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Abundance and infection rates of *Ixodes scapularis* nymphs collected from residential properties in Lyme disease-endemic areas of Connecticut, Maryland, and New York

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Ixodes scapularis, commonly known as the blacklegged tick, is responsible for transmitting Lyme disease (caused by *Borrelia burgdorferi*), the most common vector-borne disease in the United States (Centers for Disease Control and Prevention 2014). The blacklegged tick can also transmit *Anaplasma phagocytophilum* (the etiologic agent of human granulocytic anaplasmosis), *Babesia microti* (the causative agent of babesiosis), *Borrelia miyamotoi* (a relapsing fever *Borrelia*), and deer tick virus. In the northeastern U.S., the highest risk of exposure to the blacklegged tick is likely peridomestic, due to fragmented forest landscapes and other land-use characteristics, as well as the intrusion of humans into prime habitat for blacklegged ticks and their hosts (Falco and Fish 1988, Maupin et al. 1991, Nicholson and Mather 1996, Brownstein et al. 2005). Despite this, most reports of tick abundance and infection rates focus primarily on ticks collected from public lands and forested research sites (Aliota et al. 2014, Barbour et al. 2009, Diuk-Wasser et al. 2012, Hersh et al. 2014, Keesing et al. 2014).

We collected ticks from residential properties in Lyme disease-endemic areas and determined infection rates for nymphal *I. scapularis* as part of a two-year, multi-site tickborne disease intervention study involving the Centers for Disease Control and Prevention (CDC) and the Emerging Infections Programs in Connecticut (CT), Maryland (MD) and New York (NY). Here, we present tick densities and infection rates for *B. burgdorferi*, *A. phagocytophilum*, and *B. microti* from nymphal *I. scapularis*, reflecting peridomestic exposure to these pathogens.

After providing informed consent, heads of households in select endemic areas of Fairfield, Litchfield and New Haven Counties, CT; Baltimore, Carroll, Harford and Howard Counties, MD; and Dutchess County, NY, were enrolled in a randomized controlled trial to determine whether a single springtime application of pesticide reduced the incidence of human

tickborne disease. Eligible properties were at least a half-acre in size, as that is considered a reasonable surrogate for the presence of tick habitat (Maupin et al. 1991). Enrolled properties were randomized for treatment with either pesticide or water placebo. Tick habitat on randomly selected properties from both groups was drag-sampled for host-seeking nymphal blacklegged ticks during peak nymphal activity in May through July in 2011 and 2012, using methods described previously (Maupin et al. 1991, Mather et al. 1996). Briefly, a 1 m² flannel cloth was dragged over leaf litter, lawn and vegetation found on residential perimeters. Each area was sampled only once; up to forty 30 s drags were conducted for a total of up to 20 min per property. At a small number of properties in Maryland and New York that were drag-sampled, a half meter flag was used in addition to the drags (Rulison 2013) to collect ticks in particularly dense vegetation, using the same timed-sampling protocol. Nymphal tick density (the number of *I. scapularis* nymphs collected per hour) was calculated for placebo properties only and averaged for each year by study site (CT, MD and NY) and overall (across all sampled placebo properties).

Ticks were pooled by property in 95% ethanol and sent to the CDC Division of Vector-Borne Diseases in Fort Collins, Colorado for testing. Ticks collected in CT were identified at Western CT State University prior to shipment, whereas ticks from MD and NY were identified at the CDC. *I. scapularis* nymphs were tested individually for *B. burgdorferi*, *A. phagocytophilum*, and *B. microti* using real-time multiplex polymerase chain reaction (PCR) (Hojgaard et al. 2014). This assay was also used to identify other *Borrelia* genospecies by comparing PCR cycle threshold values (Ct values) for two specific *Borrelia* targets (fliD and gB31). Pathogen infection rates were calculated for all tested nymphs, regardless of treatment group, by study site and year. Difference in *B. burgdorferi* infection rates was assessed using a Z-test for proportions.

In CT, to systematically characterize *I. scapularis* nymphal emergence and activity (phenology), ticks were sampled weekly from three forested Fairfield County nature preserves using the same timed-sampling protocol. All three sites were located centrally to the CT residential study sites and also within 96 km of the Dutchess County, NY, study area. The phenology sampling locations were maple and oak dominated forests with low-lying barberry. Nymphal *I. scapularis* ticks collected at phenology sites were counted and replaced after each 30 s drag, and the average nymphal density was calculated. Nymphal ticks were replaced at the phenology sites so that tick abundance would not be affected by frequent sampling. Study approval was obtained by the Institutional Review Boards of the CDC, the state health departments in CT, MD and NY, Western CT State University, and Yale University.

