ixodes scapularis*, in Tennessee

Michelle Erin Rosen
University of Tennessee - Knoxville

To the Graduate Council:

I am submitting herewith a thesis written by Michelle Erin Rosen entitled "Investigating the Maintenance of the Lyme Disease Pathogen, *Borrelia burgdorferi*, and its Vector, *Ixodes scapularis*, in Tennessee." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Wildlife and Fisheries Science.

Graham Hickling, Major Professor

We have read this thesis and recommend its acceptance:

Carl Jones, Reid Gerhardt, Lisa Muller

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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A Thesis
Presented for the
Master of Science Degree
The University of Tennessee, Knoxville

Michelle Erin Rosen
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DEDICATION

I dedicate this thesis to my late mother and grandmother, Beverly Rosen and Gertrude Rosen. I wish they could be here to share my experiences, passion, and love for the outdoors, wildlife, and adventure.

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ABSTRACT

Lyme disease (LD), caused by the bacterium *Borrelia burgdorferi* and transmitted by blacklegged ticks (*Ixodes scapularis*), is the most commonly reported vector-borne disease in the United States. Lyme disease is endemic in northeastern states, whereas southern states report far fewer cases. This research evaluated the potential LD health risk to humans associated with blacklegged ticks in Tennessee.

I surveyed 1,018 hunter-harvested deer from 71 counties in fall 2007 and fall 2008. Of these, 160 (15.7%) from 35 counties were infested with *I. scapularis* — 30 of the counties were new distributional records for this species.

I also evaluated the seasonal phenology of *I. scapularis* at Henry Horton State Park (HHSP) in middle Tennessee by drag sampling and small mammal trapping from November 2007 to May 2009. Larval *I. scapularis* numbers per 1000m^2 dragged peaked at 4.1 ± 2.9SE in July, nymphs peaked at 5.0 ± 3.5SE in March, and adults at 12.0 ± 1.2SE in November. Overall, 191 mice (*Peromyscus* spp.) were captured on 355 occasions – *I. scapularis* ticks were present on 68 (19%) of these occasions. Larval *I. scapularis* infestation of mice peaked in June (8 of 12 mice; 67%); nymphal infestation peaked in May (3 of 16; 19%).

DNA was extracted from the *I. scapularis* collected from deer (883 samples), and at HHSP (283 samples) and tested for *B. burgdorferi* and other *Borrelia* using PCR targeting the 16s-23s intergenic spacer region of these bacteria. No *B. burgdorferi* was detected, although four samples tested positive for *B. miyamotoi*.

I conclude that *I. scapularis* is far more widespread in Tennessee than previously reported. At HHSP, the abundance of this tick reaches levels that sustain endemic cycles of

B. burgdorferi in the Northeast. Moreover, their seasonal phenology in Tennessee – whereby nymphal questing precedes larval questing – should favor *B. burgdorferi* transmission. Nevertheless, *B. burgdorferi* was not detected in these Tennessee tick populations, so the LD risk to humans posed by *I. scapularis* in Tennessee appears to be very low at the present time. Future ecological studies are needed to explain the lack of *B. burgdorferi* infection in these Tennessee ticks.

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Introduction

Lyme disease (LD), caused by the bacterium *Borrelia burgdorferi*, is the most commonly reported vector-borne disease of humans in the U.S., with around 20,000 new cases each year. Blacklegged ticks (*Ixodes scapularis*) are the main vector for *B. burgdorferi* in the eastern United States. Lyme disease is endemic in northeastern states while southern states including Tennessee have fewer reported cases (CDC 2008). Lyme disease is presently not considered to be endemic in the southeast. Nevertheless, between 1993 and 2005, an average of 28 cases of Lyme disease per year were reported from Tennessee to the Centers for Disease Control and Prevention (CDC 2004; CDC 2007).

Reported cases of several tick-borne diseases – for example Rocky Mountain Spotted Fever and Human Monocytic Ehrlichiosis – are on the rise in Tennessee (TDH 2009). Reasons include enhanced awareness and reporting by physicians, increases in the abundance and geographic range of some tick species and their associated hosts – particularly white-tailed deer (*Odocoileus virginianus*), and changes in land use and human activity in tick-infested areas. Whether Lyme disease is similarly on the rise in Tennessee is less certain. The goal of this research is to evaluate the current LD health risk to humans associated with the blacklegged tick in Tennessee, and the role of wildlife in perpetuating this risk. The project aims to assist public health officials in developing appropriate strategies for managing human health issues related to ticks in Tennessee and neighboring states.

Literature Review

Importance of studying tick-borne disease

There has been an unprecedented increase in emerging infectious diseases in the past 30 years, with the majority of these diseases being zoonotic (Jones, et al. 2008). Numerous tick-borne diseases (TBDs) are presently emerging or expanding their geographic ranges and are increasingly recognized as a threat to human health worldwide. These tick-borne infections are continuing to emerge and resurge as a result of many complex factors including climate change, urbanization, land-use changes and practices, public health policy, changes in wildlife, vector, and pathogen distributions and enhanced surveillance, and lack of prevention and control methods (Gubler 1998).

TBDs are caused by a variety of pathogens including bacteria, viruses, protozoa, and even toxins. Ticks can carry and transmit more than one disease-causing agent at a time. For example, the blacklegged tick *Ixodes scapularis* can transmit the agents of both Human Granulocytic Anaplasmosis and Lyme disease (Telford, et al. 1996). Understanding the etiology, epidemiology, and ecology of these tick-borne infections can facilitate treatment and management of disease in humans, pets, livestock, and wildlife.

Tick-borne disease in Tennessee

There is much debate and uncertainty surrounding tick-borne disease in Tennessee, although it is clear that there are a number of these diseases in the state that affect humans and other animals (Table 1.1). Five are classified presently as reportable to the state health department (Table 1.2). In the past 10 years, the number of officially reported cases of

Table 1.1: Known tick-borne diseases and pathogens in Tennessee (Haber, et al. 2007; TDH 2009; A. Moncayo, personal communication; G. Hickling, unpublished data).

Disease	**Main vector(s)**	**Pathogen**	**Type**
Ehrlichiosis	*Amblyomma amercanium* (Lone Star tick) *Dermacentor variabilis* (American Dog tick)	*Ehrlichia caffeensis*	Bacterium
Lyme disease	*Ixodes scapularis* (Blacklegged/Deer tick)	*Borrelia burgdorferi*	Bacterium
Southern Tick Associated Rash Illness	*Amblyomma amercanium* (Lone Star tick)	Unknown	Unknown
Rocky Mountain Spotted Fever	*Dermacentor variabilis* (American Dog tick)	*Rickettsia rickesttsii*	Bacterium
Type A Tularemia	*Haemophysalis leporispalustris* (Rabbit tick)	*Francisella tularensis*	Bacterium
Relapsing fever	*Ornithodoros* spp.	*Borrelia* spp.	Bacterium
Babesiosis	*Ixodes scapularis* (Blacklegged/Deer tick)	*Babesia microti*	Protoza
Cytauzoonosis	*Dermacentor variabilis* (American Dog tick)	*Cytauxzoon felis*	Protoza
Tick paraylsis	*Ixodes brunneus* (Bird tick)		Toxin
Human Granulocytic Anaplasmosis	*Ixodes scapularis* (Blacklegged/Deer tick) *Dermacentor variabilis* (American Dog tick)	*Anaplasma phagocytophilum*	Bacterium
Q Fever*	*Amblyomma amercanium* (Lone star tick) *Rhipicephalus sanguineus* (Brown Dog tick)	*Coxiella burnetii*	Bacterium

* Tick transmission is considered rare (CDC 2003).

Table 1.2: Mean, median, and range of tick-borne disease cases reported annually for the state of Tennessee, from 1995 to 2008 (TDH 2009).

Disease	Mean	Median	Range
Tularemia	2	1	0-7
Rocky Mountain Spotted Fever	101	76	31-263
Q Fever	2	1	0-10
Lyme disease	31	29	17-47
Ehrlichiosis	23	20	0-74

tick-borne diseases in Tennessee has increased (e.g., from 0 officially reported cases of Ehrlichiosis in 1995 to 74 in 2008, and from 0 reported cases of Rocky Mountain Spotted Fever in 1995 to 232 in 2008; TDH 2009), however uncertainties surrounding the diagnosis and reporting of these cases mean that such trends must be interpreted with considerable caution.

Importance of studying vector tick distribution, abundance and phenology

Information on the distribution, abundance, and seasonal phenology of ticks is key to understanding how, when, and where TBD cases occur. For example, if the only tick species capable of transmitting a certain pathogen is not present in an area, no disease will occur. Not only are seasonal timing, abundance, and distribution important in understanding disease risk, but these factors are also important in understanding how pathogens are maintained in nature. For example, in areas of the northeastern U.S., where Lyme disease is endemic, the maintenance of *B. burgdorferi* is dependant on nymphal *I. scapularis* feeding on their hosts earlier in the season than the larvae (Steere, et al. 2004). Previously infected nymphs feed on and thereby infect new hosts. Uninfected larval ticks later feed on these infected hosts and acquire *B. burgdorferi*, thereby maintaining the Lyme disease pathogen transmission cycle.

Tick distribution and abundance in Tennessee

Amblyomma americanum (Lone Star tick), *Amblyomma maculatum* (Gulf Coast tick), *Dermacentor variabilis* (American Dog tick), *I. scapularis* (Blacklegged or Deer tick), and *Rhipicephalus sanguineus* (Brown Dog tick) are the tick species most commonly observed in Tennessee, although many other hard and soft ticks have been documented in the state (Durden and Kollars 1992). *Amblyomma americanum* and *D. variabilis* are the most abundant and widespread species, being found in most counties (Gerhardt, et al. 1998).

Amblyomma americanum, D. variabilis, A. maculatum and *I. scapularis* are the four Tennessee species most likely to bite humans. For example, at three military bases in Tennessee and nearby in Kentucky, 885 ticks that attached to military personnel from 2004 to 2008 were submitted for testing; of these 86.6% were *A. americanum,* 11.2% were *D. variabilis,* 1.8% were *A. maculatum* and only 0.3% were *I. scapularis* (E. Stromdahl, U.S. Army Center for Health Promotion & Preventive Medicine, personal communication).

Ixodes scapularis *in Tennessee*

Existing data on the distribution of *I. scapularis* in Tennessee are limited and incomplete. Durden and Kollars (1992) collected passive data on tick presence in the state, summarizing collecting records from previously published literature, personal collections, and the U.S. National Tick Collection in Statesboro, GA. A distribution map of *I. scapularis* in the United States by Dennis et al. (1998) was constructed at the county level from passive, non-standardized data for the period 1907-1996; this map did not distinguish counties that were sampled but yielded no *I. scapularis* from counties where no sampling had occurred. Prior to the surveys reported here *I. scapularis* had been officially reported from only ten counties in Tennessee —

Anderson, Bedford, Campbell, Fentress, Davidson, Lake, Marion, Rutherford, Scott, and Shelby (Durden and Kollars 1992; Dennis, et al. 1998).

From 2004 to 2007, the Centers for Disease Control and Prevention funded an extensive survey to determine the risk of Lyme disease across the eastern U.S., and to update the Dennis, et al. (1998) map. Unfortunately, the semi-random sampling design employed in this study resulted in no survey sites being located in Tennessee (Diuk-Wasser, et al. 2006). The current abundance and distribution of this tick in Tennessee is thus unclear.

Lyme disease (LD)

Diagnosis and reporting of LD

The CDC provides a 'case definition' for Lyme disease; this definition was developed for national reporting of Lyme disease rather than for clinical diagnosis. It describes the clinical presentation of Lyme disease as:

> A systemic, tick-borne disease with protean manifestations, including dermatologic, rheumatologic, neurologic, and cardiac abnormalities. The best clinical marker for the disease is erythema migrans (EM), the initial skin lesion that occurs in 60%-80% of patients (CDC 2009).

For surveillance purposes, an EM is defined as a skin lesion that begins as a small macule or papule and expands over a periods of days (with a partial clearing) to greater than or equal to 5cm in diameter, with the diagnosis made by a physician. Laboratory evidence (for surveillance and recommended for people with no known exposure) is defined as either i) a positive culture of *B. burgdorferi*, ii) a 'two-tier' test interpreted using established criteria (defined below), or iii) a single-tier IgG immunoblot seropositivity interpreted using established criteria (defined below) (CDC 2009).

The established criteria for the two-tier test is to use a sensitive enzyme immunoassay (EIA) or immunofluorescent assay (IFA), followed by a Western immunoblot on all specimens positive or equivocal by the EIA or IFA. Specimens negative by an EIA or IFA are not to be tested further (CDC 1995).

The CDC guidelines state that:

> "when a Western immunoblot is used during the first 4 weeks of disease onset, both immunoglobulin M (IgM) and G (IgG) procedures should be performed. A positive IgM test result is not recommended for use in determining active disease in persons with illness greater than 1 month's duration because the likelihood of a false-positive test result for a current infection is high for these persons. If a patient with suspected early LD has a negative serology, serologic evidence of infection is best obtained by testing of paired acute- and convalescent-phase serum samples. Serum samples from persons with disseminated or late-stage LD almost always have a strong IgG response to *Borrelia burgdorferi* antigens" (CDC 1995).

An IgM test is considered positive when 2 of 5 bands are present and an IgG test is considered positive when 5 of 10 bands are present (CDC 1995).

These laboratory tests are recommended when a patient has no known exposure (see *Exposure and endemicity*, below) or for patients considered to have late manifestations (i.e. musculoskeletal, nervous, or cardiovascular conditions) of the infection (CDC 2009). The CDC notes that there are a number of commercial laboratories that conduct Lyme disease testing using methods that have not been validated and that may be misleading. The CDC urges health care providers to diagnose LD based on the patient's clinical presentation, risk of exposure to infected ticks and, if needed, of the results of validated laboratory tests interpreted using appropriate guidelines (CDC 1995).

Exposure and endemicity

The CDC (2009) defines 'exposure' as the patient having been (less than or equal to 30 days before onset of EM) in a wooded, brushy or grassy area in a county in which LD is considered endemic. Interestingly, a history of a tick bite is not required. 'Endemic' counties are those in which there have been at least two confirmed LD cases or in which there are known to be established populations of a tick vector that are infected with *B. burgdorferi* (CDC 2009).

Case classification

The CDC classifies LD cases into 3 groups: confirmed, probable and suspect. Presently (in 2009) a case is considered confirmed is if there is:

- (1) a physician-confirmed EM with a known exposure (as defined above);
- or (2) an EM without a known exposure but with laboratory evidence of *B. burgdorferi* infection (such as a positive culture, or a positive result on a two-tier EIA/IFA plus IgM Western Blot test);
- or (3) a case with at least one late manifestation and with laboratory evidence of infection (such as a single-tier IgG Western Blot positive).

Probable cases are any other physician-diagnosed case of Lyme disease with laboratory evidence of infection based on one of the tests described above. Lastly, a suspect case is an EM where there is no known exposure and no laboratory evidence, or a case with laboratory evidence but no clinical information available (CDC 2009). It is worth emphasizing that a case of Lyme disease can be 'confirmed' without any laboratory evidence (i.e., under scenario (1) above).

Changes in definition, including case classification

The above definitions of cases, exposure, and endemicity were implemented beginning January 1 2008, when the CDC revised an older definition in use from 1996 to 2008. The 2008 version revised the previous definition for a confirmed case (which was a case 'with EM or with at least one late manifestation that is laboratory confirmed') and added the definitions for probable and suspect cases.

The 1996 definition was itself a revision of the CDC's original (1990) definition. In 1996, the original clinical criteria were retained but the required laboratory evidence changed from 'a significant change in IgM or IgG antibody response in paired serum samples' (1990 version) to the two-test approach involving a sensitive enzyme immunoassay or immunofluorescence antibody assay followed by Western blot (1996 version; CDC 2009).

Isolation of *B. burgdorferi* and/or the demonstration of diagnostic levels of IgM and IgG antibodies have been included in all case definitions as one criteria for confirming a LD case.

Problems with Lyme disease surveillance

Lyme disease cases are reported to the CDC voluntarily as part of the National Notifiable Disease Surveillance System. In 1991, Lyme disease became a nationally notifiable disease and its reporting and surveillance was standardized – prior to that year LD reporting procedures varied within and among states. Today, state or local health departments are responsible for guaranteeing cases reported to the CDC meet the case definition. However, many of the LD cases reported to the CDC lack important information such as county of exposure, symptoms and signs, and laboratory results (Bacon, et al. 2008). The 2008 case definition was implemented to

give state and local health agencies more flexibility to classify Lyme disease reports, while providing for meaningful reporting of confirmed and probable cases at the national level.

Between 1991 and 2005, the number of reported LD cases nationally doubled to over 20,000; this increase is attributed to a true increase in Lyme disease incidence, plus increased detection due to increased laboratory testing (CDC 2007). These case reports require not only initial reporting but also follow up reporting to determine case classification. This has become a significant burden to many state and local health departments, and some states have consequently reduced the number of laboratory cases that are followed up, altered the stringency with which they follow the case definition, and changed their allocations of funding to Lyme disease surveillance (CSTE 2007). This has decreased Lyme disease surveillance and the number of cases reported in some states. In addition, Lyme disease case data from certain states are incomplete, unavailable, or not transferred to the CDC (Bacon, et al. 2008).

As with most vector-borne disease, LD cases are most likely under-reported in endemic areas and misdiagnosed and consequently over-reported in areas that are not endemic for Lyme disease. For example, the CDC recognizes that Lyme disease surveillance in southern states is complicated by Southern Tick Associated Rash Illness (STARI), which can resemble early Lyme disease but is associated with the bite of *A. americanum* and not caused by *B. burgdorferi* (Bacon, et al. 2008).

A further complication is that reports submitted to the CDC are based on county of residence, not exposure, so that patients with a recent travel history generate reports of LD in areas not known to be endemic for the disease (Bacon, et al. 2008).

Lyme disease in Tennessee

From 1993 to 2005, Tennessee health officials annually reported between 8 and 59 'confirmed' cases of LD to the CDC (CDC 2004; CDC 2007). Over a similar period (1995-2008) the Tennessee Department of Health annually reported 17 to 47 cases within the state (TDH 2009); these numbers include both 'confirmed' and 'probable' cases according to the 2009 case definition. Most confirmed cases involved patients with travel histories outside of Tennessee (A. Moncayo, Tennessee Department of Health, personal communication); these infections may have been acquired in known Lyme disease endemic areas in other states. Many of the 'probable' Tennessee cases were diagnosed on the basis of characteristic EM, together with indicatory but often not fully confirmatory laboratory tests for *B. burgdorferi* infection.

EM-like rashes can be caused by the bites of tick species not known to harbor *B. burgdorferi* (Wormser, et al. 2005). For example, Southern Tick Associated Rash Illness (STARI) can mimic the symptoms of Lyme disease but is associated with the bite of *A. americanum* and is of unknown etiology. It was initially suspected to be caused by *B. lonestari*, but more recent studies have concluded that this is unlikely (CDC 2008). It is also possible that some infections from spider bites are being misidentified as Lyme disease rashes. Given the continuing uncertainties surrounding laboratory procedures and test interpretation for LD, some researchers argue that there is presently a lack of convincing evidence that humans are contracting LD infections within the state of Tennessee. This uncertainty has been compounded by past entomological studies, which have suggested that the key vector for the LD pathogen – the blacklegged tick – is rare in the state.

Borrelia burgdorferi *investigations in Tennessee*

Currently, there are few published reports of *B. burgdorferi* in the state. In 2003, a skin biopsy was taken from an EM rash from a patient in Greene County (East Tennessee) and identified as being infected with *B. burgdorferi* strain B31 (Haynes, et al. 2005). However, no information on the patient's travel history was provided.

Shariat, et al. (2007) reported detecting *B. burgdorferi* in 2 of 18 pooled samples of winter ticks (*Dermacentor albipictus*) removed from white-tailed deer in Cheatham County, Tennessee. Jordan, et al. (2009) reported detection of *B. burgdorferi* in 14% of turkey and 17% of migratory waterfowl blood samples collected from a site in middle Tennessee, and reported further detections from these species in ten Tennessee counties: Bedford, Chester, Coffee, DeKalb, Dyer, Jackson, Montgomery, Robertson, Rutherford, and Stewart. In these reports, the probes and primers used to detect *B. burgdorferi* appear able to amplify and bind to multiple species of *Borrelia* (unpublished data). In addition, the test-positive samples were not confirmed by sequence analysis. Since winter ticks are a one-host tick (on deer), and deer are not reservoir-competent for *B. burgdorferi* (Telford, et al. 1988), the results of Shariat, et al. (2007) are surprising. Similarly, turkeys have not been implicated elsewhere as having a significant role in LD transmission cycles (Ostfeld and Lewis 1999; Lane, et al. 2006), so the results of Jordan, et al. (2009) are also unexpected. Detection in migratory waterfowl is consistent with other studies indicating that migratory birds can play a role in the spread of both *B. burgdorferi* and ticks (McLean, et al. 1993; Ginsberg, et al. 2005; Ogden, et al. 2008).

