Applied and Environmental Microbiology, Mar. 2011, p. 1999–2007 Vol. 77, No. 6
0099-2240/11/$12.00 doi:10.1128/AEM.02479-10

Diverse *Borrelia burgdorferi* Strains in a Bird-Tick Cryptic Cycle▽†

Sarah A. Hamer,[1]* Graham J. Hickling,[2] Jennifer L. Sidge,[1] Michelle E. Rosen,[1]‡ Edward D. Walker,[3] and Jean I. Tsao[1,4]

Department of Fisheries and Wildlife, Michigan State University, East Lansing, Michigan[1]; The Center for Wildlife Health, Department of Forestry, Wildlife, and Fisheries, The University of Tennessee, Knoxville, Tennessee[2]; Department of Microbiology and Molecular Genetics, Michigan State University, East Lansing, Michigan[3]; and Department of Large Animal Clinical Sciences, Michigan State University, East Lansing, Michigan[4]

Received 20 October 2010/Accepted 11 January 2011

The blacklegged tick *Ixodes scapularis* is the primary vector of the most prevalent vector-borne zoonosis in North America, Lyme disease (LD). Enzootic maintenance of the pathogen *Borrelia burgdorferi* by *I. scapularis* and small mammals is well documented, whereas its "cryptic" maintenance by other specialist ticks and wildlife hosts remains largely unexplored because these ticks rarely bite humans. We quantified *B. burgdorferi* infection in a cryptic bird-rabbit-tick cycle. Furthermore, we explored the role of birds in maintaining and moving *B. burgdorferi* strains by comparing their genetic diversity in this cryptic cycle to that found in cycles vectored by *I. scapularis*. We examined birds, rabbits, and small mammals for ticks and infection over a 4-year period at a focal site in Michigan, 90 km east of a zone of *I. scapularis* invasion. We mist netted 19,631 birds that yielded 12,301 ticks, of which 86% were *I. dentatus*, a bird-rabbit specialist. No resident wildlife harbored *I. scapularis*, and yet 3.5% of bird-derived ticks, 3.6% of rabbit-derived ticks, and 20% of rabbit ear biopsy specimens were infected with *B. burgdorferi*. We identified 25 closely related *B. burgdorferi* strains using an rRNA gene intergenic spacer marker, the majority (68%) of which had not been reported previously. The presence of strains common to both cryptic and endemic cycles strongly implies bird-mediated dispersal. Given continued large-scale expansion of *I. scapularis* populations, we predict that its invasion into zones of cryptic transmission will allow for bridging of novel pathogen strains to humans and animals.

The maintenance cycle of the Lyme disease (LD) pathogen *Borrelia burgdorferi* in the eastern United States involves the blacklegged tick *Ixodes scapularis*—the main enzootic and zoonotic vector—and rodents, particularly the white-footed mouse *Peromyscus leucopus* and eastern chipmunk *Tamias striatus*, which are efficient reservoir hosts for the pathogen and commonly used hosts for juvenile ticks (3, 29). The generalist feeding behavior of *I. scapularis* allows this vector to bridge the pathogen from wildlife reservoirs to humans and dogs. LD is the most frequently reported vector-borne disease in the northern hemisphere, with more than 20,000 cases reported annually in the United States and increasing annual incidence (5).

I. scapularis is expanding its geographic range in North America. For example, in 2002, a new population of *I. scapularis* was detected in the southwestern corner of Michigan's Lower Peninsula (17). These ticks subsequently invaded north through coastal dune forests along the Lake Michigan lakeshore (23, 24). In tracking this range expansion over a 5-year sampling period, we have documented the presence of multiple strains of *B. burgdorferi* not only in recently invaded *I. scapularis* ticks and their rodent hosts but also in other tick and wildlife species prior to the arrival of *I. scapularis*. We postulated that cryptic pathogen maintenance—i.e., enzootic maintenance of *B. burgdorferi* in ticks that specialize on wildlife and do not commonly bite humans—could explain this preinvasion distribution of *B. burgdorferi*. Cryptic transmission cycles of *B. burgdorferi* have been described previously. For example, *Ixodes neotomae* is a woodrat-specialist tick that rarely bites humans and is more important than *Ixodes pacificus* (the bridge vector in the western United States) in maintaining *B. burgdorferi* in nature (9, 38). Similarly, in the southeastern United States, *I. affinis* and *I. major*—both rodent-feeding ticks—are more important than *I. scapularis* in local enzootic maintenance (43). *I. dentatus*, a *B. burgdorferi*-competent vector (4, 57) that feeds almost exclusively on birds and eastern cottontail rabbits (*Sylvilagus floridanus*), has also been implicated as a cryptic vector but has been described thus far only from an area where *I. scapularis* is sympatric (35, 58). *I. dentatus* has previously been associated with antigenically variable strains of *B. burgdorferi* (2, 41, 42). Ecological studies of *I. dentatus* as a cryptic vector heretofore have not been conducted in an area where *I. scapularis* is absent, or newly invading.

The genetic diversity of *B. burgdorferi* has both ecological and epidemiological implications, since certain strains have been associated with disseminated disease in humans (50, 66). Marked geographic structure is evident (18), and host associations have been suggested at certain loci (8). In the context of a cryptic cycle, knowledge of the genetic composition of *B. burgdorferi*, particularly the relative abundance and diversity of strains in comparison to that found in areas of endemicity, may elucidate how cryptic cycles contribute to the emergence of *B. burgdorferi* in *I. scapularis*-driven systems. The range expansion of *I. scapularis* in lower Michigan provides a natural experi-

* Corresponding author. Mailing address: Department of Fisheries and Wildlife, 13 Natural Resources Bldg., Michigan State University, East Lansing, MI 48824. Phone: (517) 775-4360. Fax: (517) 432-1699. E-mail: hamer@msu.edu.

‡ Present address: Michigan Department Natural Resources and Environment, East Lansing, MI.

† Supplemental material for this article may be found at http://aem.asm.org/.

▽ Published ahead of print on 21 January 2011.

ment to study this process. In this context, the objectives of our study were to (i) characterize the tick parasites of birds and rabbits in an area outside of the *I. scapularis* expansion zone and their infection status with *B. burgdorferi*, (ii) confirm the presence or absence of *I. scapularis* by sampling frequently used hosts (mice and chipmunks), and (iii) characterize strain diversity of *B. burgdorferi* at these sites.

MATERIALS AND METHODS

Bird mist netting. In 2004 to 2007 we collaborated with bird banders at Pitsfield Banding Station (Vicksburg, MI; see Fig. S1 in the supplemental material) in southwestern Michigan, at a site ~90 km from coastal forests where *I. scapularis* ticks are now endemic. Banders mist netted birds in late-successional forest for an average of 3 days per week during the bird breeding season of May-August and 5 days/week during the fall migratory season from September to November. We aimed to investigate birds in both the breeding and the fall migratory seasons to assess both local establishment of ticks and *B. burgdorferi* and the potential for migratory imports/exports of vectors and pathogen. Between 22 and 36 12-m mist nets (Avinet, Dryden, NY) were used to capture birds. The nets were run from sunrise for approximately 6 h on fair weather days and checked hourly. From each captured bird, vital data were obtained, including species, sex, age class (hatch-year or after hatch-year), and weight, and a federally issued leg band was attached before release. Recaptures of previously banded birds were noted. All birds were checked for ticks using a magnifying head loupe and straws to blow feathers from ears. Ticks were removed and preserved in 70% ethanol. Due to time constraints and bird safety, for a small number of birds, ticks were observed but not removed. All wildlife research was approved by Michigan State University's Institutional Animal Use and Care Committee permit 02-07-13-000.

