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Regional Variation in Immature *Ixodes scapularis* Parasitism on North American Songbirds: Implications for Transmission of the Lyme Pathogen, *Borrelia burgdorferi*

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ABSTRACT *Borrelia burgdorferi*, the etiological agent of Lyme disease, is transmitted among hosts by the black-legged tick, *Ixodes scapularis*, a species that regularly parasitizes various vertebrate hosts, including birds, in its immature stages. Lyme disease risk in the United States is highest in the Northeast and in the upper Midwest where *I. scapularis* ticks are most abundant. Because birds might be important to the range expansion of *I. scapularis* and *B. burgdorferi*, we explored spatial variation in patterns of *I. scapularis* parasitism on songbirds, as well as *B. burgdorferi* infection in bird-derived *I. scapularis* larvae. We sampled birds at 23 sites in the eastern United States to describe seasonal patterns of *I. scapularis* occurrence on birds, and we screened a subset of *I. scapularis* larvae for presence of *B. burgdorferi*. Timing of immature *I. scapularis* occurrence on birds is consistent with regional variation in host-seeking activity with a generally earlier peak in larval parasitism on birds in the Midwest. Significantly more *I. scapularis* larvae occurred on birds that were contemporaneously parasitized by nymphs in the Midwest than the Northeast, and the proportion of birds that yielded *B. burgdorferi*-infected larvae was also higher in the Midwest. We conclude that regional variation in immature *I. scapularis* phenology results in different temporal patterns of parasitism on birds, potentially resulting in differential importance of birds to *B. burgdorferi* transmission dynamics among regions.

KEY WORDS Acari, Lyme disease, parasitism, pathogen transmission, zoonotic disease

Migratory birds are important agents in the dispersal of zoonotic pathogens and vectors (Hubalek 2004, Tsiodras et al. 2008), and birds may be responsible for the range expansion in North America of the Lyme disease agent, *Borrelia burgdorferi*, and its vector, the black-legged tick *Ixodes scapularis* Say (Madhav et al. 2004, Morshed et al. 2005, Ogden et al. 2008, Brinkerhoff et al. 2010). In eastern North America, the Lyme pathogen *B. burgdorferi* is maintained in a horizontal transmission cycle between immature *I. scapularis* and competent vertebrate reservoir hosts, most notably, the white-footed mouse (*Peromyscus leucopus*). *B. burgdorferi*, like its principal vector, may be associated with a variety of avian and mammalian hosts (Lane et al. 1991). Vertebrate host species vary in *B. burgdorferi* reservoir competence, or the ability to acquire and transmit infection (Fish and Daniels 1990, LoGiudice et al. 2003) and several hosts, including white-tailed deer, are incapable of transmitting spirochetes to ticks (Telford et al. 1988); such hosts are effectively dead ends for *B. burgdorferi* transmission. Thus, *I. scapularis* may be dispersed by deer, but *B. burgdorferi* must be maintained and spread by other hosts. In eastern North America, there are two population foci for *I. scapularis*, one in the Northeast and one in the upper Midwest (Diuk-Wasser et al. 2010), and the geographical range and incidence of Lyme disease are increasing around both foci (Bacon et al. 2008, Hoen et al. 2009). Birds are probably less important than rodents to the maintenance of *B. burgdorferi* in natural cycles (LoGiudice et al. 2003, but see Brisson et al. 2008), but they are potentially key drivers of *B. burgdorferi* and *I. scapularis* dispersal (Morshed et al. 2005, Ogden et al. 2008, Brinkerhoff et al. 2010).

I. scapularis has a 2-yr life cycle during which it takes three blood meals (one per life stage; larva, nymph, and adult) (Barbour and Fish 1993, Schwan and Piesman 2002). Larval ticks hatch uninfected from eggs (Patrican 1997) and acquire *B. burgdorferi* spirochetes from infected reservoir hosts. Infected larvae maintain the spirochetes through the molt into nymphs, allowing them to transmit *B. burgdorferi* to uninfected reservoir hosts during their nymphal blood meal the following summer. Because of the affinity of adult *I. scapularis* for reservoir incompetent deer (Lane et al.

