Geographical Distribution and Prevalence of Selected *Borrelia, Ehrlichia,* and *Rickettsia* Infections in *Amblyomma americanum* (Acari: Ixodidae) in New Jersey

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GEOGRAPHICAL DISTRIBUTION AND PREVALENCE OF SELECTED *BORRELIA*, *EHRLICHIA*, AND *RICKETTSIA* INFECTIONS IN *AMBLYOMMA AMERICANUM* (ACARI: IXODIDAE) IN NEW JERSEY

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ABSTRACT. We used published accounts to describe the known statewide distribution of the lone star tick, *Amblyomma americanum*, in New Jersey and field surveys to characterize the geographical range of *A. americanum* and selected *A. americanum*–transmitted pathogens in Monmouth County, the hypothesized northern limit of the species distribution. Ticks were collected using standardized methods from 50 widely dispersed public access areas within 18 municipalities to produce estimates of relative abundance among sites. Collected *A. americanum* adults were stored at −80°C until processed for DNA extraction. Individual ticks were subjected to polymerase chain reaction analyses to detect the presence of *Ehrlichia chaffeensis, E. ewingii, Rickettsia amblyommii,* and *Borrelia lonestari*. The range of *A. americanum* was generally limited to the southern half of New Jersey. Within Monmouth County, we collected *A. americanum* from 9 of 18 municipalities (50%) and 24 (48%) of the surveyed properties. We found at least 1 pathogen at 17 (70.8%) sites located within 6 of 9 municipalities, while all 4 target pathogens were detected in 5 of those 6 (83.3%) municipalities. The geographical distribution of *A. americanum* and its associated pathogens appeared to be restricted to the southern portion of the county. Possible factors governing the distribution are discussed.

KEY WORDS *Amblyomma americanum*, *Ehrlichia*, *Borrelia lonestari*, *Rickettsia amblyommii*, geographical range

INTRODUCTION

Long recognized as an important economic and nuisance pest of humans, livestock, and wildlife (Cooley and Kohls 1944, Bishopp and Trembley 1945, Clymer et al. 1970, Hair and Howell 1970, Koch and Dunn 1980), the lone star tick, *Amblyomma americanum* (L.), has only recently been recognized as an emerging public health threat (Childs and Paddock 2003). In New Jersey, the purported etiological agents of human ehrlichioses (*Ehrlichia chaffeensi* Sapulpa and *E. ewingii* sp. nov.), southern tick-associated rash illness (STARI; *Borrelia lonestari* sp. nov.) (James et al. 2001; Schulze et al. 2005, 2006), and a spotted fever group rickettsiae (SFGR; *Rickettsia amblyommii* sp. nov.) (Mixson et al. 2006) have been detected in *A. americanum.* Of the 2 human ehrlichioses, only human monocytic ehrlichiosis (HME) is reportable in New Jersey. During the period 2001–04, an average of 2.5 confirmed cases of HME were reported annually in New Jersey. During the next 4 years, the annual average was 63.5 cases, a 25-fold increase over the previous 4 years (NJDHSS 2008). Although it has been suggested that *B. lonestari* (STARI) and *R. amblyommii* (SFGR) infections cause signs and symptoms that are clinically indistinguishable from Lyme disease (Masters et al. 1992, 1998) and Rocky Mountain spotted fever (Apperson et al. 2008), respectively, their role in disease pathology is unknown.

Amblyomma americanum occurs throughout the southeastern USA as far west as central Texas and, although found on some offshore and barrier islands as far north as Maine, New Jersey marks the northern extent of significant inland populations of this tick (Schulze and Bosler 1996). A survey of 567 white-tailed deer (*Odocoileus virginianus* Zimmerman) harvested in fall 1981 suggested that the distribution of *A. americanum* was generally limited to the southern one-third of New Jersey (Schulze et al. 1984). However, since *A. americanum* is not active in the fall, this may be an underestimate of its actual geographical range.