Mammal trapping. To increase our search intensity for locally established *I. scapularis*, we sampled the small mammal community with a particular focus on white-footed mice and eastern chipmunks, as these are the most sensitive indicators of low-density *I. scapularis* in lower Michigan (24). To validate our sampling methods, we also trapped mammals 90 km due west of Pitsfield at Van Buren State Park (South Haven, MI), where *I. scapularis* was known to be established (23). Both areas were sampled in May and June, when *I. scapularis* larvae and nymphs are active simultaneously (17). In 2004 to 2009, small mammals were trapped for an average of 3 nights per summer using an average of 100 Sherman live traps (H. B. Sherman Traps, Tallahassee, FL) spaced 10 m apart and baited with sunflower seed. In addition, at Pitsfield we captured eastern cottontails, the preferred host for adult *I. dentatus*. Rabbits were trapped with wooden box traps baited with apples; these traps on occasion also captured other medium-sized mammals. Small mammals were anesthetized by using isoflurane (IsoFlo; Abbot Laboratories, Abbott Park, IL), and medium mammals were anesthetized by using ketamine hydrochloride (Ketaset; Fort Dodge, Overland Park, KS) and xylazine hydrochloride (Rompun; Bayer Health Care, Kansas City, KS), with yohimbine hydrochloride (Antagonil; Wildlife Laboratories, Fort Collins, CO) used to antagonize the xylazine. Each animal was examined for ticks, biopsied from both ears using a 2-mm (small mammals) or 4-mm (medium mammals) biopsy punch (Miltex Instruments, York, PA), and marked with a uniquely numbered ear tag (National Band and Tag, Newport, KY). Ticks and ear biopsy specimens were stored separately in 70% ethanol. Animals recaptured within the same site visit (i.e., recaptured the day after initial processing) were simply checked again for ticks. All animals were released at the site of capture.

Tick and *Borrelia* species detection. Ticks were identified to species and stage (15, 30, 54). Representative specimens are vouchered in the National Tick Collection at Georgia Southern University. Species identity for a subset of ticks was confirmed molecularly by amplifying and sequencing the tick 5.8S–28S rRNA gene internally transcribed spacer (ITS-2 [44]; GenBank accession numbers GU993304 to GU993307).

Total DNA from ticks and ear biopsy specimens was extracted by using a DNeasy blood and tissue kit (Qiagen, Valencia, CA) according to the manufacturer's animal tissue protocol, but with modifications as described previously (24). Ear biopsy specimens (one per animal) and adult and nymphal ticks were extracted individually; same-species larvae from the same individual animal were pooled for extraction. *B. burgdorferi* strain B31-infected nymphal *I. scapularis* generously provided by the Centers for Disease Control and Prevention (CDC) served as the positive extraction control, and water served as a negative control.

All tick and ear biopsy DNA extracts were tested for the presence of *Borrelia* species by using a nested PCR for the 16S-23S rRNA intergenic spacer region (IGS) of *Borrelia* spp., which amplifies an ~900-nucleotide region, by *B. burgdorferi* B31 coordinates (10). PCR enzyme kits were used throughout (PCR Supermix; [Invitrogen, Carlsbad, CA] and FailSafe PCR System [Epicentre, Madison, WI]). DNA from *B. burgdorferi* strain B31-infected ticks from the CDC served as a positive PCR control, and water served as a negative PCR control. A random subset of IGS-positive DNA samples was subjected to amplification of the 16S rRNA gene using a real-time, quantitative PCR with a probe specific to *B. burgdorferi* (59) or a standard assay using primers RS11 and S5 as published in Rudenko et al. (47).

Nucleotide sequencing. Strain typing of *Borrelia*-positive tick and ear tissue samples was attained through DNA sequencing. All IGS products ~900 bp in size were purified (Qiagen PCR purification kit), and sequences were determined by using the inner primers on an ABI Prism 3100 genetic analyzer (Applied Biosystems, Foster City, CA). Sequences for *B. andersonii* were present within some samples and removed from further analysis. *B. burgdorferi* sequences (GenBank accession numbers GU993279 to GU993303) were compared to published sequences using the basic local alignment search tool in GenBank (1). A 500-bp nucleotide segment of the IGS was aligned with the prototypical strains published in Bunikis et al. (10) using the CLUSTAL W algorithms within the program MEGA4 (56). Analysis of this fragment size allowed for identification of the 10 main IGS groups and of 20 IGS subtypes as presented in Bunikis et al. (10). In addition, sequences were identified to broad ribosomal spacer type (RST 1, 2, or 3 [36]) based on clustering topology of the IGS phylogenetic trees. In the case that a double-strand confirmed sequence did not identically match any published strain or any strain we previously found in *I. scapularis* across the Midwest, we classified it as novel IGS mutant. Mixed strain infections were noted and removed from further analysis.

To evaluate phylogenetic relationships among *B. burgdorferi* haplotypes, we constructed an unrooted minimum spanning network using TCS 1.21 (12). This method of phylogenetic analysis was selected in part due to low bootstrap support among strains within RST groups when using other traditional phylogenetic analyses, which also make assumptions that are not upheld in intraspecific genetic datasets at the population level (12). This network method determines the gene network in which the total length of the branches that connect haplotypes is minimized. Discrimination among equal-length networks required the assumption that older alleles are more common than recently derived alleles and that new mutations are more likely to be found in the same population as their ancestor.

Statistics. To determine mist netting success rate, one net hour (NH) is defined as the equivalent of one 12-m net run for 1 h. Chi-squared tests for independence were used to assess frequency of coinfestations. Logistic regression was used to assess trends in tick infestation and tick/host infection over the 4-year sampling period. Comparisons between birds captured in the migratory versus breeding season were made by calculating the z-ratio and associated probabilities for the difference between two independent proportions. Minimum infection prevalence (MIP; i.e., assuming one positive larva per pool) was used for tests conducted on pooled larvae. Statistics were performed using Statistix 8 (Analytical Software, Tallahassee, FL). The effect of sample size on strain richness was assessed by using a web-based rarefaction calculator (University of Alberta, Edmonton, Alberta, Canada [www.biology.ualberta.ca/jbrzusto/rarefact.php]). Strain richness was estimated by using the nonparametric model of Chao, which considers the number of operational taxonomic units observed, and the frequency with which each was observed, to estimate total population strain richness (11). Evidence for gene conversion, indicative of recombination among strains which may result in novel strain types, was examined using Sawyer's test in GENECONV version 1.81 (www.math.wustl.edu/~sawyer/geneconv/) (48).

RESULTS

Ticks on birds. A total of 19,631 captures of birds, representing 105 species, occurred in 48,030 net hours for an overall netting success rate of 40.9 captures per 100 NH (see Tables S1 and S2 in the supplemental material). Net success was significantly higher in October and November (53.3 birds/100 NH) versus May through August (33.4 birds/100 NH), reflecting the influx of fall migrants. Recaptures comprised 19.5% of all capture events. Gray Catbird, Myrtle Warbler, American Goldfinch, and White-throated Sparrow were the most abundant species with a sample size of over 1,000 captures of each species (see Tables S1 and S2 in the supplemental material).

Of all captures, 65.9, 30.6, and 3.5% of birds were hatch-year, after hatch-year, and of unknown age class, respectively; 30.7, 27.7, and 41.6% of the birds were male, female, and of undetermined sex, respectively.