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1991) and absence of transovarial transmission (Patrican 1997), adults are not thought to contribute significantly to the transmission cycle, and the main driver of range expansion for *B. burgdorferi* is likely to be dispersal of infected larval ticks that molt into infectious nymphs. A recent review indicates that at least 71 species of North American birds are known to be associated with *I. scapularis* and that the majority (56%) of species that have been evaluated are capable of infecting *I. scapularis* larvae with *B. burgdorferi* (Brinkerhoff et al. 2010). Several laboratory studies have also demonstrated that birds are capable of acquiring *B. burgdorferi* from infected nymphs and transmitting the infection to *I. scapularis* larvae, which then molt into infective nymphs (Anderson et al. 1990, Richter et al. 2000, Ginsberg et al. 2005). The proportion of *I. scapularis* larvae that acquire infection upon feeding on an infected avian host varies by species, but some bird species may be as competent as *P. leucopus* and capable of transmitting infection up to 60 d after inoculation (Richter et al. 2000).

Tick phenology, or the timing of seasonal activity of each developmental stage, is critical to the maintenance of many tick-borne microparasites (Randolph et al. 1999, 2000). Because the life span of *I. scapularis* is longer than that of *P. leucopus*, *B. burgdorferi* must persist throughout the majority of the year in *I. scapularis.* If larval ticks feed on hosts that have not been inoculated by infected nymphs, the transmission cycle will be interrupted, as there will be no infected nymphs to transmit pathogens to future generations of hosts in the following year. In the northeastern United States, *I. scapularis* nymphs emerge earlier in the year (May–July) than the majority of larvae (July–October), but in some locations (e.g., midwestern United States), there is a substantial number of spring larvae that emerge contemporaneously with nymphs (Gatewood et al. 2009). Recent analyses have indicated that such synchrony in host-seeking behavior of *I. scapularis* larvae and nymphs is predicted by high mean annual variation in temperature and is associated with increased relative abundance of certain *B. burgdorferi* genotypes (Gatewood et al. 2009). Because there is differential persistence of certain *B. burgdorferi* genotypes in some mammalian hosts (Hanincova et al. 2008), the duration between nymphal and larval feeding may affect genotype distributions in host-seeking nymphs (Gatewood et al. 2009).

The northward spring migration of birds (Winker et al. 1992, Hatch et al. 2010) coincides with high nymphal activity in the Northeast and high larval and nymphal activity in the Midwest (Gatewood et al. 2009). Birds in both regions may therefore become infected with *B. burgdorferi* on their way north, but birds in the Midwest are more likely to encounter host-seeking larvae during northward migration. Southward migration of birds in the fall coincides with the timing of peak larval tick activity in the Northeast and with a smaller secondary period of larval activity in the Upper Midwest (Gatewood et al. 2009). Only birds that are still infectious will pass infection onto these feeding larvae, and will likely proceed to move these infected ticks southward. The timing of both immature tick activity and bird migration could therefore be important contributors in determining the rate of pathogen spread and its resulting spatial distribution. To determine whether or not the regional variation in phenology that is detected in host-seeking ticks is also reflected in *I. scapularis* occurrence on birds, we sampled birds and collected their ticks at 23 sites throughout the eastern United States.

Materials and Methods

We collaborated with bird banders conducting ongoing studies throughout the distribution of *I. scapularis* in the eastern half of the United States to obtain opportunistic tick samples from birds. Birds were sampled in a variety of habitat types at 23 sites in 12 states (CT, MA, MD, MI, MN, NJ, NY, PA, RI, SD, VA, and WI) between 30 March 2008 and 9 November 2009. At all sites, birds were captured in mist nets (stationary, vertical, fine mesh nets designed to safely capture birds in flight) and released immediately after data and tick collection. Ticks were collected with fine forceps, stored in 70% ethanol, and identified with keys found in Clifford et al. (1961), Keirans and Litwak (1989), Durden and Keirans (1996), and Keirans and Durden (1998). Upon species identification, DNA was extracted from a subset of ticks (all *I. scapularis* larvae collected in 2008) using Qiagen DNeasy animal tissue extraction kit (Qiagen, Valencia, CA) and a modified extraction protocol in which Roche proteinase K (Roche Diagnostics, Indianapolis, IN) was substituted for the solution supplied in the kit. Tick samples were screened for the presence of *B. burgdorferi* DNA by targeting a portion of the 16S–23S rRNA intergenic spacer region for amplification by nested polymerase chain reaction (thermocycler conditions and primer sequences published in Bunikis et al. 2004). Target amplicons were visualized by gel electrophoresis and staining with ethidium bromide.

