Relative Abundance and Prevalence of Selected *Borrelia* Infections in *Ixodes scapularis* and *Amblyomma americanum* (Acari: Ixodidae) from Publicly Owned Lands in Monmouth County, New Jersey

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ABSTRACT To evaluate their potential importance in the transmission of ixodid tick-borne borrelioses in Monmouth County, NJ, we collected host-seeking *Ixodes scapularis* Say and *Amblyomma americanum* (L.) (Acari: Ixodidae) adults and nymphs to determine relative encounter frequencies and the infection prevalence of selected *Borrelia* spp. in their respective tick vectors. We also reviewed records of all ticks submitted for identification by the public in Monmouth County during 2001–2005. Relative abundance of the two species varied markedly among sites. Adult encounter frequencies for the two species were similar; however, *A. americanum* nymphs were encountered 3 times more frequently than *I. scapularis* nymphs. Of 435 ticks submitted by the public, 50.1 and 38.9% were *I. scapularis* and *A. americanum*, respectively. However, during May through August, the peak Lyme disease transmission season in New Jersey, significantly more submitted ticks were *A. americanum* (55.9%), compared with *I. scapularis* (34.1%). Polymerase chain reaction analysis of 94 *I. scapularis* and 103 *A. americanum* adults yielded infection prevalences of 31.9% for *B. burgdorferi* and 5.8% for *B. lonestari*, respectively. Although the infection prevalence of *B. burgdorferi* in *I. scapularis* was considerably higher than the infection prevalence of *B. lonestari* in *A. americanum*, the higher encounter frequencies for *A. americanum* compared with *I. scapularis* observed in this and other studies may result in increased risk of acquiring exposure to *A. americanum*-transmitted pathogens. The potential public health implications of these results are discussed.

KEY WORDS *I. scapularis*, *A. americanum*, encounter frequencies, infection prevalence, *Borrelia*

Lyme disease is the most common tick-borne disease in the United States, with nearly 154,000 confirmed cases reported in the past 10 yr and 23,763 cases diagnosed nationwide in 2002 alone (CDC 2004). The blacklegged tick, *Ixodes scapularis* Say, is the principal vector of *Borrelia burgdorferi* in the U.S. Northeast (Lane 1994). However, evidence is emerging that suggests that the lone star tick, *Amblyomma americanum* (L.), which is sympatric with the blacklegged tick in southern New Jersey (Schulze et al. 1984b), may play a role in transmission of another spirochete that causes a Lyme-like disease in several southern states (Childs and Paddock 2003). As early as 1982, *A. americanum* was implicated in the transmission of what seemed to be classic Lyme disease in New Jersey. In this case, an *A. americanum* female was removed from the site of an erythema migrans-like lesion, and spirochetes were identified in *A. americanum* adults and nymphs collected from the patient's property (Schulze et al. 1984a). Twelve years later, Barbour et al. (1996) reported an uncultivable *Borrelia* spirochete in *A. americanum* collected from several states. This new spirochete, provisionally named *B. lonestari*, is the possible etiological agent of this Lyme-like illness (Armstrong et al. 2001, Burkot et al. 2001, James et al. 2001), currently referred to as southern tick-associated rash illness (STARI) (Stegall-Faulk et al. 2003). Subsequently, *B. lonestari* DNA has been identified in *A. americanum* removed from humans in nine states, including New Jersey (Stromdahl et al. 2003), and the spirochete has recently been isolated in culture (Varela et al. 2004). It seems reasonable to hypothesize that some erythema migrans-diagnosed Lyme disease cases may indeed be STARI.

Circumstantial evidence in support of this hypothesis has been reported from the southeastern United States where *A. americanum* is widely distributed (Childs and Paddock 2003). In several southeastern states, the tick most often removed from the site of erythema migrans-like lesions, a key diagnostic indicator of Lyme disease, was *A. americanum*, rather than

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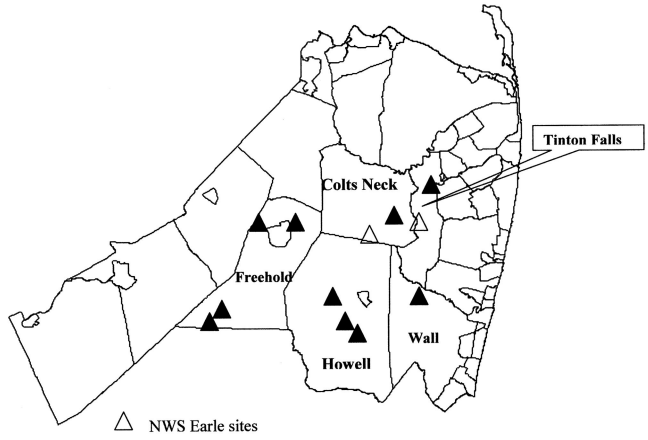

Fig. 1. Location of survey sites within Monmouth County, NJ.

