Relative Encounter Frequencies and Prevalence of Selected Borrelia, Ehrlichia, and Anaplasma Infections in Amblyomma americanum and Ixodes scapularis (Acari: Ixodidae) Ticks from Central New Jersey

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ABSTRACT  To evaluate their relative importance in tick-borne disease transmission in New Jersey, host-seeking Amblyomma americanum (L.) and Ixodes scapularis Say adults and nymphs were collected during spring activity periods in 2003 and 2004 to determine relative frequencies at which these ticks were encountered from an area known to be hyperendemic for Lyme disease. Although similar numbers of the two species were encountered during early spring of both years, A. americanum were encountered more often later in the season and exhibited a longer activity period than I. scapularis. A. americanum nymphs were collected at frequencies between 2.6 and 7.3 times higher than I. scapularis nymphs. Polymerase chain reaction (PCR) analysis of 121 A. americanum adults yielded infection prevalences of 9.1% for Borrelia lonestari, 12.3% for Ehrlichia chaffeensis, and 8.2% for E. ewingii, and coinfection prevalences of 4.1% for E. chaffeensis/E. ewingii and 0.8% for E. chaffeensis/B. lonestari. Infection prevalences in 147 I. scapularis adults were 50.3% for B. burgdorferi, 61.1% for Anaplasma (Ehrlichia) phagocytophilum, and 1.4% for a recently described novel Borrelia species, whereas the coinfection prevalences were 2.7% for B. burgdorferi/A. phagocytophilum, 0.7% for B. burgdorferi/novel Borrelia, and 0.7% for A. phagocytophilum/novel Borrelia. The B. burgdorferi infection prevalence in I. scapularis was considerably higher than that in A. americanum. However, the higher A. americanum encounter frequencies compared with I. scapularis 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  A. americanum, I. scapularis, encounter frequencies, infection prevalence

Lyme disease is the most common tick-borne illness in the United States, with >181,000 confirmed cases reported since 1990 (CDC 2003). During the same time, New Jersey ranked fourth with respect to Lyme disease reporting, with >21,000 confirmed cases or ~11.6% of the national total. The principal vector of the Lyme disease spirochete, Borrelia burgdorferi, is the blacklegged tick, Ixodes scapularis Say, which is commonly found throughout rural and suburban areas of New Jersey (Schulze et al. 1984b, 1998), Schulze et al. (2003) reported the infection prevalence of B. burgdorferi in I. scapularis adults collected from 16 of 21 New Jersey counties was 49.3%. Although the significant emphasis placed on Lyme disease prevention and control is clearly justified, a number of other tick-borne diseases have emerged, including human granulocytic ehrlichiosis (HGE), human monocytic ehrlichiosis (HME), Ehrlichia ewingii ehrlichiosis (EWE), and southern tick-associated rash illness (STARI) or Master’s disease (Childs and Paddock 2003). The public health importance of these diseases in New Jersey and elsewhere has not been adequately characterized.

In 1982, the lone star tick, Amblyomma americanum (L.), which is sympatric with I. scapularis in the southern portion of New Jersey (Schulze et al. 1984b), was implicated in the transmission of what seemed to be a classic case of Lyme disease, and spirochetes were observed in A. americanum adults and nymphs collected at the residence of a second New Jersey patient (Schulze et al.1984a). A similar A. americanum-associated Lyme disease-like illness was subsequently reported from Missouri (Masters et al. 1992, 1998; Campbell et al. 1995), but investigators failed to implicate B. burgdorferi and attempts to culture spirochetes from A. americanum were unsuccessful (Oliver 1996). Furthermore, although the incompetence of A. americana-
num as a vector of B. burgdorferi has been well-documented (Oliver et al. 1993, Piesman and Sinsky 1988, Mather and Mather 1990, Mukowie et al. 1992, Ryder et al. 1992, Sanders and Oliver 1995). Spirochetes have been found in A. americanum from New Jersey (Schulze et al. 1984a, 1986a). Indiana (Ryder et al. 1992), North Carolina (Levine et al. 1989), Alabama (Luckhart et al. 1992), Oklahoma (Kocan et al. 1992), Missouri (Feir et al. 1994), Texas (Rawlings and Teltow 1994), Iowa (Bartholomew et al. 1993), and Tennessee (Stegall-Faulk et al. 2003). In 1996, an uncultivable Borrelia was detected in A. americanum from several states and provisionally named B. lonestari (Barbour et al. 1996). This new spirochete has been proposed as the likely etiological agent of this Lyme disease-like illness (Armstrong et al. 2001, Burkot et al. 2001, James et al. 2001), currently referred to as STARI (Stegall-Faulk et al. 2003). Strondahl et al. (2003) reported B. lonestari DNA in A. americanum removed from humans in nine states, including New Jersey, and recently, B. lonestari has been isolated in culture (Varela et al. 2004). In 2001, another novel Borrelia was identified in I. scapularis nymphs collected from five states, including New Jersey (Seoles et al. 2001).