Borrelia burgdorferi has been reported from a red wolf (Penrose, et al. 2000) in the Great Smoky Mountains National Park (on the Tennessee/North Carolina border), based on enzyme-linked immunosorbent assay (ELISA) plus PCR and sequence analysis identifying the OspA

gene. Other wolves housed in the same vicinity as the PCR-positive wolf tested positive for *B. burgdorferi* antibodies but were PCR negative, which suggested these wolves also may have been exposed to the LD pathogen.

Canine serum testing (ELISA, LymeCHEK®) 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 these tests is questionable and the vaccination and travel histories of these dogs were incomplete. More recently, highly specific (99.5%) SNAP® 3Dx® and 4Dx® testing of sera from 18,891 pet dogs in Tennessee from 2001 to 2007 produced only 47 positive samples (a 0.02% prevalence) for the *B. burgdorferi* C_6 peptide (Bowman, et al. 2009), whereas the prevalence of this peptide typically exceeds 10% among dogs living in known LD-endemic parts of the U.S. (Bowman, et al. 2009). In Oklahoma, which has a similarly low test prevalence (0.2% SNAP), test-positive dogs typically had a previous travel history to Lyme-endemic areas (S. Little, Oklahoma State University, personal communication). SNAP® 4Dx® tests of blood samples taken from 20 deer from three Tennessee counties (Chester, Hardeman and Lauderdale) in 2001 identified one deer (from Chester County) as positive for *B. burgdorferi* (M. Yabsley, University of Georgia-Athens, personal communication). The sensitivity and specificity of this test when applied to deer is unknown. To date, no reports of sequence-confirmed isolates of *B. burgdorferi* from Tennessee wildlife have been published.

The LD tick/host/pathogen system in Tennessee and southeastern states.

Doubts about the endemic status of LD in Tennessee are reinforced by a belief among researchers that not only are *I. scapularis* ticks rare in the state, but the seasonal phenology and/or host preferences of the tick may be such that *B. burgdorferi* infection is not maintained in

nature. Consequently, several hypotheses (not necessarily exclusive) have been proposed as to why LD is not endemic to Tennessee and other southeastern states. These include: (1) *I. scapularis* is not sufficiently abundant to maintain the pathogen cycle; (2) the seasonal life cycle of the tick in the South differs from that in the North in a way that 'breaks' the Lyme disease transmission cycle; (3) lizards are the preferred host for nymphal *I. scapularis* in Southern states and are not competent reservoirs for the bacterium; and (4) nymphal preference for lizards means that mammals (including humans) are not as commonly bitten as in the North. These hypotheses are described in more detail below.

Drag sampling provides the best measure of potential contact between ticks and humans (Daniels, et al. 2000). In Tennessee and other southeastern states, nymphal *I. scapularis* are thought to be much more difficult to sample from vegetation using standard 'dragging' techniques than is the case for the northern populations of this tick (Diuk-Wasser, et al. 2006). Certainly, humans in the southeast are parasitized by *I. scapularis* far less than are humans in endemic areas of the north (Felz, et al. 1996).

Nevertheless, many researchers have reported finding *B. burgdorferi* in the southeast (Magnarelli, et al. 1992; Oliver, et al. 1993; Oliver, et al. 2003; Lin, et al. 2004). Magnarelli, et al. (1992) found antibodies to *B. burgdorferi* in *Peromyscus gossypinus* (cotton mouse) from Alabama, Georgia, North and South Carolina, Florida, and Mississippi; he concluded that *B. burgdorferi* may be endemic in these southeastern states. Similarly, Oliver, et al. (1993) obtained *B. burgdorferi* isolates in ticks from five locations in Florida and Georgia. These studies exemplify the need for further research on role of wildlife in maintaining potentially-cryptic cycles of Lyme disease in the south.

Because there is minimal transovarial transmission of *B. burgdorferi* (Magnarelli, et al. 1987), the 2-year lifecycle of these ticks in the northeast – whereby peak nymphal questing *precedes* peak larval questing – is key to the maintenance of the pathogen in these tick populations. There has been speculation that the longer growing season in southeastern states may mean that nymphs feed *later* in the season than larvae, reducing the feeding overlap between the two life stages, thereby breaking the transmission cycle. For example, Rodgers (1953) showed peak larval activity in May in northern Florida. He suggested that the *I. scapularis* life cycle in northern Florida is varies between 1 and 2 years depending on developmental influences such as temperature. In southeastern Missouri, however, questing larvae peaked in July and nymphs in May (Kollars, et al. 1999). In a 2-year study of ticks collected from South Carolina, Clark, et al. (1998) found that larvae peaked on rodents in August 1994 and in June 1995, where as nymphs peaked in June 1995 and August 1995. Larval and nymphal abundance on lizards in the southeastern U.S. peaked in May in a study by Oliver, et al (1993). In coastal Georgia, Durden and Oliver (1999) found that questing larvae peaked in June and that nymphs had a less distinct peak in April/May and then in August of 1993 and 1994 respectively. Larval ticks collected from cotton mice peaked in June (1993 and 1994), while nymphs peaked in April (1993 and 1994) and August (1993). In addition, there was a peak of larval ticks collected from lizards in May (1993) and June (1994) and a peak in nymphs on lizards in May and September of both years (Durden and Oliver 1999). Thus, there is considerable variation in *I. scapularis* phenology in different years and at different locations within the southern states.

Ixodes scapularis will feed on at least 14 different species of reptiles; these include southeastern five-lined skink (*Eumeces inxpectatus*), broad-headed skink (*Eumeces laticeps*), coal skink (*Eumeces anthracinus*), red-tailed skink (*Eumeces egregious*), American five-lined skink (*Eumeces fasciatus*), gound skink (*Scincella lateralis*), six-lined race runner (*Cnemidophorus sexlineatus*), northern fence lizard (*Sceloporus undulates*), western fence lizard (*Sceloporus occidentalis*), slender glass lizard (*Ophisaurus attenuatus*), island glass lizard (*Ophisaurus compressus*), mimic glass lizard (*Ophisaurus mimicus*), eastern glass lizard (*Ophisaurus ventralis*), green anole (*Anolis carolinensis*; Rodgers 1953; Apperson, et al. 1993; Oliver, et al. 1993; Keirans, et al. 1996). Many studies have concluded that most lizards are dead end or at least poorly competent reservoir hosts for *Borrelia* relative to mice and birds (Spielman, et al. 1984; Kuo, et al. 2000). However, the southeastern five-lined skink has been shown to transmit *B. burgdorferi* to 20% of xenodiagnostic larvae that were fed on infected skinks (Levin, et al. 1996). This suggests that species variation in lizard reservoir competence and life history needs to be considered when discussing their potential role in *B. burgdorferi* transmission.

It has been proposed that in the South, *I. scapularis* feed more on reptiles than in the North, thereby 'breaking' the Lyme disease transmission cycle in areas with an abundance of lizards. *Ixodes scapularis* from the north and southeast will feed on both lizards and laboratory mice, although there appeared to be a preference for mice (James and Oliver 1990). However, Rogers (1953) showed that immature *I. scapularis* primarily feed on lizards in northern Florida. Apperson, et al. (1993) found that 36.7% of lizards trapped at a site in North Carolina carried *I. scapularis* compared to only 17.8% of mice trapped at the same site. These data suggest that host selection may depend more on host availability than on host preference.

Hypotheses

To increase our understanding of LD risk and *I. scapularis* ecology in Tennessee, several specific hypotheses arising from the knowledge gaps described above were addressed in this study:

H1: Established populations of *I. scapularis* are widespread in Tennessee and, in some habitats, are at densities sufficient to maintain *B. burgdorferi* infection.

H2: Larval questing precedes the peak in nymphal questing in a manner inconsistent with the endemic cycle of *B. burgdorferi* that is seen in northeastern ticks.

H3: Borrelia burgdorferi and other *Borrelia* spp. are cycling in some Tennessee *I. scapularis* populations.

I began this project intending to test the additional hypothesis that *I. scapularis* nymphs and adults show a preference for lizard hosts vs. mammal hosts, to an extent inconsistent with the endemic cycle of *B. burgdorferi* seen in northeastern ticks. However, because lizard densities were extremely low at HHSP this hypothesis was not able to be addressed. The overall goal of this study was to help public health officials and other researchers to better understand the human risk for LD involved with *I. scapularis* and the role of wildlife in perpetuating this risk. This research also aimed to serve as a foundation for subsequent studies involving other pathogens and/or behavior differences among ticks from different regions.

Chapter 2 - Distribution and *Borrelia* spp. status of Adult Blacklegged Ticks on White-tailed Deer in Tennessee

Introduction

The primary host for the adult stage of *Ixodes scapularis* is the white-tailed deer (Wilson, et al. 1990). Therefore, collection of ticks from hunter-harvested deer at check stations provides a useful surveillance method to clarify the regional distribution of this tick species. Deer harvested during the fall hunt also provide a valuable opportunity to gather ticks that can then be tested to investigate the pathogens they may carry (Magnarelli, et al. 1995). It is important to appreciate, however, that white-tailed deer exert a zooprophylactic effect on LD spirochetes and are incompetent reservoirs for the pathogen. Non-infected ticks that feed on deer will consequently fail to acquire spirochetes, and infected ticks will lose their infection during the course of their blood meal on a deer (Telford, et al. 1988). Therefore, the prevalence of infection among ticks on deer will likely underestimate the level of infection among questing ticks.

In a 2006 pilot study, 38 *I. scapularis* were collected from 15 hunter-harvested deer at three check stations in Tennessee, confirming the presence of this tick species in two new counties — Grainger and Loudon (G. Hickling, University of Tennessee, unpublished data). In addition to identifying these new county records for *I. scapularis*, the study also identified four pathogens in these ticks — *Borrelia miyamotoi, Babesia* spp., *Theileria cervi,* and *Anaplasma phagocytophilim,* the latter three being of potential medical or veterinary importance. This chapter uses hunter check-station data to document the distributions and *Borrelia* spp. status of *I. scapularis* across Tennessee as a basis for assessing potential LD risk to humans in the state.

Methods

Tick collection from hunter-harvested deer

An extensive survey of hunter-harvested deer from selected Tennessee Wildlife Resource Agency (TWRA) deer check stations was performed in fall 2007 and fall 2008 to investigate the presence of *I. scapularis* ticks in the areas from which the deer were harvested. County was used as the common geographic unit because hunters are easily able to provide this information. In addition, past tick distribution data are available by county, and at that spatial scale, tick presence has been shown to be correlated with reported human case data (Kitron and Kazmierczak 1997; Dennis, et al. 1998).

The Tennessee Wildlife Resource Agency (TWRA) divides Tennessee into four management regions: West, Middle, East Tennessee, and the Cumberland Plateau. Each region is responsible for managing and collecting deer harvest data from hunters at agency-run check stations. These data are then reported back to the state big game biologist and posted on the agency's website for public access. TWRA biologists are assigned to check stations on the opening day of both the muzzleloader/archery season (first Saturday in November) and the gun/muzzleloader/archery season (third Saturday in November). For this study, these days were: 11/3/2007, 11/17/2007, 11/1/2008, and 11/15/2008. In addition to the state-wide check stations, some special Wildlife Management Area (WMA) quota hunts were sampled, with the data assigned to the county in which the WMA was located (e.g. Catoosa WMA was assigned to Cumberland County). TWRA biologists determined age, sex, weight, and, in some cases collected a bone sample, from the hunter-harvested deer.

Student chapters of The Wildlife Society are contracted each year by TWRA to assist state employees in running and collecting data from these check stations. In 2007, the University of Tennessee-Martin, Tennessee Technological University, and the University of Tennessee-Knoxville student volunteers were available to assist with collecting ticks at the selected check stations. In 2008, self-selected volunteers were used. Check stations were chosen based on TWRA biologist presence, volunteer availability, and anticipated *I. scapularis* distribution.

Each student or TWRA employee volunteering for tick collection watched a short training video (*http://wildlifehealth.tennessee.edu/video/ticks.html*) explaining the purpose of the research and the sampling protocol. Each check station was supplied with the necessary items to gather and store appropriate samples and data.

Volunteers worked alongside the TWRA biologists to search for ticks on deer, remove and store ticks if present, and record county of harvest. On survey days, the volunteers explained the research protocol to hunters and asked if they could examine deer for ticks. The volunteers were instructed to fill in the data sheets and verify the collected information with the hunter. If applicable, volunteers recorded the county region (northwest, northeast, southwest, southeast) or wildlife management area (WMA). However, this was not consistent across check stations, so those data were not used. Additional data collected by TWRA were transferred to the survey data sheets. Volunteers used forceps or a comb to aid in parting the hair, in order to see the skin, and collect any tick or other ectoparasites. The primary search area on the deer was from the head and face, especially around the ears, to just below the scapula (shoulders) on both sides of the animal.

All ticks from one deer were placed in one vial with 70% ethanol and labeled with the date, check station, and deer number (corresponding to the data sheet). If no ticks were present on the deer surveyed, a zero was entered on the data sheet.

Tick identification

All ticks collected were brought to the University of Tennessee's Center for Wildlife Health laboratory in Knoxville and identified to species. All ticks from one deer were separated into multiple vials for each life stage and species (i.e. one vial for adult female *I. scapularis*, one vial for nymphal *Dermacentor albipictus*). Only adult and nymphal *I. scapularis* were photographed for species verification and body length (base of the basis capitulum to the tip of the abdomen) and scutum width (at the widest point) were measured (nearest hundredth mm) for calculation of an engorgement index. Indices were calculated as the ratio of body length to scutum width (Falco, et al. 1996).

Database management

All deer survey data were entered into a Microsoft Excel worksheet with each individual deer assigned one row. After all ticks were identified, number of each species and life stage of tick found on the deer were added to the worksheet. Using ArcGIS 9.3 software, the database was brought into ArcMap 9.3 via ArcCatalogue. The database was then merged with a Tennessee counties shape file modified from United States/Counties.shp (ESRI 2007). Check station addresses were acquired from the TWRA Check Station Information Website (https://hfwa.centraltechnology.net/TNHFInternetHarvest/app/mainCheckingStation.do) and geocoded using *BatchGeoCode.com* and saved as a shape file February 2009.

Jenk's Optimization for natural breaks within ArcGIS (ESRI 2007) was used to determine four critical class intervals when mapping the proportion of deer infested with *I. scapularis* and was thereafter adjusted to fit with the proportion of deer with other tick species. Jenk's Optimization is based on the natural grouping of data and identifies break points that best group similar values. It maximizes the difference between classes while minimizing variance between them. The data collected were not normally distributed and Jenk's Optimization was the best method to classify non-normal data.

Pathogen testing

DNA was extracted from all *I. scapularis* ticks using a modified protocol from the Qiagen DNeasy Blood and Tissue Kit (Appendix 2.1). Nested Polymerase Chain Reaction (PCR) was used to detect the presence of *B. burgdorferi* and other *Borrelia* spp. using the 16s-23s rRNA intergenic spacer of *Borrelia* spp. (Bunikis, et al. 2004; Appendix 2.2). PCR amplicons were visualized by gel electrophoresis on a 1% Tris-acetate-ethylenediaminetetraacetic acid (TAE) high melting point agarose gel at 100V for 1.5 hours. Gels were visualized for 22 sec using the BioRad gel imager machine (Appendix 2.3). Positive samples were extracted from the gel, purified using the Zymoclean Gel DNA Recovery Kit (Appendix 2.4) and submitted for sequencing at the University of Tennessee's Core Sequencing Facility. Sequencing results were entered into the NCBI Blast Database and compared to known sequences for identification.

Some samples were screened using a QIAxcel machine (Quiagen, CA), which is a multicapillary electrophoresis instrument designed for automated DNA fragment analysis. Samples analyzed on this machine were run with a 15bp to 5kb alignment marker (Cat.

No. 929524) and a 100bp to 3kb size marker (Cat. No. 929553) using method AM420 (Qiagen 2008) with 30 sec additional separation time. New size markers were added to each new PCR plate and diluted to 25ng/µl using 1x PCR buffer solution. When analyzing samples, the positive threshold was set to 4% of the tallest peak. Any bands in the molecular size range of *Borrelia* spp. were considered to be positive on the QIAxel and were re-run on a traditional gel using the previously described protocol.

This assay is known to amplify multiple *Borrelia* spp., allowing us to detect pathogens other than *B. burgdorferi* that were potentially present in the ticks. Because EM rashes can be caused by tick-associated pathogens other than *B. burgdorferi,* it was relevant to assess whether any additional *Borrelia* spp. were present in the sampled ticks.

As a further check on our DNA extraction procedure, an additional PCR that amplified the mitochondrial 16S rRNA gene (Black and Piesman 1994) was run on all samples to verify DNA extraction (Appendix 2.5). For samples where this amplification was not successful, the original extraction product was diluted 1:10 and re-run both for *Borrelia* spp. and for mitochondrial DNA. For the small number of samples where no amplification of the mitochondrial DNA could be achieved, the *Borrelia* status of the sample was recorded as undetermined. In addition to the two traditional PCRs described above, a third diagnostic assay employing quantitative PCR, targeting 23s rRNA gene, was run at Michigan State University's Insect Microbiology Laboratory on all samples from 2007 (S. Hamer, Michigan State University, unpublished data).

Data analysis

Comparisons of the proportions of ticks and proportion of *I. scapularis* found on deer, and TWRA management regions were made using a chi-square test of association to determine if there was significant regional variation in blacklegged tick infestation of Tennessee deer. A Kruskal-Wallis one-way nonparametric ANOVA was used to compare the mean number of adult *I. scapularis* found on individual deer among regions.

The probability of detecting *I. scapularis* in each county was calculated based on (Thrusfield 1995) using the equation: $\mathbf{p_1 = 1 - (1 - (n /(N - d/2))^{\wedge}d}$

where **p_1:** probability of finding at least one tick-infested deer
n: no. of deer checked
N: no. of deer in County (D. Ratajczak, TWRA, personal communication)
d: no. of infested deer in County (= **0.15*N**, assuming 15% prevalence)

Engorgement was expected to decrease the likelihood of detecting *Borrelia* spp. in the ticks (through zooprophylaxisis or PCR inhibition), so a chi-test of association was used to determine if the outcome of the DNA extraction was influenced by ticks' level of engorgement.

Results

Check station coverage

Volunteers surveyed deer at 47 check stations (Figure 2.1), during November and December 2007 and 2008 (Appendix 2.6). A total of 1,018 deer was inspected, spanning 71 counties and all four TWRA management regions. The deer inspected ranged from 0.02% (Wayne, Weakley, and Hickman counties) to 15.0% (Anderson County) of the total deer harvested per county in those months (Appendix 2.7). There was relatively little overlap of survey effort in the two years, so the two years of data were pooled for analysis.

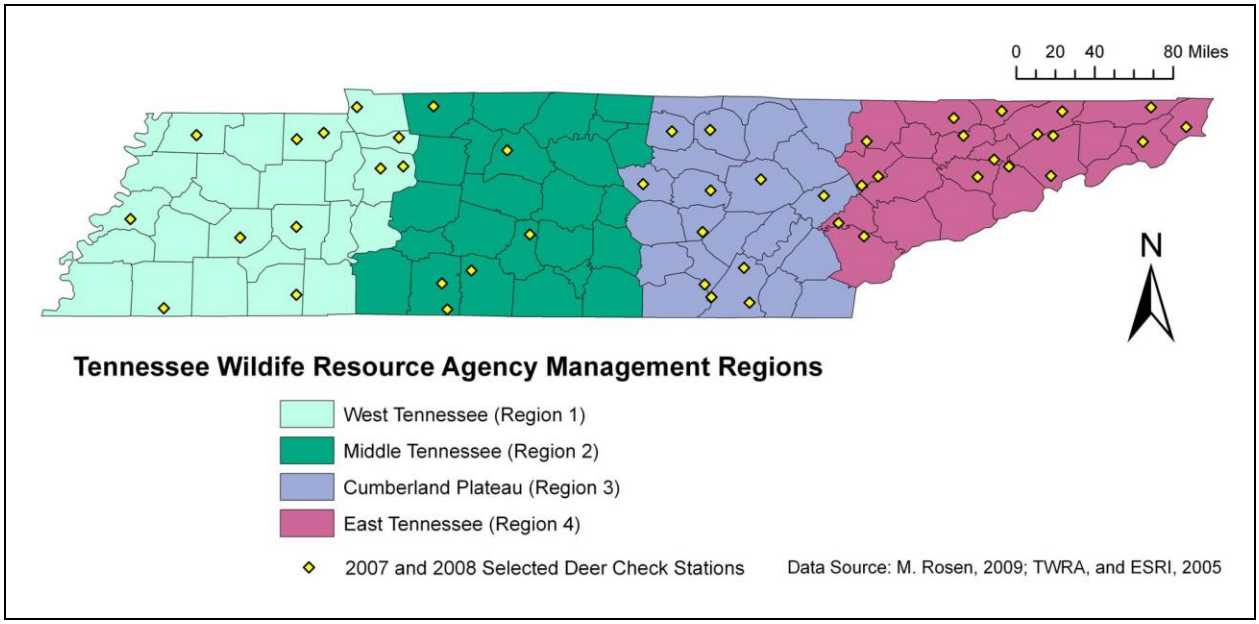

Figure 2.1: Locations of the four TWRA management regions and the 47 check stations in Tennessee where deer were inspected in 2007 and/or 2008.