Childs and Paddock (2003) speculated that the number of cases of *A. americanum*–transmitted diseases will likely increase over the next 25 years. If this prediction proves true, there will be an increasing need for a public health response, specifically in the areas of surveillance and public awareness campaigns. Critical to achieving these goals is knowledge of the geographical distribution of *A. americanum* and *A. americanum*–associated pathogens, and the identification of habitat affinities most conducive to supporting *A. americanum*.

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MATERIALS AND METHODS

Study design: We first attempted to delineate the statewide distribution of *A. americanum* through a comprehensive review of the existing scientific literature, archival databases including tick identification records from selected local/county agencies, and the administration of questionnaires to individuals having unique knowledge of tick distribution within New Jersey by virtue of their research or occupational activities. The 2nd phase of the study involved a tick survey of Monmouth County, which historically was thought to be the northern range of established populations of *A. americanum* in New Jersey (Schulze and Bosler 1996). In 1993, surveys of 610 publicly owned properties distributed throughout Monmouth County identified a number of sites that were considered high potential risk for tick-borne disease transmission based on type, amount, and access to suitable tick habitat (Schulze and Jordan 1996a). In early 2008, a number of geographically dispersed county-, state-, and federally-owned parks, forests, and wildlife management areas with habitat capable of supporting *A. americanum* (Schulze et al. 2002, Schulze and Jordan 2005) were considered as potential tick survey sites. Between 1 and 8 sites were surveyed within 18 municipalities, for a total of 50 sites. Each site was scheduled to be surveyed for a 60-min period. Smaller sites or sites with a limited amount of suitable tick habitat were sampled for 20 to 30 min, with the resulting data extrapolated to the standard 60-min period that would allow direct comparison of relative tick abundance among the 50 sites. Larger properties were divided into sections, sampled for 10 to 30 min, and the resulting data were combined to yield the 60-min survey period. In the case of a 41-km^2 military base, 60-min surveys were conducted in each of the 3 municipalities composing the facility.

Tick collections: Selected sites were sampled for host-seeking *A. americanum* during April, the peak activity period for adults (Schulze et al. 1986), using a combination of standard walking and dragging survey techniques (Ginsberg and Ewing 1989, Schulze et al. 1997). Surveys were conducted between 0900 and 1500 h (Schulze et al. 2001, Schulze and Jordan 2003). Investigators examined coveralls and drags for ticks at 20-m intervals (Schulze and Jordan 2001), and all captured ticks were placed in site-specific glass vials containing 70% ethanol. *Amblyomma americanum* adults were stored at −80°C until processed for DNA extraction.

DNA extraction: Ticks were disrupted using either of 2 methods. Manual crushing was performed using matched pestles and tubes (Bel-Art Products, Pequannock, NJ). The tick was placed in a 1.5-ml microfuge tube containing 120 μl of DNAzol Genomic DNA isolation reagent (Molecular Research Center, Inc., Cincinnati, OH) to which was added Proteinase K (Ambion, Austin, TX) at a concentration of 0.1 mg/ml. Following an overnight incubation at room temperature, the tick was crushed with the pestle. The resulting lysate was incubated at room temperature for 3 h and then heated at 95°C for 10 min. A TissueLyser (QIAGEN, Valencia, CA) was used as the other method for disrupting ticks. The tick was placed in a 2-ml safety-lock tube containing a stainless steel 5-mm bead (QIAGEN) and 125 μl of the DNAzol–Proteinase K solution mentioned above. Disruption was set at 30 Hz for 60 sec. The resulting lysate was incubated at room temperature for 3 h, then heated at 95°C for 10 min, and 120 μl of lysate was transferred to a 1.5-ml tube. Following both methods of disruption, the 120 μl of lysate was centrifuged at 10,000 × *g* for 10 min and 100 μl of the supernatant was transferred to a 1.5-ml tube for DNA isolation. The DNA was precipitated by the addition of 50 μl of 100% ethanol, followed by gentle inversion and incubation for 10 min at room temperature. The DNA precipitate was pelleted by centrifugation at 16,000 × *g* for 10 min. The DNA pellet was washed twice with 75% ethanol and dissolved in 35 μl of water before it was stored at 4°C until polymerase chain reaction (PCR) analysis. The DNA extraction was verified by amplification of tick 16S mitochondrial DNA.