Although the first human case of ehrlichiosis was recognized in the United States in 1986 (Maeda et al. 1987), Ehrlichia infections were reported from New Jersey as early as 1985 (Petersen et al. 1989). In 1991, a novel species, E. chaffeensis, was described (Anderson et al. 1991, Dawson et al. 1991) and considered the causative agent of HME. In 1992, another novel species, E. ewingii, first reported as the etiological agent of canine granulocytic ehrlichiosis (Anderson et al. 1992a), is now considered a human ehrlichiosis. A. americanum and the American dog tick, Dermacentor variabilis (Say) are the purported vectors of both E. chaffeensis (Anderson et al. 1992b, 1993) and E. ewingii (Steiert and Gilroy 2002). Although both tick species are abundant in New Jersey, only eight confirmed cases of HME have been reported over the past 3 yr (NJDHSS 2004). HGE was first recognized as a clinical entity in 1992 (Bakken et al. 1994). Although I. scapularis is the principal vector of the etiological agent A. phagocytophilum, HGE cases also are infrequently reported in New Jersey, with only 15 confirmed cases reported in the past 3 yr (NJDHSS 2004). Both HME and HGE have been reportable in New Jersey since 1995.

This preliminary study reports the frequency at which these ticks are encountered within a known hyperendemic area for Lyme disease and the infection prevalence of selected Borrelia, Ehrlichia, and Anaplasma in their respective tick vectors.

Materials and Methods

Study Area. The study was conducted at Naval Weapons Station Earle (NWS Earle), a 41-km² secured military facility located in Colts Neck, Monmouth County, NJ, an area known to be hyperendemic for Lyme disease (Bowen et al. 1984) and where both I. scapularis and A. americanum are consistently abundant (Schulze et al. 1986b, 1997; Schulze and Jordan 2003). The forest canopy is dominated by pitch pine, Pinus rigida Mill.; white oak, Quercus alba L.; red oak, Quercus rubra L.; and chestnut oak, Quercus prinus L. The understory and shrub layer is comprised of saplings and seedlings of the dominant tree species high-bush blueberry, Vaccinium corymbosum L.; lowbush blueberry, V. augustinifolium Ait.; huckleberries, Gaylussacia spp.; sweet pepperbush, Clethra alnifolia L.; spicebush, Linderia benzoin (L.) Blume; laurels, Kal- mia spp.; and greenbriar, Simalx rotundifolia L.

Tick Collections. A. americanum and I. scapularis exhibit markedly different host acquisition and questing behaviors (Schulze et al. 1997). A. americanum acquires hosts both through hunting and ambush. As hunters, A. americanum respond to certain environmental stimuli (e.g., carbon dioxide or vibrations) and are readily collected from the litter layer as they seek potential hosts. All postembryonic stages also are collected from shrub layer vegetation, where they attempt to acquire hosts through ambush. In contrast, I. scapularis acquires hosts primarily through ambush. Adults are most frequently collected while questing in the shrub layer, whereas nymphs and larvae quest at ground level within the litter. We attempted to avoid sampling bias posed by these differences in questing behaviors through the use of dragging and walking survey techniques (Ginsberg and Ewing 1989, Solberg et al. 1992). Here, dragging is better suited to sample ticks on horizontal surfaces, such as the upper surface of vegetation or litter, whereas walking surveys facilitate collection of ticks questing within the shrub vegetation. When used together, biases inherent in either sampling technique are offset. The same individuals conducted dragging and walking surveys simultaneously between 1000 and 1300 hours in an effort to minimize any investigator or temporal bias (Schulze et al. 2001a). Ticks adhering to drags and investigators’ coveralls were removed at 20-m intervals (Schulze et al. 2001b). Weather-related biases were avoided by sampling only when vegetation was dry and wind was below 10 km/h. Adult A. americanum and I. scapularis retained for infection prevalence analysis were held at 8°C and 90% RH before shipping.

