Scientific Note

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 m^2 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