Polymerase chain reaction analyses: HotStar *Taq* Master Mix (QIAGEN) was used in all reactions, according to manufacturer's instructions. All PCR reactions were run in an Eppendorf Master Gradient Thermal Cycler (Brinkmann Intruments, Inc., Westbury, NY). The thermal cycler parameters for the primary-stage PCR: 1 cycle of 95°C (15 min), 40 cycles of 95°C (30 sec), 55°C (30 sec), and 72°C (1 min), and a final extension period of 72°C (5 min). The thermal cycler parameters for the nested-stage PCR: 1 cycle of 95°C (15 min), 30 cycles of 95°C (30 sec), 55°C (30 sec), and 72°C (1 min), and a final extension period of 72°C (5 min). All primers were prepared by Invitrogen (Invitrogen Life Technologies, Carlsbad, CA) and used at 1 μM final concentration. The DNA of *B. lonestari* was detected by using a nested PCR that amplifies a region of the flagellin gene (*flaB*) (Barbour et. al. 1996). Primary PCR primers: FLaLL, 5′ACATATTCAGATGCAGACAGAGGT; and FLaRL, 5′GCAATCATAGCCATTGCAGATTGT. Nested PCR primers: FLaLS, 5′AACAGCTGAAGAGCTTGGAATG; and FLaRS, 5′CTTTGATCACTTATCATTCTAATAGC. Positive control for *B. lonestari* was provided by Susan Little (Department of Veterinary Pathobiology, Oklahoma State University, Stillwater, OK). Ticks were tested

for *R. amblyommii* using primers specific for the *rOmpA* gene (Roux et al. 1996) in the primary PCR stage, followed by a seminested PCR stage to add sensitivity (Stromdahl et al. 2008). Primary PCR primers: Rr190.70p, 5′ATGGCGAATATTTCTCCAAAA and Rr190.701, 5′GTTCCGTTAATGGCAGCATCT. Seminested PCR primers: Rr190.70p and Rr190.602n, 5′AGTGCAGCATTCGCTCCCCCT. The *R. amblyommii* positive control was provided by William Nicolson (Centers for Disease Control and Prevention [CDC], Atlanta, GA).

The DNA of *E. chaffeensis* was detected using a nested PCR that amplifies a region of the 16S rRNA gene, yielding a 389-bp product specific for *E. chaffeensis* (Dawson et. al. 1994). Primary PCR primers: ECC, 5′AGAACGAACGCTGGCGGCAAGCC; and ECB, 5′CGTATTACCGCGGCTGCTGGCA. Nested PCR primers: HE1, 5′CAATTGCTTATAACCTTTTGGTTATAAAT; and HE3, 5′TATAGGTACCGTCATTATCTTCCCTAT. The positive control for *E. chaffeensis* was provided by William Nicolson (CDC).

The 16S rRNA gene of *E. ewingii* was detected by performing a nested PCR on the primary *Ehrlichia* product produced by ECC and ECB using primers that would yield a 403-bp product specific for *E. ewingii.* Nested PCR primers: EWF1, 5′TCGAACGAACAATTCCTAAA and HE3. The specificity is imparted by the EWF1 primer. Positive *E. ewingii* controls were chosen from previous *A. amblyommii* samples that were strong positives for the *E. ewingii* genes 16S rRNA and *p28* (Gusa et al. 2001). The PCR products from the *E. ewingii* positive controls were sequenced to confirm identity.

Representative positive PCR products were purified with ExoSAP-IT (USB Corporation, Cleveland, OH) to remove unused primers and nucleotides and sent to GENEWIZ (GENEWIZ, Inc., South Plainfield, NJ) following their guidelines for sample preparation using our primers. Nucleotide sequences were read using Chromas version 2.33 (Technelysium Pty. Ltd., Helensvale, Australia) and BioEdit version 7.0.5 (Ibis Therapeutics, Carlsbad, CA), and BLASTn version 2.2.24+ (Altschul et al. 1997) searches were run to identify these sequences by comparison with sequences in the GenBank database. A subsample of *B. lonestari*, *E. chaffeensis*, *E. ewingii*, and *R. amblyommii* amplicons from *A. americanum* were sequenced using both the appropriate and reverse primer to establish and confirm identity.