Because of the differences in host-seeking behavior and phenologies between A. americanum and I. scapularis, our objective was to illustrate the potential of human encounters with both species, rather than to estimate tick abundance. 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 the appearance of a bimodal activity period. However, A. americanum adults have no fall activity period, which restricts sampling of sympatric populations to the spring. Nymphs of both species share similar May–July activity periods. To contrast the frequencies at which the two species may be encountered, we collected ticks during the respective spring activity periods of adult and nymphal A. americanum and I. scapularis (Schulze et al. 1986b). In 2003, collection of adult ticks was conducted between 15 and 21 April, whereas
nymphs were sampled on 11 and 12 June. In 2004, adults were collected between 2 and 9 April, and nymphs and adults were sampled between 2 and 9 June 2004.

Based on extensive knowledge of the study area, 20 sampling locations were selected in mixed hardwood–pine stands, which consistently yield large numbers of both species (Schulze et al. 1986b, 1998). We collected adults from a 100-m transect established at each of the 20 locations in areas with moderately dense shrub layers (generally continuous, low shrubs (<0.5 m in height) covering >90% of the area) where both A. americanum and I. scapularis tend to quest. The use of transects rather than plots allowed for a larger area to be covered during any sampling event, thus minimizing any effect due to tick aggregation. We collected nymphs from a 100-m² plot established at each of the 20 locations in proximity to its respective transect. Plots were placed in areas with patchy shrub layers (discontinuous low shrubs and >60% of area open ground), which permitted more-or-less continuous contact between the drag and litter layer, thereby facilitating the collection of nymphs of both species (Schulze et al. 1997).

Polymerase Chain Reaction (PCR) Analyses: A. americanum. Ticks were stored at −20°C until processed for DNA extraction. Individual adults were minced in a cryotube with a sterile scalpel blade, and DNA was extracted by using a QIAamp Mini kit (QIAGEN, Valencia, CA) according to the manufacturer’s instructions.

PCR assays were used to detect the presence of E. chaffeensis, E. ewingii, and B. lonestari. Amsherm Ready-to-Go PCR beads (Amsherm Biosciences Inc., Piscataway, NJ) were used in all the reaction mixtures, according to the manufacturer’s instructions. E. chaffeensis and E. ewingii PCR reactions were run in an Eppendorf Master Gradient thermocycler (Brinkmann Instruments, Westbury, NY), by using the following parameters: one cycle of 95°C (5 min), 39 cycles of 94°C (30 s), 55°C (30 s), and 72°C (1 min), and a final extension period (75°C, 5 min). B. lonestari assay parameters only differed in the annealing temperature, which was changed to 60°C for 30 s. DNA of E. chaffeensis was detected in ticks by using a nested PCR assay to amplify a segment of the variable length PCR target (VLTP) with outer primers FB3A and FB3A and inner primers FB5C and FB3 (Sumner et al. 1999). DNA extracted from the Arkansas isolate of E. chaffeensis was run on each gel as a positive control. PCR was used to amplify a 215-bp region of the flagellin (flaB) gene by using primers FlaLL and FlaRL in the primary reaction and primers FlaLS and FlaRS in the secondary reaction as described by Barbour et al. (1996). Positive control was kindly provided by Rendi Bacon and Barbara Johnson at CDC Fort Collins from purified plasmid.