A total of 2,074 captures (10.6% of all captures) were infested with ticks, representing 1,799 individual birds with ticks. A total of 12,301 ticks were removed from birds, with a mean infestation burden of 5.9 ticks per infested bird. Of all ticks, 86.4% were *I. dentatus* (10,363 larvae and 265 nymphs), 13.4% were *Haemaphysalis leporispalustris* (1,535 larvae and 118 nymphs), 0.1% were *I. scapularis* (7 larvae and 6 nymphs), and 0.06% were *Dermacentor variabilis* (6 larvae). PCR of the tick ITS-2 region resulted in *I. scapularis* sequences that matched with 100% homology to published *I. scapularis* sequences, whereas the ITS-2 regions of *I. dentatus* and *H. leporispalustris* were not previously deposited. The most commonly parasitized bird species, in which at least one-third of all individuals were infested, included Brown Thrasher, Carolina Wren, Eastern Towhee, White-throated Sparrow, Song Sparrow, Lincoln's Sparrow, Hermit Thrush, American Robin, and Yellow-breasted Chat (see Table S1 in the supplemental material). In total, 285 birds were noted to harbor ticks but were released without removing ticks. Of the remaining birds, 84.6, 23.5, 0.2, and 0.06% of parasitized birds harbored *I. dentatus*, *H. leporispalustris*, *I. scapularis*, and *D. variabilis*, comprising 7.8, 2.2, 0.02, and 0.005% of all birds, respectively. Coinfestation of birds with both *I. dentatus* and *H. leporispalustris* simultaneously was found for 8.2% of all parasitized birds; this rate is ~2.4 times lower than what would be expected by chance (χ^2 = 1,051; df = 3; $P < 0.001$). All ticks were at immature stages, with the exception of a single adult female *I. dentatus* collected from a House Wren. The mean burdens of *I. dentatus* larvae and nymphs on infested birds were 7.0 and 1.7, respectively. The mean burdens of *H. leporispalustris* larvae and nymphs on infested birds were 4.2 and 1.2, respectively.

Across all sampling, only three individual birds harbored *I. scapularis*: a Swainson's Thrush, a Hermit Thrush, and a Connecticut Warbler. All three were hatch-year birds upon their first capture during in late August and September of 2004–2005; none were recaptured subsequently. The Hermit Thrush harbored 6 larval and 5 nymphal *I. scapularis* ticks, in addition to 2 *H. leporispalustris* larvae. The Connecticut Warbler harbored a single *I. scapularis* larva. The Swainson's Thrush harbored a single *I. scapularis* nymph, in addition to 10 and 3 *H. leporispalustris* larvae and nymphs, respectively.

Ticks on mammals. At Pitsfield, no *I. scapularis* were found on 65 white-footed mice and 59 chipmunks trapped in May and June of 2005–2009. Concurrently, at Van Buren State Park, the positive control site for *I. scapularis* 90 km away, 69.3% of 179 mice and 100% of 23 chipmunks were infested with *I. scapularis. D. variabilis* was present on small mammals from both areas (Table 1). Nineteen captures of eastern cottontails at Pitsfield represented 15 individual rabbits of which 71.4% were infested by ticks. The most common species were *I. dentatus* and *H. leporispalustris*; no *I. scapularis* ticks were found on rabbits (Table 1). A total of 18 nontarget mammals, comprising seven additional species, were captured, and none harbored *I. scapularis* (Table 1).

***B. burgdorferi* in bird-associated ticks.** A total of 2,202 ticks/larval pools from 1,579 individual birds were assayed for infection with *Borrelia* species; these infested birds from which we tested ticks were a random subset of all infested birds. Of these, 78 (3.5%) ticks/larval pools were sequence confirmed positive for *B. burgdorferi*, and these infected ticks were removed from 73 different birds (4.6% of infested birds from which ticks were tested). An additional 18 samples produced IGS bands at ~900 bp in size (a finding indicative of either *B. burgdorferi* or *B. andersonii*) that were not successfully sequenced. Accordingly, the reported tick infection prevalence is a minimum. A minimum of 0.49% of all birds in our study carried *B. burgdorferi*-infected ticks (10.6% of birds carried ticks; 4.6% of infested birds carried infected ticks). *B. burgdorferi* infection prevalence in nymphs was significantly greater than that of larval pools (5.2 and 3.2%, respectively; $P = 0.02$). *B. burgdorferi* was detected in *I. dentatus*, *H. leporispalustris*, and *I. scapularis* (Table 2). Of the small number of *I. scapularis* found in our study, 1 of the 5 nymphal *I. scapularis* ticks removed from the Hermit Thrush was infected with *B. burgdorferi*.

Aggregating all 4 years of the study, *B. burgdorferi* was present in all months sampled (i.e., May-November), with the highest monthly prevalences of 11 to 12% in May and July. From mid-June through mid-August—a period that largely excludes the spring and fall migrations in our area—we detected 19 *B. burgdorferi*-positive ticks/pools, comprising 24% of all positives. Of these mid-summer positive samples, 8 (42.1%) were from hatch-year birds, which is indicative of local pathogen maintenance.

Our data on production of infected larval ticks (i.e., natural xenodiagnosis) implicate 20 species of bird as reservoir competent for *B. burgdorferi* (see Table S1 in the supplemental material). As further evidence of there being local infectious hosts, nine individual birds of four host species were each associated with multiple infected ticks/tick pools that were removed during multiple capture events (i.e., American Robin, Song Sparrow, Swainson's Thrush, and White-throated Sparrow).

***B. burgdorferi* in mammal-associated ticks.** A total of 150 adults, nymphs, and larval pools removed from white-footed mice, chipmunks, eastern cottontails, and nontarget mammals at Pitsfield were assayed for infection with *B. burgdorferi*. The only host species associated with positive ticks was the eastern cottontail, in which a minimum of 4 of 122 (3.3%) *I. dentatus* adults or nymphs were sequence confirmed to be positive for *B. burgdorferi*, and an additional 4 were positive for either *B. burgdorferi* or *B. andersonii* (no sequence obtained; Table 1). At Van Buren State Park, a total of 376 nymphs and larval pools removed from white-footed mice and chipmunks were assayed for infection with *B. burgdorferi*, 109 (29.0%) of which were positive (Table 1).

***B. burgdorferi* in mammal ear biopsy specimens.** At Pitsfield, 1 of 61 (1.6%) and 2 of 55 (3.6%) white-footed mouse and chipmunk ears, respectively, tested positive for *B. burgdorferi*, whereas the equivalent prevalences at Van Buren were 30.4 and 34.8%, respectively (Table 1). At Pitsfield, a minimum of 4 of 20 (20%) cottontail ear biopsy specimens were positive for *B. burgdorferi*, and an additional two rabbits were positive for either *B. burgdorferi* or *B. andersonii* (no sequence obtained). None of 14 ear biopsy specimens from the nontarget mammals tested positive.

