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Original Contribution

Invasion of the Lyme Disease Vector *Ixodes scapularis*: Implications for *Borrelia burgdorferi* Endemicity

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Abstract: Lyme disease risk is increasing in the United States due in part to the spread of blacklegged ticks *Ixodes scapularis,* the principal vector of the spirochetal pathogen *Borrelia burgdorferi*. A 5-year study was undertaken to investigate hypothesized coinvasion of *I. scapularis* and *B. burgdorferi* in Lower Michigan. We tracked the spatial and temporal dynamics of the tick and spirochete using mammal, bird, and vegetation drag sampling at eight field sites along coastal and inland transects originating in a zone of recent *I. scapularis* establishment. We document northward invasion of these ticks along Michigan's west coast during the study period; this pattern was most evident in ticks removed from rodents. *B. burgdorferi* infection prevalences in *I. scapularis* sampled from vegetation in the invasion zone were 9.3% and 36.6% in nymphs and adults, respectively, with the majority of infection (95.1%) found at the most endemic site. There was no evidence of *I. scapularis* invasion along the inland transect; however, low-prevalence *B. burgdorferi* infection was detected in other tick species and in wildlife at inland sites, and at northern coastal sites in years before the arrival of *I. scapularis*. These infections suggest that cryptic *B. burgdorferi* transmission by other vector-competent tick species is occurring in the absence of *I. scapularis*. Other *Borrelia* spirochetes, including those that group with *B. miyamotoi* and *B. andersonii*, were present at a low prevalence within invading ticks and local wildlife. Reports of Lyme disease have increased significantly in the invasion zone in recent years. This rapid blacklegged tick invasion—measurable within 5 years—in combination with cryptic pathogen maintenance suggests a complex ecology of Lyme disease emergence in which wildlife sentinels can provide an early warning of disease emergence.

Keywords: *Borrelia burgdorferi*, *Ixodes scapularis*, blacklegged tick, *Peromyscus leucopus*, invasion, Lyme disease

INTRODUCTION

Lyme disease accounts for more than 90% of all reported vector-borne disease in the United States with more than 20,000 cases reported annually; its current invasive spread

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from endemic foci constitutes a major public health concern (Bacon et al., 2008). In the United States, Lyme disease is caused by the bacterium *Borrelia burgdorferi* and the blacklegged tick *Ixodes scapularis* serves as the vector for most cases. Patterns of human disease mirror the geographic distribution of *B. burgdorferi*-infected *I. scapularis*, which is characterized by high-density endemic foci in the Northeast and Upper Midwestern United States. Increasing incidence is associated with both biological and nonbiological factors, including increases in abundance and range expansions of wildlife, human encroachment into tick habitat, creation of peridomestic habitats that attract wildlife hosts of ticks and pathogens, and enhanced surveillance and awareness among medical professionals. Blacklegged ticks were likely widespread before the most recent glaciation event, during which relict populations remained in refuges from which they and their vertebrate hosts later expanded (Steere et al., 2004). During the past 50 years, white-tailed deer (*Odocoileus virginiaus*) populations have undergone explosive growth due to reversion of agricultural lands to forest and restrictions on hunting. Increased deer abundance has facilitated the recent blacklegged tick expansion and Lyme disease emergence throughout the Northeast and Midwest. *I. scapularis* continues to spread from both the Northeastern and Midwestern foci (Steere et al., 2004), but the mechanisms for this spread are not established. *I. scapularis* serves as the vector for multiple zoonotic pathogens, including the agents of Lyme disease, human anaplasmosis, babesiosis, and Powassan encephalitis; therefore, its expansion constitutes increased risk of multiple diseases.

In 2002, a new population of *I. scapularis* was detected in the southwestern corner of Michigan's Lower Peninsula (Foster, 2004). This represented a significant change from the State's previous reported distribution (Walker et al., 1998), which was characterized by a probable lack of established *I. scapularis* throughout the Lower Peninsula. Established populations in the state were recognized at that time only in Menominee County of the Upper Peninsula, adjacent to endemic foci in Wisconsin (Walker et al., 1998). Nevertheless, a Midwestern model of habitat suitability (Guerra et al., 2002) predicted that sandy oak upland forests in Michigan provide highly suitable habitat for *I. scapularis,* and *I. scapularis* indeed were discovered in such areas (Foster, 2004). Suitable habitat types are widespread throughout Lower Michigan, so further invasion of *I. scapularis* is thus predicted.

Tick Versus Pathogen Invasion?

B. burgdorferi and *A. phagocytophilum* have been found within Michigan's recently invaded tick populations (Hamer et al., 2007). The early detection of these low-density infected tick populations afforded us the rare opportunity to test, in real time, three nonexclusive scenarios by which invasion of the Lyme disease vector and/or pathogen into Lower Michigan may be occurring. (1) In the "tick-first" scenario, new uninfected *I. scapularis* populations become established as a result of long distance dispersal of adult ticks by white-tailed deer. This deer-mediated invasion would introduce replete uninfected adult ticks but not *B. burgdorferi* (because deer are incompetent hosts for this pathogen; Telford et al., 1988). In this scenario, we hypothesize that *B. burgdorferi* enters the system later, as result of a slower secondary invasion mediated by infected mammalian or avian hosts. (2) In the "dual-invasion" scenario, mammalian or avian hosts introduce infected *I. scapularis* to new areas so that both *I. scapularis* and *B. burgdorferi* establish concurrently. In this scenario, *B. burgdorferi* should be detectable early in the invasion process, when *I. scapularis* densities are still very low. (3) In the "spirochete-first" scenario, enzootic transmission of the pathogen is maintained by cryptic vectors and reservoir hosts. These vectors are generally wildlife host specialists, which, unlike *I. scapularis*, do not bite humans (Telford and Spielman, 1989a, b), and thus these transmission cycles have no implications for human or canine disease risk. Invasion of *I. scapularis* (infected or uninfected) into zones of cryptic pathogen maintenance will, however, create opportunities for bridging the pathogen to humans and canines.

We initiated comprehensive sampling to investigate the spatial and temporal dynamics of the Lyme disease system in and beyond the zone of hypothesized *I. scapularis* invasion in Lower Michigan. To address invasion, we studied areas not only where all three parasitic life stages of *I. scapularis* were endemic (presence of all three stages and/or at least six individuals of a single stage constitute the Centers for Disease Control and Prevention (CDC) definition of an "established" population; Dennis et al., 1998), but also in areas beyond its detected distribution. The objectives of this study were to: (1) test whether the detected distribution of *I. scapularis* changed during the period 2004–2008; and (2) assess the pattern of occurrence of *B. burgdorferi* in relation to that of *I. scapularis*. To address the tick-first hypothesis, each field site was sampled

regularly for the presence of *I. scapularis* to detect new colonization and/or trends in tick abundance at each site. To address the dual-invasion and spirochete-first hypotheses, a diverse community of hosts and their attached ticks were assayed to assess the relationship between *I. scapularis* presence and *B. burgdorferi* infection. Thus, we include results from a diverse assemblage of ticks, including those with low or no vectorial capacity and no associated zoonotic risk (i.e., tick species that are physiologically incompetent for spirochete transmission and/or species that do not regularly feed on human hosts). Although unimportant for transmission, these incompetent ticks can serve as bioindicators of *B. burgdorferi* presence in an area if they contain an infectious blood meal or transtadially passed spirochetes from a previous meal.

Methods

Site Selection and Sampling Regime

From 2004–2008, we assessed on- and off-host ticks, and wildlife, along two sampling transects established at a spatial scale that extended beyond the known limit of the Lower Michigan *I. scapularis* population (Fig. 1). Both transects originated in the southwestern Michigan zone where *I. scapularis* was first detected in 2002 (Foster, 2004). From this origin, the coastal transect extended north along Lake Michigan (sites labeled C1–C4; 81–145 km between sites: Van Buren State Park, Van Buren Co.; Duck Lake State Park, Muskegon Co.; Orchard Beach State Park, Manistee Co.; Sleeping Bear Dunes National Lakeshore, Benzie Co.; respectively). The inland transect extended northeastward (sites labeled I1–I4; 33–76 km between sites: Fort Custer State Recreation Area, Kalamazoo Co.; Lux Arbor Reserve of Kellogg Biological Station; Barry Co.; Ionia State Recreation Area, Ionia Co.; and Rose Lake Wildlife Research Area, Clinton Co.; respectively). Both transects traversed areas where established *I. scapularis* had not been previously detected (Walker et al., 1998; Foster, 2004), or were uninvestigated. Sampling was conducted in oak-dominated, closed-canopy deciduous forest when available, because this habitat type is positively associated with *I. scapularis* presence in the endemic Midwest (Guerra et al., 2002). Otherwise, sampling was conducted in the dominant forest type at the site. All data presented are for May and June, when *I. scapularis* larvae, nymphs, and adults are simultaneously active. Each site was sampled in these months in all 5 years of the study, except for site C4 (2007 and 2008 only) and site I1 (all years except 2006).