RESULTS

Distribution of A. americanum*:* Review of the literature and unpublished archival data, and interviews of knowledgeable professionals indicated that the geographical range of *A. americanum* in New Jersey is essentially limited to the southern portion of the state (Fig. 1). With the exception of single specimens obtained from 1 hunter-killed deer each in Hunterdon and Warren counties, data available from >50 municipalities dispersed across 18 of New Jersey's 21 counties collected over a 25-year span (1981–2006) suggested that the northern range of *A. americanum* is marked by Mercer, Middlesex, and Monmouth counties.

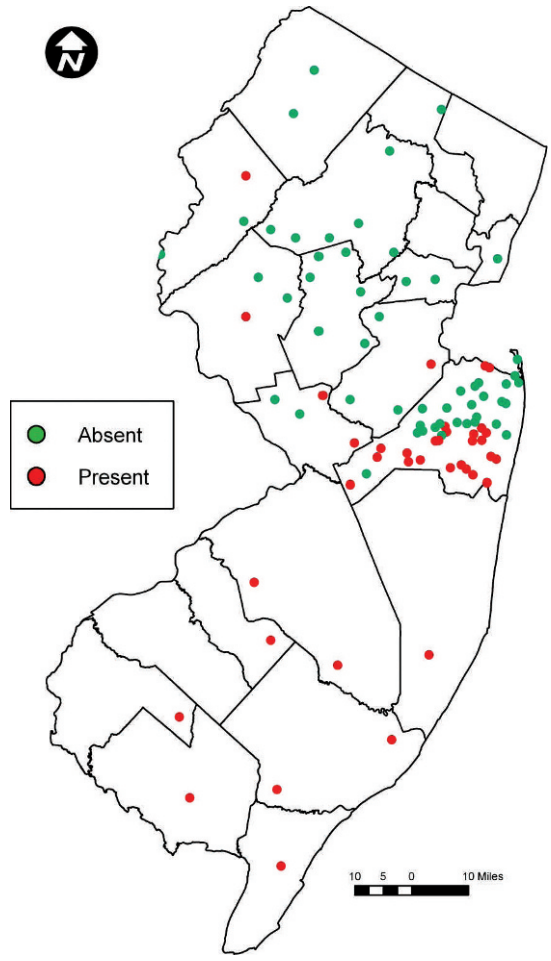

Fig. 1. Documented distribution of *Amblyomma americanum* in New Jersey.

Between April 2 and 22, 2008, surveys conducted at 50 geographically dispersed sites within 18 Monmouth County municipalities that support tick habitat yielded 290 *A. americanum* adults. *Amblyomma americanum* was collected at 24 of 50 (48%) sites located in 9 municipalities and, where found, ranged between 1 and 65 individuals. However, *A. americanum* was classified as moderately abundant (3–10 ticks/60-min survey) or abundant (>10 ticks/60-min survey)