Tick infestation and distribution

Of the 1,018 deer checked, 464 (45.6%) were infested with ticks. Cumberland Plateau (Region 3) had the highest infestation of ticks on deer (72.0%), followed by Middle Tennessee (61.3%), West Tennessee (35.4%) and East Tennessee (11.1%; Table 2.1). This difference in the percent infestation of ticks among regions was statistically significant (Table 2.1, $P < 0.0001$).

In total, 4,237 ticks of three species were collected: *I. scapularis* (on 15.7% of deer inspected), *D. albipictus* (36.2%), and *A. americanum* (4.7%; Table 2.1; Table 2.2). *D. albipictus* was by far the most abundant tick found on deer across the state (368 deer) and the most abundant tick found on individual deer (Figure 2.2a). *Amblyomma americanum* was the least abundant tick found on deer in the fall (Figure 2.2b), which was not unexpected as this is a summer-active species. A total of 160 deer (15.7%) was infested with *I. scapularis*, with infestation being most common in Middle Tennessee (45.0% of deer), followed by Cumberland

Table 2.1: Regional distribution of deer surveyed at fall check stations in Tennessee in 2007 and 2008, the number of deer parasitized and the percent infestation prevalence by three tick species. Oak Ridge Wildlife Management Area, which is partly in Anderson County, spans TWRA Regions 3 and 4 and so is included here as a separate area.

		Ticks*		***I. scapularis***		***D. albipictus***		***A. americanum***	
Region	**No. Deer Surveyed**	**Deer**	**%**	**Deer**	**%**	**Deer**	**%**	**Deer**	**%**
1	178	63	35.4%	13	7.3%	50	28.1%	9	5.01%
2	80	49	61.3%	36	45.0%	22	27.5%	13	16.3%
3	214	154	72.0%	70	32.7%	110	51.4%	11	5.1%
4	371	41	11.15%	14	3.8%	30	8.1%	3	0.8%
Oak Ridge	175	157	89.7%	27	15.4%	156	89.1%	12	6.9%
Total	**1018**	**464**	**45.6%**	**160**	**15.7%**	**368**	**36.2%**	**48**	**4.7%**

*chi-square test for association between regions; $\chi^2 = 391.2$, 4 d.f., $P < 0.0001$
**chi-square test for association between regions; $\chi^2 = 147.9$, 4 d.f., $P < 0.0001$

Table 2.2: Number and species of ticks removed from deer surveyed during November and December, 2007 and 2008, at fall check stations in Tennessee.

	Adult Female	**Adult Male**	**Nymph**	**Larvae**	**Total**
I. scapularis	492	379	0	0	871
D. albipictus	772	1193	1310	21	3296
A. americanum	10	55	3	2	70
Total	**1274**	**1627**	**1313**	**23**	**4237**

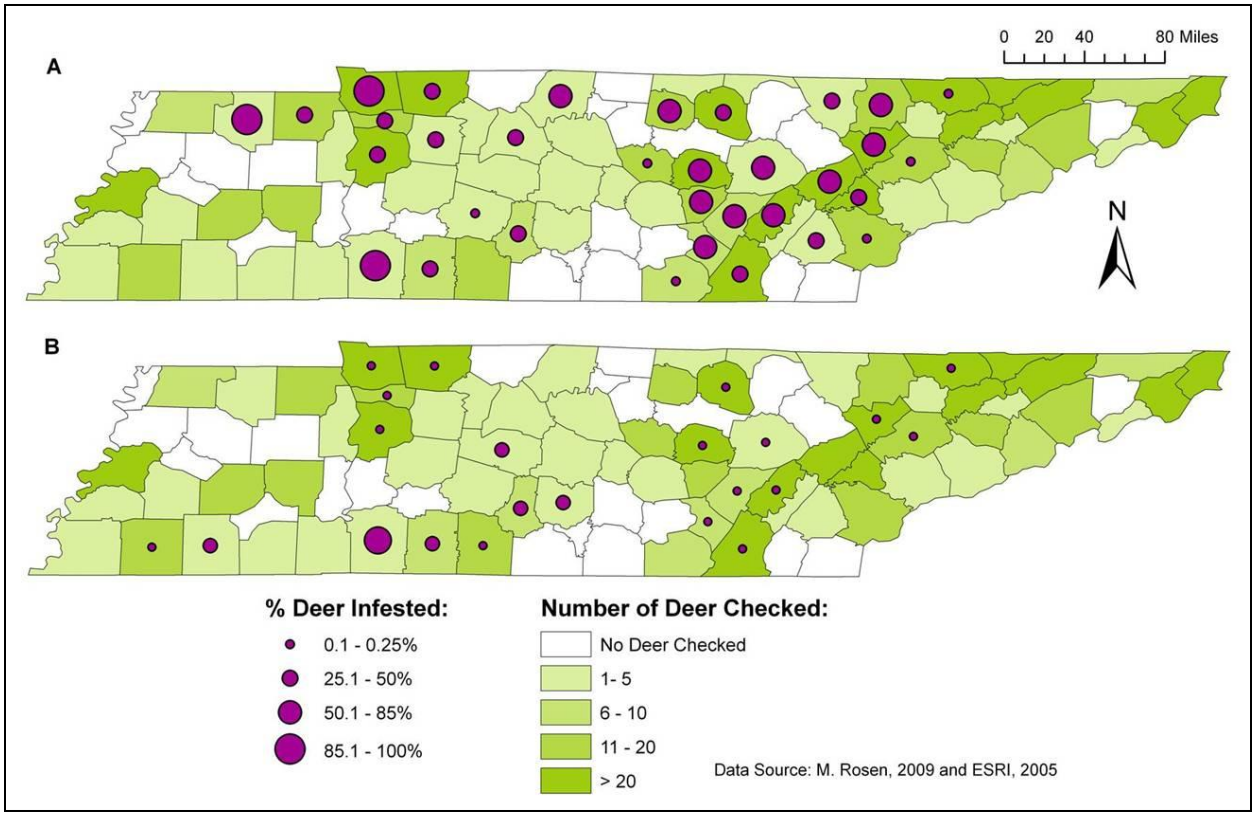

Figure 2.2: Distribution of (a) *Dermacentor albipictus* (Winter tick), and (b) *Amblyomma americanum* (Lone Star tick) from the 2007 and 2008 deer check surveys in Tennessee (N= 1,018 deer inspected).

Plateau (32.7%), West Tennessee (7.3%) and East Tennessee (3.77%; Table 2.1, Figure 2.3).

Ixodes scapularis

Deer infested with *I. scapularis* ticks were found in all four TWRA management regions (Figure 2.3) although there was a significant difference in percent infestation of deer among the different regions (Table 2.1; chi-square test of association; $\chi^2 = 147.1$, 4 d.f., $P < 0.0001$). The probably of detecting at least one *I. scapularis* infested deer, in counties where deer were checked (N= 71), ranged from 11% (Benton County) to ~100% (Anderson County) with the mean probability being 62.9% (Appendix 2.8; Figure 2.3). It is notable that *I. scapularis* was not detected in some heavily surveyed counties (Carter, Greene, Hancock, Hawkins, Jefferson,

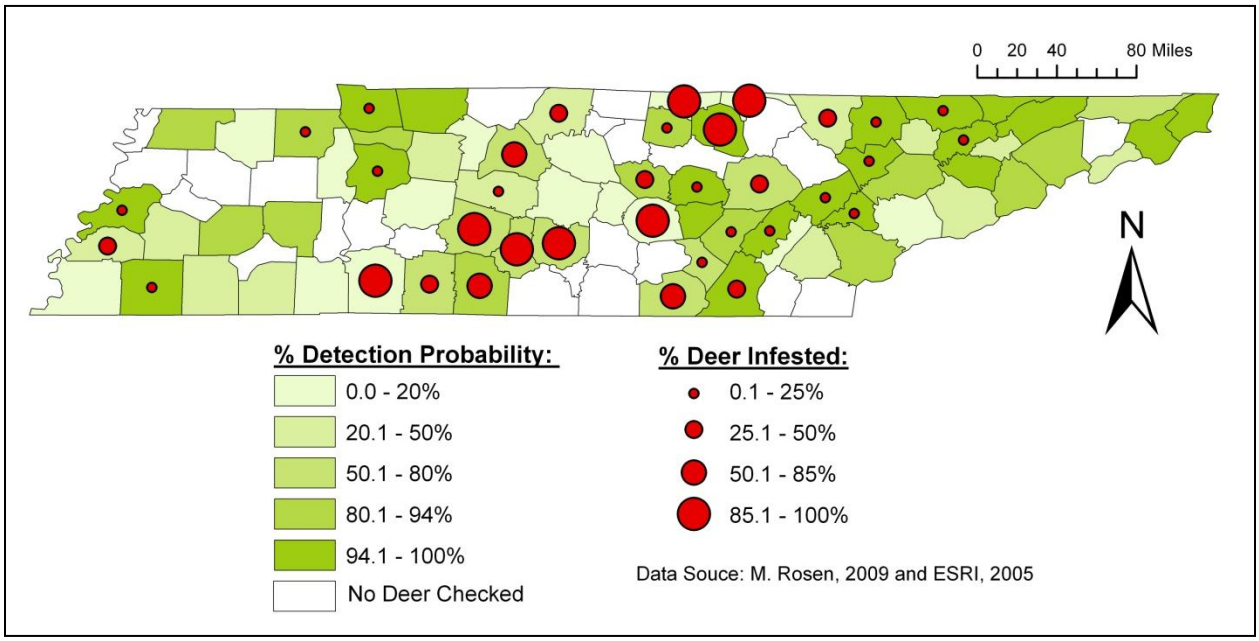

Figure 2.3: *Ixodes scapularis* distribution, percent deer infestation, and detection probability for the 2007 and 2008 deer check surveys in Tennessee (N = 1,018 deer inspected; median number per checked county = 8, range = 1 to 196).

Johnson, Monroe Counties; >90% confidence), and yet was detected in some sparsely surveyed counties (Bedford, Clay, Maury, Pickett, Sumner, Warren, Wayne; <50% confidence). This implies that the distribution of *I. scapularis* is heterogeneous – i.e., there is not a uniform 15% infestation of deer across the state. Figure 2.3 demonstrates that *I. scapularis* is more abundant on deer in Middle Tennessee (Region 2) than in other parts of the state. Overall, these ticks are clearly far more widespread and abundant throughout Tennessee than previously recognized.

The mean number of *I. scapularis* ranged from 10.75 adult ticks per individual deer (5.86 female; 4.89 male) in Middle Tennessee to 1.86 adult ticks per deer (1.29 female; 0.57 male) in East Tennessee (Table 2.3). The number of adult *I. scapularis* found on the infested deer differed significantly among regions (Table 1.1, Table 2.3).

Ixodes scapularis was confirmed present in 35 counties during this study—30 are new records (Figure 2.4).

Pathogen status of *I. scapularis* collected from deer

PCR was performed on 883 *I. scapularis* (502 females, 381 males) collected from deer

Table 2.3: Mean numbers of *I. scapularis* found on *I. scapularis*-infested deer surveyed during November and December, 2007 and 2008 at check stations in Tennessee.

		Mean ± SE		
Region	**N**	**Female[1]**	**Male[2]**	**Adult[3]**
1	13	1.00±0.16	1.15±0.37	2.15±0.48
2	36	5.86±0.86	4.89±0.83	10.75±1.63
3	70	3.10±0.44	2.20±0.37	5.30±0.78
4	14	1.29±0.32	0.57±0.17	1.86±0.43
Oak Ridge	27	1.22±0.25	0.96±0.18	2.18±0.37
Total	**160**	**3.08±0.31**	**2.37±0.28**	**5.44±0.56**

[1] Kruskal-Wallis one-way nonparametric ANOVA; H = 36.91, 4 d.f., p < 0.0001
[2] Kruskal-Wallis one-way nonparametric ANOVA; H = 31.79, 4 d.f., p < 0.0001
[3] Kruskal-Wallis one-way nonparametric ANOVA; H = 42.36, 4 d.f., p < 0.0001

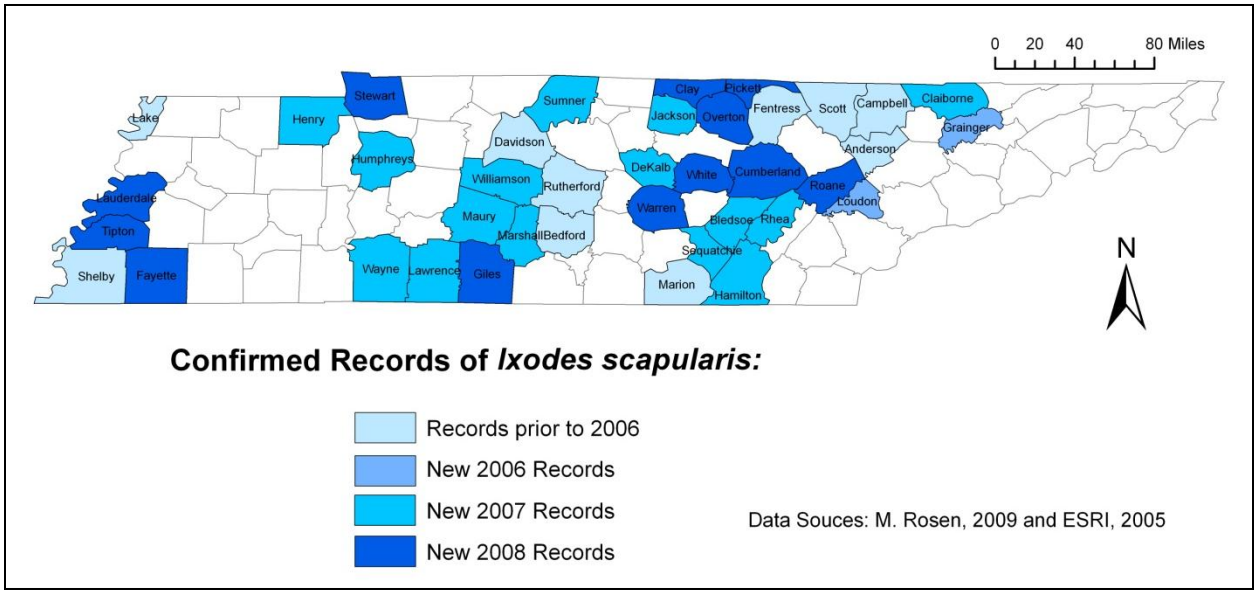

Figure 2.4: Updated distribution map for *I. scapularis* in Tennessee.

throughout Tennessee. These comprised the 871 *I. scapularis* listed in Table 2.2 plus 12 *I. scapularis* removed from deer that could not be assigned a county location. All ticks from deer tested positive for the mitochondrial 16S rRNA gene (indicating successful extraction of DNA) and 833 were sufficiently intact for an engorgement index to be calculated. Of the 833 ticks measured for engorgement, 40 (4.8 %) required a 1:10 dilution of the original DNA to produce a positive 16S result. There was no indication that a tick's level of engorgement influenced whether dilution was required to achieve positive amplification of the tick 16S mitochondrial gene (Table 2.4; $\chi^2 = 0.80$, 1 d.f., $p = 0.37$).

Borrelia burgdorferi *prevalence*

Borrelia burgdorferi was not found in any of the 883 adult *I. scapularis* tested. As a confirmatory test, 431 (247 female, 185 male) *I. scapularis* collected in 2007 were re-tested using quantitative PCR – again, no *B. burgdorferi* was detected. We expected *a priori* that

Table 2.4: DNA extraction outcomes related to tick engorgement indices (mean index = 3.13±0.06 SE).

1: 10 dilution required to extract DNA?	No. of ticks with above-average engorgement index	No. of ticks with below-average engorgement index	Mean engorgement index (±SE)*
Yes	18	22	3.75 ± 0.31
No	301	492	3.10 ± 0.06
Total*	319	514	3.13 ± 0.06

* Excluding 50 adult *I. scapularis* that were not sufficiently intact to measure scutum width and/or body length.

B. burgdorferi would be less likely to be found in engorged ticks because of the zooprophylactic effect of deer complement – in our sample 33.6% of ticks showed some degree of engorgement (E.I. > 2), however only 15.5% were heavily engorged (E.I. > 5; see Figure 2.5).

Borrelia miyamotoi *and other pathogens*

Borrelia miyamotoi was detected in one adult female *I. scapularis*; the sample was sequenced with a 262 base pair fragment that when BLASTed was 100% similar to *B. miyamotoi* as described by Bunikis, et al. (2004): NCBI accession: AY363706. The infected tick came from a 1.5 year-old male deer harvested in Overton County (Region 3) on 22 November 2008. No engorgement index was available for the tick as it was not collected intact, however from the photographic record the tick appeared to be no more than moderately engorged. An additional 13 ticks (all *I. scapularis*; 4 females; 9 males) were collected from this same deer – none tested positive for *Borrelia spp.*

Borrelia lonestari was detected in one adult female *I. scapularis* from a deer from Giles County (Region 2). The sample was sequenced to a 394 base pair fragment and was 100% similar to *B. lonestari* submitted by Bunikis, et al. (2004): NCBI accession AY363709. The infected tick was fully engorged (EI = 12.3). This 2.5 year-old male deer was harvested

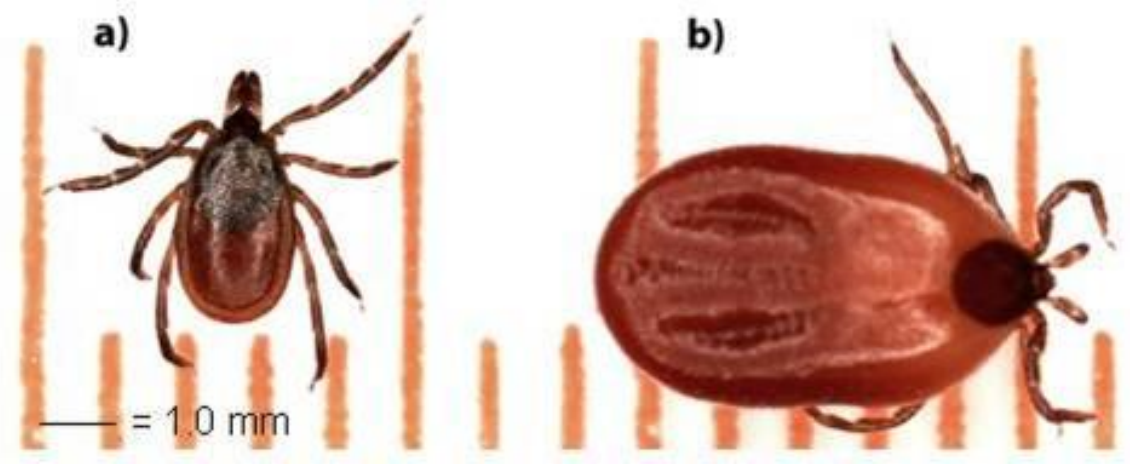

Figure 2.5: Adult female *I. scapularis* collected from white-tailed deer in Tennessee; a) slightly engorged, EI = 2.2; b) heavily engorged, EI = 5.1.

on 1 November 2008 in central Giles County (Region 2). An additional 11 ticks (all *I. scapularis;* 5 female; 6 male) were collected from the same deer – none tested positive for *Borrelia* spp.