Because we were interested in variation in tick parasitism on birds corresponding to the two Lyme disease foci in eastern North America, we divided our sites into a northeastern group (sites east of 83° west longitude) and a midwestern group (sites west of 83° west longitude) for analysis (Fig. 1). The range of *I. scapularis* is discontinuous in the eastern United States, and the two population foci are separated by a gap of ~3° of longitude where these ticks are not found (Diuk-Wasser et al. 2010). We measured overall tick prevalence in each region and compared burdens of *I. scapularis* larvae, calculated as the total number of *I. scapularis* larvae collected divided by the number of birds that were parasitized by *I. scapularis* larvae, among regions by Mann-Whitney tests. We compared proportions of larvae that co-occurred with nymphs and proportions of birds that produced *B. burgdorferi*-infected larvae between regions by log-likelihood ratio tests (*G* tests). We characterized temporal variation in larval and nymphal *I. scapularis* parasitism on birds in both regions by calculating accumulation curves in which the cumulative proportion of larvae

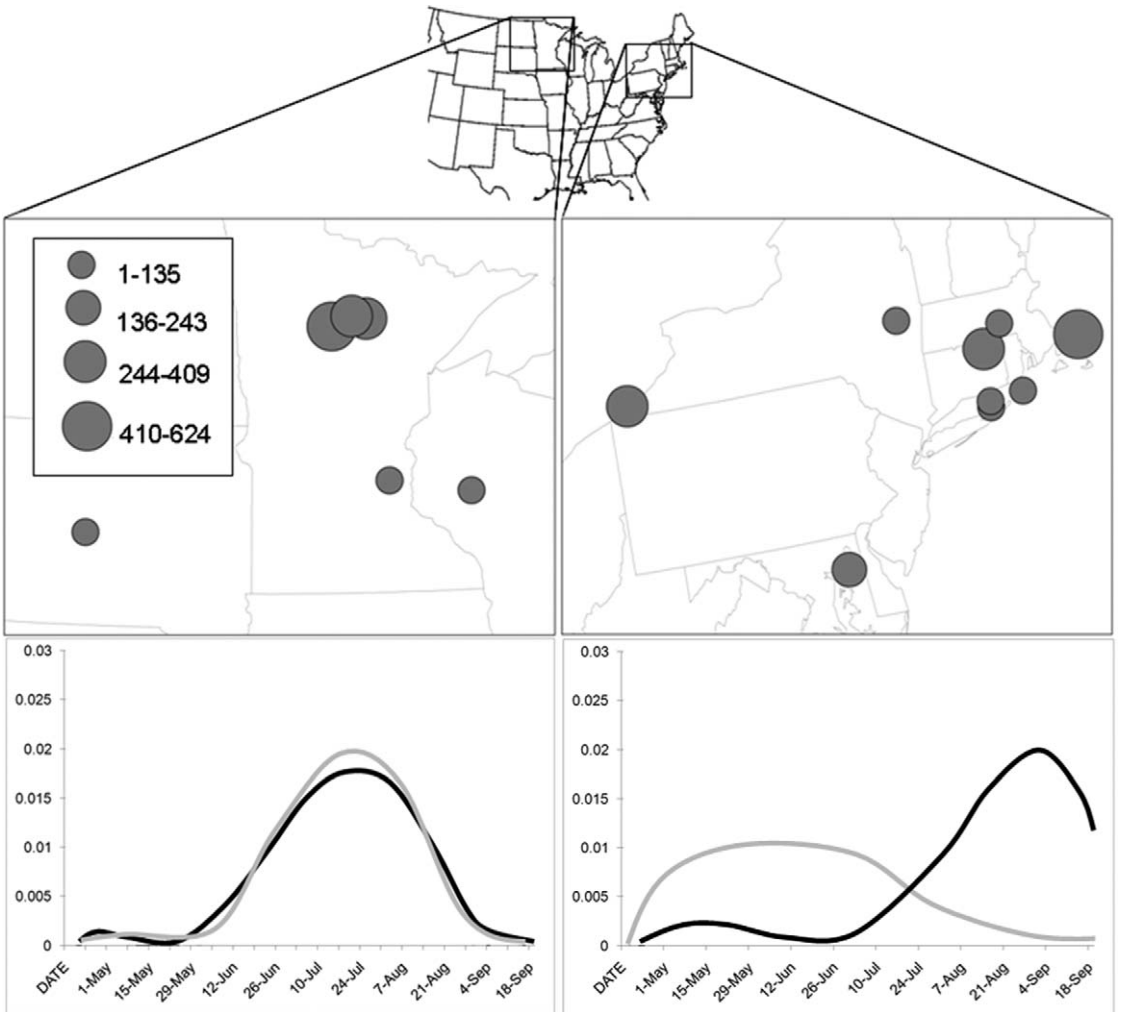

Fig. 1. Locations of sampling sites from which at least 10 bird-derived ticks were collected, with circle size proportional to the number of ticks collected from birds. Eight sites that produced fewer than 10 ticks are not shown. The number of birds from which these ticks were collected was 642 in the Midwest and 1,356 in the Northeast. Graphs are approximations of seasonal host-seeking activity of *I. scapularis* nymphs (gray lines) and larvae (black lines) in each region (Gatewood et al. 2009).

and nymphs was calculated up to a given ordinal date and then plotted over the course of the field season. From these data, we calculated the dates by which 50% of the larvae and nymphs had been collected in each region.

Results

We collected at least 10 ticks from birds at 15 of 23 sites (Fig. 1), sites where <10 ticks were collected were excluded from all analyses. A total of 6,918 ticks was collected and identified from 2,039 individuals, representing 79 bird species, during this study. Because of variation in sampling period, sampling effort, and habitat among sites, we did not make any attempts to characterize differences in bird assemblages or species-specific patterns of