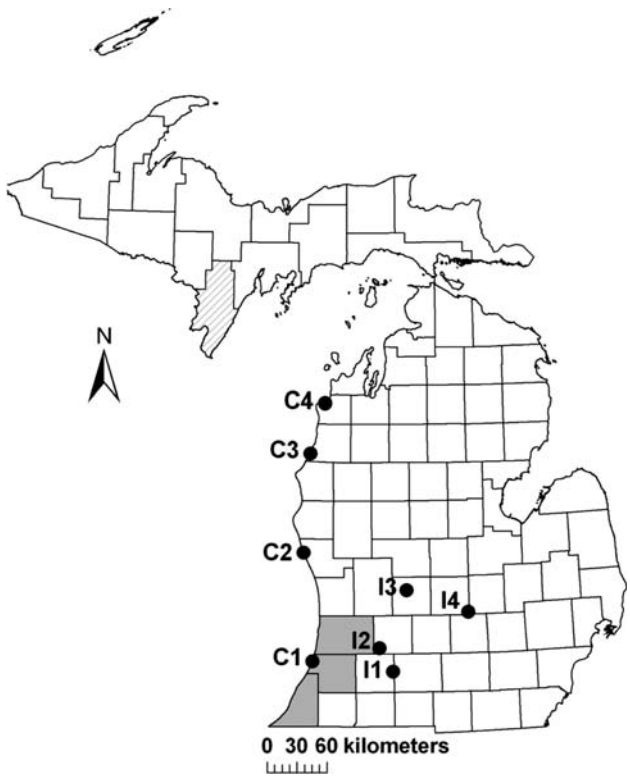

Figure 1. Locations of study sites in Lower Michigan, 2004–2008. The four sites along Michigan's west coast comprise the coastal transect (from south to north, C1–C4), and the four inland sites comprise the inland transect (from southwest to northeast, I1–I4). Shading in the Lower Peninsula represents the three-county region where *I. scapularis* were detected on small mammals in 2002–2003 (Foster 2004a). The cross-hatched county in the Upper Peninsula is Menominee County, Michigan's longstanding endemic focus of *I. scapularis* and *B. burgdorferi*.

Mammal Trapping

At each field site, small mammals were trapped along 4 to 6 transects of 25 Sherman live traps (H. B. Sherman Traps, Tallahassee, FL) spaced 10 m apart and baited with sunflower seed. Medium-sized mammals were trapped using 12–16, 32- × 10- × 12-inch live traps (Tomahawk Live Traps, Tomahawk, WI) and wooden box traps baited with peanut butter, jelly, and cat food on tortillas. Small mammals were anesthetized using Isoflurane (IsoFlo, Abbot Laboratories, Abbott Park, IL); medium-sized mammals were anesthetized using ketamine hydrochloride (Ketaset; Fort Dodge, Overland Park, KS) and xylazine hydrochloride (Rompun; Bayer Health Care, Kansas City, KS) followed by reversal with administration of yohimbine

hydrochloride (Antagonil; Wildlife Laboratories, Fort Collins, CO). Animals were identified to species and sex by inspection. Each animal was examined for ticks, biopsied in both ears using a 2-mm (small mammals) or 4-mm (medium mammals) biopsy punch (Miltex Instruments, York, PA), and ear-tagged (National Band and Tag, Newport, KY). Ticks and ear biopsies were stored separately in 70% ethanol. Additional ear biopsies were obtained from animals recaptured after an interval of 2 weeks or more; recaptures at a shorter interval were simply rechecked for ticks. All animals were released at the site of capture. Small mammal trapping success rate was discounted by the number of empty, tripped traps as follows: number mammal captures/(number traps set − 0.5*no. tripped traps); this expression assumes that on average tripped traps were open for half the night. Wildlife procedures were approved through Michigan State University's Institutional Animal Use and Care Committee permit #02-07-13-000.

Bird Mist Netting

At each site, six 12-m mist nets (Avinet, Dryden, NY) were used to capture birds in the same areas where mammals were being trapped. Nets were run from 0600–1200 h on fair weather days and checked every 45 min. Birds were weighed, identified to species and sex, measured, searched for ticks, and leg-banded with federally issued bands before release. Mist netting was performed under permit #02640 from the federal Bird Banding Laboratory.

Questing Tick Sampling

Each site was sampled for questing ticks by dragging a 1-m^2 white corduroy cloth (Falco and Fish, 1992) over the forest floor along the same six transects that were used for mammal trapping. Drag sampling was performed on rain-free days in the late morning or late afternoon to avoid the hottest and least humid times of day (Schulze et al., 2001; Diuk-Wasser et al., 2006). At least 1,000 m^2 of vegetation were sampled per visit. The cloth was inspected every 20 m, and attached ticks were stored in 70% ethanol. Drag sampling was not performed on excessively hot or wet days.

Borrelia Burgdorferi Detection

All ticks were identified to species and stage (Keirans and Clifford, 1978; Sonenshine, 1979; Durden and Keirans, 1996). Total DNA from ticks and ear biopsies was extracted using the DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA) following the manufacturer's animal tissue protocol with the following modifications. Ticks were first bisected using a sterile scalpel or were pulverized in liquid nitrogen, followed by an overnight incubation in lysis buffer. DNA was eluted using 50 µl elution solution warmed to 70°C. Ear biopsies (one per animal), adult and nymphal ticks were extracted individually, and conspecific larvae from the same individual animal or drag transect were pooled for extraction. Given our specific questions, we implemented a protocol for subsampling of ticks to assay for infection in the occasional cases of heavily parasitized hosts. In cases where more than three adult or nymphal ticks of the same species, life stage, and sex were removed from an individual host, three were randomly selected for testing (i.e., the maximum number of ticks tested from a host would be, for each tick species present, six adults (three female and three male), three nymphs, and a larval pool). By including a subset of ticks from many hosts, rather than all ticks from a smaller number of hosts (given limited resources), this sampling strategy allowed for improved coverage of the host population and also reduced statistical concerns of nonindependence that would arise if very large numbers of ticks were collected from a small number of highly infested hosts. *B. burgdorferi* strain B31-infected nymphal *I. scapularis* acquired from the CDC and water served as the positive and negative extraction controls, respectively.

B. burgdorferi was detected using: (1) a nested polymerase chain reaction (PCR) for the 16S–23S rRNA intergenic spacer region (IGS) of *Borrelia* spp. (Bunikis et al., 2004) followed by visualization with gel electrophoresis, or (2) a quantitative PCR (qPCR) of a region of the 16S rRNA of *B. burgdorferi* (Tsao et al., 2004). PCR enzyme kits were used throughout (nested PCR: PCR Supermix, Invitrogen, Carlsbad, CA and FailSafe PCR System, Epicentre, Madison, WI; qPCR: Universal PCR Master Mix, Applied Biosystems, Foster City, CA). In addition to the positive and negative DNA extraction controls for each batch of samples extracted, DNA from *B. burgdorferi* strain B31-infected ticks from laboratory colonies at the CDC and water served as positive and negative PCR controls. In qPCR, a six-point standard dilution series of DNA extracted from cultured spirochetes (10^4–10^{-1} organisms per 3 µl reaction volume) served as positive controls (*B. burgdorferi* strain C336, rRNA spacer type (RST) II strain). Reactions for qPCR were performed with an ABI Prism 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA). Preliminary experiments showed that

both tests were able to detect positive samples containing a minimum 10^0–10^{-1} organisms.

Nucleotide Sequencing

A subset of *B. burgdorferi*-positive ticks and ear biopsies were DNA sequenced to confirm pathogen identity. Samples included: (1) a random subset of positive samples from sites where *I. scapularis* is common; (2) a majority of test-positive samples from sites with an apparent absence of *I. scapularis*; and (3) any IGS amplicons of approximately 500 bp, characteristic of the relapsing-fever spirochete *B. miyamotoi* (cf. the 987 bp fragment characteristic of *B. burgdorferi*). When a sample was determined to be positive using qPCR, IGS PCR was then performed to generate the template for sequencing. The IGS product was purified (Qiagen PCR Purification Kit; Qiagen, Valencia, CA) and sequences were determined in both directions using an ABI Prism 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA). Sequences were identified as *B. burgdorferi* or *B. miyamotoi* based on comparisons to published sequences using the basic local alignment search tool in GenBank (Altschul et al., 1990). *B. burgdorferi* strains were assigned to IGS ribosomal spacer type (RST; Liveris et al., 1995) by alignment with the prototypical strains published in Bunikis et al. (2004) using the program MEGA (Tamura et al., 2007).

Statistics

Logistic regression was used to assess trends in wildlife infestation and tick infection during the 5-year sampling period. Fisher's exact test was used to assess differences in infestation among sites. Linear regression was used to assess trends in nymphal densities within sites over time. Within-year comparisons between the coastal and inland transects were made by calculating the z-ratio and associated two-tail probabilities for the difference between two independent proportions. Minimum infection prevalence (MIP; i.e., assuming only one positive larva per pool) was used for tests done on pooled larvae. Statistics were performed using Statistix 8 (Analytical Software, Tallahassee, FL).