Discussion

Tick distribution and abundance

Ixodes scapularis *distribution*

We inspected one or more deer from 71 of Tennessee's 95 counties, and recorded *I. scapularis* in 35 of those counties. It is likely that *I. scapularis* are present on deer in some of the 41 counties not surveyed (e.g., Fentress, Lincoln, Moore, and Putnam) and also in counties where we inspected minimal numbers of deer (e.g., only 1 deer each in Cannon, Rutherford and Wilson). Consequently, our present finding that 50% of the Tennessee counties surveyed

support *I. scapularis* likely underestimates the true state-wide distribution of this tick. Nevertheless, ticks were not found in some counties where a robust sample size of deer were inspected (e.g., 57 deer in Hawkins county, 29 deer each in Hancock and Johnson Counties) suggesting that there are some parts of Tennessee where these ticks are indeed absent or at very low abundance.

The distribution of *I. scapularis* in Tennessee is clearly greater than previously reported. The current published distribution of *I. scapularis* lists only ten counties in Tennessee: Anderson, Bedford, Campbell, Fentress, Davidson, Lake, Marion, Rutherford, Scott, and Shelby Counties (Durden and Kollars 1992; Dennis, et al. 1998). These earlier distribution maps were derived from sparse, passively collected, non-standardized data. Therefore the expanded distribution shown in Figure 2.4 reflects, in part, our improved surveillance effort.

The two previous studies documenting the presence of *I. scapularis* in Tennessee are between 11 and 17 years old. Due to changes in land use, wildlife distribution, climate, and other factors influencing the distribution of tick vectors, it is likely this distribution has changed. In some cases, it is possible that *I. scapularis* present in a county were not documented due to lack of search effort or sampling time. In this study, our probability estimate for detecting *I. scapularis* was greater than 80% in 49% (35 of 71) of counties searched. Therefore we are confident in our ability to detect *I. scapularis* provided they were present on deer at >10% levels of infestation. We also provide data on which counties were searched and how well they were searched, which provides information on areas where *I. scapularis* may not be present.

Despite these caveats, we consider it likely that the large number of new confirmed counties reflects true expansion of this tick's distribution. For example, Gerhardt, et al. (University of Tennessee, personal communication) have been regularly surveying tick

populations at a golf-oriented retirement community in Cumberland County since the early 1990's and have not collected *I. scapularis* in over a decade of sampling. However, this study identified *I. scapularis* on deer harvested close to that retirement community and our research group dragged adult *I. scapularis* there in spring 2009 (J. Harmon, University of Tennessee, unpublished data).

Ixodes scapularis *abundance*

On average, 15.7% of wild deer in Tennessee were infested with *I. scapularis* in the fall, although the numbers of ticks found on individual deer were generally low (mean = 5.4 ± 0.6SE, median = 3, range 1 – 44).

There was significant variation in the proportions of deer infested with *I. scapularis* in different parts of Tennessee, with the tick being most prevalent in Middle Tennessee. Based on spatial analyses of *I. scapularis* distribution in other parts of the U.S. (Brownstein, et al. 2005; Diuk-Wasser, et al. 2006), this variation likely reflects the ticks' association with certain preferred microclimates, vegetation, land uses, elevations, soil types, precipitation, and other similar factors. Fine-scale investigation would be expected to reveal these habitat effects in Tennessee, however given our uneven search effort we confine our discussion of *I. scapularis* distribution to the county level.

One useful source of information on county-level 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 both abiotic and biotic factors that influence ecosystem characters – including geology, physiography, vegetation, climate soils, land use, wildlife and hydrology

(Griffith, et al. 1997). Comparison of our deer infestation prevalence data with a county-level map of Tennessee's level III ecoregions (Figure 2.6) indicates that *I. scapularis* is most abundant on deer in the Interior Plains (TWRA Region 2: Middle TN), an ecoregion characterized by areas of low elevation. Hills and plains in this ecoregion are composed of a diverse mixture of sandstone, siltstone, and shale. Natural vegetation in this region is primarily 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. 1997).

Conversely, *I. scapularis* ticks were least abundant in the Blue Ridge Mountains ecoregion, and in the eastern parts of the Ridge and Valley ecoregion, suggesting avoidance of higher-elevation habitats as has been reported elsewhere (Jouda, et al. 2004). *Ixodes scapularis* was also largely absent from the Southeastern Plains ecoregion, perhaps because agricultural land use dominates in this part of the state. Temperature, precipitation, and deer abundances could also have a role in tick distribution. Temperature and precipitation means are relatively uniform throughout the state, with the exception of the eastern most ecoregion, which is cooler and wetter. Presently there is no reliable map of deer distribution and population in Tennessee.

Nationally, it is clear that this tick species adapts to highly diverse habitats, ranging from conifer, boreal forests to subtropical areas (Dennis, et al. 1998). Brownstein, et al. (2005) showed that there was a significant relationship between landscape structure, climatic factors, and the density of *I. scapularis.* A more detailed habitat suitability index model should be applied to Tennessee, specifically looking at land cover, temperature, precipitation, soil type, and host abundance. Analysis of this tick's ecological associations would serve as a guide for additional studies on other pathogens like *Ehrlichia* spp. and *Babesia* spp. that are transmitted by *I. scapularis* (Dennis, et al. 1998).

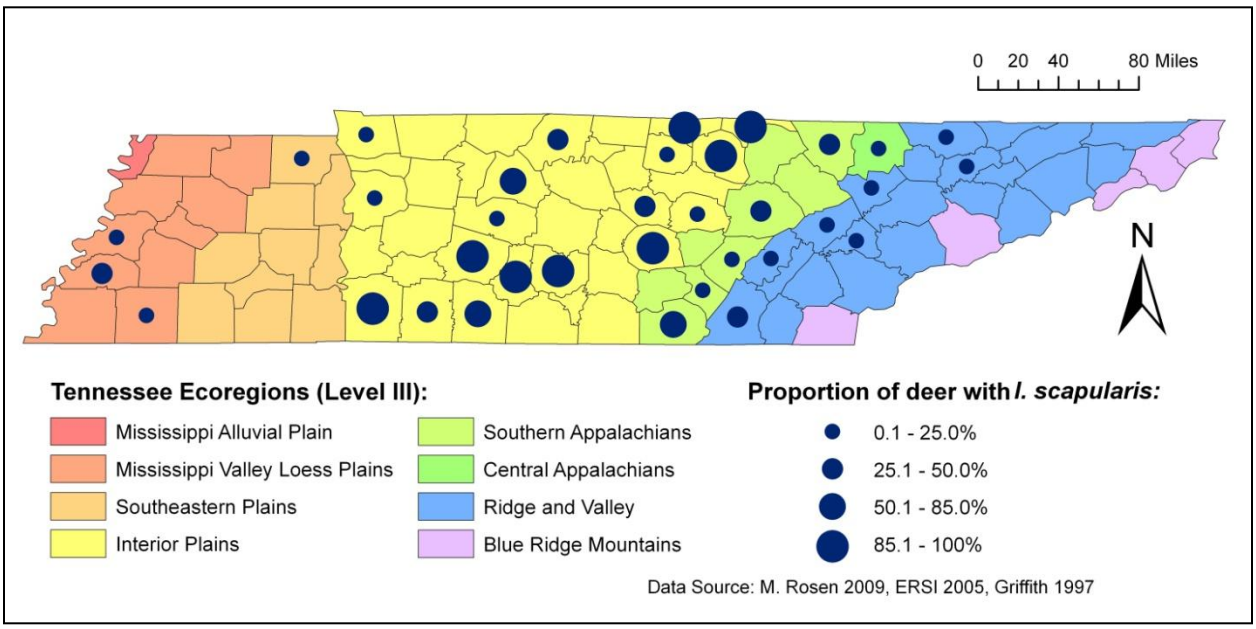

Figure 2.6: Proportion of deer infested with *I. scapularis* compared with Tennessee's Level III ecoregions. Each county was assigned its dominant ecoregion, based on Griffith's (1997) Level III map.

Other tick species

Dermacentor albipictus and *A. americanum* were found on numerous deer from across the state. *Amblyomma americanum* was the least abundant of the tick species removed from deer in the fall. The minimal number of *A. americanum* on deer during our surveys is unsurprising, given its seasonal phenology in Tennessee: adults are most active from April to early July, nymphs in April/May and then again in August/September, and larvae from late June to October (Bloemer, et al. 1988; Marsland 1997; Kollars, et al. 2000; Goddard 2007). *Amblyomma americanum* is not a competent vector for *B. burgdorferi* as it has a borreliacidal effect on the spirochetes (Piesman and Happ 1997; Ledin, et al. 2005).

Dermacentor albipictus was very common on Tennessee deer in the fall – our results for this tick are similar to other recent studies (Cortinas and Kitron 2006). *Dermacentor albipictus*

is a one-host tick, meaning that after larvae attach to a host, all subsequent life stages are completed on that host. Its primary hosts are members of the family Cervidae and so this tick is found throughout the range of white-tailed deer (Baldridge, et al. 2009). Because *D. albipictus* is a one host tick, it rarely feeds on other hosts and transmission of pathogens to a new host is minimal.

Limitations to these distribution and abundance data

On opening day of muzzleloader and gun season, TWRA biologists are required to collect biological information at higher throughput check stations; these stations are unequally distributed throughout the state. These stations are more efficient to use as survey locations because (1) more deer are seen throughout the day and (2) hunters are required to stay while biological information is collected providing an excellent opportunity to survey deer for ticks.

In addition to biologist presence at scattered check stations, uneven volunteer contribution is another explanation for the unequal survey effort. The student chapters of The Wildlife Society are contracted with TWRA to aid biologist as check stations. However, their main task is to aid biologists, not search for ticks. Time allocated for tick searching varied between check stations and volunteers; this created a difference in the number of deer surveyed at a check station (in addition to the difference in number actually brought to each station) and how thoroughly, accurately, and completely each deer was surveyed.

Based on the unequal search effort at multiple levels, we can only conclude and determine the presence of *I. scapularis* at the county level. If no ticks were found in counties where multiple deer were checked, we can only conclude that this tick may or may not be present.

Pathogen status of ticks on deer

Finding *Borrelia* spp. other than *B. burgdorferi* in our tick sample emphasizes the importance of using species-specific pathogen probes, and the need to carry out sequencing and/or culture as a confirmatory step for any PCR-based analysis of pathogen status of these ticks and hosts. Both *B. miyatotoi* and *B. lonestari* are part of the relapsing fever group of *Borrelia,* a separate group from the Lyme disease *Borrelia* (Bunikis, et al. 2004). Little is known about the etiology of these two spirochetes, and their pathogenicity to humans is unknown. *Borrelia lonestari* was tentatively implicated as the causative agent for Southern Tick Associated Rash Illness (James, et al. 2001), however this is no longer believed to be the case (CDC 2008).

Shariat, et al. (2007) reported 6 of 18 (33%) pooled *D. ablipictus* samples from Cheatham County[1] as being infected with *Borrelia* spp. She reports four (22%) of these samples as being *B. lonestari* and the other two (11%) as *B. burgdorferi*. Two individual deer were implicated for each pathogen (4 of 18 deer). These two *B. burgdorferi* samples were *i)* 8 nymphs from four separate deer — two on each, and *ii)* engorged adults from two individual deer — two from one deer and one from the other deer (n=3). These findings need to be interpreted with caution, as the primers and probes used by Shariat, et al. (2007) for PCR amplification and hybridization of *B. burgdorferi* have the potential to bind to multiple species of *Borrelia* (unpublished data).

Non-vector competent tick species do at times test positive for *B. burgdorferi* (Luckhart, et al. 1992; Piesman and Happ 1997; Schulze, et al. 2006) and several other studies have reported ticks removed from deer as being infected with this pathogen (Lacombe, et al. 1993; Magnarelli, et al. 1995). In one study on deer in Alabama, *D. albipictus* was recovered but

[1] In this present study, only one deer, with no ticks found, was checked from Cheatham County.

Borrelia spp. was not detected by IFA, however it was detected in one male *I. scapularis* from that same deer (Luckhart, et al. 1992). In another Alabama study, 68 *D. albipictus* and 39 *D. variablis* tested negative by IFA and DFA for *B. burgdorferi*, while 3% of *I. scapularis* (5 of 165) and 4% of *A. americanum* (6 of 150) tested positive (Luckhart, et al. 1991). In LD endemic areas of Connecticut, where 10.5% of *I. scapularis* ticks tested positive, only 0.6% (1 of 157) *D. albipictus* tested positive by fluorescein isothiocyanate-labeled rabbit antibodies to *B. burgdorferi* (Magnarelli, et al. 1986).

In 2006, *B. miyamotoi* was detected in 15 of 36 (42%) adult *I. scapularis* collected opportunistically from deer at three east Tennessee check stations in Anderson, Grainger and Loudon (TWRA Region 4; G. Hickling, University of Tennessee, unpublished data). However, in my study, in 2007 and 2008, 883 adult *I. scapularis,* collected from across the state, were tested for *Borrelia* spp. and only one adult female collected in 2008 tested positive — none of the other 13 *I. scapularis* removed from that same deer tested positive. Thus the prevalence of this pathogen in Tennessee ticks appears to be highly variable in both time and space.

Little is known about the etiology of *B. miyamotoi,* other than it has the potential to be transmitted to humans and it has been recorded in all three vectors of *B. burgdorferi* (Mun, et al. 2006). We are not aware of any published data on its pathology in humans or other animals. This spirochete has been reported from *I. ricinus* in Europe, *I. scapularis* in the eastern U.S. and *I. pacificus* in the western U.S (Mun, et al. 2006). Mun, et al. (2006) report a 0.7%-1.7% infection prevalence for host-seeking adult and nymphal *I. pacificus*, similar to what is reported for host-seeking *I. scapularis* (1.9%-2.5%) in the eastern U.S. However, in my current study area the proportion of infected ticks collected from deer was locally high in 2006, raising the possibility that deer may be reservoirs for *B. miyamotoi.* One possible factor contributing to this

pathogen vanishing from our survey in 2007 and 2008 was a state-wide die off of deer in 2007 due to an outbreak of Epizootic Hemorrhagic Disease (EHD) exacerbated by the 2007 drought (TWRA 2009).

Implications for national Lyme disease risk and risk maps

The reported LD case rate in Tennessee is very low compared to endemic areas of the northeastern U.S. Previously, this has been thought due to an absence of *I. scapularis* throughout much of the state. Our findings suggest, however, that *I. scapularis* are in fact widespread in Tennessee, although at relatively low abundance and without measurable infection with the LD pathogen. This latter finding is surprising – it is unusual to find established *I. scapularis* tick populations without concurrent *B. burgdorferi* infection. Tick bite data for humans in Tennessee indicate that most bites are from *A. americanum* (the Lone Star tick) and very few are from *I. scapularis* (E. Stromdahl, U.S. Army Center for Health Promotion & Preventive Medicine, personal communication). One possible explanation for this pattern is that southern *I. scapularis* prefer other hosts to humans and have different questing behaviors compared to that of northern populations of this species. Another possibility is that *I. scapularis* numbers are on the increase, so that bites by these ticks will become more common in coming years. If so, and if *B. burgdorferi* becomes established in these tick or reservoir populations, there would be implications for human health.

At a national level, one approach taken to help provide the public with information and increase awareness has been the creation of national LD "risk maps" (CDC 1999). The CDC's current national LD risk map is based on information on vector distribution, abundance, *B. burgdorferi* infection prevalence, and human exposure. This has been compiled by county to

generate four LD risk categories — high, moderate, low, and minimal to no risk. The vector distribution data used were from Dennis, et al. (1998) which identifies *I. scapularis* as 'reported' in a county if at least one tick is collected from any time period, and 'established' if six or more ticks of one life stage, or two of the three life stages, are collected in a single visit. However, information from counties that were sampled and did not produce *I. scapularis* was purposely left out (Dennis, et al. 1998; CDC 1999) and subsequently went under the category of "absence of ticks or missing data." Therefore risk may be underestimated in counties for which no data were available. The CDC recognized this flaw and used a neighborhood analysis procedure in ArcGIS that smoothed absent data and minimized reporting gaps (CDC 1999).

The nymphal stage of *I. scapularis* is believed to be the only stage with a significant role in LD transmission to humans. Drag sampling provides a useful estimate of the likelihood that humans will come into contact with these vector nymphs (Piesman 2002). Mapping the spatial and temporal distribution of host-seeking nymph densities collected by drag sampling can therefore provide a measure of disease risk to humans if the maps are combined with information on the ticks' pathogen status. Diuk-Wasser, et al. (2006) report on a large survey that mapped the spatial and temporal distribution of host-seeking nymphs across the eastern half of the U.S. Because of random sampling, however, Tennessee was void of any sampling sites in this survey. Furthermore, the seasonal timing of their sampling – between May and August – may have missed the peak questing period of nymphs in southern states (Durden and Oliver 1999), so this survey likely underestimated nymphal abundance in states surrounding Tennessee.

Earlier studies on the distribution of *I. scapularis* in Tennessee used passive, non-standardized data to determine the distribution of the tick. The next step in determining the human health risk of LD in Tennessee should be to undertake additional fall surveys of deer in

counties not sampled adequately during this study. We also recommend studies that investigate other LD risk factors, tick abundance, host interactions, and pathogen status, similar to studies undertaken in other states (for example Schulze, et al. (1991) and Guerra, et al. (2002)).

Conclusion

This chapter provides updated distribution maps for *I. scapularis* in Tennessee and highlights the apparent absence of the LD pathogen among these tick populations. Confirming the presence of this tick (in thirty new counties) emphasizes its endemic status within the state. Further tick and pathogen surveys at additional sites not sampled here are needed to more fully understand LD risk in Tennessee.

Chapter 3 - Seasonal Phenology of *I. scapularis* ticks in Middle Tennessee

Introduction

Tick phenology is critical to the epidemiology of tick-borne disease, as seasonal population dynamics impact the transmission of many tick-borne pathogens (Randolph, et al. 2002). Sampling of small mammals and lizards is a sensitive way to investigate the larval and nymphal life stages of *I. scapularis*, in part because immature *I. scapularis* are difficult to sample from vegetation (Piesman 2002). Sampling the blood and tissue of these host species can also provide information on *B. burgdorferi* infection in the ticks. Meanwhile, vegetation dragging for questing ticks provides a useful measure of epidemiological risk -- i.e. the number of questing ticks available to bite humans, and their pathogen prevalence (CDC 1999).

In Tennessee and other southeastern states, *I. scapularis* are thought to be much more difficult to sample from vegetation using standard 'dragging' techniques than is the case for northern populations of this tick (Tedders 1994; Clark, et al. 1998). This may reflect lower population densities and perhaps behavioral differences between *I. scapularis* populations in the north and south. We anticipated that the success of vegetation dragging could be improved by targeting areas with high densities of adult *I. scapularis* identified from the fall deer/tick collection survey described in Chapter 2. One such area was in the vicinity of Henry Horton State Park (HHSP) in Marshall County, where all deer checked in fall 2007 were found to be infested with *I. scapularis* – this chapter presents the finding of a 19-month investigation of *I. scapularis* ecology and phenology at HHSP.

Methods

Study site

Henry Horton State Park (N35° 35' 55"; W86° 41' 58") is located 40 miles south of Nashville, TN on U.S. Hwy 31A between the towns of Chapel Hill and Lewisburg, Tennessee (Figure 3.1). The 1,140-acre park contains a golf course, campgrounds, and three hiking trails (Wild Turkey, Hickory Ridge and Wilhoite Mill) traversing forested areas. The park is located within the Interior Plateau (Level III), Inner Nashville Basin (Level IV) ecoregion of Tennessee. This ecoregion is characterized by outcrops of Ordovician-age limestone, lower gradient streams than surrounding areas, often flowing over large expanses of limestone bedrock. Parts of the park are rocky with sink holes and deep crevices. The park is dominated by an oak-hickory forest with areas of bluestem prairie and cedar glade. The most characteristic hardwoods within the inner basin are a maple-oak-hickory-ash association (Griffith, et al. 1997). The limestone cedar glades of Tennessee are a unique mix of grassland/forest/cedar glades vegetation type with many endemic species and are located in this ecoregion. Residential and commercial land use is increasing in the surrounding area (Griffith, et al. 1997). A number of vertebrate species seen in the park -- including wild turkeys (*Meleagris gallopavo*), white-tailed deer (*Odocoileus virginianus*), gray squirrels (*Sciurus carolinensis*), Eastern cottontails (*Sylvilagus floridanus*), raccoons (*Procyon lotor*), and Virginia opossums (*Didelphis virginiana*) -- may play a role in *I. scapularis/B. burgdorferi* dynamics but were not sampled by our methods.