tick parasitism. We collected a total of 3,279 immature *I. scapularis* (1,698 nymphs and 1,581 larvae). In addition to *I. scapularis*, *Amblyomma americanum* (six nymphs), *Amblyomma maculatum* (six nymphs, 75 larvae), *Dermacentor variabilis* (one larva), *Hemaphysalis leporispalustris* (484 nymphs, 2,413 larvae), *Ixodes brunneus* (13 adults, 13 nymphs), *Ixodes dentatus* (10 nymphs, 604 larvae), and *Ixodes muris* (one adult, four nymphs) were recovered from birds at our sampling sites. The most commonly parasitized bird species for both regions are listed in Table 1. Most bird banders were not able to thoroughly check every bird for ticks, and many did not indicate which specific birds had and had not been checked, thus making thorough evaluations of tick prevalence impossible. However, ticks were detect on approximately equivalent proportions of birds among those that were reported as being carefully checked

Table 1. Bird species from which the most ticks of all species were recovered, with values pooled among sites and years

Bird species	*Amblyomma maculatum*	*Haemaphysalis leporispalustris*	*Ixodes brunneus*	*Ixodes dentatus*	*Ixodes scapularis*	Total ticks
Gray Catbird (327)	1	234		23	592	850
Veery (178)		389		2	422	813
White-throated Sparrow (142)		196			394	590
Ovenbird (198)		254	3	238	95	590
Hermit Thrush (79)		302	1	117	106	526
Common Yellowthroat (184)	12	140		2	297	451
Song Sparrow (101)	15	184	1	33	181	414
Eastern Towhee (47)		93	2	5	175	275
House Wren (46)	2	117		5	109	233
Carolina Wren (41)	10	116	1	5	64	196

for ticks (23.5% of 2,871 birds and 21.2% of 3,034 birds for the Northeast and Midwest, respectively), suggesting that there were not substantial regional biases in tick detection. Overall, 67.7% of birds (1,381 of 2,039) that were parasitized by ticks were carrying at least one immature *I. scapularis*, and *I. scapularis* were collected from every site that produced ticks. Ten bird species contributed 74% of the immature *I. scapularis* collected (2,433 of 3,279) with Gray Catbird (*Dumetella carolinensis*), Veery (*Catharus fuscescens*), and Ovenbird (*Seiurus aurocapillus*), most commonly parasitized by immature *I. scapularis* during this period. In addition to the species listed in Table 1, Mourning Warblers (63 nymphs, 87 larvae) and American Robins (71 nymphs, 57 larvae) contributed substantial numbers of immature *I. scapularis.*