Results

Wildlife Captures

We achieved a total of 1,667 mammal captures, comprised of 1,472 small and 195 medium mammal captures. Small mammals were captured in a total of 7,998 adjusted trap nights (18.4% overall capture rate). White-footed mouse (*Peromyscus leucopus*) was the most commonly caught species (84.9% of all small mammal captures); their capture rates did not differ between coastal and inland transect sites ($P = 0.95$). Medium mammals were captured in a total of 777 adjusted trap nights (25.1% overall capture rate), with similar rates along both transects ($P = 0.93$). Raccoon (*Procyon lotor*) was the most abundant species (65.6% of all medium mammal captures). Despite escaped animals and the release of lethargic shrews (*Blarina brevicauda*), 99.8% of captured mammals were processed, 82.5% of which were first captures, and 6.5% were recaptures after a minimum 2-week interval; these animals were processed in full and used for computing the infestation and infection prevalences reported below. The remaining 11% represent white-footed mice recaptured within 2 weeks. These latter mice are not considered further in infestation analyses; however, ticks removed from these animals are considered in infection analyses. In total, 14 mammal species were parasitized by 8 species of ticks (Table 1).

A total of 747 bird captures, comprised of 55 species, occurred in 2,180 net hours for an average of 34.3 birds per 100 net hours. Due to escaped birds, 99.2% were processed, of which 91.6% represent first captures and 6.7% were bird recaptured after a minimum 2-week interval; these birds were processed in full and used in computing infestation and infection prevalences below. The remaining 1.6% represents birds recaptured within 2 weeks—none harbored ticks. Birds were parasitized by three species of ticks (Table 1; Supplement 1).

I. scapularis on Wildlife

Along the inland transect, *I. scapularis* were rarely found on wildlife, with only 3 of 574 white-footed mice parasitized. Two of these were trapped at site I3 in 2007 (3% infestation), with one harboring a single larva and nymph, and one harboring four larvae. The other mouse was trapped at site I1 in 2008 (3.9% infestation) and harbored a single nymph. None of the 73 eastern chipmunks, 133 individuals of other mammal species, or 504 birds inspected on this inland transect harbored *I. scapularis*.

In contrast, a steep gradient of *I. scapularis* infestation of wildlife was evident along the coastal transect. In all years the level of white-footed mouse infestation was highest at the southernmost C1 site (75–100% with no significant difference among years; $P = 0.08$), and larval and nymphal

Table 1. Tick-Host Associations and Infestation Prevalence on the Inland and Coastal Transects in Lower Michigan, May–June, 2004–2008

Host species	Transect	No. captures	No. animals infested with L/N/A (% infested with any stage)							
			D. variabilis	*I. scapularis*	*I. texanus*	*I. cookei*	*I. dentatus*	*I. marxi*	*H. leporispalustris*	*A. americanum*
Northern short-tailed shrew	Inland	10								
Blarina brevicauda	Coastal	3								
Virginia opossum	Inland	22	0/0/15 (68.2)			1/1/1 (9.1)				
Didelphis virginiana	Coastal	13	0/0/6 (46.2)	6/8/2 (61.5)		0/3/0 (23.1)				
Southern flying squirrel	Inland	1								
Glaucomys volans	Coastal	13	1/0/2 (23.1)	0/2/0 (15.4)	1/0/0 (7.7)			0/0/1 (7.7)		
Striped skunk										
Mephitis mephitis	Coastal	2		0/1/0 (50)		1/1/1 (100)				
Meadow vole										
Microtus pennsylvanicus	Coastal	1		1/0/0 (100)						
Long-tailed weasel										
Mustela frenata	Inland	2	1/0/0 (50)			1/1/0 (50)				
White-footed mouse	Inland	665	293/58/0 (46.2)	2/2/0 (0.5)						
Peromyscus leucopus	Coastal	570	106/42/0 (20.3)	175/53/0 (33.5)	1/1/0 (0.4)					
Raccoon	Inland	64	1/4/37 (59.4)		6/26/32 (68.8)	1/6/1 (10.9)				
Procyon lotor	Coastal	62	2/1/39 (62.9)	3/11/2 (21.0)	10/34/34 (72.6)	0/5/2 (9.7)	0/1/0 (1.6)	0/0/1 (1.6)		0/0/1 (1.6)
Eastern gray squirrel										
Sciurus carolinensis	Coastal	5	1/0/1 (20)	2/4/0 (80)						
Fox squirrel										
Sciurus niger	Coastal	1		1/1/0 (100)						
Eastern cottontail	Inland	4	0/0/1 (25)				0/1/4 (100)		0/0/1 (25)	
Sylvalagus floridanus	Coastal	1					0/0/1 (100)		0/1/1 (100)	
Eastern chipmunk	Inland	74	3/2/0 (6.8)							
Tamias striatus	Coastal	84	1/0/0 (1.2)	16/36/0 (46.4)	1/0/0 (1.2)					
Red squirrel	Inland	16	0/1/0 (6.3)		0/0/1 (6.3)	0/1/0 (6.3)		0/1/1 (12.5)		
Tamiasciurus hudsonicus	Coastal	2		1/0/0 (50)	0/0/1 (50)					
Meadow jumping mouse	Inland	12	6/1/0 (50)				4/0/0 (33.3)			
Zapus hudsonius	Coastal	16		1/2/0 (12.5)			0/0/1 (6.3)			
Birds[a]	Inland	504					57/29/0 (12.9)		1/5/6 (1.2)	
All species	Coastal	237		5/14/0 (5.9)			4/4/2 (3.8)		2/2/0 (1.3)	

[a]Species-specific bird captures and tick associations are presented in Supplement 1.

N nymph, *A* adult, *L* larva.

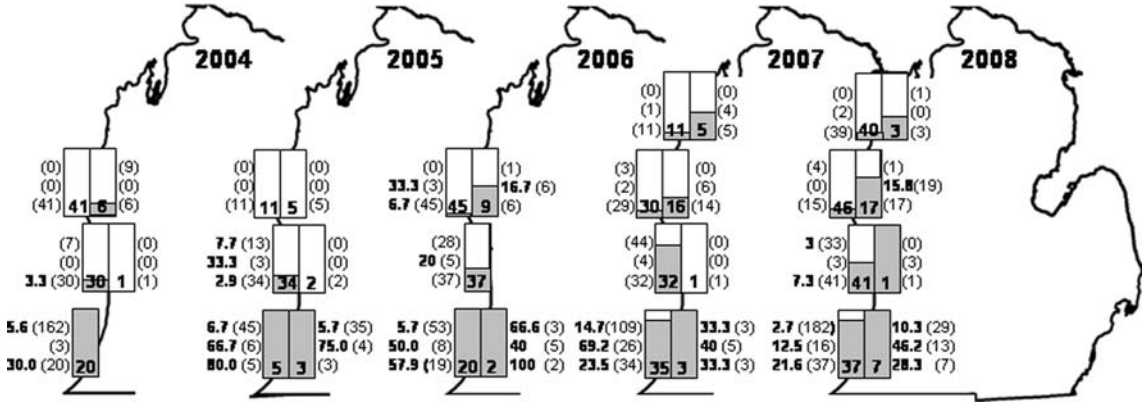

Figure 2. Infestation of white-footed mouse (*left rectangle*) and eastern chipmunk (*right rectangle*) with *I. scapularis* at the four sites of the coastal transect, May and June, 2004–2008. Level of shading indicates the proportion of animals infested, with the number of animals screened for ticks inside the symbol. Adjacent to each symbol, *B. burgdorferi* infection prevalence (%) in (1) *I. scapularis* larvae (minimum infection prevalence), (2) nymphs removed from hosts, and (3) host ear biopsies are listed from top to bottom, each followed by sample size in parenthesis. At site C4, no traps were set in 2004–2006. At site C1 in 2004 and C2 in 2006, no chipmunks were captured despite traps being set. Information on the inland transect sites, where infestation and infection rates were close to zero, appears in the text.

burdens averaged 4.7 and 0.6 per mouse, and 5.6 and 2.3 per infested mouse (maxima of 37 and 11, respectively). Infestation at the more northern sites was much lower but increased progressively during the study (Fig. 2). At site C2, *I. scapularis*-infested mice were trapped in all 5 years, with infestation increasing significantly over time ($P < 0.001$). At site C3, no *I. scapularis*-infested mice were observed until the third summer after which infestation increased ($P = 0.76$). Site C4 harbored a low proportion of *I. scapularis*-infested mice in both 2007 and 2008 (9% and 10%, respectively); these were the only 2 years when May–June sampling was conducted at this site (in July 2005 none of 21 mice trapped at C4 harbored *I. scapularis*, whereas 60% of 110, 13% of 54, and 0% of 16 mice were infested at sites C1, C2, and C3 respectively).

Overall, 48.2% of 81 eastern chipmunks harbored *I. scapularis* along coastal sites. As with the mice, infestation was highest at site C1, with all chipmunks there ($n = 15$) infested in all years. Fewer chipmunks were infested at the three more northern sites (Fig. 2; $P < 0.0001$); however, infestation levels were increasing over time ($P = 0.01$ for sites C2 and C3 pooled). *I. scapularis* burdens on chipmunks were greatest at site C1; larval burdens there were similar to those of mice (average 4.7 per chipmunk and 5.8 per larval-infested chipmunk, maximum of 20), whereas nymphal burdens were much higher (average 7.2 per chipmunk, and 7.7 per nymphal-infested chimpunk, maximum of 36). Of 117 other mammals caught on the coastal transect, 28.2% comprising ten species harbored *I. scapularis* (Table 1); most were from C1 (54.5%) or C2 (24.2%). Of 237 bird captures, *I. scapularis* was found on 14, of which 13 were from C1 and one was from C2. These 14 birds comprised five species: American robin, chipping sparrow, Eastern towhee, indigo bunting, and Northern cardinal (Supplement 1).