TABLE 1. Mammal infestation prevalence with various tick species, mammal infection prevalence with *B. burgdorferi*, and mammal-derived tick prevalence of infection with *B. burgdorferi* at the Pitsfield Banding Station and Van Buren State Park, May to October, 2004–2009[a]

Mammal	Source	Pitsfield		Van Buren	
		% Infested (*n*)	% Infected (*n*)	% Infested (*n*)	% Infected (*n*)
White-footed mouse (*Peromyscus leucopus*)	*I. scapularis*	0 (65)		69.3 (179)	33.9 (168)
	D. variabilis	9.2 (65)	0 (6)	39.1 (179)	9.2 (119)
	Ear biopsy specimens		1.6 (61)		30.4 (171)
Eastern chipmunk (*Tamias striatus*)	*I. scapularis*	0 (59)		100 (23)	46.1 (89)
	D. variabilis	8.5 (59)	0 (3)	8.7 (23)	
	Ear biopsy specimens		3.6 (55)		34.8 (23)
Eastern cottontail (*Sylvilagus floridanus*)	*I. dentatus*	61.9 (21)	3.3 (122)[b]		
	H. leporispalustris	23.8 (21)	0 (9)		
	D. variabilis	4.8 (21)			
	Ear biopsy specimens		20 (20)[b]		
Raccoon (*Procyon lotor*)	*D. variabilis*	33.3 (3)			
	Ear biopsy specimens		0 (3)		
Fox squirrel (*Sciurus niger*)	Tick infestation	0 (1)			
	Ear biopsies		0 (1)		
Meadow jumping mouse (*Zapus hudsonius*)	*I. dentatus*	66.6 (3)	0 (1)		
	Ear biopsy specimens		0 (3)		
Red squirrel (*Tamiasciurus hudsonicus*)	Tick infestation	0 (1)			
	Ear biopsies		0 (1)		
Northern short-tailed shrew (*Blarina brevicauda*)	Tick infestation	0 (1)			
	Ear biopsy specimens				
Virginia opossum (*Didelphis virginiaina*)	*D. variabilis*	25 (4)			
	Ear biopsy specimens		0 (3)		
Woodchuck (*Marmota monax*)	*D. variabilis*	33.3 (3)	0 (2)		
	I. cookei	66.6 (3)	0 (7)		
	Ear biopsy specimens		0 (3)		

[a] Values indicate (i) the percentage of mammals infested by ticks of various species, (ii) the percentage of adults, nymphs, or larval pools of ticks removed from mammals that are infected with *B. burgdorferi*, and (iii) the percentage of mammals with infected ear biopsy specimens. Each is followed by the sample size (*n*) in parentheses (not all ticks were tested for infection).

[b] Infection prevalence is considered a minimum due to additional samples that were PCR positive for either *B. burgdorferi* or *B. andersonii* but were not sequenced.

***B. burgdorferi* genotypes.** *B. burgdorferi* IGS PCR products were successfully sequenced from 80 samples, including nine mammal-associated samples and 71 ticks/larval pools removed from birds. Of the 80 sequences, 10% were interpreted as mixed strain infections due to the presence of double-nucleotide peaks at polymorphic sites (six tick samples removed from birds, one chipmunk ear, and one tick removed from a rabbit). Among all IGS strains, we found no evidence for recombination using Sawyer's test. Within the 500-nucleotide IGS fragment that we analyzed, 58 sites were found to be polymorphic, including one indel block of 7 nucleotides (treated as a single polymorphism).

TABLE 2. Prevalence of infection with *B. burgdorferi* in ticks removed from birds, 2004–2007, at the Pitsfield Banding Station[a]

Species	Larval pools		Nymphs	
	No. of samples tested	% Infected	No. of samples tested	% Infected
I. dentatus	1,452	3.0	262	5.0
H. leporispalustris	364	3.9	114	5.3
I. scapularis	2	0.0	6	16.7
All species	1,818	3.2	382	5.2

[a] In addition to the samples listed, a single adult female *I. dentatus* and a pool of 6 *D. variabilis* larvae tested negative.

In all, 25 IGS strains were found among 72 samples, including strains from all three RST groups. Of all of the strains, 32% were "ubiquitous" (i.e., also found in association with classic, *I. scapularis*-driven transmission cycles in the Northeast [10] and/or the Midwest [S. A. Hamer, unpublished data]), and 68% were "unique" (i.e., novel IGS mutants thus far found only at Pitsfield in cryptic transmission; Fig. 1). The only strains found more than once were ubiquitous strains; all unique strains were singletons, many of which were single- or double-nucleotide polymorphisms of ubiquitous strains. A minimum spanning network of Pitsfield strains shows, for example, that there were many unique strains very similar to the ubiquitous strain IGS 2D, including six different single nucleotide polymorphism mutants and three different double nucleotide polymorphism mutants (Fig. 1). The strain evenness, a

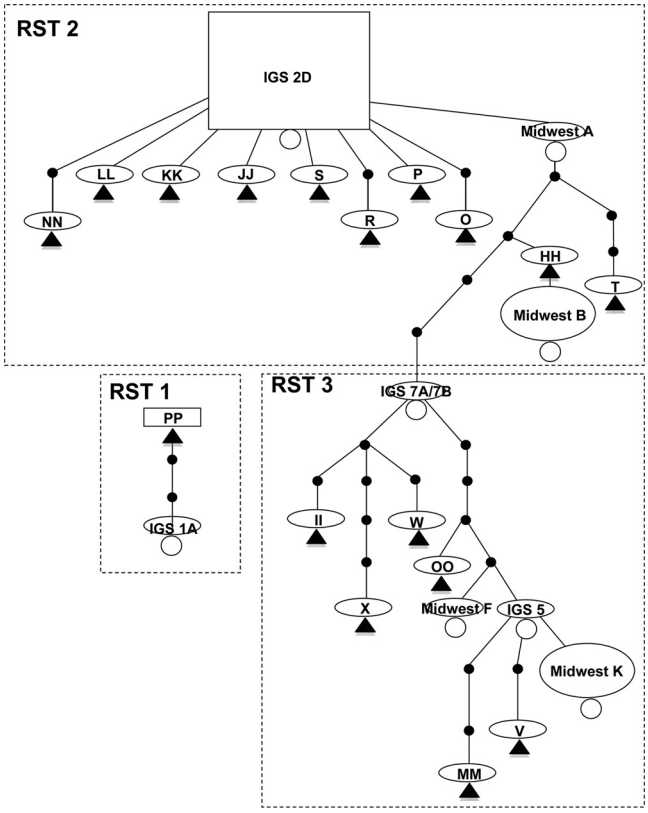

FIG. 1. Minimum spanning network of *B. burgdorferi* IGS haplotypes collected from Pitsfield Banding Station, 2004–2007. Dashed rectangles delineate the three main RST groups. Haplotypes are represented by solid ovals or rectangles; the solid rectangles represent the haplotypes with the highest outgroup weights, which correlate with haplotype age. The size of each haplotype is proportional to its frequency within the sampled population. Each black circular node connecting haplotypes represents one mutational change. In addition to the point mutations, there was also a 7-bp indel that was considered one mutation. Unique strains (novel IGS mutants not previously reported) are indicated with a black triangle; ubiquitous strains (haplotypes previously reported by Bunikis et al. (10) or found in *I. scapularis* across the Midwest (Hamer, unpublished) are indicated with an open circle.

component of diversity, is illustrated by the relative sizes of the symbols in Fig. 1, in which the most abundant strains are depicted by the largest symbols (IGS 2D, Midwest B, and Midwest K). A rarefaction curve to assess strain richness suggests that true *B. burgdorferi* strain richness at Pitsfield is vast, since the rate at which new strains were found per unit of individuals sequenced was not yet asymptotic. We detected 25 IGS strains within 72 samples derived from investigations of over 19,000 birds and a small number of mammals; from these data, the Chao-1 nonparametric estimator of true species richness is 246 ± 101 strains.