Ixodes scapularis nymphs were the most common tick and life stage collected at all sites and in both years, accounting for 864 (91%) of 952 total ticks collected from 267 properties. *Dermacentor variabilis* adults were the second most common tick (n=69), collected both years in all sites. Six *I. scapularis* adults were collected in 2011 in CT. Other ticks collected include *Amblyomma americanum* (one adult and two nymphs in MD and one nymph in CT, all in 2012), *Haemaphysalis leporispalustris* (six nymphs in MD in 2012), and *Ixodes dentatus* nymphs (one in MD and two in NY in 2012). The average *I. scapularis* nymphal rate across all sampled placebo properties was 27.4 nymphs/hour (range 0-174, standard

deviation 37.0) in 2011 and 7.6 nymphs/hour (range 0-60, standard deviation 12.7) in 2012 (Table 1). In 2011 at the CT phenology sites, peak nymphal activity was detected the second week of June with an average of 414 nymphs/hour (range 0-981, standard deviation 239.01) (Figure 1). In 2012, peak activity was detected the first week of June with an average of 113 nymphs/hour (range 0-276, standard deviation 87.06).

The overall nymphal infection rate for *B. burgdorferi* in 2011 was 18.5%, with site-specific infection rates ranging from 16.4% (CT) to 23.2% (NY). In 2012, the overall nymphal infection rate was 15.3%, with site-specific infection rates ranging from 9.3% (CT) to 23.1% (NY) (Table 1). The overall rates were not significantly different by study year at the 95% confidence level ($Z=1$, $p=0.33$). *Anaplasma phagocytophilum* was detected from at least one sampling location in each state, with infection rates of 1.5%-4.8% across all properties in a state. The *B. microti* infection rate ranged from 5.9% in CT in 2011 to 15.4% in NY in 2012. *B. microti* was not detected in MD nymphs. Two nymphs collected in NY in 2012 were determined to be infected with a non-Lyme *Borrelia* genospecies, one of which was co-infected with *B. burgdorferi*.

Co-infections with *B. burgdorferi* and *B. microti* were identified in 22 nymphs collected in CT and NY. One CT nymph was coinfecting with *B. burgdorferi* and *A. phagocytophilum*. Overall, approximately one half to two thirds of *B. microti*-infected nymphs were co-infected with *B. burgdorferi* and approximately one quarter of *B. burgdorferi*-infected nymphs were co-infected with *B. microti*.

We report nymphal tick densities and infection rates from residential properties in endemic regions of CT, MD, and NY, reflecting actual potential for peridomestic exposure. The overall nymphal density for residential properties in 2011 was much greater than that in 2012, and this finding was echoed in the densities determined at the phenology sites in CT. Nymphal densities are known to vary by year of collection, even at the same study site, and this has been attributed to the variability of host populations (e.g., deer densities) and climatic conditions (Eisen et al. 2004). Despite the difference in nymphal densities, the overall *B. burgdorferi* infection rates for 2011 and 2012 were not significantly different and are consistent with previously published reports of both peridomestic and non-peridomestic infection rates (Falco and Fish 1988, Maupin et al. 1991, Barbour et al. 2009, Diuk-Wasser et al. 2012). Similarly, the infection rates of *A. phagocytophilum* and *B. microti* are consistent with previous findings (Aliota et al. 2014, Keesing et al 2014, Krause et al. 2014). Our data demonstrate not only a high variability in tick abundances and pathogen infection rates between residential properties, but also between years. Most previous findings have focused on non-residential properties, and therefore our findings increase the existing knowledge of infection rates on the fragmented landscapes of residential properties, where homeowners are most likely to encounter ticks.

Previous reports have suggested risk of infection as a function of tick density and tick infection prevalence (Connally et al. 2006, Nicholson and Mather 1996, Pepin et al. 2012). However, the high variability in tick abundance at a residential level, as in this study, makes predictions based on entomological factors alone complicated (Pardanani and Mather 2004). Furthermore, as properties were sampled a single time over many weeks each year, it is

possible that the true picture of tick abundance at each location is not represented in our findings, given the seasonal activity of this species of tick. Our findings were potentially influenced by changing day-to-day climatic and other environmental conditions. Nonetheless, nymphal blacklegged ticks were present on approximately 60% of all control properties sampled. Given the likelihood of exposure to ticks in these endemic areas, the prevalence of pathogens found in *I. scapularis* nymphs suggests peridomestic risk, not only for Lyme disease, but also for anaplasmosis, babesiosis, and other emerging pathogens.

The nymphal coinfection rates that we detected are also consistent with previous reports (Swanson et al. 2006, Hersh et al. 2014) although the proportion of *B. microti*-infected nymphs coinfecting with *B. burgdorferi* is striking. The coinfection rates underscore the need to educate healthcare providers to consider coinfections when patients present with tick exposure or likely tickborne disease. Our findings should also serve as a reminder to clinicians to consider emerging tickborne diseases, such as non-Lyme *Borrelia* infections, including *B. miyamotoi*, when patients with likely tick exposure but a clinical presentation (such as relapsing fever) different from common tickborne diseases.