For regional comparisons in immature *I. scapularis* parasitism on birds over time, we restricted our analysis to include the date range during which ticks (all species) were collected from birds in both the Northeast and Midwest; between 18 April and 20 September, 3,087 immature *I. scapularis* (1,539 larvae and 1,548 nymphs) were collected from 1,763 birds (Table 2). We collected a total of 1,861 immature *I. scapularis* (899 larvae and 962 nymphs) from sites in the northeastern United States and 1,326 immature *I. scapularis* (640 larvae and 686 nymphs) from sites in the midwestern United States. The time lag between date by which 50% of larvae and 50% of nymphs were collected was substantially shorter in the Midwest (1 d) than in the Northeast (56 d; Fig. 2).

More birds were sampled in the Northeast, and thus more immature *I. scapularis* were collected in this region (Table 2). Furthermore, there was greater early- and late-season sampling effort in the Northeast; we estimate that 96.6% of midwestern birds were banded between 16 May and 26 August. However, the number of *I. scapularis* larvae per bird parasitized by *I. scapularis* larvae (i.e., burden) was equivalent in the Northeast (2.3 larvae per bird) and Midwest (2.5 larvae per bird), and the median values were not significantly different ($W = 85{,}358.5$, $P = 0.96$). Mean nymphal burdens were identical (1.85 nymphs per bird) and not statistically significant between regions. Birds in the Midwest were more likely to be contemporaneously infested with larvae and nymphs; 341 of 640 (53.3%) larvae in the Midwest were collected from birds that yielded at least one nymph, whereas 218 of

Table 2. Regional and temporal variation in mean numbers of *I. scapularis* larvae and nymphs per tick-parasitized bird

Date range	Northeast			Midwest		
	Larvae	Nymphs	Birds	Larvae	Nymphs	Birds
1–15 April	0.00	0.48	25	–	–	0
16–30 April	0.02	0.57	49	0.00	0.00	2
1–15 May	0.09	0.96	98	0.00	0.00	1
16–31 May	0.25	1.51	148	0.69	1.38	13
1–15 June	0.06	1.63	33	0.58	1.13	40
16–30 June	0.40	3.30	55	0.44	1.04	25
1–15 July	0.34	2.44	74	1.64	1.43	124
16–31 July	0.50	1.38	24	1.41	1.32	164
1–15 Aug.	1.65	0.53	149	0.74	0.83	149
16–31 Aug.	1.09	0.16	137	0.43	0.67	120
1–15 Sept.	1.53	0.18	199	–	–	0
16–30 Sept.	0.59	0.20	155	0.00	0.00	4
1–15 Oct.	0.29	0.23	130	–	–	0
16–31 Oct.	0.06	0.11	62	–	–	0
1–15 Nov.	0.00	0.06	18	–	–	0
Total individuals	941	1,012	1,356	640	686	642

Numbers of birds from which ticks were collected per 2-wk period are also indicated. All birds represented in this table were parasitized by ticks, but were not necessarily parasitized by *I. scapularis.* The absence of late-season tick detection in the Midwest results from a sharp decline in sampling effort at midwestern sites after 26 August.

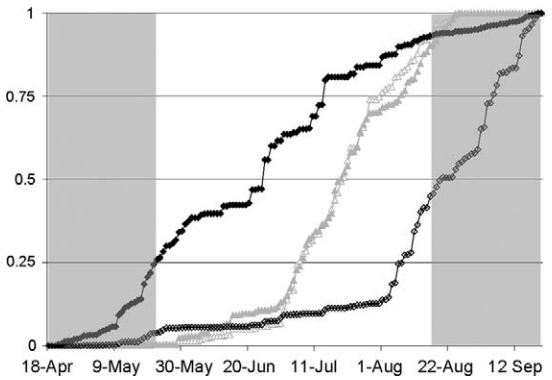

Fig. 2. Accumulation over time of *I. scapularis* larvae (open shapes) and nymphs (filled shapes) collected from birds at northeastern (black lines) and midwestern (gray lines) sampling sites. Shaded regions represent approximate songbird migration periods.

