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Evaluating acarological risk for exposure to *Ixodes scapularis* and *Ixodes scapularis*-borne pathogens in recreational and residential settings in Washington County, Minnesota

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ABSTRACT

The distribution of *I. scapularis*, the tick vector of the bacteria that cause Lyme disease, has been expanding over the last two decades in the north-central United States in parallel with increasing incidence of human cases of Lyme disease in that region. However, assessments of residential risk for exposure to ticks are lacking from this region. Here, we measured the density of host-seeking *I. scapularis* nymphs in two suburban and two rural public recreational sites located in Washington County, Minnesota as well as in nearby residential properties. We sought to compare tick densities across land use types and to identify environmental factors that might impact nymphal density. We also assessed the prevalence of infection in the collected ticks with Lyme disease spirochetes (*Borrelia burgdorferi* sensu stricto, *B. mayonii*), and other *I. scapularis*-borne pathogens including *B. miyamotoi*, *Babesia microti* and *Anaplasma phagocytophilum*. Similar to studies from the eastern United States, on residential properties, *I. scapularis* nymphal densities were highest in the ecotonal areas between the forest edge and the lawn. Residences with the highest densities of nymphs were more likely to have a higher percentage of forest cover, log piles, and signs of deer on their property. In recreational areas, we found the highest nymphal densities both in the wooded areas next to trails as well as on mowed trails. Among the 303 host-seeking *I. scapularis* nymphs tested for pathogens, *B. burgdorferi* sensu stricto, *A. phagocytophilum* and *B. miyamotoi* were detected in 42 (13.8%), 14 (4.6%), and 2 (0.6%) nymphs, respectively.

1. Introduction

Lyme disease is the most commonly reported vector-borne disease in the United States (Adams et al., 2014). In recent decades, counties reporting the presence of *Ixodes scapularis*, the primary vector of Lyme disease spirochetes (*Borrelia burgdorferi* sensu stricto and *B. mayonii*), and those classified as high incidence for Lyme disease have increased in number with the most notable expansion in the upper Midwest and in the Northeast (Eisen et al., 2016; Kugeler et al., 2015). Lyme disease prevention strategies have largely focused on 1) avoiding tick habitat, 2) reducing the risk of tick bites by using repellents on skin or clothing or wearing permethrin-treated clothing, 3) reducing the risk of tick-borne pathogen transmission through prompt detection and removal of ticks, and 4) reducing the abundance of infected ticks through landscape modification and/or use of chemical or biological controls on tick-questing substrates or hosts (Eisen and Dolan, 2016). Success of these interventions relies, in part, on knowledge of where humans and zoonotic hosts are most likely to encounter ticks.

A limited number of studies from the northeastern United States that assessed where humans are most likely to encounter *I. scapularis* nymphs and adults implicated peridomestic settings for the majority of exposures, but also noted the importance of exposure to ticks in recreational settings (Carroll et al., 1992; Falco and Fish, 1989, 1988; Maupin et al., 1991; Stafford and Magnarelli, 1993). In an effort to better target prevention efforts, several studies aimed to identify where host-seeking nymphs and adults are most abundant in residential settings. Overall, the highest numbers of host-seeking ticks were typically found in the woods and in ecotones comprised of woods and lawn and less commonly in lawns that were distant from woodlands (Carroll et al., 1992; Maupin et al., 1991; Stafford and Magnarelli, 1993).

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Studies assessing where humans frequently encounter ticks or the distribution of host-seeking *I. scapularis* nymphs on residential properties and in comparison to nearby recreational sites are lacking for the north-central United States (Kitron and Kazmierczak 1997).

In this study, we measured the density of host-seeking *I. scapularis* in two suburban and two rural public recreational sites located in Washington County, Minnesota as well as in nearby residential properties. We used a stratified sampling approach to assess the distribution of host-seeking nymphs by land use type (e.g. forest, ecotone, lawn, and ornamental), and we used observational surveys and remotely sensed land cover data to collect additional information about environmental factors that might impact nymphal density. Our goals were to 1) statistically compare the density of host-seeking nymphs between land use types and residential properties and to describe patterns in nymphal density within recreational areas, 2) identify environmental predictors of elevated host-seeking *I. scapularis* nymphal density on residential properties, and 3) report the prevalence in nymphs of Lyme disease spirochetes (*Borrelia burgdorferi* sensu stricto, *B. mayonii*), and other *I. scapularis*-borne pathogens including *B. miyamotoi*, *Babesia microti* and *Anaplasma phagocytophilum*.