The single *B. burgdorferi*-positive from an *I. scapularis* nymph removed from a bird was type "Midwest A" of RST group 2 (Fig. 1), a ubiquitous strain with a wide Midwestern distribution. There was no difference in the proportion of unique strains in bird-associated ticks during the migratory (1 May to 15 June; 15 August to 31 November) versus nonmigratory (15 June to 15 August) seasons (23.6 and 30%, z-ratio, 043; $P = 0.67$). Of the seven *B. burgdorferi* sequences identified in mammal tissues or mammal-derived ticks, all but one were ubiquitous strains, with all three RST groups represented (Fig. 1). The single infected white-footed mouse ear was infected with Midwest A, the chipmunk ear was infected with Midwest B, and the *I. dentatus* adults removed from rabbits were infected with IGS 2D ($n = 2$), IGS 1A ($n = 1$), and Midwest K ($n = 1$).

DISCUSSION

Our 4-year data set provides evidence for maintenance of *B. burgdorferi* by bird-associated ticks in the apparent absence of *I. scapularis*. The focal site where we trapped birds is outside the detected range of established *I. scapularis* populations, based on both historic records and our findings over the 2004–2007 sampling period (24). Whereas birds in general have previously been considered to dilute the force of infection in areas of endemicity, by diverting tick bites away from white-footed mice which are generally able to infect more ticks than do birds (19, 37), we present an ecological scenario whereby birds may accelerate increasing LD risk to humans through their maintenance of *B. burgdorferi* in the absence of classic *I. scapularis*/white-footed mouse transmission. The acceleration of disease risk may occur in zones of cryptic transmission into which the bridge vector *I. scapularis* invades and feeds upon wild birds or rabbits that are already infected. Recently, Humphrey et al. (28) determined that the historical demographic, migratory, and population genetic patterns of *B. burgdorferi* do not reflect those of sympatric *I. scapularis.* In particular, while *I. scapularis* ticks within Wisconsin showed population structure, *B. burgdorferi* did not. Humphrey et al. postulate that reservoir hosts may be moving *B. burgdorferi* around [more than they do vector ticks]. Similarly, working in the LD system in Europe, Vollmer et al. (60) demonstrate that population structure of different *B. burgdorferi sensu lato* species, which exhibit different host associations, are a function of the mobility of their respective host species. Our data suggest that birds are a mechanism by which diverse *B. burgdorferi* types may be spread from zones of cryptic transmission to zones of epidemiological importance.

Absence of *I. scapularis*. Our data demonstrate that the distribution of *B. burgdorferi* is not necessarily congruent with that of *I. scapularis*. *I. scapularis* was not encountered on any mammals or resident birds at our study site and was rarely encountered on fall migrants, with three birds found to carry *I. scapularis* during late August through October. Given the species-specific breeding and migratory distribution and timing of these species (7), combined with the endemicity of *I. scapularis* in the southern Upper Peninsula (62) and other zones in the upper Midwest, we posit that these migratory birds picked up *I. scapularis* prior to arrival at Pitsfield. Furthermore, all three infested birds were captured during the first 2 years of this 4-year study, suggesting that the infestation of these birds did not represent the incipient stage of successful establishment.

Migratory birds are known to be an important source of adventitious *I. scapularis* in areas beyond the range of established *I. scapularis* and may be responsible for extending the geographic range of *I. scapularis* (32, 40, 52) and *B. burgdorferi*

sensu lato (14, 65). Ogden et al. (40) found that northbound migratory birds, during spring migration, are very likely to be important in expanding the northern geographic range of *I. scapularis* across a wide front in northern Canada, with 0.35% of birds infested and an average of 0.007 *I. scapularis* per migratory bird. In contrast, we did not find *I. scapularis* ticks on birds during the spring migration in Michigan, albeit our sample size (988 birds) in the spring months was small. Furthermore, it is unclear whether *I. scapularis* occurs in locations from which these birds winter or stopover during migration. During the mid-August to November fall migratory period over 4 years, we searched a total of 14,667 birds, and found 3 (0.02%) to harbor a total of 13 *I. scapularis* ticks. Given this average of 0.0009 *I. scapularis* per migratory bird, and given that not all *I. scapularis* are likely to drop off while their host is on site, it seems unlikely at this time that southbound migrants alone would seed a new population of *I. scapularis* at this site. Future *I. scapularis* colonization of this site will likely result from both bird-derived engorged *I. scapularis* larvae and nymphs on spring or fall migrants, as well as a continuation of the invasion process occurring to the west (24).

Preponderance of *I. dentatus*. A total of 10.6% of birds at our site were infested with ticks, the vast majority of which were *I. dentatus* (86.4%), followed by *H. leporispalustris* (13.4%) and *I. scapularis* (0.1%). Upon review of tick species assemblages on avian hosts across sites endemic and not endemic for *I. scapularis*, a similar preponderance of *I. dentatus* has not been found. For example, no *I. dentatus* ticks were identified among the 1,643 ticks removed from birds in the Midwest where >90% were *H. leporispalustris* (39). In zones where *I. scapularis* is endemic, no *I. dentatus* ticks were documented among 883 ticks removed from birds in the central Maryland Piedmont (49), and *I. dentatus* comprised only a small proportion of ticks (<4%) removed from birds relative to *I. scapularis* (>94%) in Westchester County, NY (6), and Lyme, CT (55). As a local comparison, 90 km to the west of Pitsfield in a zone of recent *I. scapularis* establishment, in May and June of 2004–2008, we found 20.7% of the birds to be infested with ticks, of which 95.6% were *I. scapularis*, 3.1% were *H. leporispalustris*, and 1.3% were *I. dentatus* (24). Only in a study in southern Canada where *I. scapularis* is expanding its range were *I. dentatus* and *I. scapularis* found nearly equal in abundance on birds (34 and 29.3% of ticks, respectively [40]), although it is difficult to assess whether this reflects the locally acquired tick fauna versus the tick community composition from where the birds originated. The preponderance of *I. dentatus* in the present study reflects the tick community composition prior to *I. scapularis* invasion. *I. dentatus* itself may be undergoing range expansion, having been detected in Michigan only since 1992, despite an established passive surveillance program since 1968 (63).

Cryptic *B. burgdorferi* maintenance. This research underscores the potential importance of birds in *B. burgdorferi* maintenance and dispersal in the absence of *I. scapularis*. Given the demonstrated vector competence of *I. dentatus* for *B. burgdorferi* (57) and its abundance on birds, we have identified it as a cryptic vector in our study region. Local transmission of this pathogen is evident in that hatch-year birds, known to have been born onsite, harbored infected ticks. Furthermore, eastern cottontails, which are important hosts for feeding adult *I. dentatus* ticks, were present onsite and infected with *B. burgdorferi*. The extent to which *I. dentatus*-mediated cryptic cycling occurs in other areas is unknown, as studies of the sample size required to detect a low infection prevalence are lacking.

The diversity of avian hosts able to infect ticks highlights the complex transmission dynamics of this pathogen. Many bird species have been proposed as competent *B. burgdorferi* reservoir hosts using laboratory studies and natural xenodiagnostic investigations (4, 20, 45, 46). Using natural xenodiagnosis, we implicate 20 avian species as competent reservoirs for *B. burgdorferi*, including 9 species which have not before been reported: Brown-headed Cowbird, Blue Jay, Eastern Towhee, Fox Sparrow, Magnolia Warbler, Slate-colored Junco, Tufted Titmouse, White-throated Sparrow, and Yellow-breasted Chat. Given that infective birds were found during both the summer breeding months and the fall migratory months, we posit that host movement from sites of cryptic transmission may provide a mechanism for dispersal of cryptic *B. burgdorferi* to new sites. Conversely, using a data set of ticks removed from spring migratory birds arriving in Canada, Ogden et al. (40) detected no infected larval *I. scapularis* ticks. This result may have been due to waned infection contracted the previous year, or lack of exposure of these birds to infectious ticks.