The data reported in this paper provide additional entomological evidence for peridomestic risk for tickborne diseases in highly endemic regions. However, it is yet unclear how tick abundance and infection rates correlate with human disease outcomes. Additional investigation, including consideration of social and recreational behaviors and high-risk, high-use areas of the peridomestic environment is warranted to determine how these entomologic findings correlate with risk of human disease.

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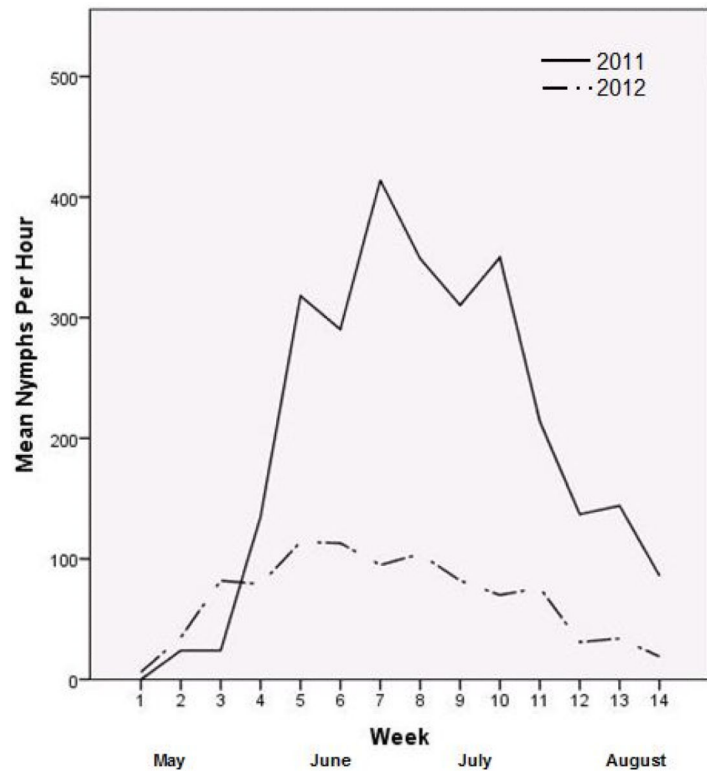


Figure 1.
Ixodes scapularis nymphal activity, Fairfield County, Connecticut, 2011-2012.

Ixodes scapularis nymphal densities and infection rates by study site and year, Connecticut, Maryland, and New York, 2011–2012.

Table 1

2011													
Site	<i>I. scapularis</i> nymphal densities ¹				<i>I. scapularis</i> nymphal pathogen infection rates ²				<i>I. scapularis</i> nymphal pathogen coinfection rates				
	Collection Dates	Number of properties	Avg nymphs per hr	Std dev nymphs per hr	Number of properties	<i>B. burg</i> infection rate	<i>A. phag</i> infection rate	<i>B. micro</i> infection rate	Number <i>B. burg</i> / <i>B. micro</i> coinfectd	<i>B. burg</i> / <i>B. micro</i> coinf. rate	Proportion <i>B. burg</i> / <i>B. micro</i> of all <i>B. burg</i>	<i>B. burg</i> / <i>A. phag</i> / <i>B. micro</i> coinf. rate	
CT	6/15-7/20	25	46.1	45.7	50	16.4%	3.0%	5.9%	11	2.5%	42.3%	15.3%	0.0%
MD	6/20-7/1	13	22.0	32.2	29	20.6%	1.5%	0.0%	0	0.0%	n/a	n/a	0.0%
NY	6/21-7/28	29	13.6	21.8	58	23.2%	4.8%	7.1%	8	4.8%	66.7%	20.5%	0.0%
All sites		67	27.4	37.0	137	18.5%			19				

2012													
Site	<i>I. scapularis</i> nymphal densities ¹				<i>I. scapularis</i> nymphal pathogen infection rates ²				<i>I. scapularis</i> nymphal pathogen coinfection rates				
	Collection Dates	Number of properties	Avg nymphs per hr	Std dev nymphs per hr	Number of properties	<i>B. burg</i> infection rate	<i>A. phag</i> infection rate	<i>B. micro</i> infection rate	Number <i>B. burg</i> / <i>B. micro</i> coinfectd	<i>B. burg</i> / <i>B. micro</i> coinf. rate	Proportion <i>B. burg</i> / <i>B. micro</i> of all <i>B. burg</i>	<i>B. burg</i> / <i>A. phag</i> / <i>B. micro</i> coinf. rate	
CT	5/23-6/22	32	5.1	8.1	64	9.3%	4.0%	0.0%	0	0.0%	n/a	n/a	1.3%
MD	5/16-6/7	9	10.2	12.8	20	17.9%	0.0%	0.0%	0	0.0%	n/a	n/a	0.0%
NY	6/6-7/6	24	10.0	16.9	46	23.1%	0.0%	15.4%	3	7.7%	50%	33%	0.0%
All sites		65	7.6	12.7	130	15.3%			3				

¹ Nymphal density was calculated for placebo properties only.

² Pathogen infection rates were calculated for all tested nymphs, regardless of treatment group.