I. scapularis (Masters et al. 1992, 1998). Furthermore, the spirochete prevalence in *I. scapularis* from the southeastern United States is typically low, apparently because the tick feeds primarily on reservoir-incompetent lizards (James and Oliver 1990, Clark et al. 2005), and although *I. scapularis* has been reported from many southeastern states (Dennis et al. 1998), encounters with humans are infrequent (Sonenshine 1993, Felz et al. 1996, Diuk-Wasser et al. 2006).

Although common on some barrier and offshore islands from Massachusetts to New York (Means and White 1997), New Jersey marks the northern extent of significant inland populations of *A. americanum* (Schulze and Bosler 1996). In contrast to the southeastern United States, subadult *I. scapularis* often feed on reservoir-competent small mammals (Schulze et al. 1986a), and as a result, New Jersey is one of the few states with sympatric populations of *A. americanum*- and *B. burgdorferi*-infected *I. scapularis* (Schulze et al. 1984b). We have previously reported on the relative encounter frequencies of these two tick species at a known hyperendemic area for Lyme disease and the infection prevalence of selected *Borrelia*, *Ehrlichia*, and *Anaplasma* in their respective tick vectors (Schulze et al. 2005). In that study, infection prevalence of *B. burgdorferi* in *I. scapularis* was considerably higher than the *B. lonestari* infection prevalence in *A. americanum*. However, it seemed that, at least at our study area, higher encounter frequencies observed for *A. americanum* compared with *I. scapularis*, might result in increased risk of acquiring exposure to *A. americanum*-transmitted pathogens. In the present broader survey of public lands at the northern limit of *A. americanum* distribution in New Jersey, we report the frequency at which sympatric *I. scapularis* and *A. americanum* were encountered and the infection prevalence of selected *Borrelia* in their respective tick vectors and suggest that abundant and aggressive *A. americanum* may pose a significant but under-recognized public health risk for tick-borne illness.

Materials and Methods

Study Area. The study was conducted in Monmouth County, NJ, an area known to be hyperendemic for Lyme disease (Bowen et al. 1984). Surveys were conducted at 12 sites, including two sites within Naval Weapons Station Earle (NWS Earle), a secured military facility where both *I. scapularis* and *A. americanum* are consistently abundant (Schulze et al. 1986a, 1997; Schulze and Jordan 2005), and individual sites in 10 public parks surrounding NWS Earle (Fig. 1). All sites were located in forested areas suitable for both tick species (Schulze and Jordan 1996). Forest canopies were predominantly oak (*Quercus* spp.)-dominated mixed hardwoods or mixed hardwoods and pitch pine, *Pinus rigida* Mill., with understories and shrub layers composed of saplings and seedlings of the dominant tree species; highbush blueberry, *Vaccinium corymbosum* L.; lowbush blueberry, *Vaccinium angustifolium* Ait.; huckleberries (*Gaylussacia* spp.); sweet pepperbush, *Clethra alnifolia* L.; spicebush, *Lindera benzoin* (L.) Blume; laurels (*Kalmia* spp.); and greenbriar, *Smilax rotundifolia* L.

Tick Collections. We established six 100-m transects at each survey site in habitats expected to yield ticks (Schulze and Jordan 1996). We collected adult ticks along three of these 100-m transects placed in areas with moderately dense, more-or-less uniform shrub layers where both *I. scapularis* and *A. americanum* adults tend to quest. The three remaining 100-m transects were established in nearby areas with patchy to sparse shrub layers, which permitted more frequent contact between drags and litter layer, to facilitate the collection of nymphs of both species (Schulze et al. 1997). We used transects rather than plots to cover a larger area during any sampling event in an attempt to minimize the effect of tick aggregation.