Questing Ticks

A total of 58,459 m^2 of drag sampling was conducted, with 26,800 and 31,650 m^2 along the inland and coastal transects, respectively. A total of 1,984 ticks were collected of which 87% were *I. scapularis*. Four other tick species were collected on drag cloth: *Dermacentor variabilis* ($n = 252$), *Amblyomma americanum* ($n = 4$), *Haemaphysalis leporispalustris* ($n = 2$), and *I. dentatus* ($n = 5$).

All but one *I. scapularis* came from coastal sites. Along the inland transect, a single larva was dragged (at I3 in 2007). Conversely, along the coastal transect, 86 adults, 246 nymphs, and 1,388 larvae were collected, of which 95.3%, 91.5%, and 93.3% respectively were from site C1. A significant gradient of *I. scapularis* density was detected, with the greatest abundance at site C1, where all life stages were present in all five summers of sampling (peak May/June densities were 8.2 adults, 29.7 nymphs, and 90.8 larvae per 1000 m^2) and nymphal abundance did not change significantly during the 5 years ($R^2 = 0.14$; $P = 0.26$). Many fewer, yet increasing, numbers of *I. scapularis* occurred at the two sites to the north (Fig. 3): at site C2, no *I. scapularis* were dragged at the start of the study whereas all stages were present by the end with a significant increase in

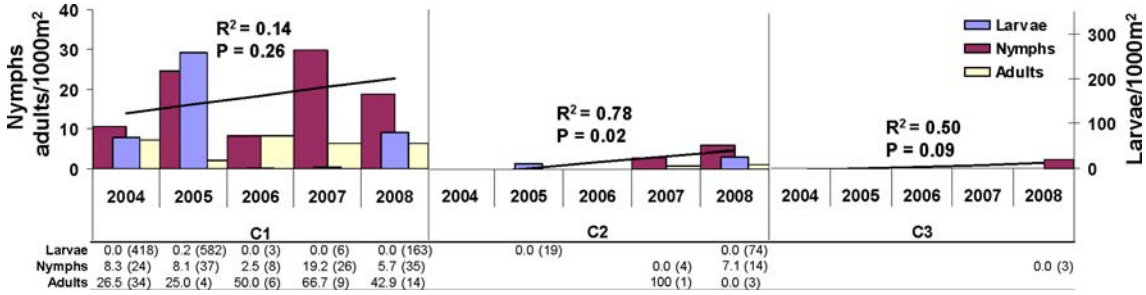

Figure 3. Average densities and *B. burgdorferi* infection prevalences of drag-sampled *I. scapularis* at the three sites on the coastal transect in May and June, 2004–2008. No *I. scapularis* were dragged at site C4. Regression lines and coefficients for nymphal densities are shown. Infection prevalence for each life stage (minimum infection prevalence for larvae) is expressed as the percent positive, with the total number tested in parenthesis below each year. C = coastal transect site.

nymphal density ($R^2 = 0.78$; $P = 0.02$). At site C3, an increase in nymphal density occurred ($R^2 = 0.05$, $P = 0.09$), and this was the only life stage found on drag cloths. No questing *I. scapularis* were collected at site C4.

B. burgdorferi Infection in Drag-sampled Ticks

Along the coastal transect, 36.6%, 9.3%, and a minimum of 0.1% of adult, nymphal, and larval *I. scapularis* respectively were infected with *B. burgdorferi*, with the majority of infected ticks (95.1%) collected at site C1 (Fig. 3). There was no significant change in infection prevalence among years at site C1 ($P = 0.38$–0.71 for the three age classes); too few positive ticks were collected at other coastal sites to test for equivalent trends. Along the inland transect, the single *I. scapularis* larva that was dragged at site I3 in 2007 tested positive for *B. burgdorferi* (Table 2). Of all *D. variabilis* collected on drag cloth, *B. burgdorferi* was found in 8 of 147 adults, 0 of 4 nymphs, and a minimum of 1 of 59 larvae processed in 15 pools, again with no apparent temporal trends. All infected drag-sampled *D. variabilis* came from site C1, with the exception of two infected adults from sites I1 and I2. *B. burgdorferi* detection in other drag-sampled tick species across the years was as follows: 0 of 4 *A. americanum*, 0 of 1 *A. maculatum*, 0 of 1 *I. cookei*, 2 of 2 *H. leporispalustris* (both nymphs from site C1), and 0 of 3 *I. dentatus*.

Host Infection with *B. burgdorferi*

Of the 1,056 white-footed mouse ear biopsies that were collected from 2004–2008, only 2.1% of 576 tested positive from inland sites (I1: 0/71; I2: 9/213; I3: 3/169; I4: 0/121, with no apparent trend over time), whereas 11.9% of 480 coastal mice tested positive ($P < 0.0002$). Infection prevalence was high in all years at site C1 (21.6–80%), and low and inconsistent at sites further north (Fig. 2). A total of 148 chipmunk biopsies were assayed for infection, of which 4.2% of 72 were positive along the inland transect (I1: 0/9; I2: 0/20; I3: 0/4; I4: 3/39), and 6.6% of 76 (all at site C1) were positive along the coastal transect (Fig. 2; no difference between transects, $P = 0.72$). A total of 240 ear biopsies from alternative mammal species were assayed for infection: 6.5% of 124 and 4.3% of 116 were positive along the inland and coastal transects, respectively (no difference between transects, $P = 0.57$). Positive alternative mammals comprised four species (raccoon, red squirrel (*Tamiasciurus hudsonicus*), meadow jumping mouse (*Zapus hudsonius*), and Virginia opossum (*Didelphus virginiana*) at two inland sites (I3 and I4) and three species (eastern gray squirrel (*Sciurus carolinensis*), raccoon, and meadow jumping mouse)) at two coastal sites (C1 and C3).

B. burgdorferi infection in *I. scapularis* removed from hosts

Along the inland transect, all five larvae removed from two mice at site I3 in 2007 were negative, whereas the single nymph from the same site and year was positive (Table 2), and the single nymph from site I1 in 2008 was negative. Along the coastal transect, infection in *I. scapularis* removed from mice and chipmunks was highest at site C1 and decreased at sites to the north in all years, with no infection in the ticks from site C4 (Fig. 2). Alternative mammalian hosts harboring *I. scapularis* were found at all coastal sites, but *B. burgdorferi* infection in these ticks was found only at sites C1 and C2 (Supplement 2). Birds harboring *I. scapularis* were found only at sites C1 and C2, and *B. burgdorferi* infection in bird-derived *I. scapularis* occurred only at site C1 (Supplement 2).

Table 2. Sequence Confirmation and Accession Numbers of *B. burgdorferi* Infection in Various Sample Types from Coastal and Inland Sites in Lower Michigan, 2004–2008

Site	Year	Sample type	Host species or drag	RST (1, 2, 3)	Acc. No
C1	2004	*D. variabilis* AF	Drag	2	GQ856591
		Ear biopsy	WFMO	1	GQ856630
		Ear biopsy	WFMO	2	GQ856631
		I. marxi AF	SFSQ	1	GQ856615
		I. scapularis L	WFMO	2	GQ856598
		I. scapularis N	EAGS	2	GQ856614
	2005	*D. variabilis* L	Drag	1	GQ856596
		D. variabilis L	WFMO	3	GQ856600
		I. scapularis N	VIOP	2	GQ856594
		I. scapularis N	WFMO	3	GQ856595
		I. texanus L	SFSQ	2	GQ856601
	2006	*D. variabilis* N	WFMO	2	GQ856639
		I. scapularis N	Drag	2	GQ856592
		I. scapularis N	EACH	1	GQ856612
		I. scapularis N	WFMO	2	GQ856611
	2007	*D. variabilis* N	WFMO	3	GQ856643
		Ear biopsy	EACH	3	GQ856632
		H. leporispalustris N	Drag	3	GQ856609
		I. scapularis L	NOCA	3	GQ856619
		I. scapularis N	AMRO	3	GQ856616
C2	2005	Ear biopsy	WFMO	2	GQ856620
	2006	*I. scapularis* L	WFMO	2	GQ856593
	2008	Ear biopsy	WFMO	2	GQ856610
C3	2006	Ear biopsy	WFMO	3	GQ856623
		Ear biopsy	WFMO	3	GQ856622
		Ear biopsy	WFMO	3	GQ856621
I2	2004	*D. variabilis* L	WFMO	1	GQ856638
	2005	*D. variabilis* L	WFMO	1	GQ856602
	2006	Ear biopsy	WFMO	2	GQ856628
		Ear biopsy	WFMO	2	GQ856629
		Ear biopsy	WFMO	2	GQ856627
		Ear biopsy	WFMO	2	GQ856626
		Ear biopsy	WFMO	2	GQ856625
		Ear biopsy	WFMO	2	GQ856624
	2007	*D. variabilis* L	WFMO	3	GQ856603
I3	2004	*D. variabilis* L	WFMO	2	GQ856599
		D. variabilis L	WFMO	2	GQ856597
		I. texanus N	COON	3	GQ856640
	2006	Ear biopsy	WFMO	2	GQ856642
		Ear biopsy	MJMO	2	GQ856641
	2007	*I. scapularis* N	WFMO	2	GQ856606
		I. scapularis L	Drag	2	GU190359
		D. variabilis L	WFMO	2	GQ856608
		D. variabilis L	WFMO	2	GQ856607
		D. variabilis L	WFMO	2	GQ856605
		D. variabilis L	WFMO	2	GQ856604

Table 2. continued

Site	Year	Sample type	Host species or drag	RST (1, 2, 3)	Acc. No
		Ear biopsy	VIOP	2	GQ856636
		Ear biopsy	COON	2	GQ856634
		Ear biopsy	COON	2	GQ856635
		Ear biopsy	WFMO	2	GQ856633
	2008	*I. dentatus* AF	EACO	2	GQ856618
		I. dentatus AM	EACO	2	GQ856617
I4	2004	*I. texanus* N	COON	1	GQ856613
	2006	Ear biopsy	EACH	2	GQ856637

RST = 16S–23S rRNA spacer type of *B. burgdorferi.*
I inland transect site, *C* coastal transect site, *AF* adult female, *AM* adult male, *N* nymph, *L* larvae, *WFMO* white-footed mouse, *SFSQ* southern flying squirrel, *EAGS* eastern gray squirrel, *VIOP* Virginia opossum, *EACH* eastern chipmunk, *NOCA* Northern cardinal, *AMRO* American robin, *SOSP* song sparrow, *COON* raccoon, *MJMO* meadow jumping mouse, *EACO* eastern cottontail.