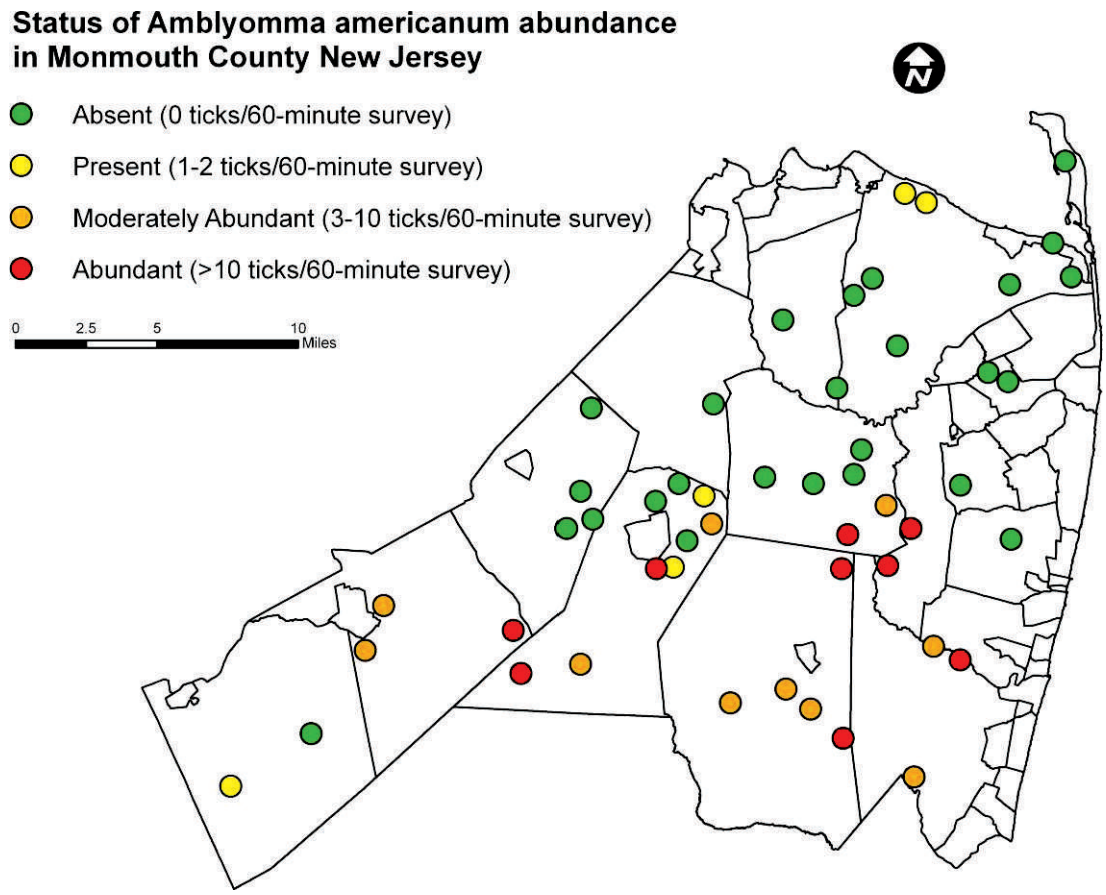

Fig. 2. Distribution of *Amblyomma americanum* in Monmouth County, NJ.

(classifications modified from Schulze et al. 1991) in only 7 municipalities located in the southern portion of the county (Fig. 2). Single specimens were collected at 2 sites in Middletown Township and 1 site in Upper Freehold Township. The survey results for *A. americanum* are summarized in Table 1.

Geographic coordinates of tick collection sites were georeferenced to digitized state coverages of environmental data to examine ecological factors that govern distribution of *A. americanum* in Monmouth County. The distribution of *A. americanum* appeared to be closely related to the geomorphologic fall line marking the transition between the Inner and Outer Coastal Plain Provinces (Fig. 3). A line of cuesta hills in Monmouth County separates lowlands and rolling hills underlain by Cretaceous deposits on the Inner Coastal Plain from the unconsolidated tertiary deposits of acidic and droughty sands, silt, and gravels of the Outer Coastal Plain (Collins and Anderson 1994). The soils and hydrology on the Outer Coastal Plain create a distinctive complex of pitch pine (*Pinus rigida* Mill) and oak communities and interspersed wetlands found across the southeastern USA and in disjunct patches covering the coastal plain of New Jersey, much of the southern half of Long Island, north along the Atlantic Coast to Cape Cod, MA, and in disjunct patches west to near Albany, NY (Christensen 1988).

Distribution of A. americanum *pathogens:* Of the 24 properties yielding *A. americanum*, at least one of the target pathogens was found at 17 (70.8%) sites located within 6 of the 9 (66.7%) municipalities. At sites and municipalities failing to produce positive ticks, only 1–3 and 1–5 specimens were available for testing, respectively. All 4 target pathogens were detected in 5 of the 6 (83.3%) municipalities. Five *A. americanum* were coinfected, while a single tick was infected with 3 pathogens. Infection prevalence data are summarized in Table 2. Nucleotide sequences from *B. lonestari*–positive ticks (randomly selected using a simple random number generator from an Excel [Microsoft Corporation, Redmond, WA] spreadsheet) were found to be 99% homologous to published GenBank sequences for *B. lonestari* as follows: GenBank accession nos. AY850063 and U26705 (Barbour et al. 1996). Randomly selected

Table 1. Summary of *Amblyomma americanum* collections at selected publicly owned lands in Monmouth, NJ, April 2008.