Sampling was performed during the respective spring activity periods of adult and nymphal *I. scapularis* and *A. americanum* (Schulze et al. 1986a). In New Jersey, *I. scapularis* adults are active between October and April, but they become quiescent during winter

when temperatures are consistently below freezing, resulting in an apparently bimodal activity pattern. Although *I. scapularis* adults are more numerous in the fall, *A. americanum* adults have no fall activity period, restricting simultaneous sampling of the two species to the spring. Nymphs of both species share similar May–July activity periods. Adults were surveyed twice between 9 and 21 April 2004, whereas nymphs were sampled twice between 2 and 9 June 2004.

I. scapularis and *A. americanum* exhibit markedly different host acquisition and questing behaviors (Schulze et al. 1997). The more aggressive *A. americanum* acquires hosts through both hunting and ambush (Stromdahl et al. 2003), and all active stages may be collected from both the forest litter as well as from shrub layer vegetation. In contrast, because *I. scapularis* acquires hosts primarily through ambush, adults are most frequently collected while questing in shrub layer vegetation. However, unlike subadult *A. americanum*, *I. scapularis* nymphs and larvae quest at ground level within the litter. We attempted to avoid possible sampling bias posed by the differences in host acquisition behaviors of the two species through the use of a combination of dragging and walking surveys (Ginsberg and Ewing 1989, Solberg et al. 1992, Schulze et al. 1997) conducted simultaneously between 0900 and 1300 hours when vegetation was dry and wind speed was <10 km/h. Ticks adhering to drags and investigators' coveralls were removed at 20-m intervals. Adults of both species retained for infection prevalence analysis were held at 8°C and 90% RH and subsequently stored in 70% ethanol and held at −80°C until analyzed. We analyzed adult ticks because of we anticipated higher infection prevalence in this stage, resulting from an earlier second feeding as nymphs, and to allow direct comparisons to earlier New Jersey studies (Schulze et al. 2003, 2005).

Tick Submissions. Both the Monmouth County Mosquito Extermination Commission (MCMEC) and the Monmouth County Health Department (MCHD) offer tick identification services to the public. As another means of comparing relative encounter frequencies for the two tick species, each agency was asked to provide the collection location, species, and stage of all ticks submitted for the period 2001–2005. In 2004, the Freehold Area Health Department (FAHD) contacted all general practitioners, pediatricians, and internists within the municipalities surrounding NWS Earle and offered tick identification services. Physicians wishing to participate were provided tick submission kits containing a self-addressed and stamped padded mailer.

DNA Extraction. DNA was isolated from *I. scapularis* and *A. americanum* by using a standardized extraction protocol (Schulze et al. 2003). Briefly, adult ticks were crushed with disposable pestles in the presence of 120 μl of DNAzol Reagent (Invitrogen, Carlsbad, CA). The resulting lysates were heated at 95°C for 10 min. Insoluble tissue fragments were pelleted by centrifugation at 10,000 × *g* at room temperature. DNA was precipitated by the addition of 50 μl of 100% ethanol to the supernatant. After mixing and a 3-min incubation at room temperature, the DNA precipitate was pelleted by centrifugation at 16,000 × *g*. The pellet was washed twice with 75% ethanol and resuspended in 35 μl of water. Tick lysate DNA was stored at 4°C until PCR analysis.

PCR Analyses: *I. scapularis*. The PCR assay for *B. burgdorferi* has been described previously (Schulze et al. 2003). Briefly, primers (FLA1, 5′CACATATTCAGATGCAGACAGAGGTTCTA3′; FLA2, 5′GAAGGTGCTGTAGCAGGTGCTGGCTGT3′) defining a 390-bp inner region of the bacterial flagellin (fla) gene, were purchased from Invitrogen. Recombinant *Taq* polymerase, 10× polymerase chain reaction (PCR) buffer, and dNTPS were supplied by TaKaRa Biomedicals (Shiga, Japan). PCR reactions of 50 μl contained 0.5 μM each primer, 200 μM each dNTP, 10.0 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.25 U of TaKaRa *Taq*, and 10 μl of tick lysate DNA. Reactions were kept on ice until transferred to an Eppendorf Master Gradient thermal cycler (Brinkmann Instruments, Westbury, NY). The thermal cycler program consisted of an initial 1-min 94°C denaturation step followed by 30 cycles of 30-s denaturation at 94°C, 30 s of annealing at 55°C, and 30 s of extension at 72°C. The positive control reactions contained 2 ng of *B. burgdorferi* genomic DNA (ATCC 35210D, American Type Culture Collection, Manassas, VA).