941 (23.2%) larvae co-occurred with nymphs in the Northeast ($G = 149.6$, $P < 0.001$). Because we lack capture data on unparasitized birds from many sites, we are not able to make comparisons in *I. scapularis* prevalence among sites or regions. Infection prevalence in birds was calculated as the number of birds that produced *B. burgdorferi*-infected larvae divided by the number of birds that were parasitized by *I. scapularis* larvae, and was significantly greater in the Midwest (22 of 58 birds [37.9%]) than in the Northeast (19 of 153 birds [12.4%]; $G = 14.6$, $P = 0.005$).

Discussion

Many North American songbird species serve as hosts for immature *I. scapularis* ticks, and migratory behavior of birds has been identified as a possible mechanism of range expansion of *B. burgdorferi* and its vector (Morshed et al. 2005, Ogden et al. 2008, Brinkerhoff et al. 2009). Our results indicate that there is spatial variation in patterns of *I. scapularis* parasitism on songbirds, and that these patterns are generally consistent with phenological differences observed in *I. scapularis* host-seeking activity from the northeastern and upper midwestern United States (Fig. 1; Gatewood et al. 2009). We found greater temporal overlap in parasitism by nymphal and larval *I. scapularis* in the Midwest than in the Northeast (Fig. 2), and larvae removed from birds in the Midwest were more likely to be infected with *B. burgdorferi* than larvae in the Northeast. Regional differences in host-seeking activity of immature *I. scapularis* and patterns of *B. burgdorferi* infection in birds suggest that the role of birds in local transmission dynamics may vary spatially. Additionally, the temporal variation in host seeking of immature ticks and tick parasitism on birds could influence the dispersal of this vector species, and these effects may differ regionally.

The long-distance migration of many songbird species is an attractive candidate mechanism to explain the observed range expansion of *I. scapularis* and increasing incidence of Lyme disease. Northward migration of songbirds in North America typically occurs during April and May, and southward migration occurs from late August through September (Winker et al. 1992, Swanson and Palmer 2009, Hatch et al. 2010). Spring migration precedes the periods of highest observed larval and nymphal *I. scapularis* parasitism in both regions, although southward migration occurs during peak larval parasitism in the Northeast (Table 2). Ogden et al. (2008) demonstrated that northward-migrating birds sampled in eastern Canada are occasionally parasitized by *I. scapularis* nymphs, but rarely by larvae, a finding that is consistent with the patterns of *I. scapularis* parasitism we see on birds, particularly in the Northeast. We do not have sufficient early season data to determine whether there are regional differences in *I. scapul*aris parasitism on northward-migrating birds, but because a substantial proportion (>50%) of *I. scapularis* larvae in the Northeast occurs on birds in August and September (Fig. 2), it is reasonable to conclude that birds might play a role in southward dispersal from this region.

Nymphal burdens greater than one nymph per parasitized bird occurred in both regions in May and June and declined in the late summer. Southward dispersal of nymphs during fall migration is therefore relatively unlikely in either region (Table 2). In our data set, there was an early season sampling bias toward the Northeast (Table 2), and as a result, fewer birds were sampled, and therefore, fewer ticks were collected in the Midwest in April and May. The result of this bias is an artificial rightward shift of the nymphal, and potentially larval, accumulation curves for the Midwest. Similarly, because sampling effort in the Midwest declined earlier (few birds were sampled after 26 August) than in the Northeast, the accumulation of larvae in the Midwest may be less abrupt than is indicated in Fig. 2. It is regrettable that we were unable to control for sample size and sampling effort over time and among regions, and the accumulation curves in Fig. 2 should be interpreted cautiously. However, the regional differences in larval and nymphal burdens, which are largely independent of sampling effort, suggest that the observed temporal patterns in parasitism are robust even with unequal sampling effort. Gatewood et al. (2009) report a decline in host-seeking activity of *I. scapularis* nymphs and larvae after July in the Midwest, which is consistent with the observed pattern of parasitism on birds and suggests that the lack of late-season sampling in this region should not dramatically affect our conclusions.