Figure 3.1: Typical forest habitat on Wild Turkey trail at Henry Horton State Park, Tennessee, in August 2008. Pitfall traps and a drift fence for lizards were installed at this site, near transect B.

Climate records

Monthly average temperature and precipitation data from the Shelbyville climate station, the closest climate station, were obtained for the past 30 years (to May 2009) from the National Oceanic and Atmospheric Administration (NOAA) website at *http://www.weather.gov/climate/xmacis.php?wfo=ohx* (accessed June 2009).

Vegetation dragging

Standardized sampling of ticks questing on vegetation was conducted by dragging 3500m^2 of forested habitat at HHSP at monthly intervals from November 2007 to May 2009. Seven 250m drag transects originating from Wild Turkey trail (N = 4) and Hickory Ridge trail (N = 3) were established. Drag sampling involved walking out and back on each transect (500m total) while dragging a 1x1m corduroy cloth that was checked for attached ticks every 20m

(Figure 3.2). Ticks were removed with forceps and stored by transect in vials containing 70% ethanol. Drag locations were varied by a few meters on each outward and return leg to avoid resampling the same vegetation.

Small mammal and lizard trapping

Small mammals and lizards were live trapped to determine the host preference and relative abundance of ticks on these potential host species. All animal procedures were approved by the University of Tennessee Institutional Animal Care and Use Committee (UT-IACUC #1201). Sherman live traps (3.5" x 3" x 9") were placed at 10m intervals in protected areas along six of the dragging transects (i.e., 25 traps per transect for a total of 150 traps per survey). Traps were baited with sunflower seeds at dusk and checked for captures at sunrise the following morning.

Figure 3.2: Drag sampling at Henry Horton State Park, Tennessee, in January 2008.

Eight dry pitfall traps and 10 funnel traps were set for lizards at opportunistic sites within the park. Each pitfall trap consisted of a 10-gallon plastic bucket sunk into the soil with the mouth of the bucket level with the soil surface. Many ground-dwelling animals fall, unharmed, into the trap, but are unable to escape. Pitfall and funnel traps were operated from the time we arrived at the park (approximately 1pm) until the following afternoon, for ~24 hours per trap per survey. Funnel traps were removed from the park and pitfall traps were covered with a tight fitting lid upon completion of that months sampling.

We identified, weighed, and determined sex on all animals trapped. Isoflurane was administered to animals when blood was collected to reduce pain from the tail clip. We then collected an ear biopsy, blood, and all ticks from trapped animals. Two 2mm ear biopsies were taken from each mammal upon its initial capture; one further ear biopsy was taken upon each subsequent capture. Hofmeister and Childs (1995) found that the ear biopsy is an efficient method for obtaining tissue samples from rodents infected with *B. burgdorferi*. Blood samples were obtained from rodents using a standard tail clip procedure, with a sample constituting 1% or less of body weight taken from an animal. Each rodent received a numbered, aluminum 5/16 inch Monel ear tag so that recaptures could be identified.

Pathogen testing

All collected samples were brought the University of Tennessee's Center for Wildlife Health. Blood and tissue samples were stored at -80°C for future analysis. If a *Borrelia*-positive tick was identified, blood and tissue samples collected from the corresponding host were tested.

Ticks collected at the park were identified to species and separated into individual vials based on dragging transect or mammal number, species, and life stage. All *I. scapularis* were

photographed for species verification and adult and nymphal ticks were measured for engorgement as described in Chapter 3. DNA extraction, PCR amplification, purification, and sequencing were performed as described in Chapter 3.

In addition to traditional PCR, novel pathogen testing technology under development by IBIS Biosciences (Carlsbad, CA) was used opportunistically on some ticks collected from January to March 2009, as a supplemental test for *B. burgdorferi* and other pathogens. Ticks were submitted in 70% alcohol – IBIS Biosciences extracted nucleic acids and returned 50µl of the DNA/RNA extract to the University of Tennessee for other testing. The material retained by IBIS was analyzed using broad-range primers and mass spectrometry to identify bacteria species by size and GC content (Ecker, et al. 2008).

Data analysis

Seasonal phenology curves were generated by summing monthly data on the relative abundance of ticks collected off mammals (*I. scapularis* per 100 trap nights) with monthly data on their relative abundance on vegetation (*I. scapularis* per 1000m^2 dragged). Differences in the number of ticks collected by dragging at our two sites (i.e. Wild Turkey on the eastern side of the park and Hickory Ridge to the west) were analyzed using a 2-way ANOVA, with transects as replicates and site and month as predictor variables.

The proportion of infested mice by sex was tested using a 1-tailed Fisher Exact test (1-tailed because I hypothesized males would be infested by ticks more often than females). A 2-tailed Fisher Exact test was used to test for a difference in mouse infestation between the eastern and western sites within the park (2-tailed as I had no prior expectation as to which sites would produce more infested mice).

An unweighted least squares linear regression was used to test for a relationship between the number of mice per transect infested with larval *I. scapularis* during spring/summer versus the number of adult *I. scapularis* dragged on those transects during fall/winter. A two-sample t-test was used to test for a difference in the engorgement index (EI) of nymphs collected from mammals versus nymphs dragged from the vegetation.

Results

Climate data

In 2007, there was a much greater deviation from 30-year climate normals than was the case in 2008 and 2009 (Figure 3.3) due to a period of severe drought (NOAA 2009). Monthly average temperatures were above the 30 year normals for most of 2007, and monthly rainfall was well below normals for the entire year. In contrast, 2008 and 2009 were both relatively normal years in terms of temperature and overall precipitation.

Seasonal abundance of questing *I. scapularis*

Dragging at HHSP from November 2007 to May 2009 indicated that larval *I. scapularis* activity peaked in July 2008, and that nymphs peaked in March 2008 and again in May 2008 and 2009. Adults peaked in November 2007 and again in April 2009. There were also minor peaks in adult activity in February/March 2008 and December 2008 (Appendix 3.1).

There was a significant difference in the overall abundance of adults between the two sites (Figure 3.4; mean of 1.82 adults/1000m^2 dragged on the Wild Turkey transects; mean of 0.89 adults/1000m^2 on the Hickory Ridge transects; 2-way ANOVA by site $F=8.99$, 1,93 df, $p = 0.0035$).

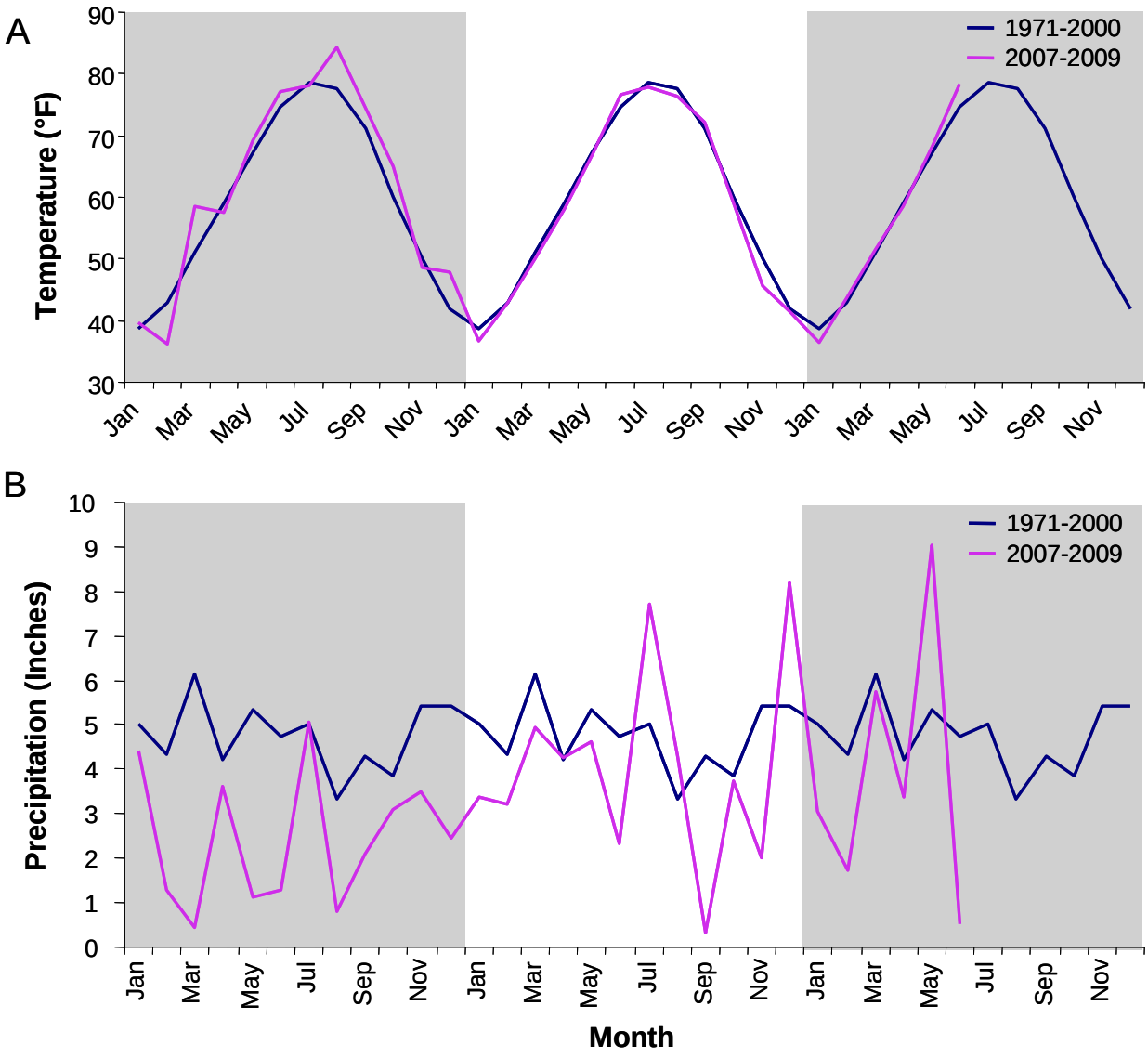

Figure 3.3: (A) Monthly average temperature for this study (2007-2009) compared with monthly temperature normals (i.e., 30-year averages for 1971-2000) at Shelbyville Water climate station, 14 miles southeast of Henry Horton State Park. (B) Monthly average precipitation for this study (2007-2009) compared with monthly precipitation normals (i.e., 30-year averages for 1971-2000) at Shelbyville Water Climate Station.

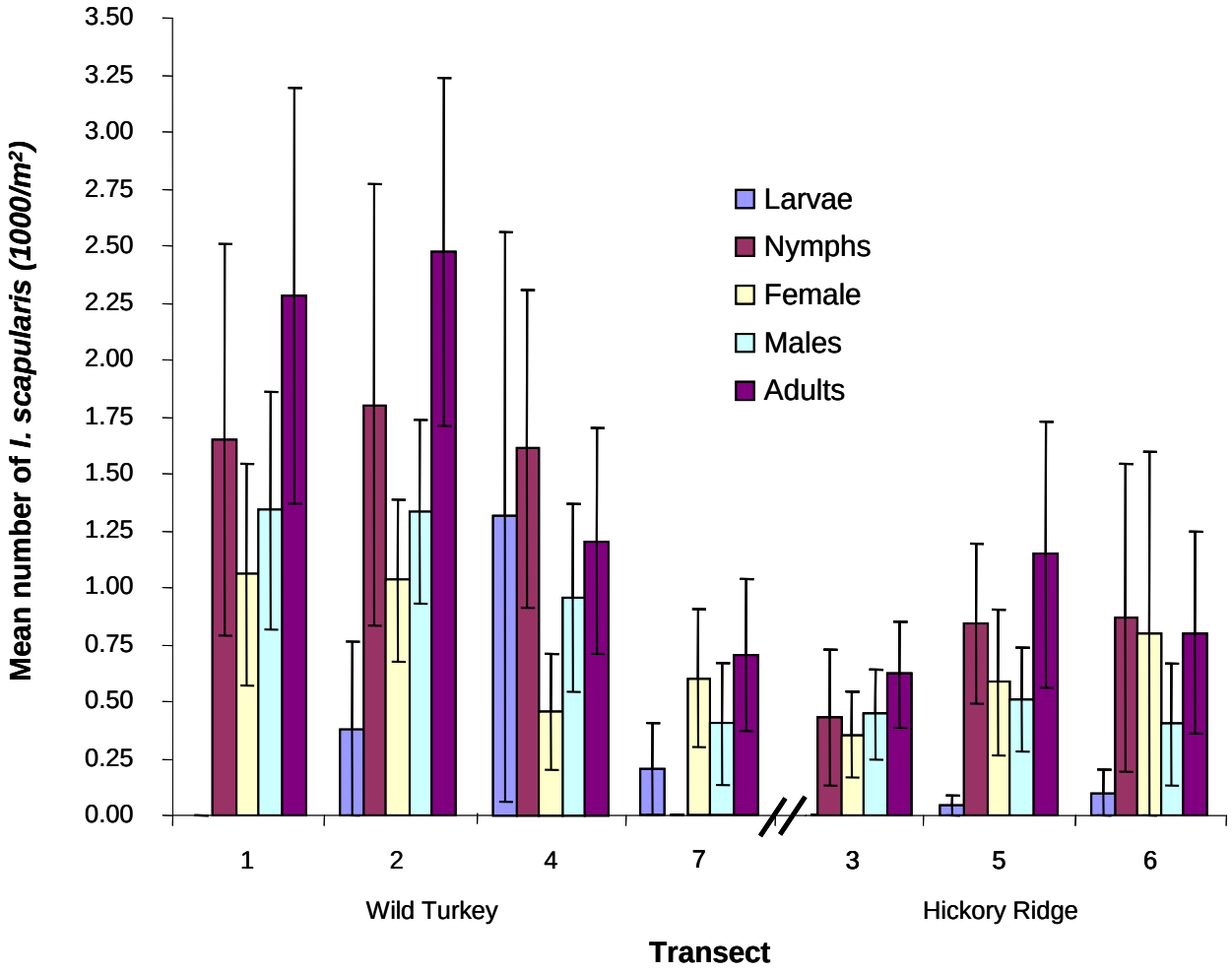

Figure 3.4: Mean number of *I. scapularis* (±SE) dragged per 1000m^2 from each transect at Henry Horton State Park (means are for N = 19 drag samples at monthly intervals, from November 2007 to May 2009).

The mean abundance of nymphs on the Wild Turkey and Hickory Ridge transects showed the same trend (1.43 and 0.70 nymphs/1000m^2, respectively) but for this age-class the difference between sites was not statistically significant (F = 2.07, 1, 93 df, p = 0.15). Nymphal abundance peaked in March 2008 and May 2008 and 2009 at Wild Turkey and peaked in May 2008 and 2009 and Aug 2008 at Hickory Ridge (Appendix 3.1).

Overall, there was a significant difference in the abundance of blacklegged ticks dragged at Wild Turkey (mean = 3.87 ± 0.68SE) versus Hickory Ridge (mean = 1.85 ± 0.33SE; F = 6.43, 1, 93 df, p = 0.013). The number of nymphs (F = 2.94, 1, 93 df, p = 0.0004) and adults (F = 15.80, 1, 93 df, p < 0.0001) varied significantly by month at both sites, as was expected given the highly seasonal life cycle of these ticks. *Dermacentor variabilis* and *A. americanum* were also collected by dragging at both sites, although those data are not reported here.

Small mammal and lizard trapping

Small mammal trapping

There was a total of 357 mammal captures from January 2008 to May 2009; 355 were *Peromysus* spp. (191 different individuals, likely all *P. leucopus*) and 2 were *Blarina* spp. (2 different individuals). *Ixodes scapularis* larvae were found on mice from June 2008 to October 2008 and in March 2009, with the peak being in June 2008 with 67% (8 of 12) of mice infested with 1-10 (median = 4) larvae per mouse. *Ixodes scapularis* nymphs were found on mice in April, May, and October of 2008 and in March and April of 2009. Peak nymphal infestation occurred in May 2008, with 19% (3 of 16) mice infested (with 1 nymphal tick in all three cases; Table 3.1). None of the mice captured were simultaneously infested with nymphal and larval *I. scapularis.*

Table 3.1: Number and percentage infestation, and larval and nymphal burdens, of *I. scapularis* and *D. variabilis* on *Peromyscus* spp. at Henry Horton State Park from January 2008 to May 2009.

			No. infested (%)							***I. scapularis* burden**		***D. variabilis* burden**	
			I. scapularis			***D. variabilis***			**Co-infested**	**Larval range; median**	**Nymphal range; median**	**Larval range; median**	**Nymphal range; median**
Month	**No.**	**No. /100 trap nights**	**Larvae**	**Nymphs**	**Both**	**Larvae**	**Nymphs**	**Both**	**(Nymph or Larvae)**				
Jan-08	0	0.00											
Feb-08	11	14.7				6 (54.6)						1-2; 1	
Mar-08	13	17.3				10 (76.9)						1-8; 2	
Apr-08	11	14.7		1 (9.1)		10 (90.9)	1 (9.1)	1 (9.1)	1 (9.1)		1-1; 1	1-27; 4	2-2; 2
May-08	16	21.3		3 (18.8)		14 (87.5)	6 (37.5)	5 (31.3)	3 (18.8)		1-1; 1	1-22; 2	1-1; 1
Jun-08	12	12.0	8 (66.7)			4 (33.3)			2 (16.6)	1-10; 4		1-2; 1	
Jul-08[1,2,3]	28	18.7	17 (60.7)			5 (17.9)			3 (10.7)	1-7; 2		1-2; 1	
Aug-08[1]	37	24.7	21 (56.8)			9 (24.3)	4 (10.8)		8 (21.6)	1-4; 1		1-5; 1	1-1; 1
Sep-08[2,3]	29	19.3	7 (24.1)			4 (13.8)			1 (3.5)	1-1; 1		1-1; 1	
Oct-08	22	14.7	2 (9.1)	1 (4.6)		5 (22.7)				1-2; 1.5	1-1; 1	1-2; 1	
Nov-08	15	10.0											
Dec-08	20	13.3											
Jan-09	6	4.0											
Feb-09	15	10.0				1 (6.7)						1-1; 1	
Mar-09	49	32.7	1 (2.0)	2 (4.1)		19 (38.8)			1 (2.0)	1-1; 1	1-1; 1	1-26; 2	
Apr-09	34	22.7		2 (5.9)		20 (58.8)	1 (2.9)	1 (2.9)	2 (5.9)		1-3; 2	1-12; 2	1-1; 1
May-09	38	25.3		3 (7.9)		3 (7.9)	2 (5.3)				1-1; 1	1-7; 1	1-2; 1.5

[1]Two trapping nights; all mice checked were counted on day 1 and only new mice were counted from day 2.

[2] Two *Blarina* spp. were trapped; 1 in July with 77 *I. scapularis* larvae and 1 in Sept. with no ticks.

[3] Two *Peromyscus* spp. were trapped (1 in July; 1 in Sept) with 1 *A. americanum* larvae on each.

Larval *D. variabilis* were found on mice from February to October 2008 and again in February to May 2009. Peak *D. variabilis* infestation occurred in April 2008 with 90% (10 of 11) mice infested with 1-27 (median = 4) larvae per mouse. The following spring larval *D. variabilis* again peaked in April with 59% (20 of 34) mice infested with 1-12 (median = 2) larvae per mouse. Peak nymphal infestation occurred in May 2008 with 38% (6 of 16) mice infested with 1-22 (median = 2) nymphs, with this life stage being found on mice in April, May, and August 2008 and again in April and May 2009. Co-infestation of *D. variabilis* nymphs and larvae on individual mice was observed in April and May 2008 and again in April 2009. The peak of this co-infestation occurred in May 2008 with 31% (5 of 16) of mice infested with both life stages (Table 3.1).

Co-infestations of *I. scapularis* and *D. variabilis* were found on mice from April to September 2008 and again in March and April 2009. Peak co-infestation occurred in August 2008 with 22% (8 of 37) of mice infested with both tick species (Table 3.1).

Male mice were caught on 203 occasions and females were caught on 150 occasions (the sex of two mice were not determined). On 21.2% (42 of 203) of these occasions, male mice were infested with *I. scapularis*, compared with 16.7% (25 of 150) of occasions for female mice; this difference between sexes was in the direction expected but was not statistically significant (Fisher Exact test, $p = 0.17$ on a 1-tailed test).

The overall *I. scapularis* infestation of mice at Henry Horton State Park from January 2008 to May 2009 was 19.2% (68 of 355). This level of infestation did not vary significantly between our two sites (Wild Turkey = 17.6%, Hickory Ridge = 21.0%; Fisher Exact Test, $p = 0.42$ for a two-tailed test).