Municipality	No. sites surveyed	No. sites with *A. americanum*	No. sites by *A. americanum* abundance category[1]			
			Absent	Present	Moderately abundant	Abundant
Atlantic Highlands	1	0	1	0	0	0
Colts Neck Township	6	2	4	0	1	1
Eatontown	1	0	1	0	0	0
Freehold	1	0	1	0	0	0
Freehold Township	8	6	2	2	2	2
Highlands	1	0	1	0	0	0
Holmdel Township	1	0	1	0	0	0
Howell Township	5	5	0	0	3	2
Little Silver	2	0	2	0	0	0
Manalapan Township	4	0	4	0	0	0
Marlboro Township	1	0	1	0	0	0
Middletown Township	8	2	6	2	0	0
Millstone Township	3	3	0	0	2	1
Neptune Township	1	1	0	0	0	1
Ocean Township	1	0	1	0	0	0
Tinton Falls Township	2	2	0	0	0	2
Upper Freehold Township	2	1	1	1	0	1
Wall Township	2	2	0	0	2	0

[1] Absent = 0 ticks/60-min survey; Present = 1–2 ticks/60-min survey; Moderately abundant = 3–10 ticks/60-min survey; Abundant = >10 ticks/60-min survey (classifications modified from Schulze et al. 1991).

nucleotide sequences from *E. chaffeensis*–positive ticks were found to be 99% homologous to published GenBank sequences for *E. chaffeensis* as follows: GenBank accession nos. EU826516 (Tomassone et al. 2008) and AF416764 (Massung et al. 2002). Randomly selected nucleotide sequences from *E. ewingii*–positive ticks were found to be 99% homologous to published GenBank sequences for *E. ewingii* as follows: GenBank accession nos. U96436 (Goldman et al. 1998), DQ365880, and AY093440 (Yabsley et al. 2002). Randomly selected nucleotide sequences from *R. amblyommii*–positive ticks were found to be 99% homologous to the published GenBank sequence, accession no. EF194096 (Zanettii et al. 2008), for *R. amblyommii*.

DISCUSSION

Because of the preeminence of Lyme disease, the potential public health importance of *A. americanum*–transmitted pathogens has often received less attention (Childs and Paddock 2003). Although more extensive research specifically targeting *A. americanum* is required, we did not identify a significant expansion of the range of this species in New Jersey that has been observed in other states (Mather and Mather 1990, Ginsberg et al. 1991, Means and White 1997) or that was observed with the blacklegged tick, *Ixodes scapularis* Say, in New Jersey during the 1980s (Goldstein et al. 1990, Risley and Hahn 1994, Schulze et al. 1998). However, once established, local populations of *A. americanum* often exceed those of *I. scapularis* and, combined with its aggressive host-seeking behavior, it is reasonable to expect human–*A. americanum* encounters will be commonplace (Schulze et al. 2005, 2006).

Overall, the infection prevalence in the current study for *B. lonestari* (6.8%) and *E. ewingii* (7.1%) in *A. americanum* adults was similar to that for *B. lonestari* (5.8–9.1%) and *E. ewingii* (8.2%) reported in earlier studies in Monmouth County (Schulze et al. 2005, 2006). However, the infection prevalence for *E. chaffeensis* (5.3%) was <50% of that (12.3%) from an earlier study (Schulze et al. 2005), while the infection prevalence for *R. amblyommii* (12.8%) was nearly double that (6.6%) from a single site in central Monmouth County (Mixson et al. 2006). Only one tick was coinfected with *E. chaffeensis*/*E. ewingii* in this study compared to a 4.1% infection prevalence reported in an earlier study, while *B. lonestari*/*E. chaffeensis* coinfections were similar in the current (0.4%) and earlier (0.8%) studies (Schulze et al. 2005). In this study, coinfection prevalence of 0.7% and 0.4% were reported for *B. lonestari*/*R. amblyommii* and *E. chaffeensis*/*R. amblyommii*, respectively, while a single tick was infected with *B. lonestari*/*E. chaffeensis*/*R. amblyommii*.