PCR Analyses: *A. americanum*. The PCR assay used was a modification of the method of Stegall-Faulk et al. (2003). Primers (BBBLS1, 5′CAAAAATTAATACACCAGCA3′ and BBBLS2, 5′GCAATCATAGCCATTGCAGA3′), defining a 476-bp region of the *B. lonestari* fla gene and 499-bp region of the *B. burgdorferi* fla, gene were purchased from Invitrogen. The PCR reaction and thermal cycler program as described above for *I. scapularis*. The positive control reactions contained 2 ng of *B. burgdorferi* genomic DNA and 2 ng of *B. lonestari* genomic DNA (Susan Little, Department of Veterinary Pathobiology, Oklahoma State University, Stillwater, OK).

Gel Electrophoresis. Samples were stored at 4°C until gel electrophoresis could be accomplished. Ten microliters of each PCR amplification was analyzed by gel electrophoresis in 2.5% Tris-acetate EDTA (TAE) agarose gels. Agarose and 10× TAE running buffer were purchased from Fisher (Suwanee, GA); 100-bp DNA ladders (Promega, Madison, WI) were included in each gel for reference. Gels were stained with ethidium bromide and photographed. An *I. scapularis* sample was considered positive if the predicted 390-bp DNA fragment was present in the gel. An *A. americanum* sample was considered positive if the predicted 476-bp (*B. lonestari*) and/or 499-bp (*B. burgdorferi*) DNA fragment was clearly present in the gel.

DNA Purification and Sequencing. The amplicons of interest were electrophoresed through 1.25% agarose gels that were cast and run in 1× modified TAE buffer (Millipore Corporation, Billerica, MA). The amplicons were visualized on a UV light table and excised using a razor blade. The DNA fragments were separated from the gel and purified using a Montage

Table 1. Summary of adult *I. scapularis* and *A. americanum* collections from selected publicly owned lands in Monmouth County, NJ

Location	*I. scapularis*			*A. americanum*		
	Σ	Mean	Range	Σ	Mean	Range
NWS Earle	64	10.7 ± 1.3	6–14	42	7.3 ± 2.2	3–17
Wayside Training Area	54	9.0 ± 1.9	5–16	25	4.2 ± 1.0	1–8
Turkey Swamp Park (county)	7	1.2 ± 0.5	0–3	0	0	0
Turkey Swamp Park (state)	3	0.5 ± 0.3	0–2	28	4.7 ± 0.7	3–7
Dorbrook Park	10	1.7 ± 0.7	0–4	0	0	0
East Freehold Park	5	0.8 ± 0.5	0–3	0	0	0
Monmouth Battlefield State Park	11	1.8 ± 0.7	0–4	1	0.2 ± 0.2	0–1
Manasquan Reservoir	66	11.0 ± 2.1	5–17	6	1.0 ± 0.4	0–2
Oak Glen Park	8	1.3 ± 0.3	0–2	42	7.0 ± 2.0	0–12
Allaire State Park	2	0.3 ± 0.2	0–1	78	13.0 ± 2.5	6–21
Shark River Park	38	6.3 ± 1.3	2–11	30	5.0 ± 1.4	1–11
Obre Road Park	10	1.7 ± 0.6	0–4	9	1.5 ± 0.9	0–6
Total	276	3.8 ± 0.6	0–17	261	3.6 ± 0.6	0–21

DNA gel extraction kit (Millipore Corporation) following the manufacturer's protocol.

The purified product was sequenced on an ABI PRISM 3100 Automated DNA Sequencer (Applied Biosystems, Foster City, CA) at the sequencing facility located in the Biotechnology Center for Agriculture and the Environment at Rutgers University (New Brunswick, NJ). Sequences were read using Chromas version 2.23 (Technelysium Pty. Ltd., Helensvale, Australia) and BioEdit version 7.04.1 (Ibis Therapeutics, Carlsbad, CA), and BLASTn version 2.2.10 searches were run to identify these sequences by comparison with sequences in the GenBank database. All *B. lonestari* amplicons from *A. americanum* and a subsample of *B. burgdorferi* amplicons from *I. scapularis* were sequenced using both the appropriate forward and reverse primer to establish and confirm identity.

Results

Tick Collections: Adults. We collected *I. scapularis* from all 12 survey sites (Table 1). Surveys yielded 276 adults, with a mean ± SE of 23.0 ± 7.1 ticks per site (range, 2–66 ticks per site). In total, 261 *A. americanum* adults were collected from nine survey sites (21.8 ± 6.9 ticks per site; range, 0–78 ticks per site). Abundance of the two species did not differ significantly at the 12 sites ($t = 0.13$, df = 11, $P = 0.90$). Overall, *I. scapularis* and *A. americanum* adults represented 51.4 and 48.6% of the adults collected, respectively.