No significant relationship was evident between the percent of mice infested with larval *I. scapularis* during peak infestation and the number of adult dragged *I. scapularis* during peak months on the transects (Figure 3.5; unweighted least squares linear regression: $F = 0.38$, 1,5 df, $p = 0.56$).

Lizard trapping

During the months that lizard trapping was performed (June 2008-Aug 2008), no lizards were captured or seen around traps. On a few occasions, 1-2 individual *Eumeces* spp. were seen around maintenance buildings in open areas of the park; however no lizards were sighted in any forested areas during the 19 months of this study.

Phenology curves

Based on our combined dragging data and small mammal sampling, relative larval activity peaked at Henry Horton State Park in July 2008. Nymphs had distinct peaks in March and May 2008 and in May 2009. Adult *I. scapularis* peaked in November 2007, February 2008 and again in December 2008 (Figure 3.6; Appendix 3.2).

Pathogen testing

Of the ticks collected at Henry Horton State Park from November 2007 to May 2009, 212 questing *I. scapularis* and 71 *I. scapularis* removed from mammals (Table 3.2) were tested for the presence of *Borrelia* spp. With the exception of one larval pool collected by dragging[2], all ticks tested positive for the 16s mitochondrial gene, indicating successful DNA extraction.

[2] *B. burgdorferi* is not transmitted transovarially, so I would not expect to detect this pathogen in dragged larvae.

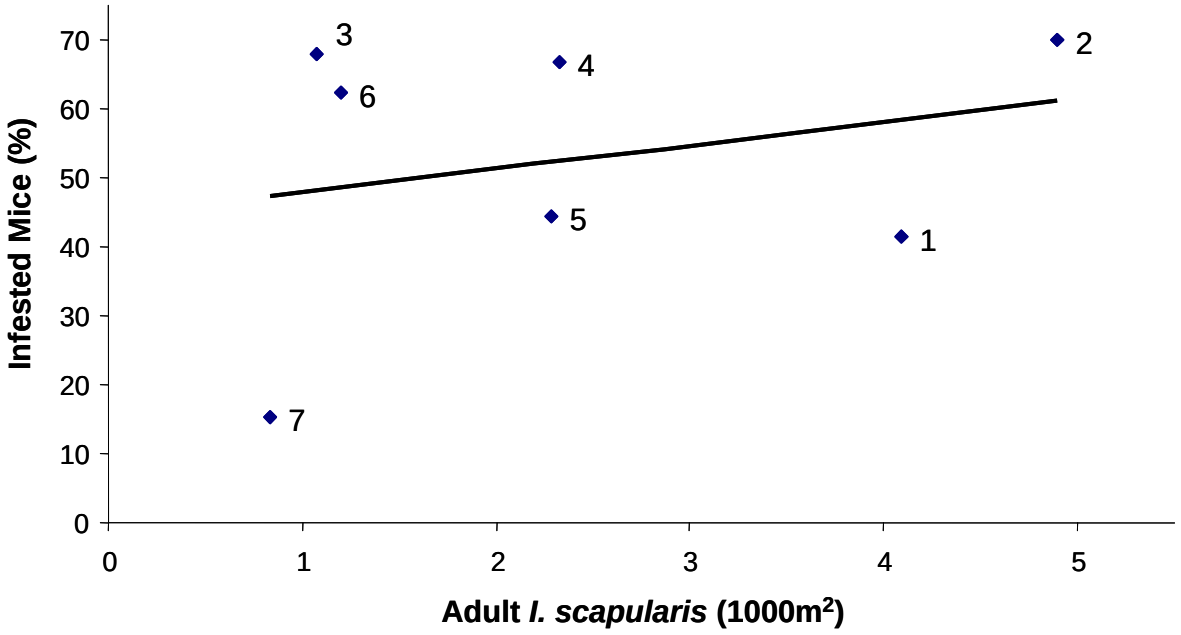

Figure 3.5: Relationship between the percentage of mice infested with larval *I. scapularis* (June-September 2008) and the number of adult *I. scapularis* dragged per 1000m^2 (November 2008 to March 2009) on seven transects at Henry Horton State Park. Wild Turkey = transects 1, 2, 4, and 7; Hickory Ridge = transects 3, 5, and 6.

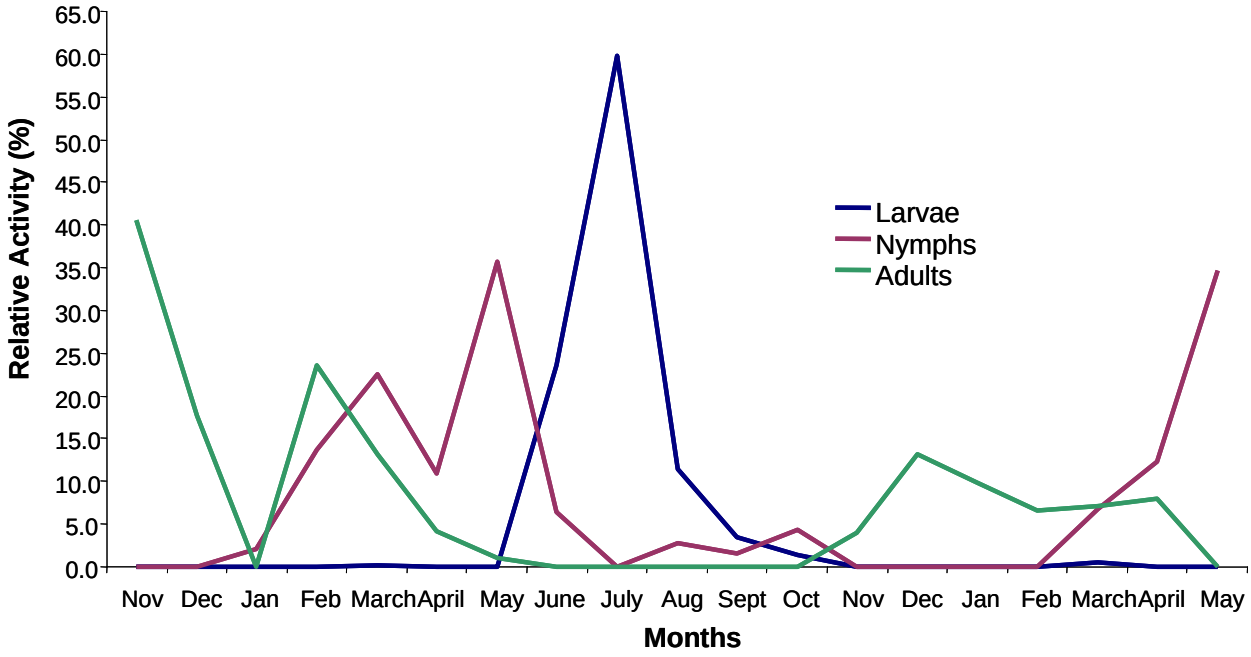

Figure 3.6: Relative seasonal abundance of *I. scapularis* at Henry Horton State Park, combining data from small mammals and drag sampling from November 2007 to May 2009 (see Appendix 3.2 for contributing data).

Table 3.2: Numbers of *I. scapularis* and *I. brunneus* tested for *Borrelia* spp. using the 16s-23s intergenic spacer region. Larval ticks from one transect or from one mammal were pooled for testing; numbers in the larval column indicate the number of pools tested.

Method	Larva	Nymph	Female	Male	Total
Dragging	3	45	89[1]	80	217
Mammal	61	10	0	0	71

[1] includes 5 *I. brunneus* adult females

Engorgement indices

Engorgement indices were measured for 83 female and 74 male *I. scapularis* collected from the vegetation. These data are not presented as no adult *I. scapularis* were collected from mice and adults collected from the vegetation are presumed to be unengorged. A total of 53 nymphs were measured and no significant difference in engorgment index was found between the 8 nymphs collected from the *Peromyscus* spp. and the 43 nymphs collected from the vegetation (Table 3.3) suggesting that most nymphs collected from the mice were not yet heavily engorged.

Borrelia burgdorferi *prevalence*

Borrelia burgdorferi was not detected in any of the 288 HHSP samples tested by standard PCR in our laboratory. In addition, 57 *I. scapularis* dragged in 2007 (27 male, 30 female) were re-tested at Michigan State University using real-time PCR, and again no *B. burgdorferi* were identified. Finally, 38 (3 from mammals; 2 nymphs and 1 larval pool and 35 from dragging; 11 female, 18 male, 1 nymph) *I. scapularis* samples and 5 *I. brunneus* (all dragged) collected from January to March in 2009 were tested in California using IBIS technology — none tested positive for any *Borrelia* spp.

Table 3.3: Engorgment indices (EI) and standard error (SE) for 53 *I. scapularis* nymphs collected from Henry Horton State Park from November 2008 to May 2009, and tested for *Borrelia* spp.

Collection Method	No. Measured	EI ±SE*
Dragged	45	2.21±0.15
Mammal trapping	8	2.21±0.11

*Two sample t-test; 0.01, 44,7 d.f., p = 0.99)

Borrelia miyamotoi *and other* Borrelia *spp.*

Borrelia miyamotoi was detected by standard PCR in three samples from Henry Horton State Park. The first positive sample was a pool of *I. scapularis* larvae collected in July 2008 from a 14g female *Peromyscus* spp. This mouse was captured only once at the Wild Turkey site (transect 1), and was infested with two *D. variabilis* larvae and five *I. scapularis* larvae. The latter tested positive for *B. miyamotoi* with an IGS sequence of 262 base pair that was 96% similar to a published *B. miyamotoi* sequence in Genbank (accession number: AY363706.1). Ear tissue and whole blood from this mouse were extracted and tested, with no positive result.

The second positive sample was a nymphal *I. scapularis* collected on April 4, 2009 from a 21g male *Peromyscus* spp. At the time of capture, three ticks (one nymphal *I. scapularis* and two *D. variabilis* larvae) were collected from this mouse, which had been previously captured on March 8, 2009 at the same site (Hickory Ridge, transect 3); on that occasion it had two *D. variabilis* larvae attached. Ear tissue from both March and April was tested, as well as whole blood from March (no blood was collected in April) but no *Borrelia* spp. were detected. The sequence from the one positive tick was 100% similar to the *B. miyamotoi* sequence in Genbank (accession number: AY531879.1).

The third tick infected with *B. miyamotoi* was a flat adult male *I. scapularis* collected in April 2009 from Wild Turkey site (transect 7). This sample produced an IGS sequence that was 99% similar to the *B. miyamotoi* sequence published in Genbank (accession number AY531879.1). Other ticks collected on this dragging transect that day were: one adult male *D. variabilis*, and one adult female, one adult male and two nymphal *A. americanum.*

Other pathogen species

IBIS Biosciences assayed 30 unengorged *I. scapularis* ticks (11 adult female, 18 adult male, 1 nymph) and five adult female *I. brunneus* (all collected by dragging between January and March 2009). Three samples of *I. scapularis* collected from mammals were also tested (two nymphs and one larva). Five of the 38 *I. scapularis* samples (13.1%; one adult female, three adult males and one larva) tested positive for Spotted Fever Group Rickettsia[3]. The Rickettsia-positive larva was collected from a female *Peromyscus* spp. in March 2009 — and was the only tick removed from that mouse. The same mouse was initially captured in February 2009 with no ticks and later recaptured in April with three *D. variabilis* larvae attached. Two of the Rickettsia-positive adult ticks were collected in January, one in February, and one in March. One was collected from Hickory Ridge (transect 5) and three were collected from Wild Turkey (two from transect 2 and one from transect 4).

Discussion

In forest habitat at Henry Horton State Park – where every deer that was inspected carried *I. scapularis* – both adult and nymphal *I. scapularis* were readily draggable at a rates similar to those for *I. scapularis* populations in southwestern Michigan that are known to maintain substantial endemic levels of *B. burgdorferi* (Hamer, et al. 2007). Furthermore, the level of *I. scapularis* infestation of *Peromyscus* spp. at HHSP is similar to that seen in these areas in Michigan with endemic levels of this pathogen (S. Hamer, unpublished data). The fact that we were able, in two successive years, to reliably drag questing nymphal *I. scapularis* suggests that

[3] The genus Rickettsia is classified into two groups, typhus (TG) and spotted fever (SFR); the later mainly associated with ticks and range from an unknown pathogenicity to extremely pathogenic to humans (Fournier, P. E. and D. Raoult (2009). Current Knowledge on Phylogeny and Taxonomy of Rickettsia spp, Blackwell Publishing.)

these ticks in Tennessee exhibit the same host-seeking behavior that results in elevated risk of human LD in the northeastern U.S.

Because there is minimal transovarial transmission of *B. burgdorferi*, it has been argued that the 2-year lifecycle of these ticks in the Northeast – whereby peak nymphal questing *precedes* peak larval questing – is the key to the maintenance of the pathogen in these tick populations. These observations led to a consequent hypothesis that the longer growing season in Tennessee would mean that nymphs feed *later* in the season than larvae, thereby breaking the *B. burgdorferi* transmission cycle (Rodgers 1953; Apperson, et al. 1993; Oliver, et al. 1993). However, this study indicates that the nymphal *I. scapularis* at HHSP do in fact feed on mice *before* the peak in larval ticks occurs, as is seen in the Northeast. Although the longer growing season may indeed affect seasonal timing of the host seeking ticks – such that immature ticks quest somewhat earlier in the year than is the case in the Northeast – it does not appear to alter the *relative* timing of nymphal versus larval feeding and so may have little effect on *B. burgdorferi* transmission potential.

Year 1 of this study (2007) was substantially hotter and drier than 2008 and 2009, and this appears to have advanced the seasonal phenology of *I. scapularis* that year – this likely explains why there was a larger peak in adult activity in November and December 2007 than in 2008, and why nymphs were found questing in February and March of 2008. Rodgers (1953) and Mount, et al. (1997) both indicate that higher temperatures accelerate tick development, so that weather differences will induce variation in *I. scapularis* phenology from year to year. According to Clark, et al. (1998), adult *I. scapularis* were most abundant in October in South Carolina, which is slightly earlier than we found in this study, but similar to Rodgers' (1953) findings. Jones and Kitron (2000) showed rainfall was the key factor in regulating *I. scapularis*

populations at a study site in Illinois. Their data showed that during drought years parasitism on mice was reduced that year and also in the following year (Jones and Kitron 2000). If the Tennessee system is similar to that of Illinois, we would expect to see an increase in *I. scapularis* population beginning in fall 2009.

Significantly more *I. scapularis* were collected from the Wild Turkey site versus Hickory Ridge at HHSP, perhaps because Wild Turkey is a damper and more humid site[4]. Collection of humidity data, and vegetation and soil sampling, could be undertaken to test this hypothesis.

Borrelia miyamotoi was detected in 1.0% of 288 samples tested at HHSP. This is similar to the 0.7-1.7% prevalence of *B. miyamotoi* found in host-seeking *I. pacificus* in California (Mun, et al. 2006) and 1.9-2.5% prevalence for *I. scapularis* in the eastern U.S (Scoles, et al. 2001). This supports previous suggestions that *B. miyamotoi* is widely distributed, at low prevalence, in North American *Ixodes* ticks. However, this low prevalence is in striking contrast to the high local prevalence of this pathogen detected in 2007 in East Tennessee deer (see Chapter 3); this transient high prevalence is intriguing and deserves further study.

[4] Wild Turkey is at lower elevation and holds rainfall longer; Hickory Ridge is at higher elevation and is rocky, with shallow soil.

Chapter 4 - Conclusions

Lyme disease risk in Tennessee

Lyme disease is a newly emerging disease, first identified in the U.S. in 1977. Despite extensive research and efforts to manage the problem, LD incidence has increased rapidly so that it is now the most common vector-borne disease in North America. Perhaps because we do not have a well-understood tradition of prevention and treatment of Lyme disease in the U.S., there is huge public uncertainty and concern about the disease that extends well beyond the geographic regions where it is known to be endemic. This concern is fueled by problems with LD diagnosis, and disagreement about treatment, particularly for the proportion of infected patients who develop chronic symptoms that they attribute to unresolved or undiagnosed LD.

There are no scientific data to suggest that there are high, or even moderate, rates of LD transmission occurring in Tennessee. Indeed, until recently there was no evidence demonstrating the presence of the *B. burgdorferi* spirochete in ticks or wildlife in the state. A small number of human and canine cases are diagnosed in Tennessee each year, but this is to be expected given frequent travel by humans and companion animals to and from known LD-endemic areas in the Northeast. Nevertheless, public concern about the disease in Tennessee has grown, fed by a number of factors including:

- Reports from the Northeast that blacklegged ticks and *B. burgdorferi* are expanding in abundance and geographic range;
- Increases in Tennessee abundance of other ticks, particularly *A. americanum*;
- Inability of many people to recognize different tick species, coupled with lack of awareness that most tick species cannot transmit *B. burgdorferi*, and that tick-

associated rashes may be a consequence of other tick-borne pathogens, or hypersensitivity reactions, rather than being LD-associated EM's[5];

- Failure by some heath providers to provide consistent information about the eco-epidemiology of LD in Tennessee[6]

We conclude that at present the risk of acquiring Lyme disease in Tennessee is very low; not because blacklegged ticks are absent from Tennessee, but because they are at relatively low abundances and rarely bite humans in the state, and because those ticks that do bite humans are very unlikely to be carrying *B. burgdorferi.* It is possible this situation could change in the future, and so ongoing surveillance and health provider vigilance for increasing LD case rates is recommended.

Meanwhile, the human population of Tennessee is clearly at risk of at least two other tick-borne diseases – Ehrlichiosis and Rocky Mountain Spotted Fever – both of which are potentially lethal and increasing in incidence. We recommend that prevention and education efforts for tick-borne infections should prioritize these two diseases over LD at the present time.

[5] Armstrong, P. M., L. R. Brunet, A. Spielman and S. R. Telford (2001). "Risk of Lyme disease: perceptions of residents of a Lone Star tick-infested community." Bulletin of the World Health Organization **79**(10): 916-925.Residents of an area in Maryland infested with both *A. americanum* and *I. scapularis* exaggerated the perception of LD risk because they were being bitten by an aggressive tick; *A. americanum* accounted for 95% of the human-attached ticks submitted in that study. Armstrong, P. M., L. R. Brunet, A. Spielman and S. R. Telford (2001). "Risk of Lyme disease: perceptions of residents of a Lone Star tick-infested community." Bulletin of the World Health Organization **79**(10): 916-925..

[6] Anecdotal examples include patients being told "there is no Lyme Disease in Tennessee" despite the Tennessee Department of Health reporting an annual average of ~30 cases on their public website (http://health.state.tn.us/CEDS/WebAim/interactive.htm), and patients being told "there are no blacklegged ticks in Tennessee" when the species is documented as being established in 10 Tennessee counties on the Lyme Disease Risk map available on the CDC's public website (http://www.cdc.gov/ncidod/dvbid/lyme/riskmap.htm).

Future research needs

What limits blacklegged tick population distribution and abundance in Tennessee?

Although widespread in Tennessee, *I. scapularis* are certainly not as abundant here as they are in northeastern states such as New York and southern Maine, where densities of questing nymphal *I. scapularis* of >12 per 1000m^2 are common (Diuk-Wasser, et al. 2006). Possible explanations for lower density in Tennessee include (*i*) perhaps *I. scapularis* is emerging in the southern states and has not reached its carrying capacity; (*ii*) perhaps the climate and/or habitat is not suitable to sustain large populations of this ticks; or (*iii)* perhaps *I. scapularis* behaves differently in southern states, for example preferring to feed on different hosts (e,g. lizards) and consequently altering its questing behavior so that it is not easily collected by dragging.

These hypotheses could be investigated by performing intensive field studies sampling multiple hosts (i.e. lizards, mammals, and birds) and ticks from vegetation across a spatial gradient. In addition, studies could compare the northern and southern questing behaviors of these tick populations (e.g., diurnal timing of peak questing) including climate data such as temperature and humidity. Lastly, to gain a better understanding of which species are acting as primary hosts, blood meal analysis (Humair, et al. 2007) may be a useful alternative to wildlife capture to determine the extent to which Tennessee *I. scapularis* ticks feed on various vertebrate hosts.

Computer simulations of tick populations require a density dependent step in the tick life-cycle if they are to avoid 'runaway' exponentially-growing populations. Most models (Mount, et al. 1997; Ogden, et al. 2005) incorporate an 'on host competition" assumption to introduce this density dependence – however this mechanism is not likely applicable at HHSP where there are

many mice (more than Michigan) carrying few ticks (fewer than Michigan; S. Hamer, Michigan State University, unpublished data). Some other factor may be regulating *I. scapularis* density in Tennessee, but it is presently unclear what that factor might be.