Strain diversity of cryptic *B. burgdorferi*. We detected an unprecedented level of diversity at the 16S-23S ribosomal intergenic spacer of cryptic *B. burgdorferi* within host and tick populations not typically investigated because they are often assumed to be unimportant for enzootic maintenance or human risk, respectively. Rarefaction analysis suggests that our sampled richness reflects only a small proportion of true strain richness. Intergenic spacer regions generally accumulate higher degrees of sequence variation between related species than do coding regions, because spacers do not produce a functional gene product and are free of selective constraint (with the exception of a tRNA gene that occurs within the IGS of *Borrelia* species). Furthermore, because no detectable recombination is found within the IGS (which is chromosomally located), in contrast to other *B. burgdorferi* typing schemes (such as *ospC*, which is located on a plasmid), IGS is a sensitive marker of evolutionary change (36) and has been used to differentiate among strains of *B. burgdorferi* (10).

The pattern of genetic diversity we observed among the cryptic *B. burgdorferi* was characterized by a population of approximately one-third ubiquitous strains that are also found in LD-endemic sites and two-thirds of unique strains that are novel mutants. Such a pattern may result from ecological opportunity for the bacterium since it associated with different vector, hosts, or geographical regions. Among others, strain IGS 2D was likely a founder at Pitsfield and/or is the most adapted to birds and rabbits, since it is the most common strain, comprising 40.3% of all sequences, and is present in ticks removed from both birds and rabbits. Founders may then undergo clonal expansion and diversification, evident as a star phylogeny in the minimum spanning network in which each novel strain is derived independently from its common ancestor. Although these novel variants are apparently permitted within birds, rabbits, and *I. dentatus*, our data suggest that they are not exposed to the mouse or chipmunk immune systems at our cryptic cycle site as they would in most *I. scapularis*-driven cycles. Furthermore, multiple-niche polymorphism, a form of frequency-dependent balancing selection, can maintain diver-

sity within the population when the environment is heterogeneous and no single genotype has the highest fitness in all environments (8). This selection does not occur on the IGS region itself but on other areas of the genome that are under selection and linked to the IGS region, such as *ospC*. For example, host associations, facilitated by selective killing of strains by host-specific innate immune factors (34), have been postulated as a factor leading to the diversifying selection of LD spirochetes at the interspecies level in Europe (14) and have also been suggested at the intraspecies level of *B. burgdorferi* in some (8) but not other (25) studies.

The frequency with which mixed-strain infections were present in this cryptic system (10% of all infected samples) is comparatively less than that which characterizes *I. scapularis* in areas where LD is endemic and yet surprising given the low overall prevalence of *B. burgdorferi* infection in this cryptic system (3.5% of bird-associated ticks/pools). Using the same IGS gene target, Gatewood et al. (18) found the proportion of mixed-strain *B. burgdorferi* infections to be ca. 20% in nymphal *I. scapularis* ticks collected in areas of LD endemicity across the northeast and Midwest, where the overall infection prevalence was 20.8%. The presence of mixed infections within the cryptic system suggests that the degree of interaction among infected ticks and hosts is high and that hosts are exposed to multiple strains.

Epidemiological significance of a cryptic cycle. Cryptic cycles may serve as additional sources or repositories of pathogen genetic diversity. The epidemiological significance of a cryptic cycle is likely to depend upon (i) the infectivity of cryptic strains to humans, (ii) the frequency with which infected cryptic vectors directly feed on humans, (iii) the presence or imminent invasion of a bridging vector, (iv) the frequencies with which the cryptic vector and bridging vector share hosts, and/or (v) the extent to which the cryptic *B. burgdorferi* serves as additive infection or replaces invading strains already circulated by the bridging vector. The following discussion expands upon each of these factors.

Some RST 1 strains are associated with a higher frequency of disseminated infection in humans, more efficient transmission to mice, or more invasive disease in experimental animals (13, 26, 50, 64, 66). At our cryptic site, we document the presence of two RST 1 strains (IGS 1A and "Novel PP"), a finding which suggests the potential epidemiological significance of this cryptic cycle, although these particular strain types represented only a minority (8%) of strain types found in cryptic circulation. The pathogenicity in humans of the large number of novel IGS mutant strains is unknown. To begin to assess the epidemiological risk associated with novel strains found within this cryptic cycle, 11 of the bird-derived samples with novel IGS types were typed at the *ospC* locus (this was kindly performed by B. Travinsky and A. Barbour). Of these, *ospC* was successfully amplified and sequenced from 10 samples, resulting in the detection of *ospC* A, K, and I3 (associated with RST 1, 2, and 3, respectively), which represent three of the four *ospC* major groups associated with disseminated human LD (50).

Biting of humans by *I. dentatus* is extremely rare in comparison to the numbers of bites people receive from *I. scapularis* and *D. variabilis* in the United States (16, 51) and has been reported for at least 19 people across Michigan (61, 63); North Carolina (27); West Virginia (22); Washington, DC (53); Connecticut (2); and Maine (31). Given the paucity of direct human biting by the cryptic vector, epidemiological significance of a cryptic cycle is contingent upon the presence of a bridging vector that will share hosts with the cryptic vector and bridge the pathogen to humans. Within our study region, *I. scapularis* is undergoing a range expansion along Michigan's coastal dune forests less than 90 km to the west of our study site (24), and the Pitsfield Banding Station is on the trajectory for continued invasion with habitat features that are predicted to support *I. scapularis* populations should they become introduced (21).

Since mice are key hosts for the bridging vector *I. scapularis*, adventitious feeding events by the cryptic vector on atypical hosts, including mice, may offer a mechanism for the transmission of cryptic strains to bridging vectors and subsequently humans. Although *I. dentatus* is typically associated with birds and rabbits, we documented this tick species feeding on two meadow jumping mice of a total of three captured at Pitsfield. This represents an expansion of the documented host range for this tick species and may afford the opportunity for small mammals to become infected with cryptic *Borrelia* spp. Similarly, an *I. dentatus* larva was found on a white-footed mouse in Tennessee (33). Conversely, the bridge vector may feed directly on cryptically infected birds and rabbits, thereby merging cryptic and classic transmission cycles. While the frequency at which *I. scapularis* feeds on rabbits in areas of endemicity is not widely studied, Telford and Spielman (58) found that 41, 38, and 3% of rabbits were infested with *I. scapularis* larvae, nymphs, and adults, respectively, and that 85, 62, and 85% were simultaneously infested with the equivalent life stages of *I. dentatus*.

It is currently unknown whether the infection prevalence in invading *I. scapularis* is elevated due to the existence of cryptic *B. burgdorferi* in the region undergoing invasion. In southwest Michigan, *B. burgdorferi* was found within recently invaded *I. scapularis* populations at a prevalence of 9.3% of drag-sampled nymphs (24). While *I. scapularis* and *B. burgdorferi* may invade simultaneously, infection prevalence within an invasion zone may increase at a faster rate when cryptically infected wildlife serve as hosts for recently invaded ticks. The degree to which invading strains may replace cryptic strains, or how host associations of strains may affect their persistence and the blending of the invading and cryptic cycles, will remain uncertain until longer-term data are gathered from within invasion zones.