B. burgdorferi Infection in Alternative Tick Species

Assaying samples of the seven species of ticks other than *I. scapularis* removed from mammals and birds revealed relatively high levels of *B. burgdorferi* infection in alternative ticks at sites with sympatric *I. scapularis* (Supplement 2). Additionally, some alternative tick species from inland sites—in the apparent absence of *I. scapularis*—were associated with low-level infection prevalence. Infected *D. variabilis* removed from mice were found at sites I2 and I3, where a total of 7.1% of 98 nymphs (positives from 6 animals) and a minimum of 0.7% of 2006 larvae (positives from 14 animals) collected at these two sites together were infected. This contrasts with site C1, where sympatric *I. scapularis* occur in high density: 17.6% of 74 nymphal (positives from 12 animals) and a minimum of 2.5% of 672 larval (positives from 17 animals) *D. variabilis* removed from white-footed mice were infected.

Of the alternative tick species collected from vertebrates, *I. texanus* was the most abundant and was ubiquitous across our study sites, commonly found parasitizing raccoons (Table 1). Infected attached *I. texanus* were found not only at all coastal transect sites, but also in all three tick life stages from inland sites (2.4–2.6%; Supplement 2). In summary, infected *D. variabilis, I. texanus, I. cookei, I. dentatus, I. marxi*, and *H. leporispalustris* were removed from coastal wildlife, and infected *D. variabilis, I. texanus*, and *I. dentatus* were removed from inland wildlife (Supplement 2).

Nucleotide Sequencing

Pathogen identity was confirmed by nucleotide sequencing of the 16S-23S rRNA IGS region of a subset of the PCR positive samples reported earlier (i.e., from host ear tissue, *I. scapularis* ticks, and other tick species). We obtained sequence confirmation of 26 samples from coastal sites where *I. scapularis* is abundant and 28 samples from inland sites where *I. scapularis* is absent or at a very low density. Representatives of all three *B. burgdorferi* RST groups were found at sites characterized by both the presence and absence of *I. scapularis* (Table 2).

Opportunistic Detection of other *Borrelia* Species

Using IGS PCR followed by DNA sequencing, we detected spirochetes that group with *B. miyamotoi*, an organism genetically similar to relapsing fever group spirochetes, in ears from 12 white-footed mice at sites C2–C4. Two tick samples (a single nymph and a pool of 20 larval *I. scapularis*) removed from the same eastern chipmunk in 2005 at site C1 also tested positive for *B. miyamotoi*-like spirochetes. Representative *B. miyamotoi*-like sequences from these ears and ticks are deposited in Genbank (accession numbers GQ856588–GQ856589). We also detected spirochetes that group with *B. andersonii*, a Lyme borreliosis group spirochete associated with *I. dentatus* ticks, birds, and rabbits (Marconi et al., 1995), in one *I. dentatus* nymph removed from a song sparrow from site I3 in 2007 (GQ856590) and in ear tissue from one white-footed

mouse from cite C1 in 2006. Because the IGS region of *B. andersonii* was not previously characterized in Genbank, we identified this species based on characterization of the 16S gene (data not shown).

Discussion

Lyme disease is emerging in the United States largely due to the spread of the bridging vector, *I. scapularis*. Both in the Northeast and the Upper Midwest, the spread of this tick has been documented at a gross scale, but few have studied this spread in real time (Falco et al., 1995, Schulze et al., 1998, and Cortinas and Kitron, 2006 are exceptions). By monitoring ticks and hosts for 5 years, we assessed the temporal and spatial dynamics of *I. scapularis* populations along coastal and inland transects in western Lower Michigan. Our goal was to investigate three nonmutually exclusive hypotheses explaining how the disease system is emerging across the landscape; we refer to these as the tick-first, dual-invasion, and spirochete-first hypotheses. Our results suggest that there are distinct but overlapping processes that determine *I. scapularis/B. burgdorferi* dynamics. In some areas, observations of newly arrived ticks and spirochetes in host/vector communities where *B. burgdorferi* had not been detected support the tick-first and/or dual-invasion hypotheses. Conversely, in other areas, the detection of spirochete-infected wildlife and alternative tick species preceded detection of *I. scapularis*, which is consistent with the spirochete-first hypothesis. We summarize our key findings and consider their support for these hypotheses.

I. scapularis Invasion

At a broad spatial scale, a continuum of endemicity of *I. scapularis* exists in the Midwest, whereby Wisconsin, Minnesota, and a focal area in Michigan's Upper Peninsula harbor long-established tick populations (Jackson and Defoliart, 1970; Drew et al., 1988; Strand et al., 1992). Illinois and Indiana harbor ticks that colonized later (Pinger and Glancy, 1989; Bouseman et al., 1990), and Michigan's Lower Peninsula represents the most recent invasion front (Foster, 2004; Hamer et al., 2007). We postulate that these Michigan ticks represent an expanding focus from recently described populations in northern Indiana (near the Michigan border and Lake Michigan; Pinger et al., 1996). The density of questing nymphs at coastal site C1, where *I. scapularis* was most common, averaged 16.8/1000 m^2, which is higher than the average density of 6.6/1000 m^2 in *I. scapularis*-positive sites from throughout the eastern United States (Diuk-Wasser et al., 2006). Densities of nymphs at the other *I. scapularis*-positive sites in our study were much lower.

As in many other states that have witnessed an emergence of Lyme disease during the past four decades, white-tailed deer populations have increased sharply in Michigan's southern Lower Peninsula for the past 50 years, from near extirpation in the 1960s to nearly one million animals presently as a consequence of harvest restrictions and reforestation (MDNR, 2002). Deer densities throughout the invasion zone are clearly above the threshold necessary to support blacklegged tick populations. The reforestation that supported the deer expansion also created an abundance of suitable habitat for other woodland hosts and *I. scapularis*. Thus, the observed tick invasion may be a consequence of a sufficient number of introductions and a large enough propagule size deposited in southwestern Michigan to achieve the density needed to establish and disperse.

The progressive increase in tick density along the coastal sites from south to north among years is suggestive of rapid tick invasion and colonization northward along the coastal dune forests of Lake Michigan. *I. scapularis* could only be detected at the southernmost site at the beginning of the study, whereas all four sites harbored established populations by the end of the study period. Numerous authors have proposed that climate change may facilitate tick invasion of new areas due to a warming of the microclimate experienced by the ticks and shorter periods of freeze (e.g., Githeko et al., 2000; Lindgren and Gustafson, 2001). The prospects for such shifts are debatable (Randolph, 2004), and *I. scapularis* in Lower Michigan is well south of its distributional limits, so we presently have no evidence to suggest climate change as a driver for this invasion.

The disparity in tick distribution between our two transects suggests that there are important ecological differences between coastal and inland sites (Sonenshine et al., 1995), with the former allowing for many more introductions of ticks, and/or higher success in establishment after introduction. Hosts with high tick burdens and large home ranges, such as deer and migratory birds, are critical for long distance dispersal of *I. scapularis*, whereas small mammals with small home ranges may limit invasion by diverting ticks from more vagile hosts (Madhav et al.,

2004). Deer densities were not quantified in our study, but densities of other mammalian and nonmigratory birds during the study period, as indexed by our trap and mist-net success, were similar between coastal and inland sites. Migratory birds tend to concentrate along shorelines (Alerstam, 1978) and fly parallel to the long axis of Lake Michigan (Diehl et al., 2003), which may allow for more drop-offs of ticks from birds at coastal compared with inland sites.