An alternative is that these populations have only recently invaded and will increase in density in coming years. *I. scapularis* densities at HHSP are comparable to densities seen in some areas with emerging tick populations, such as in southwestern Michigan (S. Hamer, Michigan State University, unpublished data) and Indiana (Pinger, et al. 1996). This may indicate that Tennessee is itself an emerging area. The mild climate in Tennessee may result in a rapid growth of this tick population once they become established, hence the need for vigilance. However, most southern states appear to have low but persistent *I. scapularis* populations, so regional climate conditions, habitat, or host ecology may be responsible for these low populations of southern *I. scapularis.* It is interesting to speculate on the potential of *A. americanum* versus. *I. scapularis* interspecific competition or interference, given that *A. americanum* is so common in most southern states.

What limits *B. burgdorferi* infection of blacklegged ticks in Tennessee?

General epidemiological theory predicts there is a 'threshold' density of *I. scapularis* below which *B. burgdorferi* infection will not be self-sustaining in these tick populations. If *I. scapularis* abundance is below this level in Tennessee, this could explain why no *B. burgdorferi* were identified in this study The abundance of blacklegged ticks at HHSP was similar, however, to that in an emerging population in southwestern Michigan, which is known to sustain a high (25-50%) prevalence of *B. burgdorferi* (Hamer, et al. 2007). This suggests several possibilities:

- Perhaps Tennessee *I. scapularis* populations *do* already support *B. burgdorferi* – we simply failed to detect it. We acknowledge that our intensive HHSP study (Chapter 3) investigated the seasonal phenology of *I. scapularis* at only one location in middle Tennessee. Because of variation in climate during the sampled months and because only one location was sampled, long term studies such as this should be initiated at other locations throughout Tennessee and the southeast to clarify both temporal and spatial fluctuations. Nevertheless, our deer surveys (Chapter 2) were spread widely across the state and help to address this criticism.
- Perhaps Tennessee *I. scapularis* populations have, in recent years, increased above the necessary threshold density and so *could* support *B. burgdorferi;* however the pathogen simply has not yet had time to arrive. The recent Jordan, et al. (2009) paper reporting *B. burgdorferi* in migratory waterfowl in mid-Tennessee gives some support to this hypothesis. We do believe the distribution and abundance of *I. scapularis* is presently expanding and we hypothesize that as these populations continue to grow, we may indeed see emergence of *B. burgdorferi* in the state.
- Perhaps Tennessee *I. scapularis* populations cannot support *B. burgdorferi* for reasons other than the numerical abundance of these ticks. The 'unsuitable phenology' hypothesis has not been supported by our HHSP study, which demonstrates Tennessee nymphs feeding before larvae, as in the northeast. However, more research is needed on the 'host seeking behavior' hypothesis (whereby southern populations of this tick may seek hosts differently than northern populations).

Are there health implications of other *Borrelia* species in Tennessee?

Little is known about *B. miyamotoi* and *B. lonestari* (both detected in these tick populations) and it may be worth pursuing further ecological studies that explore the etiology of these pathogens. The high prevalence of *B. burgdorferi* and *B. lonestari* reported in Tennessee birds (Jordan, et al. 2009) requires further study – this work is now underway. In addition, studies exploring the link between *A. americanum* (on and off multiple hosts), the *Borrelia* spp. and other pathogens they may carry, and tick-borne illness in Tennessee may help to explain some of the 'Lyme disease' cases reported each year in Tennessee and other southern states.

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APPENDICES

Appendix 2.1: Tick DNA Extraction Protocol.

Modified from: Qiagen DNeasy Blood and Tissue Handbook: Protocol: Purification of Total DNA from Animal Tissues (Spin-Column Protocol) Catalog No. 69506

Tick Prep:
1. Remove ticks from identification/measurement vials and blot dry on a Kimwipe.
2. "Zero" a clean 1.5ml centrifuge tube, labeled with extraction identification number.
3. Place 1 tick per vial and recorded tick extraction ID and weight.
4. Include 1 positive and 1 negative control tick per batch.

Phase 1- Lysis:
1. Turn on incubator and set to 56˚C. Put beaker for ATL/Pro-K solution and ATL solution in incubator to warm.
2. Place each individual tick/vial into liquid Nitrogen without submerging the vial.
3. Use a pestle to pop and grind the tick in the vial. Leave the pestle in the vial until after the lysis buffer has been added.*
4. Create a master mix of lysis solution. (180µL of Buffer ATL and 20µL Pro-K per sample-solutions provided). Prepare enough for 5% extra ticks due to loss from transfer.
5. When samples have warmed (room temp), add 200µL of Buffer ATL and Pro-K solution to each 1.5ml microcentrifuge tube.** Be careful not to shoot tick particles out of vial. If you direct the pipette tip to the side of the vial instead of straight down, this can be prevented.
6. Mix thoroughly by vortexing for 5-15 seconds and incubate at 56˚C overnight, rocking. Make certain no tick pieces are stuck to the vial where the lysis buffer can't reach.
*Use a clean scalpel to position the tick for cutting within its vial if the tick does not pop with liquid N_2. Cut tick into several (at least 4) pieces, with attention to cutting open the midgut. It may help to lean the vial against the tube tray while cutting.
**You can add 20µl of additional ATL/pro-K to large engorged ticks at a time until the tick is completely submerged.

Phase 2- Extraction
1. Place enough Buffer AE (provided) for final elution to 70˚C. (100ul per sample)
2. Pre-label 1 set of spin-columns and 2 sets of 1.5ml centrifuge tubes with final extraction ID.
3. Create a master mix of 200µl Buffer AL (provided) and 200µl of EtOH (95%-100%) per sample.
4. Remove samples from incubator and vortex for 15 seconds
5. Add 400µl of Buffer AL/EtOH master mix to each sample and mix again thoroughly by vortexing.
6. Pipette the sample mixture from step 5 (including any precipitate) in the corresponding spin-column.
7. Centrifuge each spin-column at ≥6,000 x g (8,000rpm) for 1 min. Discard flow through collection tube and place in a clean collection tube.
8. Add 500µl of Buffer AW1 (provided) and centrifuge at ≥6,000 x g (8,000rpm) for 1 min. Discard flow through collection tube and place in a clean collection tube.
9. Add 500µl of Buffer AW2 (provided) and centrifuge at 20,000 x g (14,000rpm) for 3 min. Discard flow through collection tube and place the corresponding (final) 1.5 microcentrifuge tube. Incubate at 45˚C for 10 minutes.
10. Pipet 50µl of Buffer AE directly onto the spin-column membrane. Incubate at room temperature for 10 min.
11. Centrifuge each sample at ≥6,000 x g (8,000rpm) for 1 min to elute.
12. Place spin-column in the second labeled microcentrifuge tube and repeat step 10.
13. Be sure all microcentrifuge tubes are properly labeled (elution 1 or 2, place a cardboard box and stored in the freezer (if not amplified immediately after).

Appendix 2.2: *Borrelia burgdorferi* PCR Protocol.

Modified from: Bunikis, J., U. Garpmo, J. Tsao, J. Berglund, D. Fish, and A. G. Barbour. 2004. Sequence typing reveals extensive strain diversity of the Lyme borreliosis agents *Borrelia burgdorferi* in North America and *Borrelia afzelii* in Europe. Microbio. 150:1741 – 1755. And Sarah Hamer, Michigan State University, Unpublished, 2007.

PCR I – Outer Primers (Use hood I)

I. Prepare a mastermix containing PCR Supermix (Invitrogen) and outer-forward and outer-reverse primers[1]. Standardize primers such that 1µL contains ~0.166pmoles. Each sample will need 43 µL Supermix plus 1 uL of each primer[7]. Account for number of samples and controls plus a ~5% error buffer.
   a. If 96 samples, then calculate for 100 samples as follows:
      IGS 1: 1 * 100 = 100µL
      IGS 2: 1 * 100 = 100µL
      Supermix: 43 * 100 = 4300µL

II. Combine the mastermix reagents in a multi-channel pipettor boat. Mix thoroughly. Using a multi-channel pipettor set to 45µL,fill the appropriate number of wells on a 96-well PCR plate on ice.

III. Mix in 5µL of each sample DNA (microcentrifuged for 30sec at 8,000rpm) into individual wells, using extraction controls as well as PCR controls (5uL previously-positive extract for positive; no-template control (NTC) for negative).

IV. Run **outer** PCR program on thermocycler as follows:
   a. Initial denature: 1 min at 80°C, 3 minutes at 94°C
   b. 35 cycles as follows:
      i. Denature: 30 sec at 94°C
      ii. Anneal: 30 sec at 56°C
      iii. Extend: 1 min at 74°C
   c. Final extension: 7 min at 72°C

V. Store samples at 4°C until prepared for PCR II

PCR II- Inner Primers (Use hood II)

I. Follow above protocol for Mastermix preparation, but use IGS Fn and IGS Rn primers.

II. Mix in 5µL of PCR I product into each well using a multi-channel pipettor.

III. Run **inner** PCR program on thermocycler as follows:
   a. Initial denature: 1 min at 80°C, 90 sec at 94°C and repeat once
   b. 40 cycles as follows:
      i. Denature: 30 sec at 94°C
      ii. Anneal: 30 min at 60°C
      iii. Extend: 1 min at 74°C
   c. Final extension: 7 min at 72°C
   d. Store samples at 4°C until prepared for gel electrophoresis

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[7] Primers purchased from IDET. Sequences as follows:
IGS F: 5' – GTA TGT TTA GTG AGG GGG GTG – 3' = 21 bases. **IGS R**: 5' – GGA TCA TAG CTC AGG TGG TTA G – 3' = 22 bases
IGS Fn: 5' – AGG GGG TGA AGT CGT AAC AAG – 3' = 21 bases. **IGS Rn**: 5' – GTC TGA TAA ACC TGA GGT CGG A – 3' = 22 bases
Lyophilized primers were initially hydrated with tris buffer (10mM, pH 8.0), 10x the amount of nmoles given to create a 100nmole/uL solution. A dilution was then prepared and used in reactions as a shortcut. This resulted in a 0.166pmole/uL concentration which has worked great for a 50ul reaction. The following calculation can be used to standardize: Target: 8.3pmoles/ul in a 50uL reaction. X = (.166pmoles*50ul)/1ul
   i. I X = (.166pmoles*50ul)/1ulf IGS F primer was synthesized to 25.8nmoles
      1. 258uL of tris was added = 100pmole/uL solution
      2. c1v1 = c2v2 = 100pmole/uL * XuL = 8.3pmoles/uL * (1000uL)
         X = 83uL, concentrated stock + 917uL of sterile, pure water to create 1.0mL w. stock (8.3pmoles/uL)

Appendix 2.3: 1% Agarose Gel Protocol.

Protocol:

Gel:
2.0g Agarose
200mL 1x TAE
20uL EtBr

1. Dissolve 2.0g of agarose into 200mL of 1X TAE in microwave.
2. Once cool, add 20µL of EtBr to a 200mL gel (1µL of EtBr (10mg/mL) for every 10mL of gel)
3. Pour gel. Place place comb and remove any bubbles
4. Once solid, pour 1000mL of 1X TAE over gel (Add EtBr to buffer; 100µl EtBr)
5. Remove comb carefully

Loading:
Loading dye (4^oC)
Gel ladder (4^oC)
Mixing plate

1. In a mixing plate add: Making sure each well is completely mixed
    a. Sample wells: 3µL loading dye and 10µL PCR amplicon
    b. Marker wells: 10µl of DNA ladder (contains dye)
2. Load gel from bottom to top, with 1 ladder well at the beginning and end each row
3. Create a "map" of what samples are loaded into each well
4. Load 11µL of mixture into each well
5. Set electrophoresis machine to 100 volts and run gel from left to right (black to red, - to +) for 1.5 hours

Appendix 2.4: DNA Purification and Sequencing Protocol.

Modified from: Zymoclean Gel DNA Recovery Kit (Catalog No. D4001)

Materials Needed:
1.5µl Microcentrifuge tubes—weighed, and final set for purified sample
Autoclaved (sterile) pure water (heated to 55 °C)
Razer blades

Sample Prep:
1. Weigh clean, dry, 1.5µl microcentrifuge tubes (number of samples to be excised)
2. Turn on hot plate to 55 °C

Purification:
1. Excise the DNA fragment from the gel using a new razor blade or scalpel and transfer it to a weighed microcentrifuge tube. **Make sure to make the excision as precise as possible and to cut out as little gel as possible.**
2. Weigh microcentrifuge tubes again to obtain sample weight.
3. Add 3 volumes of ADB (provided) to each volume of agarose excised from the gel (i.e. for every 100µl of gel, add 300µl of ADB)
4. Incubate at 55 °C for 5 to 10 minutes or until the gel is completely dissolved
5. Transfer the melted agarose solution to a Zymo-Spin I column (provided) in a collection tube
6. Centrifuge at ≥10,000 x g for 60 seconds. Discard flow-through and place in a new collection tube
7. Add 200µl of Wash Buffer to the column and centrifuge at ≥10,000 x g for 30 seconds. Discard flow-through and place in a new collection tube.
8. Repeat step 7.
9. Spin samples for 30 seconds at ≥10,000 x g to remove any additional liquid. Discard collection tube and place into final 1.5µl microcentrifuge labeled with sample name.
10. Add 20µl of 55°C sterile pure water to the column and incubate at room temperature for 1 minute.
11. Spin at ≥10,000 x g for 60 seconds to elute DNA.
12. Discard minicolumn and store sample at -20 °C or prepare for sequencing.

Sequencing:
1. Primers: primers must be at 5pmole/µl for sequencing. Bring these labeled primers and samples (on ice), and submission sheet to Joe May (A211) in Life Sciences. 4-6231; jmay@utk.edu
2. Follow the directions to measure DNA concentration for all samples. Submission must be complete by 12 noon (latest) for next day results. Last copy goes into lab notebook!
3. When picking up samples, remember to take, print out, samples, and primers (on ice).

Appendix 2.5: 16s mitochondrial rRNA gene PCR Protocol.

Modified from: Black, William C., and J. Piesman. 1994. Phylogeny of hard- and soft-tick taxa (Acari-Ixodida) based on mitochondrial 16S rDNA sequences. Pro. Natl. Acad. Sci. 91:10034-10038.

Protocol: (Use hood I)

I. Prepare a mastermix containing PCR Supermix (Invitrogen) and 16S-1 and 16S+2 primers[1]. Standardize primers such that 1µL contains ~0.2pmoles. Each sample will need 21.5 µL Supermix plus 1µL of each primer[8]. Account for number of samples and controls plus a ~5% error buffer.
   a. If 96 samples, then calculate for 100 samples as follows:
      16S-1: 1 * 100 = 100µL
      16S+2: 1 * 100 = 100µL
      Supermix: 21.5 * 100 = 2150µL

II. Combine the mastermix reagents in a multi-channel pipettor boat. Mix thoroughly. Using a multi-channel pipettor set to 23.5µL, fill the appropriate number of wells on a 96-well microtiter plate on ice.

III. Mix in 2.5µL of each sample DNA into individual wells, using extraction controls as well as PCR controls (2.5µL previously-positive extract); no-template control (NTC) for negative).

IV. Run PCR program on thermocycler as follows:
   a. Initial denature: 5 min at 95°C
   b. 10 cycles as follows:
      i. Denature: 60 sec at 92°C
      ii. Anneal: 60 sec at 48°C
      iii. Extend: 90 sec at 72°C
   c. 32 cycles as follows:
      i. Denature: 60 sec at 92°C
      ii. Anneal: 35 sec at 52 (4)°C
      iii. Extend: 90 sec at 72°C
   d. Final extension: 7 min at 72°C

V. Store samples at 4°C until prepared for gel electrophoresis.

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[8] Primers purchased from IDET. Sequences as follows:
16S-1: 5' – CCG GTC TGA ACT CAG ATC AAG T – 3' = 22 bases. 16S+2: 5' – TTG GGC AAG AAG ACC CTA TGA A – 3' = 22 bases
Lyophilized primers were initially hydrated with tris buffer (10mM, pH 8.0), amount of tris added was based upon the amount of primer (nmol) to create a 100pmol/ul solution as the concentrated stock. A second dilution was then prepared, alloquated and used in reactions as a shortcut. This resulted in a 0.2pmole/uL concentration which has worked for the 26ul reaction. The following calculation can be used to standardize:
   ii. I X = 1.0pmole/ul = X/50ul)= 50pmole concentration of working stock
   iii. 100pmole/ul(X) = (1000ul final volume)(50pmole/ul)
      1. X = 500ul of concentrated stock, 500ul of tris buffer or sterile mill-q water
      2. 50ul of 100pmoles/ul + 50ul of sterile water = 100ul of 50pmole/ul

Appendix 2.6: Selected 2007 and 2008 TWRA Big Game Check Stations.

Name	Address	City	ZIP code	Latitude	Longitude
ADAMS TAXIDERMY	102 SHIPE RD	POWELL	37849	36.033112	-84.128313
ANDERSON'S SPORTS HQTRS	873 WEST CHURCH ST	LEXINGTON	38351	35.660356	-88.424062
BUCKS N BASS	664 E MEETING ST	DANDRIDGE	37725	36.029929	-83.394345
BYBEE MARKET	1554 HWY 160	BYBEE	37713	36.10656	-83.163429
Catoosa WMA	4650 GENESIS RD	CROSSVILLE	38571	36.012026	-84.994845
CLINCH RIVER MARKET	127 TAZEWELL HWY	SNEEDVILLE	37869	36.516886	-83.2163
COFFMANS GROCERY	714 HILHAM HWY	LIVINGSTON	38570	36.376095	-85.368141
COPE'S CUSTOM SLAUGHTERING	307 GOFF RD	SPARTA	38583	35.929882	-85.36541
CREEKSIDE MARKET 2	8691 ASHEVILLE HWY	GREENEVILLE	37743	36.037262	-82.852776
DEKALB MKT	3250 NASHVILLE HWY	SMITHVILLE	37166	35.976324	-85.863566
DREADEN'S ONE STOP	9517 HWY 70	MCEWEN	37101	36.107759	-87.635011
FLOWERS DERR PROCESSING	4550 EATONS CREEK RD	NASHVILLE	37218	36.227004	-86.867349
FORREST LANDING MARKET	4448 NASHVILLE HWY	CHAPEL HILL	37034	35.605424	-86.699929
GLENN'S DEER PROCESSING	160 CHARLESTON ST	MOSCOW	38057	35.060696	-89.402742
GOODMAN'S TAXIDERMY & PROCESSING	384 JACOBS RD	MORRISTOWN	37813	36.158683	-83.272783
HAMPTON BAIT SHOP	126 1ST AVE	HAMPTON	37658	36.289866	-82.173463
HARGETT'S PROCESSING	2526 JUNIUS LEE RD	RIPLEY	38063	35.719046	-89.647702
HUNTER'S HARVEST DEER PROCESSOR	435 DOVER RD	CLARKSVILLE	37042	36.551233	-87.410948
HUNTS OUTDOORS	330 CLYDETON RD	WAVERLY	37027	36.092608	-87.802617
INMAN'S STOP N CHAT	10785 CAMPBELLSVILLE RD	PULASKI	38478	35.338601	-87.130876
JOYCE'S TAXIDERMY & MEAT PROCESSING	9875 HWY 131	WASHBURN	37888	36.333927	-83.498985
Land Between the Lakes WMA*				36.54598	-87.97593
LITTLE GENERAL # 57	429 EAST HARPER	TROY	38260	36.338279	-89.160338
MASHBURNS GENERAL MERCHANDISE	24 MOCKERSON RD	FIVE POINTS	38457	35.051122	-87.309527
MINORS MARKET	1022 HWY 11W	CHURCH HILL	37642	36.512868	-82.769481
Oak Ridge WMA*				35.96595	-84.25022
OUTDOOR COUNTRY	524 N GRUNDY QUARLES HWY	GAINESBORO	38562	36.366281	-85.651187
POP'S BAIT SHOP	1003 WEST GAINES ST	LAWRENCEBURG	38464	35.242605	-87.348499
PORTER'S GROCERY, INC	6635 HORTON HWY	GREENEVILLE	37745	36.334228	-82.836977
Prentice Cooper WMA*				35.14276	-85.36013

Appendix 2.6 (continued).