Tick Collections: Nymphs. Nymphs of both species were present at all survey sites (Table 2). We collected 323 *I. scapularis* nymphs (mean = 26.8 ± 7.5 nymphs per site, range, 3–91 nymphs per site) and 1,064 *A. americanum* nymphs (mean = 88.7 ± 30.8 nymphs per site, range, 1–353 nymphs per site). Nymphal *A. americanum* were significantly more abundant at the 12 sites than nymphal *I. scapularis* ($t = 2.38$, df = 11, $P = 0.03$). *A. americanum* represented 76.8% and *I. scapularis* 23.2% of the total collection.

Tick Submissions. In total, 435 ticks were submitted for identification by the public and health care providers, comprising 218 (50.1%) and 169 (38.9%) *I. scapularis* and *A. americanum*, respectively (Table 3). *Dermacentor variabilis* (Say) (10.8%) and *Rhipicephalus sanguineus* (Latrielle) (0.2%) accounted for the remainder of submissions. For 262 submitted ticks, identification reports included date of submission, allowing us to examine temporal distribution of tick encounters. In total, 145 (55.3%) ticks were submitted between May and August, the peak Lyme disease transmission season in New Jersey (Goldoft et al. 1990). During this 4-mo period, submissions included 35 (34.1%) *I. scapularis*, 81 (55.9%) *A. americanum*,

Table 2. Summary of nymphal *I. scapularis* and *A. americanum* collections from selected publicly owned lands in Monmouth County, NJ

Location	*I. scapularis*			*A. americanum*		
	Σ	Mean	Range	Σ	Mean	Range
NWS Earle	50	8.3 ± 2.9	0–21	180	30.0 ± 14.4	3–53
Wayside Training Area	91	15.1 ± 3.0	3–23	353	58.8 ± 16.3	12–96
Turkey Swamp Park (county)	11	1.8 ± 0.7	0–5	40	6.7 ± 2.1	2–15
Turkey Swamp Park (state)	24	4.0 ± 0.4	3–5	163	27.2 ± 11.2	4–75
Dorbrook Park	8	1.3 ± 0.2	1–2	7	1.2 ± 0.5	0–3
East Freehold Park	3	0.5 ± 0.3	0–2	1	0.2 ± 0.2	0–1
Monmouth Battlefield State Park	17	2.8 ± 0.8	1–6	4	0.7 ± 0.3	0–2
Manasquan Reservoir	12	2.0 ± 0.7	0–5	29	4.8 ± 0.7	2–6
Oak Glen Park	16	2.7 ± 0.8	0–5	42	7.0 ± 0.9	4–11
Allaire State Park	3	0.5 ± 0.5	0–3	169	28.2 ± 8.8	5–58
Shark River Park	40	6.7 ± 2.1	1–15	48	8.0 ± 3.3	0–21
Obre Road Park	48	8.0 ± 1.9	3–14	28	4.7 ± 0.5	3–6
Total	323	4.5 ± 0.6	0–23	1064	14.8 ± 2.8	0–96

Table 3. Summary of Monmouth County ticks submitted to the MCHD, MCMEC, and FAHD for identification

Source	Date	No. ticks	Tick species (% total)			
			I. scapularis	*A. americanum*	*D. variabilis*	*R. sanguineus*
MCHD	2001–2003	173	102 (59.0)	61 (35.2)	10 (5.8)	
MCMEC	2001–2005	210	104 (49.5)	75 (35.7)	30 (14.3)	1 (0.5)
FAHD	2004–2005	52	12 (23.1)	33 (63.5)	7 (13.4)	
Σ	2001–2005	435	218 (50.1)	169 (38.9)	47 (10.8)	1 (0.2)

and 29 (20.0%) *D. variabilis.* Significantly greater numbers of *A. americanum* than *I. scapularis* were removed from people and submitted for identification ($\chi^2 = 8.01$, df = 1, $P < 0.01$) during the peak of the Lyme disease transmission season.

Infection Prevalence: *I. scapularis.* PCR analysis of 94 *I. scapularis* adults yielded 30 ticks positive for *B. burgdorferi* (31.9%). Infected ticks were found in 10 of 12 survey sites. The nucleotide sequences from a subset of *B. burgdorferi*-positive ticks were 98–99% homologous to the published GenBank sequence for *B. burgdorferi* (GenBank accession no. AE001126) (Fraiser et al. 1997).