B. burgdorferi Distribution, Abundance, and Diversity

Overall infection prevalences in drag-sampled *I. scapularis* in the invasion zone were 8.6% and 36.6% in nymphs and adults, respectively; these infection prevalences are lower than those characteristic of *I. scapularis* in the endemic Northeast (15% in nymphs (Gatewood et al., 2009); 49% in adults (Schulze et al., 2003)). The density of infected nymphs (DIN) is correlated with the incidence of human Lyme disease in a given area (Stafford et al., 1998). Across our study, the DIN at site C1 was 1.5/1000 m^2, which is similar to DINs in endemic sites of the Northeast (1.3/1000 m^2) and Midwest (1.0/1000 m^2; Gatewood et al., 2009). The overall prevalence of infection of white-footed mice at site C1 was 32.2%, which is lower than that reported from endemic foci (for example, 76% by the end of summer in Connecticut; Barbour et al., 2009). In addition to the lower nymphal infection prevalence as an explanation for lower prevalence of mouse infection, the difference may be due in part to sampling prior to the end of the nymphal host-seeking, and therefore transmission, period.

We detected *B. burgdorferi* not only in sites where *I. scapularis* is established or apparently recently invaded, but also within alternative tick species and wildlife hosts occurring at sites in the apparent absence of *I. scapularis*. Cryptic transmission of *B. burgdorferi* sensu lato has been documented in various host and tick species (Anderson et al., 1989; Telford and Spielman, 1989b; Brown and Lane, 1992; Maupin et al., 1994; Oliver et al., 2003) but has not previously been investigated in an area of *I. scapularis* invasion. The requirements for demonstration of a cryptic transmission cycle include the presence and interactions of (1) *B. burgdorferi*, (2) a vector-competent tick species, and (3) reservoir-competent hosts. Although our data confirm the presence of many reservoir competent hosts, and infection with several strains of *B. burgdorferi* in such hosts and in various tick species—all suggestive of cryptic cycles—it remains unclear which tick species are maintaining transmission of *B. burgdorferi* in the absence of *I. scapularis*. We documented infection in both questing and on-host *D. variabilis*, as did Walker et al. (1998) at a highly endemic area in Michigan's Upper Peninsula, yet this tick species is not considered a competent vector (Piesman and Sinsky, 1988). *Ixodes cookei* and *I. marxi* both had a low prevalence of infection in our study, but these infected ticks were removed from animals (southern flying squirrel, raccoon) only at site C1, where *I. scapularis* and *B. burgdorferi* were at relatively high prevalence; infections in these alternative tick species thus may represent spillover from feeding on hosts infected by *I. scapularis*. Furthermore, *I. cookei* is an incompetent vector (Ryder et al., 1992). Two infected tick species removed from wildlife at inland sites included *I. texanus* and *I. dentatus*. Although the vector competency of *I. texanus* is unknown, its most important host—raccoons—are known to be competent reservoirs, although only 35% as efficient as white-footed mice at infecting xenodiagnostic larvae (Fish and Daniels, 1990). Both *I. dentatus* and eastern cottontails are competent for transmission and maintenance of *B. burgdorferi* sensu lato (Telford and Spielman, 1989a); however, parasitism of *I. dentatus* on hosts other than birds and rabbits was low during our study and is unlikely to explain infections in these hosts. Lord et al. (1994) documented a site in Pennsylvania at which isolations of *B. burgdorferi* from mice were common, yet *I. scapularis* was not found, and suggested that some other mechanism besides transmission by a tick vector was responsible.

We detected three different *Borrelia* species within ticks and hosts (*B. burgdorferi*, *B. miyamotoi,* and *B. andersonii*), including a diverse assemblage of *B. burgdorferi* strains. The diversity of *B. burgdorferi* RST types along the coast suggests high rates of pathogen flow and/or multiple introduction events, rather than isolated founder effects. Similarly, all three RST types were found in alternative tick vectors or ear biopsies from sites beyond the invasion front. Studies of the population genetic structure of *B. burgdorferi* will be useful for determining the dynamics of *B. burgdorferi* in the presence and absence of *I. scapularis,* and how cryptic cycles affect *B. burgdorferi* infection prevalence and strain types in the bridging vector *I. scapularis*.

Intriguingly, a single *I. scapularis* larva dragged at an inland site was infected with *B. burgdorferi*. Sequencing of the IGS region identified this organism as RST 2, which is the most common type in the Midwest. Evidence for transovarial passage of *B. burgdorferi*, which would produce infected

questing *I. scapularis* larvae, is rare (but see Magnarelli et al., 1987 who found that transovarial transmission in *I. dammini* occurs at a variable rate). Although the single infected questing larva that we report herein is of little epidemiological significance, our finding was especially surprising considering the extremely low density of *I. scapularis* in the area. One possible explanation is that this infection was from spirochetes acquired during a partial larval bloodmeal on an infected host that groomed the larva off.

Implications for Hypotheses

Our detections of *B. burgdorferi* shed light on our hypotheses about the emergence of the Lower Michigan *I. scapularis/B. burgdorferi* system. At four sites (C3, C4, I1, I3), we detected *I. scapularis* on mice after previous years of surveillance had failed to detect infestation on mice, and we interpret this as evidence for tick invasion into these sites. At site C4, no *B. burgdorferi* has yet been found, despite finding *I. scapularis* in both 2007 and 2008, supporting a tick-first process. At site C3, the first detection of *B. burgdorferi* coincided with that of *I. scapularis*, supporting a dual invasion process. At sites I1 and I3, *B. burgdorferi* was found in other sample types before detecting *I. scapularis*, supporting a spirochete-first process whereby *B. burgdorferi* is being maintained in cryptic transmission cycles. An alternative explanation is that a very low-density *I. scapularis* population—below our detection threshold—exists at I1 and I3. Such low-density *I. scapularis* populations would not normally be considered capable of maintaining *B. burgdorferi* (Madhav et al., 2004; Ogden et al., 2008).

Implications for Detection of *I. scapularis/ B. burgdorferi* Invasion

Studies of invasion most often are initiated once the invader has reached densities that have negative consequences (such as human disease) because resources for active surveillance and research are not usually appropriated in the absence of such impacts. Rarely are standardized investigations of wildlife undertaken in areas of no or low tick density that can provide a sensitive real-time warning system for invading ticks, spirochetes, and impending Lyme disease. One exception is Schulze et al. (1998), who reported a significant increase in human Lyme disease cases in 1990–1995 in Hunterdon County, NJ, that occurred after geographic expansion of ticks on deer in the county between 1981 and 1987. Falco et al. (1995) monitored a 2.6-fold increase in questing *I. scapularis* nymphal density (from 13 to 34 nymphs/1,000 m^2) in endemic Westchester Co., NY, from 1984 to 1991, which coincided with a 4.2-fold increase in reported human cases of infection. In contrast, at our study site C2, where nymphal drag data demonstrate invasion most clearly, densities increased from 0 to 5.3 nymphs/1,000 m^2 from 2004 to 2008 (Fig. 2.) Thus, we are assessing an invasion during its earliest stages, and human disease incidence is still very low (see below).

We found that prevalence of infestation of *I. scapularis* on white-footed mice provides a sensitive index of invasion (as did Lord et al., 1992); detection of ticks on mice generally preceded detection of ticks on drag cloths, alternative mammal species, and birds. Mouse sampling also was more useful than two other methods of surveillance for ticks and Lyme disease foci. Assessment of ticks on hunter-harvested deer in November across the study area was hampered by low densities of adult ticks in this early-stage invasion, and by a mismatch in the timing of the fall hunting season in relation to adult questing phenology in the invasion zone (their major activity peak is in the spring; S. Hamer, unpublished data). The efficacy of canine serosurveillance in the area was likely reduced by widespread use of anti-parasite prophylaxis on pet dogs living in the invasion zone (Hamer et al., 2009).

Alternative tick species and wildlife hosts enabled detection of *B. burgdorferi* in the apparent absence of *I. scapularis.* Detection of cryptically cycling *B. burgdorferi* decouples its invasion from that of *I. scapularis,* and may hasten *B. burgdorferi* transmission dynamics among an invading *I. scapularis* ticks. Cryptically cycling *B. burgdorferi* thus may decrease the time lag between first detection of *I. scapularis* and first detection of *B. burgdorferi*-infected *I. scapularis*.

Study Limitations

The results we present are based on sampling within the May and June period only, which does not include all months of blacklegged tick activity. The most commonly cited *I. scapularis* phenology—based on studies in Westchester Co., NY (Fish, 1993)—is characterized by bimodal peaks in adult activity in the spring and fall and nymphal activity in the early summer, which precedes peak larval activity in the late summer. Gatewood et al. (2009) noted that whereas the Northeast is generally characterized by distinctly separate peaks in nymphal and larval activity in

June and August, respectively, as indicated above, the Midwest is characterized by synchronous feeding of nymphs and larvae in June through July, with a much smaller late-season peak in larval activity. Whereas we present 5 years of data from May and June only (early summer), all our sites also were sampled during July–August (late summer) in at least one year with similar rodent trapping and drag sampling effort (unpublished data). At all sites where we found *I. scapularis* in the late summer, this species also was found in the early summer period reported here. Furthermore, in 2008–2009 we conducted a longitudinal study of *I. scapularis* phenology at monthly intervals at site C1 (unpublished data). Larval infestation of mice was greatest in June (83.8%), followed by a decline (44.1%, 48%, 24.5%, and 19.4% monthly infestation prevalence in July–October, respectively), and nymphal infestation followed a similar trend (24.3%, 8.5%, 2.0%, 2.0, and 0% in June–October, respectively). These patterns support the synchronized feeding reported by Gatewood et al. (2009) and are similar those reported by Godsey et al. (1987) in Wisconsin and Strand et al. (1992) in Michigan's Upper Peninsula. Thus, we conclude that our sampling focused on the period during which we had the best chance of detecting infested mice each year and that this period was appropriate for answering our research questions.