2. Materials and methods

2.1. Study site

This study took place in Washington County, Minnesota, which lies on the eastern edge of the Twin Cities metropolitan area (Fig. 1). The county population was almost 252,000 in 2015. Approximately 56% of the county land area is devoted to agriculture, 20% is residential development, 11% is designated as parks or recreational areas, 10% is covered by fresh water, and less than 5% is commercial or industrial development (Minnesota Metropolitan Council, 2010). Washington County forests are dominated by aspen, birch, maple, basswood, and oak, but there are considerable mixed conifer forests that host a variety of pine species intermingled with deciduous canopy trees (Almendinger, 1989). The eastern and southern edges of the county are bounded by the St. Croix and Mississippi rivers, respectively. We selected Washington County for this study because *I. scapularis* is established in the area (Eisen et al., 2016), much of the county contains suitable habitat for the tick vector (Johnson et al., 2016), and there is a high incidence of Lyme disease cases (36 cases/100,000 population between 2008 and 2013 compared to 22 cases/100,000 for the state over the same time period) reported to the Minnesota Department of Health (MN Department of Health Vectorborne Disease Program, 2016; Robinson et al., 2015).

2.2. Recreational site selection

We selected four recreational areas in Washington County for sampling (Fig. 1). We created the sampling frame for the recreational sites by extracting the boundaries of parks, recreational, and preserve areas from the Metropolitan Council Generalized Land Use dataset (Minnesota Metropolitan Council, 2010) and selecting land units that contained more than 20% forested area based on the USGS National Land Cover dataset (Homer et al., 2015) using ArcGIS 10.3 (ESRI, Redlands, CA). Next, we overlaid these recreational areas on a map of census block human population density (U.S. Census Bureau, 2010). We selected two recreational sites that were in or near suburban areas of high human population density and two recreational sites that were surrounded by lower density, more rural areas. Recreational sites surrounded by high population density urban areas were excluded from the site selection because the majority of the residential households in these areas did not meet the selection criteria described below, in particular the yards were too small and there was not sufficient tree cover for tick sampling.

The two suburban recreational sites were Lake Elmo Park Reserve (LEPR) and Katherine Abbott Park (KAP). LEPR is 2165 acres with 80% of this area set aside for habitat preservation and restoration. The park contains prairie, wetlands, and tracts of mixed northern hardwoods, predominately oaks, elms, and maples (Washington County Parks and Open Spaces, 2010). KAP is a 76-acre community park comprised mostly of mixed oak forests, grassland, and several wetlands (City of Mahtomedi, 2013). The two rural recreational sites were William O'Brien State Park (WOSP) and St. Croix Bluffs Regional Park (SCBRP). WOSP is located on the St. Croix National Scenic Riverway, and is approximately 2200 acres (MN Department of Natural Resources, 2008). The land cover ranges from prairie, savanna, and wetlands to hardwood and floodplain forests. Prescribed burns and periodic flooding of the St. Croix River affect the plant communities in the park. SCBRP is 579 acres, bounded in the east by the St. Croix River, and contains upland prairies, mixed-conifer forests, and forested bluffs that descend to the river shoreline.

The tick sampling location in each recreational site was selected through discussion with the Minnesota Department of Health and the park management staff to identify a high-use trail near a forested area. Prior to field work, we used GoogleEarth to identify sampling transects in three land use types at each recreational site: on trail, next to trail (e.g., the 1 m wide drag is placed on the outside edge of the trail), and > 10 m off trail (in adjacent woods).

2.3. Residential site recruitment

After selecting the recreational sites, we overlaid the MetroGIS Regional Parcel Dataset (MetroGIS, 2016) and selected all parcels zoned as 1-unit residential properties within 5 km of the recreational sites. We retained parcels from 1 to 5 acres that contained some forest based on the USGS National Land Cover dataset (Homer et al., 2015) to ensure sufficient forested area for sampling. We extracted mailing addresses for all parcels that met these selection criteria and mailed a letter of invitation to participate in the study.

After a three week recruitment period, we mapped the location of households who responded to the letter and decided to focus our residential study around LEPR and WOSP. From those who responded to the recruitment letter and were located within 5 km of one of these recreational sites, we randomly selected 12 households for a total of 24 residential properties. Each selected household was contacted via telephone to schedule property visits and delineate property boundaries. Prior to field work, we identified four land use types on each property using GoogleEarth and Washington County aerial imagery (Washington County Public Works, 2014): forest (closed-canopy forest with leaf litter), ecotone (up to 5 m on either side of the intersection of forest with lawn), lawn (maintained cultivated grasses > 5 m from forest edge), and ornamental (area < 1 m from annual and perennial ground cover, flower gardens, shrubs, and hedges below chest height).