Cryptic cycles have the potential to accelerate the increase in human disease risk within an invasion zone of the bridge vector. Infection of birds in cryptic cycles may allow bird dispersal of *B. burgdorferi* strains such that the movement and maintenance of *B. burgdorferi* is unlinked from that of the main bridging vector. Further studies are needed to clarify the role of birds in generating and maintaining genetic diversity of *B. burgdorferi* and the consequences this diversity may have for infection of humans or canines with these bird-derived strains.

ACKNOWLEDGMENTS

This study was supported by Doctoral Dissertation Improvement Grant DEB-0910025 from the National Science Foundation and G. H. Lauff and T. Wayne and Katherine Porter scholarships from Kellogg Biological Station (S.A.H.).

This study is Kellogg Biological Station contribution number 1556.

Rich and Brenda Keith conducted all sample collections at Pitsfield Banding Station; Aubrey Rankin, Emily Koppel, Todd Lickfett, and

Natalie Ochmanek assisted in the field and laboratory. Bridgit Travinsky and Alan Barbour performed the *ospC* typing. We thank the Michigan Department of Natural Resources for permission to work at Van Buren State Park.

REFERENCES

1. **Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. J. Lipman.** 1990. Basic local alignment search tool. J. Mol. Biol. **215:**403–410.
2. **Anderson, J. F., et al.** 1996. Novel *Borrelia burgdorferi* isolates from *Ixodes scapularis* and *Ixodes dentatus* ticks feeding on humans. J. Clin. Microbiol. **34:**524–529.
3. **Anderson, J. F., and L. A. Magnarelli.** 1993. Natural history of *Borrelia burgdorferi* in vectors and vertebrate hosts, p. 11–24. *In* H. S. Ginsberg (ed.), Ecology and environmental management of Lyme disease. Rutgers University Press, New Brunswick, NJ.
4. **Anderson, J. F., L. A. Magnarelli, and K. C. Stafford.** 1990. Bird-feeding ticks transstadially transmit *Borrelia burgdorferi* that infect Syrian hamsters. J. Wildl. Dis. **26:**1–10.
5. **Bacon, R. M., K. J. Kugeler, K. S. Griffith, and P. S. Mead.** 2007. Lyme disease–United States, 2003–2005. MMWR Morb. Mort. Wkly. Rep. **56:**573–576.
6. **Battaly, G. R., and D. Fish.** 1993. Relative importance of bird species as hosts for immature *Ixodes dammini* (Acari, Ixodidae) in a suburban residential landscape of southern New York state. J. Med. Entomol. **30:**740–747.
7. **Brewer, R., G. A. McPeek, and R. J. Adams(ed.).** 1991. The atlas of breeding birds of Michigan. Michigan State University Press, East Lansing, MI.
8. **Brisson, D., and D. E. Dykhuizen.** 2004. *ospC* diversity in *Borrelia burgdorferi*: different hosts are different niches. Genetics **168:**713–722.
9. **Brown, R. N., and R. S. Lane.** 1992. Lyme disease in California: a novel enzootic transmission cycle of *Borrelia burgdorferi*. Science **256:**1439–1442.
10. **Bunikis, J., et al.** 2004. Sequence typing reveals extensive strain diversity of the Lyme borreliosis agents *Borrelia burgdorferi* in North America and *Borrelia afzelii* in Europe. Microbiol. SGM **150:**1741–1755.
11. **Chao, A., and S. Tsung-Jen.** 2003. Nonparametric estimation of Shannon's index of diversity when there are unseen species in sample. Environ. Ecol. Stat. **10:**429–443.
12. **Clement, M., D. Posada, and K. A. Crandall.** 2000. TCS: a computer program to estimate gene genealogies. Mol. Ecol. **9:**1657–1659.
13. **Derdakova, M., et al.** 2004. Interaction and transmission of two *Borrelia burgdorferi* sensu stricto strains in a tick-rodent maintenance system. Appl. Environ. Microbiol. **70:**6783–6788.
14. **Dubska, L., I. Literak, E. Kocianova, V. Taragelova, and O. Sychra.** 2009. Differential role of passerine birds in distribution of *Borrelia* spirochetes, based on data from ticks collected from birds during the postbreeding migration period in central Europe. Appl. Environ. Microbiol. **75:**596–602.
15. **Durden, L. A., and J. E. Keirans.** 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, Lanham, MD.
16. **Falco, R. C., and D. Fish.** 1988. Ticks parasitizing humans in a Lyme disease endemic area of southern New York State. Am. J. Epidemiol. **128:**1146–1152.
17. **Foster, E. S.** 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, MI.
18. **Gatewood, A. G., et al.** 2009. Climate and tick seasonality are predictors of *Borrelia burgdorferi* genotype distribution. Appl. Environ. Microbiol. **75:**2476–2483.
19. **Giardina, A. R., K. A. Schmidt, E. M. Schauber, and R. S. Ostfeld.** 2000. Modeling the role of songbirds and rodents in the ecology of Lyme disease. Can. J. Zool. **78:**2184–2197.
20. **Ginsberg, H. S., et al.** 2005. Reservoir competence of native North American birds for the Lyme disease spirochete, *Borrelia burgdorferi*. J. Med. Entomol. **42:**445–449.
21. **Guerra, M., et al.** 2002. Predicting the risk of Lyme disease: habitat suitability for *Ixodes scapularis* in the north central United States. Emerg. Infect. Dis. **8:**289–297.
22. **Hall, J. E., et al.** 1991. Parasitization of humans in West Virginia by *Ixodes cookei* (Acari: Ixodidae), a potential vector of Lyme borreliosis. J. Med. Entomol. **28:**186–189.
23. **Hamer, S. A., et al.** 2007. Zoonotic pathogens in *Ixodes scapularis*, Michigan. Emerg. Infect. Dis. **13:**1131–1133.
24. **Hamer, S. A., J. I. Tsao, E. D. Walker, and G. J. Hickling.** 2010. Invasion of the Lyme disease vector *Ixodes scapularis*: implications for *Borrelia burgdorferi* endemicity. EcoHealth **7:**47–63.
25. **Hanincova, K., K. Kurtenbach, M. Diuk-Wasser, B. Brei, and D. Fish.** 2006. Epidemic spread of Lyme borreliosis, northeastern United States. Emerg. Infect. Dis. **12:**604–611.
26. **Hanincova, K., et al.** 2008. Fitness variation of *Borrelia burgdorferi* sensu stricto strains in mice. Appl. Environ. Microbiol. **74:**153–157.
27. **Harrison, B. A., B. R. Engber, and C. S. Apperson.** 1997. Ticks (Acari: Ixodidae) uncommonly found biting humans in North Carolina. J. Vector Ecol. **22:**6–12.
28. **Humphrey, P. T., D. A. Caporale, and D. Brisson.** 2010. Uncoordinated phylogeography of *Borrelia burgdorferi* and its tick vector, *Ixodes scapularis*. Evolution **64:**2653–2663.
29. **James, A. M., and J. H. Oliver.** 1990. Feeding and host preference of immature *Ixodes dammini*, *I. scapularis*, and *I. pacificus* (Acari, Ixodidae). J. Med. Entomol. **27:**324–330.
30. **Keirans, J. E., and C. M. Clifford.** 1978. The genus *Ixodes* in the United States: a scanning electron microscope study and key to the adults. J. Med. Entomol. **15**(Suppl. 2)**:**1–149.
31. **Keirans, J. E., and E. H. Lacombe.** 1998. First records of *Amblyomma americanum*, *Ixodes* (*Ixodes*) *dentatus*, and *Ixodes (Ceratixodes) uriae* (Acari: Ixodidae) from Maine. J. Parasitol. **84:**629–631.
32. **Klich, M., M. W. Lankester, and K. W. Wu.** 1996. Spring migratory birds (Aves) extend the northern occurrence of blacklegged tick (Acari: Ixodidae). J. Med. Entomol. **33:**581–585.
33. **Kollars, T. M.** 1992. New host of *Ixodes dentatus* (Acari, Ixodidae), and first record in Tennessee. J. Med. Entomol. **29:**715–716.
34. **Kurtenbach, K., et al.** 2002. Differential survival of Lyme borreliosis spirochetes in ticks that feed on birds. Infect. Immun. **70:**5893–5895.
35. **Levine, J. F., D. E. Sonenshine, W. L. Nicholson, and R. T. Turner.** 1991. *Borrelia burgdorferi* in ticks (Acari, Ixodidae) from coastal Virginia. J. Med. Entomol. **28:**668–674.
36. **Liveris, D., A. Gazumyan, and I. Schwartz.** 1995. Molecular typing of *Borrelia burgdorferi* sensu lato by PCR-restriction fragment length polymorphism analysis. J. Clin. Microbiol. **33:**589–595.
37. **LoGiudice, K., R. S. Ostfeld, K. A. Schmidt, and F. Keesing.** 2003. The ecology of infectious disease: effects of host diversity and community composition on Lyme disease risk. Proc. Natl. Acad. Sci. U. S. A. **100:**567–571.
38. **Maupin, G. O., et al.** 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. J. Infect. Dis. **170:**636–643.
39. **Nicholls, T. H., and S. M. Callister.** 1996. Lyme disease spirochetes in ticks collected from birds in midwestern United States. J. Med. Entomol. **33:**379–384.
40. **Ogden, N. H., et al.** 2008. Role of migratory birds in introduction and range expansion of *Ixodes scapularis* ticks and of *Borrelia burgdorferi* and *Anaplasma phagocytophilum* in Canada. Appl. Environ. Microbiol. **74:**1780–1790.
41. **Oliver, J. H., Jr., et al.** 1996. Unusual strain of *Borrelia burgdorferi* isolated from *Ixodes dentatus* in central Georgia. J. Parasitol. **82:**936–940.
42. **Oliver, J. H., Jr., et al.** 1998. First isolation and cultivation of *Borrelia burgdorferi* sensu lato from Missouri. J. Clin. Microbiol. **36:**1–5.
43. **Oliver, J. H., Jr., et al.** 2003. An enzootic transmission cycle of Lyme borreliosis spirochetes in the southeastern United States. Proc. Natl. Acad. Sci. U. S. A. **100:**11642–11645.
44. **Poucher, K. L., H. J. Hutcheson, J. E. Keirans, L. A. Durden, and W. C. Black.** 1999. Molecular genetic key for the identification of 17 *Ixodes* species of the United States (Acari: Ixodidae): a methods model. J. Parasitol. **85:**623–629.
45. **Rand, P. W., E. H. Lacombe, R. P. Smith, and J. Ficker.** 1998. Participation of birds (Aves) in the emergence of Lyme disease in southern Maine. J. Med. Entomol. **35:**270–276.
46. **Richter, D., A. Spielman, N. Komar, and F. R. Matuschka.** 2000. Competence of American robins as reservoir hosts for Lyme disease spirochetes. Emerg. Infect. Dis. **6:**133–138.
47. **Rudenko, N., M. Golovchenko, L. Grubhoffer, and J. H. Oliver.** 2009. *Borrelia carolinensis* sp. nov., a new (14th) member of the *Borrelia burgdorferi* sensu lato complex from the southeastern region of the United States. J. Clin. Microbiol. **47:**134–141.
48. **Sawyer, S.** 1989. Statistical tests for detecting gene conversion. Mol. Biol. Evol. **6:**526–538.
49. **Scharf, W. C.** 2004. Immature ticks on birds: temporal abundance and reinfestation. Northeast. Nat. **11:**143–150.
50. **Seinost, G., et al.** 1999. Four clones of *Borrelia burgdorferi* sensu stricto cause invasive infection in humans. Infect. Immun. **67:**3518–3524.
51. **Smith, R. P., Jr., E. H. Lacombe, P. W. Rand, and R. Dearborn.** 1992. Diversity of tick species biting humans in an emerging area for Lyme disease. Am. J. Public Health **82:**66–69.
52. **Smith, R. P., et al.** 1996. Role of bird migration in the long-distance dispersal of *Ixodes dammini*, the vector of Lyme disease. J. Infect. Dis. **174:**221–224.
53. **Sollers, H.** 1955. *Ixodes dentatus* (Marx) collected from a human (Acarina, Ixodidae). Proc. Entomol. Soc. Wash. **57:**120.
54. **Sonenshine, D. E.** 1979. Ticks of Virginia. Virginia Polytechnic Institute and State University, College of Agriculture and Life Sciences, Blacksburg, VA.
55. **Stafford, K. C., V. C. Bladen, and L. A. Magnarelli.** 1995. Ticks (Acari, Ixodidae) infesting wild birds (Aves) and white-footed mice in Lyme, CT. J. Med. Entomol. **32:**453–466.
56. **Tamura, K., J. Dudley, M. Nei, and S. Kumar.** 2007. MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol. Biol. Evol. **24:**1596–1599.
57. **Telford, S. R., and A. Spielman.** 1989. Competence of a rabbit-feeding *Ixodes*