A longer duration of sampling at all sites would have provided greater sensitivity of detection of stable *I. scapularis* populations. Whereas neither our data nor that of Gatewood et al. (2009) show significant larval activity in the late summer, late summer larval activity of greater (Northeast) or equivalent (Midwest) magnitude to early summer activity has been reported (Daniels et al., 1996; Jones and Kitron, 2000; Kitron et al., 1991). In the Northeast, the early peak in May–June represents the remainder of the cohort of larvae that hatched the previous July and successfully overwintered, whereas the second peak of higher magnitude in August represents the subsequent cohort of newly hatched larvae from recently laid eggs by adults that were active in spring as well as the previous fall (after ovipositional diapause; Daniels et al., 1996). In the Midwest, the separate peaks observed by some researchers at some sites are posited to result from the separate oviposition periods of the spring and fall adults (Jones and Kitron, 2000; Kitron et al., 1991), whereby the early larvae may result from oviposition by fall adults and the late summer larvae result from oviposition by spring adults. Furthermore, there has been a suggestion that this bimodality may be more pronounced in newly establishing populations (Jones and Kitron, 2000). Therefore, if blacklegged tick invasion in Michigan is driven by adult ticks moving in during the spring (e.g., on deer), our sampling may miss the resulting larvae emerging late that first summer.

Assaying ticks of all stages and species removed from hosts increased our capability to detect the presence of *B. burgdorferi* at a site compared with assaying *I. scapularis* or host tissue alone. Our protocol, however, did not maximally allow us to detect *B. burgdorferi* infection because we did not assay all ticks removed from hosts. We selected a random subset of three per species/stage/sex combination to assay for infection, sometimes resulting in over ten ticks tested from some hosts that were infested my multiple tick species and stages. Given limited resources, this protocol allows us to gather infection data from ticks removed from a greater number and diversity of hosts as well as to avoid overrepresenting heavily parasitized animals in the overall results of tick infection. Testing of all ticks from such animals would have increased the odds of both detecting a positive tick on a given animal and detecting different genospecies that coinfect the same vertebrate host (although that latter was not a main study objective). Although comprehensive tick testing may have resulted in a finer temporal resolution of invasion dynamics, we do not believe it would have altered our qualitative conclusions.

Implications for Disease and Prevention

Data collected from careful study of the underlying ecology and transmission of zoonotic pathogens within animal reservoirs before and during invasion can provide an early warning of increasing disease risk to human and companion animals. Cryptic cycling of *B. burgdorferi* does not imply human risk of Lyme disease in these areas but may hasten the establishment of transmission cycles that involve *I. scapularis*. Such cycling also may introduce different strains of *B. burgdorferi* to *I. scapularis*, which may lead to variable clinical manifestations (Wormser et al., 1999).

The tick/spirochete invasion we document in this paper is associated with a significant recent increase in confirmed cases of human Lyme disease within the Southwest/Western Michigan zone of invasion. Incidence in this 14-county region increased during the period from 1996 (when Lyme disease became reportable in Michigan) to 2008 from 0.16 cases per 100,000 people to a peak incidence of 0.63 ($R^2 = 0.28$; 1-tailed $P = 0.03$; E. Foster, Michigan Department of Community Health, personal

communication). Incidence remains substantially lower than the average annual incidence in the ten endemic states from which > 93% of human Lyme disease across the United States is reported (average of 29.2 cases per 100,000 population for 2003–2005; Bacon et al. 2008). In contrast with the significant peridomestic exposures that occur in the Northeastern United States, recreational exposure is probably common in the Michigan invasion zone, which is dominated by recreational areas, camps, vacation homes, and rural communities. Recreational exposure may produce low reported disease incidence locally, because recreational visitors will return home (often to other counties or states) before developing symptoms and seeking medical attention. Spatial epidemiology and trace-back of human Lyme disease cases reported elsewhere in Lower Michigan would help shed light on this possibility.

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References

Alerstam T (1978) Re-oriented bird migration in coastal areas: dispersal to suitable resting grounds. *Oikos* 30:405–408

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. *Journal of Molecular Biology* 215:403–410

Anderson JF, Magnarelli LA, Lefebvre RB, Andreadis TG, McAninch JB, Perng GC, Johnson RC (1989) Antigenically variable *Borrelia burgdorferi* isolated from cottontail rabbits and *Ixodes dentatus* in rural and urban areas. *Journal of Clinical Microbiology* 27:13–20

Bacon RM, Kugeler KJ, Griffith KS, Mead PS (2008) Surveillance for Lyme disease - United States, 1992–2006. *Morbidity and Mortality Weekly Report* 56:1–9

Barbour AG, Bunikis J, Travinsky B, Hoen AG, Diuk-Wasser MA, Fish D, Tsao JI (2009) Niche partitioning of *Borrelia burgdorferi* and *Borrelia miyamotoi* in the same tick vector and mammalian reservoir species. *American Journal of Tropical Medicine and Hygiene* 81:1120–1131

Bouseman JK, Kitron U, Kirkpatrick CE, Siegel J, Todd KS (1990) Status of *Ixodes dammini* (Acari, Ixodidae) in Illinois. *Journal of Medical Entomology* 27:556–560

Brown RN, Lane RS (1992) Lyme disease in California: a novel enzootic transmission cycle of *Borrelia burgdorferi*. *Science* 256:1439–1442

Bunikis J, Garpmo U, Tsao J, Berglund J, Fish D, Barbour AG (2004) Sequence typing reveals extensive strain diversity of the Lyme borreliosis agents *Borrelia burgdorferi* in North America and *Borrelia afzelii* in Europe. *Microbiology* 150:1741–1755

Cortinas MR, Kitron U (2006) County-level surveillance of white-tailed deer infestation by *Ixodes scapularis* and *Dermacentor albipictus* (Acari: Ixodidae) along the Illinois River. *Journal of Medical Entomology* 43:810–819

Daniels TJ, Falco RC, Curran KL, Fish D (1996) Timing of *Ixodes scapularis* (Acari: Ixodidae) oviposition and larval activity in southern New York. *Journal of Medical Entomology* 33:140–147

Dennis DT, Nekomoto TS, Victor JC, Paul WS, Piesman J (1998) Reported distribution of *Ixodes scapularis* and *Ixodes pacificus* (Acari : Ixodidae) in the United States. *Journal of Medical Entomology* 35:629–638

Diehl RH, Larkin RP, Black JE (2003) Radar observations of bird migration over the Great Lakes. *Auk* 120:278–290

Diuk-Wasser MA, Gatewood AG, Cortinas MR, Yaremych-Hamer S, Tsao J, Kitron U, et al. (2006) Spatiotemporal patterns of host-seeking *Ixodes scapularis* nymphs (Acari : Iodidae) in the United States. *Journal of Medical Entomology* 43:166–176

Drew ML, Loken KI, Bey RF, Swiggum RD (1988) *Ixodes dammini* - occurrence and prevalence of infection with *Borrelia* species in Minnesota. *Journal of Wildlife Diseases* 24:708–710

Durden LA, Keirans JE (1996) *Nymphs of the genus* Ixodes *(Acari: Ixodidae) of the United States: taxonomy, identification key, distribution, hosts, and medical/veterinary importance.* Thomas Say Publications in Entomology, Entomological Society of America

Falco RC, Daniels TJ, Fish D (1995) Increase in abundance of immature *Ixodes scapularis* (Acari: Ixodidae) in an emergent Lyme disease endemic area. *Journal of Medical Entomology* 32:522–526

Falco RC, Fish D (1992) A comparison of methods for sampling the deer tick, *Ixodes dammini*, in a Lyme disease endemic area. *Experimental & Applied Acarology* 14:165–173

Fish D (1993) Population ecology of *Ixodes dammini*. In: *Ecology and Environmental Management of Lyme Disease*, Ginsberg HS (editor), New Brunswick, NJ: Rutgers University Press

Fish D, Daniels TJ (1990) The role of medium-sized mammals as reservoirs of *Borrelia burgdorferi* in Southern New York. *Journal of Wildlife Diseases* 26:339–345

Foster ES (2004) *Ixodes scapularis* (Acari: Ixodidae) and *Borrelia burgdorferi* in southwest Michigan: population ecology and verification of a geographic risk model. Masters Thesis, Michigan State University, East Lansing

Gatewood AG, Liebman KA, Vourc'h G, Bunikis J, Hamer SA, Cortinas R, et al. (2009) Climate and tick seasonality are predictors of *Borrelia burgdorferi* genotype distribution. *Applied and Environmental Microbiology* 75:2476–2483

Githeko AK, Lindsay SW, Confalonieri UE, Patz JA (2000) Climate change and vector-borne diseases: a regional analysis. *Bulletin of the World Health Organization* 78:1136–1147

Godsey MS, Amundson TE, Burgess EC, Schell W, Davis JP, Kaslow R, et al. (1987) Lyme disease ecology in Wisconsin: distribution and host preferences of *Ixodes dammini*, and prevalence of antibody to *Borrelia burgdorferi* in small mammals. *American Journal of Tropical Medicine and Hygiene* 37:180–187