In our data set, *I. scapularis* larvae collected from midwestern birds were more likely to be infected than larvae collected from northeastern birds. This phenomenon likely results from the shorter average duration between nymphal and larval parasitism in this region. A laboratory study of *B. burgdorferi* infection in American Robins indicated that spirochete acquisition by *I. scapularis* larvae diminishes after 2 mo and that spirochetes are no longer transmissible to larvae after 6 mo (Richter et al. 2000). Northeastern birds may be more likely to clear *B. burgdorferi* infection

before parasitism by larvae, thus reducing the number of infected larvae generated by birds in this region. Because nymphal activity tends to occur after spring migration, it is unlikely that northward-migrating birds will be infected with *B. burgdorferi.* Cofeeding transmission, whereby a pathogen is transmitted from one vector to another without causing systemic infection in the host, might also account for the observed regional differences in infection prevalence in bird-derived larvae. Cofeeding transmission has been demonstrated in several tick-borne pathogens (Randolph et al. 1999, 2000), including *B. burgdorferi* (Gern and Rais 1996), but is not thought to be an important mechanism of infection for ticks feeding on white-footed mice (Piesman and Happ 2001). The greater contribution of bird-infected larvae may result in a greater contribution by birds to enzootic *B. burgdorferi* transmission and potentially more within-region dispersal in the Midwest than in the Northeast. With relatively few samples, we are unable to perform rigorous regional comparisons of the timing of occurrence of bird-infected larvae, and thus do not draw any conclusions regarding the dispersal of these ticks.

In mammals, different *B. burgdorferi* strains have different infection kinetics, with some persisting longer in hosts than others (Hanincova et al. 2008). Therefore, the duration between inoculation and parasitism by *I. scapularis* larvae could influence the likelihood of spirochete acquisition by larvae. Such comparative studies have not been performed with North American bird species, but if similar phenomena occur, the role of birds in selectively transmitting different strains or genotypes could vary regionally. Because of the substantially greater lag between the median dates of nymphal and larval parasitism in the Northeast than in the Midwest, short-duration infections in birds would have a lower likelihood of transmission to larvae at northeastern sites. A similar hypothesis was proposed by Gatewood et al. (2009) to account for differences in *B. burgdorferi* genotype frequencies in host-seeking nymphs between northeastern and midwestern sites. If transmissibility of some or all *B. burgdorferi* strains/genotypes from birds to *I. scapularis* larvae is dependent on the time since inoculation, a shorter lag between nymphal and larval feeding could result in higher probability of bird (and mammal)-mediated transmission of *B. burgdorferi.*

Our results indicate that regional variation in the phenology of host-seeking immature *I. scapularis* ticks is reflected in patterns of *I. scapularis* parasitism on North American songbirds. The consequences of this variation could be important to the range expansion of this vector and to the epidemiology of Lyme disease. Because birds in the Midwest are more likely than birds in the Northeast to be parasitized by nymphs and larvae at the same time, short-lived *B. burgdorferi* infections in midwestern birds have a greater chance of transmission to larvae. As a result, there may be regional variation in the role of birds in local *B. burgdorferi* transmission dynamics, with birds infecting relatively more *I. scapularis* larvae in the Midwest than in the Northeast. Furthermore, birds in the Midwest may be capable of infecting larvae much earlier in the year than northeastern birds, potentially yielding a relatively higher avian contribution to enzootic *B. burgdorferi* transmission. Significant differences in *B. burgdorferi* genotype frequencies have been observed in host seeking between these two regions (Gatewood et al. 2009, Barbour and Travinsky 2010); because birds are capable of transmitting most *B. burgdorferi* genotypes, including some that are rarely observed in mammals (Brinkerhoff et al. 2010), it is possible that a higher number of bird-infected ticks in the Midwest could account for some of this variation.

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