Positive PCR products were purified with QIAquick-Spin PCR column (QIAGEN) according to the manufacturer’s instructions. Purified products were sequenced using a BigDye Terminator Cycle Sequencing Ready Reaction kit according to the manufacturer’s recommended protocol (Applied Biosystems, Foster City, CA). Unincorporated fluorescence-labeled deoxynucleoside triphosphates were removed with Dye Ex Spinkit columns (QIAGEN) according to the manufacturer’s recommendations. Both strands were analyzed by using an Applied Biosystem model 3100 automated DNA sequencer. Nucleotide sequences were edited and assembled with the Pregap and Gap programs of the STADEN sequence analysis package (Staden Package, Staden et al. 2003). Nucleotide comparisons were made using the Netblast function of the GCG package (Wisconsin Package version 10.2, Genetics Computer Group, Madison, WI).

PCR Analyses: I. scapularis. DNA was extracted from I. scapularis adults by snap-freezing in liquid nitrogen and grinding to a powder with a sterile pestle (Kontes Glass, Vineland, NJ) in sterile 1.5-mL microcentrifuge tubes. A solution containing 100 µl each of Tris-EDTA buffer, pH 8, and IsoQuick lysis buffer was added to each vial. The remaining extraction steps were performed according to the modified protocol of Schwartz et al. (1997). DNA was resuspended in 50 µl of sterile deionized water and stored at 4°C. All ticks were analyzed for B. burgdorferi, A. phagocytophilum, and a novel Borrelia species. The following primers were used.

B. burgdorferi. To detect the presence of B. burgdorferi DNA, a nested PCR reaction was performed using two sets of primers (BOR PAF, BOR P95R and BOR PBNF, BOR P97NR) designed by Liveris et al. (1999) to amplify the 941-bp gene fragment of the 16s-23s rDNA spacer region. IsoQuick extraction kits were used in this assay (Orca Research, Bothell, WA).

Anaplasma phagocytophilum. Primers EHR 521 and EHR 747 were used to amplify a 247-bp fragment of 16S ribosomal DNA as described by Pancholi et al. (1995).

Novel Borrelia species. Primers used to detect this novel Borrelia species were designed by Scoles et al. (2001). FLA181 F and FLA 400R specifically amplify a 219-bp gene fragment of the novel Borrelia, but not the homologous fragment of B. burgdorferi.

Results

Tick Collections: Adults. In 2003, sampling of the 20 transects yielded a total of 157 A. americanum adults. The mean ± SE number of A. americanum was 7.8 ± 2.2 adults per transect (range: 0—46). During the same sampling period, 191 I. scapularis were collected, with a mean of 9.6 ± 2.1 adults per transect (range: 0—37). The first round of sampling for adults in 2004 yielded 139 A. americanum, with a mean of 6.9 ± 1.4 per
transect (range 0–25) and 218 *I. scapularis* adults for a mean of 10.9 ± 2.4 adults per transect (range 0–38). The second round of sampling in 2004, conducted nearly 2 mo later, produced 91 *A. americanum* adults, with a mean of 4.6 ± 0.8 per transect and a range of 0–17 adults per transect. Only 13 *I. scapularis* adults were collected at the same period (mean of 0.7 ± 0.2 per transect, range 0–2).

**Tick Collections: Nymphs.** In total, 978 *A. americanum* nymphs were obtained in 2003, with a mean of 48.9 ± 11.7 nymphs per plot (range 2–174). Surveys of the same plots produced 133 *I. scapularis* nymphs with a mean of 6.7 ± 1.7 nymphs per plot (range 0–25). In total, 438 *A. americanum* nymphs were collected in 2004, with a mean of 21.9 ± 5.1 per plot (range 2–91), and 166 *I. scapularis* nymphs, with a mean of 8.3 ± 1.7 per plot and a range of 0–34 nymphs per plot.

**Infection Prevalence: *A. americanum***. Of the 121 *A. americanum* adults subjected to PCR analysis, 11 (9.1%) were positive for *B. lonestari*. The infection prevalence for *E. chaffeensis* and *E. ewingii* was 12.3 and 8.2%, respectively. The coinfection prevalence for *E. chaffeensis/E. ewingii* was 4.1% and *E. chaffeensis/B. lonestari* was 0.8% (Table 1). Representative positives sequenced for verification were 100% identical to GenBank deposits for the flagellin gene *B. lonestari*, the outer membrane P28 gene for *E. ewingii*, and the VLPT for *E. chaffeensis*.