Guerra M, Walker E, Jones C, Paskewitz S, Cortinas MR, Stancil A, Beck L, Bobo M, Kitron U (2002) Predicting the risk of Lyme disease: habitat suitability for *Ixodes scapularis* in the north central United States. *Emerging Infectious Diseases* 8:289–297

Hamer SA, Roy PL, Hickling GJ, Walker ED, Foster ES, Barber CC, Tsao JI (2007) Zoonotic pathogens in *Ixodes scapularis*, Michigan. *Emerging Infectious Diseases* 13:1131–1133

Hamer SA, Tsao JI, Walker ED, Mansfield LS, Foster ES, Hickling GJ (2009) Use of tick surveys and serosurveys to evaluate pet dogs as a sentinel species for emerging Lyme disease. *American Journal of Veterinary Research* 70:49–56

Jackson JO, DeFoliart GR (1970) *Ixodes scapularis* say in Northern Wisconsin. *Journal of Medical Entomology* 7:124–125

Jones CJ, Kitron UD (2000) Populations *of Ixodes scapularis* (Acari : Ixodidae) are modulated by drought at a Lyme disease focus in Illinois. *Journal of Medical Entomology* 37:408–415

Keirans JE, Clifford CM (1978) The genus *Ixodes* in the United States: a scanning electron microscope study and key to the adults. *Journal of Medical Entomology Supplements* 2:1–149

Kitron U, Jones CJ, Bouseman JK (1991) Spatial and temporal dispersion of immature *Ixodes dammini* on *Peromyscus leucopus* in northwestern Illinois. *The Journal of Parasitology* 77:945–949

Lindgren E, Gustafson R (2001) Tick-borne encephalitis in Sweden and climate change. *The Lancet* 358:16–18

Liveris D, Gazumyan A, Schwartz I (1995) Molecular typing of *Borrelia burgdorferi* sensu lato by PCR restriction fragment length polymorphism analysis. *Journal of Clinical Microbiology* 33:589–595

Lord R, Humphreys J, Lord V, McLean R, Garland C (1992) *Borrelia burgdorferi* infection in white-footed mice (*Peromyscus leucopus*) in hemlock (*Tsuga canadensis*) habitat in western Pennsylvania. *Journal of Wildlife Diseases* 28:364–368

Lord RD, Lord VR, Humphreys JG, McLean RG (1994) Distribution of *Borrelia burgdorferi* in host mice in Pennsylvania. *Journal of Clinical Microbiology* 32:2501–2504

Madhav NK, Brownstein JS, Tsao JI, Fish D (2004) A dispersal model for the range expansion of blacklegged tick (Acari : Ixodidae). *Journal of Medical Entomology* 41:842–852

Magnarelli LA, Anderson JF, Durland F (1987) Transovarial transmission of *Borrelia burgdorferi* in *Ixodes dammini* (Acari:Ixodidae). *The Journal of Infectious Diseases* 156:234–236

Marconi RT, Liveris D, Schwartz I (1995) Identification of novel insertion elements, restriction fragment length polymorphism patterns, and discontinuous 23S ribosomal RNA in Lyme disease spirochetes: phylogenetic analyses of ribosomal RNA genes and their intergenic spacers in Borrelia japonica sp nov and genomic group21038 (Borrelia andersonii sp. nov) isolates. *Journal of Clinical Microbiology* 33:2427–2434

Maupin GO, Gage KL, Piesman J, Montenieri J, Sviat SL, Vanderzanden L, Happ CM, Dolan M, Johnson BJ (1994) Discovery of an enzootic cycle of *Borrelia burgdorferi* in *Neotoma mexicana* and *Ixodes spinipalpis* from northern Colorado, an area where Lyme disease is nonendemic. *Journal of Infectious Diseases* 170:636–643

MDNR (2002) Deer management history in Michigan. Michigan Department of Natural Resources. Available: http://www.michigan.gov/dnr/0,1607,7-153-10363_10856_10905-28543–,00.html. Accessed 1 Dec 2009

Ogden NH, Lindsay RL, Hanincova K, Barker IK, Bigras-Poulin M, Charron DF, Heagy A, Francis CM, O'Callaghan CJ, Schwartz I, Thompson RA (2008) Role of migratory birds in introduction and range expansion of *Ixodes scapularis* ticks and of *Borrelia burgdorferi* and *Anaplasma phagocytophilum* in Canada. *Applied and Environmental Microbiology* 74:3919–3919

Oliver JH, Lin T, Gao L, Clark KL, Banks CW, Durden LA, James AM, Chandler FW Jr (2003) An enzootic transmission cycle of Lyme borreliosis spirochetes in the southeastern United States. *Proceedings of the National Academy of Sciences of the United States of America* 100:11642–11645

Piesman J, Sinsky RJ (1988) Ability of *Ixodes scapularis, Dermacentor variabilis*, and *Amblyomma americanum* (Acari, Ixodidae) to acquire, maintain, and transmit Lyme disease spirochetes (*Borrelia burgdorferi*). *Journal of Medical Entomology* 25:336–339

Pinger RR, Glancy T (1989) *Ixodes dammini* (Acari, Ixodidae) in Indiana. *Journal of Medical Entomology* 26:130–131

Pinger RR, Timmons L, Karris K (1996) Spread of Ixodes scapularis (Acari: Ixodidae) in Indiana: collections of adults in 1991–1994 and description of a Borrelia burgdorferi-infected population. *Journal of Medical Entomology* 33:852–855

Randolph SE (2004) Evidence that climate change has caused 'emergence' of tick-borne diseases in Europe? *International Journal of Medical Microbiology Supplements* 293:5–15

Ryder JW, Pinger RR, Glancy T (1992) Inability of *Ixodes cookei* and *Amblyomma americanum* nymphs (Acari, Ixodidae) to transmit *Borrelia burgdorferi*. *Journal of Medical Entomology* 29:525–530

Schulze TL, Jordan RA, Hung RW (1998) Comparison of *Ixodes scapularis* (Acari: Ixodidae) populations and their habitats in established and emerging Lyme disease areas in New Jersey. *Journal of Medical Entomology* 35:64–70

Schulze TL, Jordan RA, Hung RW (2001) Effects of selected meteorological factors on diurnal questing of *Ixodes scapularis* and *Amblyomma americanum* (Acari : Ixodidae). *Journal of Medical Entomology* 38:318–324

Schulze TL, Jordan RA, Hung RW, Puelle RS, Markowski D, Chomsky MS (2003) Prevalence of Borrelia burgdorferi (Spirochaetales : Spirochaetaceae) in Ixodes scapularis (Acari : Ixodidae) adults in New Jersey, 2000–2001. *Journal of Medical Entomology* 40:555–558

Sonenshine DE (1979) *Ticks of Virginia*, Blacksburg, VA: Virginia Polytechnic Institute and State University. College of Agriculture and Life Sciences

Sonenshine DE, Ratzlaff RE, Troyer J, Demmerle S, Demmerle ER, Austin WE, et al. (1995) *Borrelia burgdorferi* in Eastern Virginia: comparison between a coastal and inland locality. *American Journal of Tropical Medicine and Hygiene* 53:123–133

Stafford KC 3rd, Cartter ML, Magnarelli LA, Ertel SH, Mshar PA (1998) Temporal correlations between tick abundance and prevalence of ticks infected with *Borrelia burgdorferi* and increasing incidence of Lyme disease. *Journal of Clinical Microbiology* 36:1240–1244

Steere AC, Coburn J, Glickstein L (2004) The emergence of Lyme disease. *Journal of Clinical Investigation* 113:1093–1101

Strand MR, Walker ED, Merritt RW (1992) Field studies on *Ixodes dammini* in the Upper Peninsula of Michigan. *Vector Control Bulletin of North Central States* 1:11–18

Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. *Molecular Biology and Evolution* 24:1596–1599

Telford SR, Mather TN, Moore SI, Wilson ML, Spielman A (1988) Incompetence of deer as reservoirs of the Lyme disease spirochete. *American Journal of Tropical Medicine and Hygiene* 39:105–109

Telford SR, Spielman A (1989a) Competence of a rabbit feeding *Ixodes* (Acari, Ixodidae) as a vector of the Lyme disease spirochete. *Journal of Medical Entomology* 26:118–121

Telford SR, Spielman A (1989b) Enzootic transmission of the agent of Lyme disease in rabbits. *American Journal of Tropical Medicine and Hygiene* 41:482–490

Tsao JI, Wootton JT, Bunikis J, Luna MG, Fish D, Barbour AG (2004) An ecological approach to preventing human infection: Vaccinating wild mouse reservoirs intervenes in the Lyme disease cycle. *Proceedings of the National Academy of Sciences of the United States of America* 101:18159–18164

Walker ED, Stobierski MG, Poplar ML, Smith TW, Murphy AJ, Smith PC, Schmitt SM, Cooley TM, Kramer CM (1998) Geographic distribution of ticks (Acari : Ixodidae) in Michigan, with emphasis on *Ixodes scapularis* and *Borrelia burgdorferi*. *Journal of Medical Entomology* 35:872–882

Wormser GP, Liveris D, Nowakowski J, Nadelman RB, Cavaliere LF, McKenna D, Holmgren D, Schwartz I (1999) Association of specific subtypes of *Borrelia burgdorferi* with hematogenous dissemination in early Lyme disease. *The Journal of Infectious Diseases* 180:720–725