These data suggest that, where abundant, *A. americanum* may be expected to be infected with 1 or more pathogens. With the increased likelihood of tick bites from *A. americanum*, the associated tick-borne illnesses, including STARI, SFGR, and ehrlichioses due to *E. chaffeensis* and *E. ewingii* should be considered for inclusion among

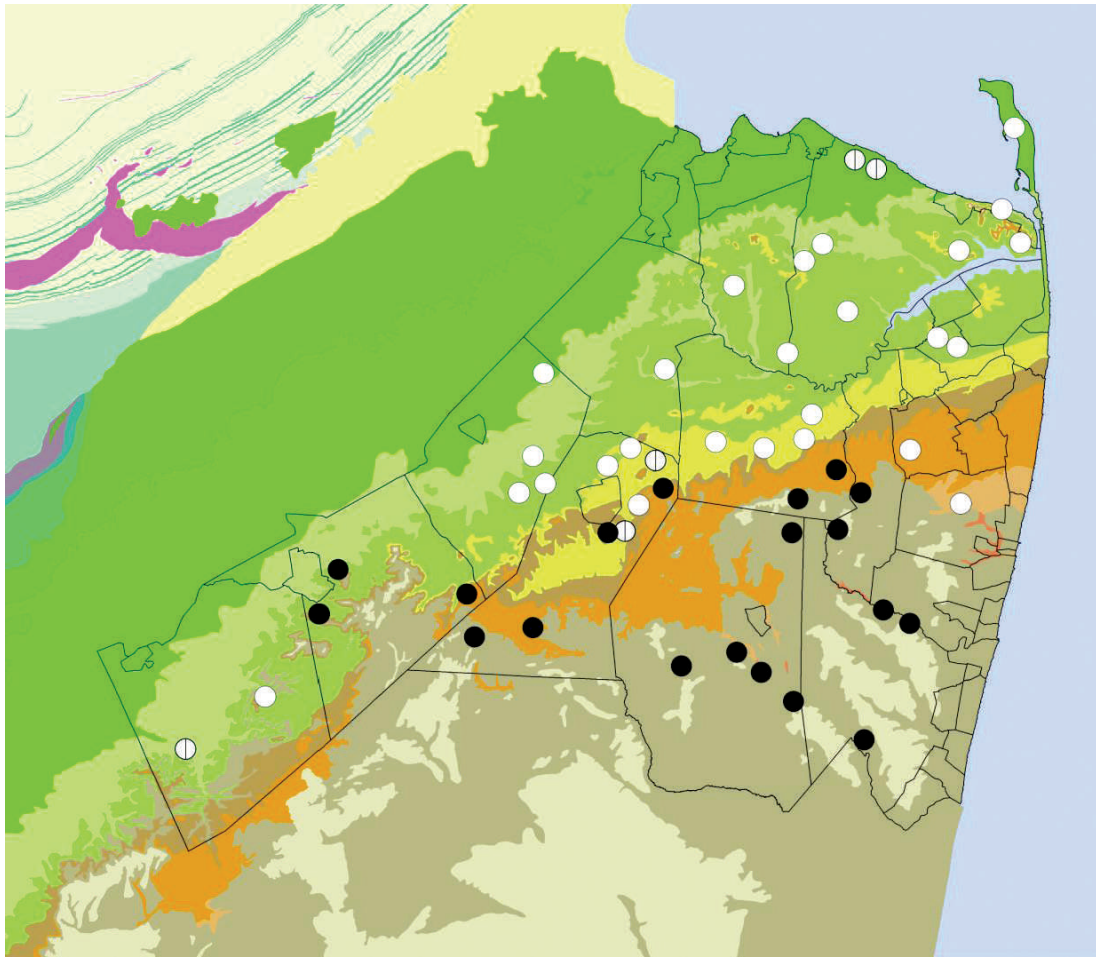

Fig. 3. Location of tick sampling sites superimposed on Monmouth County, NJ, bedrock geology. The brown and orange band represents gneissic fall line between Inner and Outer Coastal Plain deposits. Sampling sites: ○, *Amblyomma americanum* absent; ⦶, *A. americanum* present; ●, *A. americanum* moderately abundant/abundant.