(Acari: Ixodidae) as a vector of the Lyme disease spirochete. J. Med. Entomol. **26:**118–121.
58. **Telford, S. R., and A. Spielman.** 1989. Enzootic transmission of the agent of Lyme disease in rabbits. Am. J. Trop. Med. Hyg. **41:**482–490.
59. **Tsao, J. I., et al.** 2004. An ecological approach to preventing human infection: vaccinating wild mouse reservoirs intervenes in the Lyme disease cycle. Proc. Nat. Acad. Sci. U. S. A. **101:**18159–18164.
60. **Vollmer, S. A., et al.** 2011. Host migration impacts on the phylogeography of Lyme borreliosis spirochaete species in Europe. Environ. Microbiol. **1:**184–192.
61. **Walker, E. D., M. L. Poplar, and H. L. Russell.** 1992. *Ixodes dentatus* (Acari: Ixodidae) in Michigan: first state records and occurrence on a human. Great Lakes Entomol. **25:**303–304.
62. **Walker, E. D., T. W. Smith, J. Dewitt, D. C. Beaudo, and R. G. McLean.** 1994. Prevalence of *Borrelia burgdorferi* in host-seeking ticks (Acari, Ixodidae) from a Lyme disease endemic area in northern Michigan. J. Med. Entomol. **31:**524–528.
63. **Walker, E. D., et al.** 1998. Geographic distribution of ticks (Acari: Ixodidae) in Michigan, with emphasis on *Ixodes scapularis* and *Borrelia burgdorferi*. J. Med. Entomol. **35:**872–882.
64. **Wang, G. Q., et al.** 2002. Dis. severity in a murine model of Lyme borreliosis is associated with the genotype of the infecting *Borrelia burgdorferi* sensu stricto strain. J. Infect. Dis. **186:**782–791.
65. **Weisbrod, A. R., and R. C. Johnson.** 1989. Lyme disease and migrating birds in the Saint Croix River Valley. Appl. Environ. Microbiol. **55:**1921–1924.
66. **Wormser, G. P., et al.** 2008. *Borrelia burgdorferi* genotype predicts the capacity for hematogenous dissemination during early Lyme disease. J. Infect. Dis. **198:**1358–1364.