**Infection Prevalence: *I. scapularis***. Analysis of 147 *I. scapularis* adults for *B. burgdorferi* yielded 74 positive ticks (50.3%). The infection prevalence for *A. phagocytophilum* was 6.1%, whereas 1.4% of adults were infected with the novel *Borrelia*. Coinfection prevalences were 2.7% for *B. burgdorferi/A. phagocytophilum*, 0.7% for *B. burgdorferi/novel Borrelia*, and 0.7% for *A. phagocytophilum/novel Borrelia* (Table 2).

**Discussion**

The results of this study showed that during the spring activity period, *I. scapularis* and *A. americanum* adults were encountered at similar frequencies. However, in earlier studies at NWS Earle, *A. americanum* were generally collected with greater frequency compared with *I. scapularis* by a ratio of ≈3:1 (Schulze et al. 1997, 2000, 2001a). The data also suggest that the spring activity of *I. scapularis* adults preceded that of *A. americanum* but that the activity period of *A. americanum* tended to be of longer duration. There was a large difference in encounter frequencies for nymphs during both years. In 2003, the mean encounter frequency for *A. americanum* was 7.3 times greater than that for *I. scapularis*, whereas in 2004, *A. americanum* nymphs were encountered 2.6 times more often than *I. scapularis* nymphs. The nymphal tick populations seem to be more synchronous and may provide a better model for comparing encounter frequencies, given that the nymphs of both species are active during May–July.

Analysis of *I. scapularis* adults yielded a *B. burgdorferi* infection prevalence of 50.3%, which was similar to the 49.3% infection rate recently reported from a statewide survey of *I. scapularis* adults (Schulze et al. 2003) and that previously reported from NWS Earle nearly two decades earlier (Schulze et al. 1986a). The *B. lonestari* infection prevalence in *A. americanum* adults collected at NWS Earle during spring 2003 (9.1%) also was similar to spirochete infection prevalence observed in *A. americanum* adults (5.4%) from the earlier study and recently reported in Missouri (Bacon et al. 2003). The infection prevalences of *E. chaffeensis* (12.3%) and *E. ewingii* (8.2%) in *A. americanum* were similar to those reported from Missouri (Steiert and Gilroy 2002) and New York (Mixson et al. 2004), although HME is infrequently reported in New Jersey (CDC 2003).

The results of this study showed that the infection prevalence of *B. burgdorferi* in *I. scapularis* adults was approximately fivefold higher than the *B. lonestari*, *E. chaffeensis*, and *E. ewingii* infection prevalences in *A. americanum*. However, lower infection prevalence may be offset by the higher likelihood of encountering *A. americanum*, relative to *I. scapularis*, from this study and historically from the same location (Schulze et al. 1997, 2000, 2001a). Larger numbers of *A. americanum* encounters may reflect greater numbers of this species compared with *I. scapularis* and/or the fact that although *I. scapularis* most often acquires hosts through ambush, *A. americanum* is far more aggressive, acquiring hosts through both ambush and hunting (Stromdahl et al. 2003). Thus, when both species are active, humans are far more likely to encounter *A. americanum*. This is particularly true of *A. americanum* larvae, which are apparently infected with *B. lonestari* via
transovarial transmission (Stromdahl et al. 2003) and frequently encountered in clusters of hundreds of individuals (Schulze et al. 1986a, Stromdahl et al. 2003). Furthermore, 73% of Lyme disease cases in New Jersey have a date of onset in May through August, with a peak in June, suggesting an epidemiological link to the activity period of I. scapularis nymphs (Goldoft et al. 1990). However, this peak in transmission also occurs during the peak activity periods of all postembryonic stages of A. americanum (Schulze et al. 1986b). Given these factors, it is reasonable to speculate that where both I. scapularis and A. americanum are active, some proportion of reported B. burgdorferi infections may, in fact, be B. lonestari and that human ehrlichioses may be significantly underdiagnosed or underreported.

These findings suggest a potential shortcoming in public health intervention against tick-borne diseases. Most educational materials focus exclusively on the prevention of Lyme disease and tick management strategies tend to be directed toward the control of I. scapularis. Unfortunately, many of the current tick control strategies are either untested against, or not effective against tick species.

References Cited

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