Infection Prevalence: *A. americanum.* Six (5.8%) of the 103 *A. americanum* adults subjected to PCR analysis were positive for *B. lonestari.* Six of the nine survey sites yielded *B. lonestari*-positive *A. americanum* adults. The nucleotide sequences from the six *B. lonestari*-positive ticks were 99% homologous to published GenBank sequences for *B. lonestari* as follows: GenBank accession nos. AY442142 (Varela et al. 2004), U26075 (Barbour et al. 1996), AF298653 (Burkot et al. 2001), AY166716 (Bacon et al. 2003), and AY237710 (Stromdahl et al. 2003). None of the PCR-analyzed *A. americanum* adults were positive for *B. burgdorferi.*

Discussion

Overall, host-seeking *I. scapularis* and *A. americanum* adults showed considerable variability in abundance among sites, but they were encountered at similar frequencies. The relative encounter frequencies observed in this study do not reflect the differences in tick encounters described previously at NWS Earle, where *A. americanum* adults were generally 3 times more abundant than *I. scapularis* (Schulze et al. 1997, 2001, 2002), but they are consistent with those found in a more recent study (Schulze et al. 2005). Both *I. scapularis* and *A. americanum* nymphs were found at all survey sites and also exhibited significant variability in abundance among sites. However, *A. americanum* nymphs were ≈3 times more likely to be encountered than *I. scapularis* nymphs, a frequency similar to that previously described for NWS Earle (Schulze et al. 2005). It remains unclear whether this disparity in encounter frequency reflects greater numbers of *A. americanum* compared with *I. scapularis* or the more aggressive nature of *A. americanum.* Regardless, it seems that when both species are active, humans may be more likely to encounter *A. americanum.*

Our data suggest that both *I. scapularis* and *A. americanum* are well established at the publicly owned lands we surveyed, although the relative numbers of the two tick species varied markedly among sites. We have previously shown the relative abundance of the two species to vary in different forest types (Schulze and Jordan 1996, Schulze et al. 2002). The differences in abundance observed here may reflect differences in habitat suitability among or within the survey sites and argue for more extensive surveys when assessing relative risk for tick-borne diseases (Schulze et al. 2002; Schulze and Jordan 2005). Although the limited surveys performed in this study are useful in demonstrating spatial differences in tick encounter rates and infection prevalence, the data should not be considered indicative of relative risk at any particular site.

Analysis of *I. scapularis* adults yielded an overall *B. burgdorferi* infection prevalence of 31.9%, which was somewhat lower that the 49.3–50.3% infection prevalence recently reported from surveys of *I. scapularis* adults across New Jersey and at NWS Earle, respectively (Schulze et al. 2003, 2005). The *B. lonestari* infection prevalence in *A. americanum* adults collected in this study (5.8%) was similar to those (5.4–9.1%) previously reported from NWS Earle (Schulze et al. 1986b, 2005) and recently reported in Missouri (Bacon et al. 2003). Nevertheless, as observed in previous comparative studies, the infection prevalence of *B. burgdorferi* in *I. scapularis* adults was approximately five-fold higher than the *B. lonestari* infection prevalence in *A. americanum* (Schulze et al. 1986b, 2005). However, the lower *B. lonestari* infection prevalence may be offset by the higher encounter frequencies observed for *A. americanum* relative to *I. scapularis* observed here and historically from the same location (Schulze et al. 1997, 2002, 2005).

Indeed, public submissions of ticks made for identification and the field collection of ticks from 12 sites indicated that when both species are active, humans may be encountering at least as many *A. americanum* as *I. scapularis*, particularly during the peak Lyme disease transmission season. Because 73% of Lyme disease cases in New Jersey have dates of onset in May through August, peaking in June, disease acquisition has been epidemiological linked to the seasonal activity of *I. scapularis* nymphs (Goldoft et al. 1990). However, this peak in transmission also occurs during the primary activity periods of both *A. americanum* adults and nymphs (Schulze et al. 1986a). Thus, it is reasonable to speculate that where *I. scapularis* and *A. americanum* are sympatric, some proportion of reported erythema migrans-diagnosed Lyme disease cases might be the result of *B. lonestari* infections. If true, we feel that greater emphasis in public health

awareness efforts should be placed on the potential role of *A. americanum* in human tick-borne illness as well as in and the development of effective methods to control this tick species.

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