Name	Address	City	ZIP code	Latitude	Longitude
QUICK STOP BBQ MARKET AND DELI	276 JOHN MCGHEE HWY	CARYVILLE	37714	36.29318	-84.213237
R & D CUSTOM SLAUGHTERING	27015 US 127	DUNLAP	37327	35.2355	-85.410119
ROAN CRK MRT	4862 ROAN CREEK RD	MOUNTAIN CITY	37683	36.398821	-81.854566
ROCK HILL GROCERY	1635 HWY 70 N	ROGERSVILLE	37857	36.34418	-82.952418
ROCKY TOP MARKETS #37	104 BLUFF RD	KINGSTON	37763	35.889972	-84.529557
SALE CREEK GARDEN CENTER	14108 OLD DAYTON PK	SALE CREEK	37373	35.357792	-85.119362
SIMMONS BP	10624 POND CREEK RD	PHILADELPHIA	37846	35.690536	-84.421517
SLOANS LLC	107 HWY 360	VONORE	37885	35.591441	-84.233578
SOUTHERN OUTDOORS	2089 HWY 25 E	TAZEWELL	37879	36.465045	-83.569983
SOUTHSIDE PARKWAY MARKET	59 PINEY RD	SPENCER	38585	35.62288	-85.424699
STANTONVILLE AMOCO	8528 HWY 142	STANTONVILLE	38379	35.158688	-88.424163
SUPER DISCOUNT TOBACCO AND GAS	1435 RIVERSIDE DR	JACKSON	38301	35.581425	-88.838761
THE STORE	3588 HWY 421	BRISTOL	37620	36.544399	-82.114791
THE TACKLE BOX	6160 HWY 79 NORTH	PARIS	38242	36.355349	-88.220761
Volunteer Army Ammunition Plant WMA*				35.10193	-85.07813
WELLS CREEK MARKET	6565 HWY 13	ERIN	37061	36.319962	-87.667099
YODER BROTHERS MEAT MARKET	1650 BRIAR PATCH RD	PARIS	38242	36.308271	-88.420494

*****No address was provided for these check stations, so a GPS coordinate was identified within the WMA to serve as the check station point.

Appendix 2.7: Deer survey data (November and December 2007 and 2008) per county for other tick species.

Name	Region	2007 Population Estimate	2007 & 2008 Harvest	2007 & 2008 Checked	% harvested checked	Deer w/ ticks	Deer w/ *D. albi*	Deer w/ *A. amer*	Deer w/ other	% w/ ticks	% w/ *D. albi*	% w/ *A. amer*	% w/ other
Anderson	4	5500	1310	196	14.96%	163	159	12	160	83.16%	81.12%	6.12%	81.63%
Hawkins	4	18700	3603	57	1.58%	0	0	0	0				
Claiborne	6	11000	1975	48	2.43%	3	1	1	2	6.25%	2.08%	2.08%	4.17%
Stewart	1	14500	4283	31	0.72%	29	29	4	29	93.55%	93.55%	12.90%	93.55%
Hancock	4	6600	1274	29	2.28%	0	0	0	0				
Johnson	4	8800	1663	29	1.74%	0	0	0	0				
Roane	3	11550	4579	29	0.63%	25	23	0	23	86.21%	79.31%		79.31%
Hamilton	3	5775	2346	28	1.19%	17	8	2	9	60.71%	28.57%	7.14%	32.14%
Overton	3	11550	2212	28	1.27%	27	12	1	13	96.43%	42.86%	3.57%	46.43%
Humphreys	1	16000	4145	25	0.60%	13	11	2	12	52.00%	44.00%	8.00%	48.00%
Loudon	4	4950	940	25	2.66%	13	11	0	11	52.00%	44.00%		44.00%
Carter	4	7700	1464	24	1.64%	0	0	0	0				
Grainger	4	8250	1296	24	1.85%	1	0	0	0	4.17%			
White	3	8925	2332	24	1.03%	15	13	1	13	62.50%	54.17%	4.17%	54.17%
Montgomery	2	20000	5311	23	0.43%	10	10	3	10	43.48%	43.48%	13.04%	43.48%
Lauderdale	1	6825	2549	21	0.82%	3	0	0	0	14.29%			
Rhea	3	10500	2843	21	0.74%	17	17	1	17	80.95%	80.95%	4.76%	80.95%
Fayette	1	19000	8360	19	0.23%	3	0	1	1	15.79%		5.26%	5.26%
Campbell	4	8250	1383	18	1.30%	12	10	0	10	66.67%	55.56%		55.56%
Jefferson	4	6050	929	18	1.94%	0	0	0	0				
Van Buren	3	6300	1627	18	1.11%	11	11	0	11	61.11%	61.11%		61.11%
Greene	4	7700	1652	17	1.03%	0	0	0	0				
Monroe	4	4950	884	16	1.81%	1	1	0	0	6.25%	6.25%		
Henry	1	22000	8062	14	0.17%	5	4	0	4	35.71%	28.57%		28.57%
Houston	1	6500	1804	13	0.72%	5	5	1	5	38.46%	38.46%	7.69%	38.46%
DeKalb	3	9450	1670	12	0.72%	4	1	0	1	33.33%	8.33%		8.33%
Giles	2	22000	7839	12	0.15%	10	0	1	1	83.33%		8.33%	8.33%
Henderson	1	11025	2202	12	0.54%	0	0	0	0				
Jackson	3	12075	2915	12	0.41%	8	7	0	7	66.67%	58.33%		58.33%

Appendix 2.7 (continued).

Name	Region	2007 Population Estimate	2007 & 2008 Harvest	2007 & 2008 Checked	% harvested checked	Deer w/ ticks	Deer w/ *D. albi*	Deer w/ *A. amer*	Deer w/ other	% w/ ticks	% w/ *D. albi*	% w/ *A. amer*	% w/ other
Knox	4	2200	901	12	1.33%	3	3	1	3	25.00%	25.00%	8.33%	25.00%
Madison	1	17500	5334	12	0.22%	0	0	0	0				
Bledsoe	3	4200	1454	10	0.69%	8	7	1	7	80.00%	70.00%	10.00%	70.00%
Cocke	4	6050	1127	10	0.89%	0	0	0	0				
Obion	1	9975	3260	10	0.31%	0	0	0	0				
Sullivan	4	8800	1914	9	0.47%	0	0	0	0				
Unknown				9		4	1	1	2	44.44%	11.11%	11.11%	22.22%
Lawrence	2	14000	4321	8	0.19%	4	3	3	4	50.00%	37.50%	37.50%	50.00%
Sequatchie	3	4725	971	8	0.82%	6	5	1	5	75.00%	62.50%	12.50%	62.50%
Marshall	2	10500	4606	7	0.15%	7	2	2	4	100.00%	28.57%	28.57%	57.14%
Marion	4	7000	1991	6	0.30%	6	1	0	1	100.00%	16.67%		16.67%
Bedford	2	9000	3264	5	0.15%	5	0	2	2	100.00%		40.00%	40.00%
Cumberland	3	7875	3072	5	0.16%	3	3	1	3	60.00%	60.00%	20.00%	60.00%
Davidson	2	6500	1368	5	0.37%	3	2	0	2	60.00%	40.00%		40.00%
Maury	2	16500	3595	5	0.14%	5	1	0	1	100.00%	20.00%		20.00%
Hardeman	1	26000	9055	4	0.04%	1	0	1	1	25.00%		25.00%	25.00%
Tipton	1	3675	1339	4	0.30%	2	0	0	0	50.00%			
Williamson	2	11000	2516	4	0.16%	1	0	1	1	25.00%		25.00%	25.00%
Dickson	2	15000	3198	3	0.09%	1	1	0	1	33.33%	33.33%		33.33%
Hamblen	4	1650	522	3	0.57%	0	0	0	0				
Haywood	1	11550	2903	3	0.10%	0	0	0	0				
McMinn	3	7875	3527	3	0.09%	1	1	0	1	33.33%	33.33%		33.33%
McNairy	1	14500	5410	3	0.06%	0	0	0	1				33.33%
Sevier	4	2750	627	3	0.48%	0	0	0	0				
Sumner	2	12000	3594	3	0.08%	2	2	0	2	66.67%	66.67%		66.67%
Scott	3	11000	1513	2	0.13%	2	1	0	1	100.00%	50.00%		50.00%
Unicoi	4	3300	529	2	0.38%	0	0	0	0				
Union	4	3850	675	2	0.30%	0	0	2	0			100.00%	
Benton	1	13650	2837	1	0.04%	0	0	0	0				
Blount	4	4400	662	1	0.15%	0	0	0	0				

Appendix 2.7 (continued).

Name	Region	2007 Population Estimate	2007 & 2008 Harvest	2007 & 2008 Checked	% harvested checked	Deer w/ ticks	Deer w/ *D. albi*	Deer w/ *A. amer*	Deer w/ other	% w/ ticks	% w/ *D. albi*	% w/ *A. amer*	% w/ other
Cannon	2	5000	1305	1	0.08%	0	0	0	0				
Cheatham	2	9000	1862	1	0.05%	0	0	0	0				
Clay	3	6825	1272	1	0.08%	1	0	0	0	100.00%			
Hardin	1	13125	3839	1	0.03%	0	0	0	0				
Hickman	2	17000	4420	1	0.02%	0	0	0	0				
Meigs	3	8400	2625	1	0.04%	0	0	0	0				
Pickett	3	2750	228	1	0.44%	1	0	0	0	100.00%			
Rutherford	2	10000	2883	1	0.03%	0	0	0	0				
Shelby	1	12075	1949	1	0.05%	0	0	0	0				
Warren	3	7350	1166	1	0.09%	1	0	0	0	100.00%			
Wayne	2	13500	5419	1	0.02%	1	1	1	1	100.00%	100.00%	100.00%	100.00%
Weakley	1	19000	5967	1	0.02%	1	1	0	1	100.00%	100.00%		100.00%
Wilson	2	11000	2973	1	0.03%	0	0	0	0				
Total				**1018**	**1**	**464**	**368**	**48**	**383**	**45.58%**	**36.15%**	**4.72%**	**37.62%**

Appendix 2.8: Deer survey data (November and December 2007 and 2008) per county for *I. scapularis.*

Name	Region	2007 & 2008 Checked	Deer w/ I. scap	% w/ I. scap	Mean I. scap/deer	Mean Female I. scap/deer	Mean Male I. cap/deer	Detection Probability
Anderson	4	196	30	15.31%	2.37	1.37	1	100.00%
Hawkins	4	57	0					99.99%
Claiborne	6	48	1	2.08%	1	1	0	99.96%
Stewart	1	31	2	6.45%	2.5	1.5	1	99.35%
Hancock	4	29	0					99.10%
Johnson	4	29	0					99.10%
Roane	3	29	2	6.90%	5	3.5	1.5	99.10%
Hamilton	3	28	11	39.29%	5.82	3.09	2.73	98.95%
Overton	3	28	26	92.86%	5.62	3.15	2.46	98.94%
Humphreys	1	25	2	8.00%	1	1	0	98.27%
Loudon	4	25	5	20.00%	1.4	1.2	0.2	98.28%
Carter	4	24	0					97.97%
Grainger	4	24	1	4.17%	1	1	0	97.97%
White	3	24	5	20.83%	2.2	1.4	0.8	97.97%
Montgomery	2	23	0					97.61%
Lauderdale	1	21	3	14.29%	1.33	0.33	1	96.70%
Rhea	3	21	4	19.05%	1.5	1	0.5	96.69%
Fayette	1	19	2	10.53%	2	1	1	95.42%
Campbell	4	18	3	16.67%	1.33	0.67	0.67	94.62%
Jefferson	4	18	0					94.63%
Van Buren	3	18	0					94.63%
Greene	4	17	0					93.67%
Monroe	4	16	0					92.57%
Henry	1	14	1	7.14%	2	1	1	89.68%
Houston	1	13	0					87.88%
DeKalb	3	12	4	33.33%	2.25	1.25	1	85.73%
Giles	2	12	10	83.33%	7.2	3.8	3.4	85.72%
Henderson	1	12	0					85.73%
Jackson	3	12	3	25.00%	2.33	1.67	0.67	85.73%
Knox	4	12	0					85.80%
Madison	1	12	0					85.73%
Bledsoe	3	10	1	10.00%	5	2	3	80.28%
Cocke	4	10	0					80.27%
Obion	1	10	0					80.26%
Sullivan	4	9	0					76.78%
Unknown		9	3	33.33%	1	0.33	0.67	
Lawrence	2	8	3	37.50%	11.33	6.33	5	72.68%
Sequatchie	3	8	2	25.00%	1	0.5	0.5	72.71%
Marshall	2	7	7	100.00%	12.29	7.14	5.14	67.88%
Marion	4	6	5	83.33%	3.2	2	1.2	62.22%

Appendix 2.8 (continued).

Name	Region	2007 & 2008 Checked	Deer w/ I. scap	% w/ I. scap	Mean I. scap/deer	Mean Female I. scap/deer	Mean Male I. scap/deer	Detection Probability
Bedford	2	5	5	100.00%	17.8	8.8	9	55.56%
Cumberland	3	5	2	40.00%	20	13.5	6.5	55.56%
Davidson	2	5	3	60.00%	8	4.67	3.33	55.57%
Maury	2	5	5	100.00%	11	5.8	5.2	55.56%
Hardeman	1	4	0					47.73%
Tipton	1	4	2	50.00%	5	1.5	3.5	47.74%
Williamson	2	4	1	25.00%	23	15	8	47.73%
Dickson	2	3	0					38.52%
Hamblen	4	3	0					38.55%
Haywood	1	3	0					38.53%
McMinn	3	3	0					38.53%
McNairy	1	3	0					38.53%
Sevier	4	3	0					38.54%
Sumner	2	3	1	33.33%	1	1	0	38.53%
Scott	3	2	1	50.00%	11	8	3	27.70%
Unicoi	4	2	0					27.71%
Union	4	2	0					27.71%
Benton	1	1	0					10.99%
Blount	4	1	0					14.97%
Cannon	2	1	0					14.97%
Cheatham	2	1	0					14.97%
Clay	3	1	1	100.00%	4	3	1	14.97%
Hardin	1	1	0					14.97%
Hickman	2	1	0					14.97%
Meigs	3	1	0					14.97%
Pickett	3	1	1	100.00%	14	8	6	14.97%
Rutherford	2	1	0					14.97%
Shelby	1	1	0					14.97%
Warren	3	1	1	100.00%	25	14	11	14.97%
Wayne	2	1	1	100.00%	3	1	2	14.97%
Weakley	1	1	0					14.97%
Wilson	2	1	0					14.97%
Total		**1018**	**160**	**15.72%**	**5.44**	**3.08**	**2.37**	

* Counties not surveyed are not listed.

Appendix 3.1: Mean and standard error of *I. scapularis* per 1000m^2 at Henry Horton State Park and within sampling sites (Wild Turkey and Hickory Ridge) from November 2007 to May 2009. L: larvae; N: nymph, AF: adult females, AM: adult male, A: adults (both male and female).

HHSP

Visit	No.	L	N	AF	AM	A
Nov-07	3			5.95±0.19	4.43±0.52	12.01±1.16
Dec-07	2			1.58±1.05	2.89±0.26	5.89±1.74
Jan-08	5		0.17±0.17			
Feb-08	5		3.13±2.16	2.31±1.08	3.69±1.03	5.29±1.74
Mar-08	5	0.20±0.20	4.95±3.45	0.86±0.57	2.17±0.89	2.77±1.14
Apr-08	6		1.27±0.68	0.67±0.42	0.50±0.34	0.83±0.30
May-08	7		4.22±1.44	0.29±0.29		0.14±0.14
Jun-08	7		1.67±1.09			
Jul-08	7	4.14±2.86				
Aug-08	7	0.29±0.29	0.95±0.95			
Sep-08	6	0.17±0.17	0.33±0.33			
Oct-08	7		0.29±0.29			
Nov-08	7			0.29±0.29	0.73±0.36	0.87±0.34
Dec-08	5			3.33±1.27	0.37±0.23	2.77±0.80
Jan-09	6			1.29±0.44	0.75±0.48	1.75±0.87
Feb-09	8			0.50±0.50	1.25±0.75	1.50±0.73
Mar-09	8		0.07±0.07	0.57±0.32	0.99±0.40	1.36±0.50
Apr-09	7			0.29±0.29	1.71±0.52	7.86±0.63
May-09	5		4.93±1.27			

Wild Turkey							Hickory Ridge					
Visit	No.	L	N	AF	AM	A	No.	L	N	AF	AM	A
Nov-07	2			5.89±0.31	4.96±0.004	11.96±2.0	1			6.08±0.0	3.38±0.0	12.38±0.0
Dec-07	1			2.63	2.63	7.63	1			0.53±0.0	3.16±0.0	4.16±0.0
Jan-08	3						2		0.42±0.42			
Feb-08	3		4.92±3.39	3.85±0.96	4.88±1.34	7.55±1.94	2		0.45±0.45		1.91±0.09	1.91±0.09
Mar-08	3	0.33±0.33	7.14±5.71	1.43±0.83	3.33±0.95	4.33±1.11	2		1.67±1.67		0.42±0.42	0.42±0.42
Apr-08	3		1.33±1.33	0.67±067	1.0±0.58	1.33±0.33	3		1.20±0.72	0.67±0.67		0.33±0.33

Appendix 3.1 (continued).

Wild Turkey							Hickory Ridge					
Visit	**No.**	**L**	**N**	**AF**	**AM**	**A**	**No.**	**L**	**N**	**AF**	**AM**	**A**
May-08	4		4.79±2.30	0.5±0.5		0.25±0.25	3		3.48±1.88			
Jun-08	4		2.92±1.72				3					
Jul-08	4	7.0±4.73					3					
Aug-08	4	0.5±0.5					3		2.22±2.22			
Sep-08	3		0.67±0.67				3	0.33±0.33				
Oct-08	4						3		0.67±0.67			
Nov-08	4				0.78±0.48	0.78±0.48	3			0.67±0.67	0.67±0.67	1.0±0.58
Dec-08	3			2.56±0.73	0.61±0.31	2.94±1.16	2			4.50±3.50		2.5±1.5
Jan-09	3			1.50±0.76	0.83±0.83	2.17±1.69	3			1.80±0.58	0.67±0.67	1.33±0.88
Feb-09	4				2.0±1.41	2.0±1.41	4			1.0±1.0	0.50±0.50	1.0±0.58
Mar-09	5		0.11±0.11	0.91±0.46	1.28±0.55	1.88±0.68	3				0.51±0.51	0.51±0.51
Apr-09	4			0.50±0.50	2.0±0.82	2.5±1.03	3				1.33±0.67	1.33±0.67
May-09	3		6.0±2.0				2		3.33±0.0			

Appendix 3.2: Relative activity of *I. scapularis* from ticks collected off mammals (per 100 traps nights) and from dragging (per 1000m^2) at Henry Horton State Park, TN from November 2007 to May 2009.

Month	Number/transect			Relative activity		
	L	N	A	L	N	A
Nov-07	0.0	0.0	10.3	0.0	0.0	40.5
Dec-07	0.0	0.0	4.5	0.0	0.0	17.7
Jan-08	0.0	0.5	0.0	0.0	2.1	0.0
Feb-08	0.0	3.0	5.9	0.0	13.7	23.5
Mar-08	0.3	4.9	3.3	0.2	22.5	13.2
Apr-08	0.0	2.4	1.1	0.0	11.0	4.2
May-08	0.0	7.7	0.2	0.0	35.7	1.0
Jun-08	34.0	1.4	0.0	23.6	6.4	0.0
Jul-08	86.3	0.0	0.0	59.9	0.0	0.0
Aug-08	16.6	0.6	0.0	11.5	2.8	0.0
Sep-08	5.0	0.3	0.0	3.5	1.5	0.0
Oct-08	2.0	1.0	0.0	1.4	4.4	0.0
Nov-08	0.0	0.0	1.0	0.0	0.0	4.1
Dec-08	0.0	0.0	3.3	0.0	0.0	13.2
Jan-09	0.0	0.0	2.5	0.0	0.0	9.9
Feb-09	0.0	0.0	1.6	0.0	0.0	6.5
Mar-09	0.7	1.5	1.8	0.5	6.8	7.1
Apr-09	0.0	2.7	2.0	0.0	12.3	7.9
May-09	0.0	7.5	0.0	0.0	34.7	0.0
12 month total	144.2	21.7	25.3			

VITA

Michelle Rosen is originally from Farmington Hills, MI and attended Michigan State University, where she earned her Bachelor of Science degree in Fisheries and Wildlife. Michelle has worked for the Catskill Outdoor Education Corps, U.S. Fish and Wildlife Service, and the Michigan Department of Natural Resources Wildlife Disease Laboratory. In 2005, she worked as a technician assisting a Michigan State field investigation of the emergence of Lyme disease in lower Michigan. Michelle completed her Master of Science in Wildlife and Fisheries Science with a minor in Entomology and Plant Pathology at the University of Tennessee, Knoxville in August 2009.