Table 2. Infection prevalence of selected known and potential tick-borne pathogens in *Amblyomma americanum* adults collected at public access properties in Monmouth County, NJ, April 2008.

			No. infected (% infected)				
Township	No. sites	No. ticks	*Borrelia lonestari*	*Ehrlichia chaffeensis*	*E. ewingii*	*Rickettsia amblyommii*	Coinfections[1]
Colts Neck	2	42	1 (2.4)	3 (7.1)	4 (9.5)	3 (7.1)	None
Freehold	6	35	2 (5.7)	2 (5.7)	3 (8.6)	5 (14.3)	*B.l./R.a.*; *B.l./E.c.*; *E.c./R.a.*
Howell	5	74	7 (9.5)	4 (5.4)	6 (8.1)	10 (13.5)	*E.c./E.e.*; *B.l./R.a.*
Middletown	2	2	0 (0)	0 (0)	0 (0)	0 (0)	None
Millstone	3	32	2 (6.3)	1 (3.1)	0 (0)	6 (18.8)	None
Neptune	1	19	2 (10.5)	1 (5.3)	2 (10.5)	3 (15.8)	*B.l./E.c./R.a.*
Tinton Falls	2	71	5 (7.0)	4 (5.6)	5 (7.0)	9 (12.7)	None
Upper Freehold	1	1	0 (0)	0 (0)	0 (0)	0 (0)	None
Wall	2	5	0 (0)	0 (0)	0 (0)	0 (0)	None
Total	24	281	19 (6.8)	15 (5.3)	20 (7.1)	36 (12.8)	6 (2.1)

[1] *B.l.*, *B. lonestari*; *E.c.*, *E. chaffeensis*; *E.e.*, *E. ewingii*; *R.a.*, *R. amblyommii*.

the differential diagnoses of tick-borne diseases in areas where *A. americanum* is present. Further, based on our preliminary investigation, the geographical distribution of *A. americanum* in Monmouth County seems to be restricted to the southern portion of the county. We believe that the hydrologic and edaphic conditions and the resulting vegetation types that characterize the Atlantic coastal pine forest complex appear to also characterize the range of *A. americanum* in New Jersey. We have previously shown that relative abundance of sympatric *I. scapularis* and *A. americanum* vary widely among different forest types and forest type appears to influence patterns of annual variation in tick numbers (Schulze and Jordan 1996b, Schulze et al. 1998). These differences appear to be related to microclimate conditions created by vegetation and local microtopography (Schulze and Jordan 2005; Schulze et al. 2001, 2002). Recently expanding populations of *A. americanum* in New York (Ginsberg et al. 2002) and in Rhode Island and Connecticut (Ijdo et al. 2000) also occur in these coastal pine–oak habitats established in sandy, droughty soils distinguishing them from surrounding ecoregions.

In contrast to questing *I. scapularis*, all 3 stages of questing *A. americanum* exhibited poor relationships with microclimate variables recorded in the litter and shrub layers (Schulze and Jordan 2005). Compared to *I. scapularis*, *A. americanum* appears to be successful in a range of habitats that are both drier and warmer than those tolerated by sympatric *I. scapularis* (Schulze and Jordan 2003, 2005; Schulze et al. 2001, 2002). Further research is required to determine which environmental conditions and which habitats are most likely to support this species in other parts of its range. However, these sorts of habitats may contribute to the range extension of this aggressive tick species into the midcontinent, such as its emergence in the sandy, well-drained relict dune region of northwestern Ohio (Irving et al. 2000), and mapping these environmental factors may provide a more precise way of predicting the potential threat of *A. americanum*–associated disease.

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