2.4. Tick sampling and observational surveys

We drag sampled all recreational sites twice and all residential sites once between 31 May and 20 June 2016. We chose these dates to coincide with expected peak *I. scapularis* nymphal activity based on previous phenological tick sampling conducted by the Minnesota Department of Health and on a time-lagged peak occurrence of reported Lyme disease cases in the state. We sampled for ticks by dragging a 1-m^2 cloth made of rubber-bonded cotton fabric with a rope attached to a 48" dowel inside the top edge. Weighted "fingers" were sewn to the bottom half of the drag to ensure sampling occurred near the ground. We dragged up to 750 m^2 in each land use type at the recreational sites and on each of the residential properties. If there was not 750 m^2 of area available in a particular land use type on a residential property, the entire land use type was sampled. Every 15 m, samplers stopped and removed all ticks from themselves and the drag to minimize the likelihood of ticks falling off the drag before being collected. Ticks were

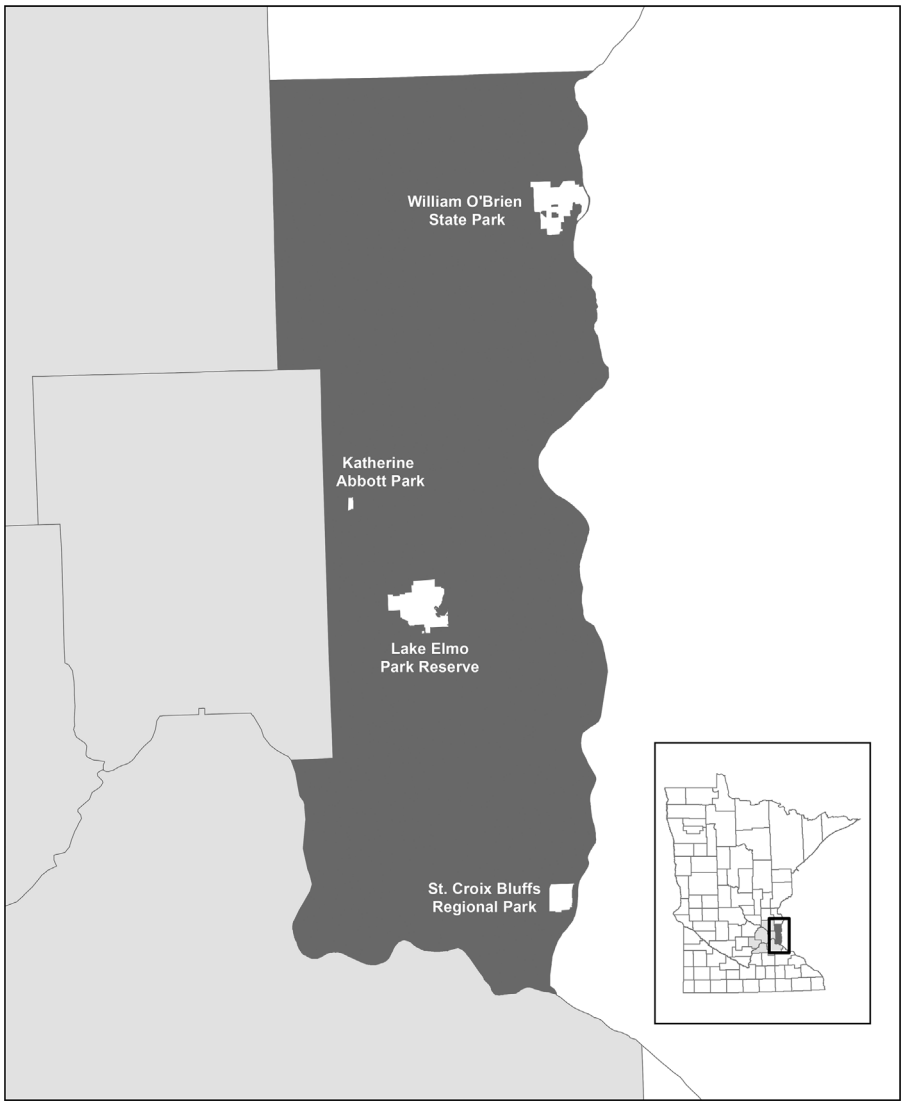

Fig. 1. Map of study location. Inset: Minneapolis/St. Paul metropolitan counties are shown light gray, and Washington County is shown in dark gray. Primary figure: Washington County is shown in dark gray, and the four public recreational sampling sites are shown in white.

placed in pre-labeled vials of 70% ethanol. All ticks were identified to species at the Centers for Disease Control and Prevention, Fort Collins, CO. Ticks were then stored in 70% ethanol at −20 °C pending DNA extraction.

In addition to tick sampling, we collected basic weather data and completed an observational survey at each sampling location to collect information on landscape features that may influence the density of ticks. We collected temperature, humidity, and wind speed at the time of tick dragging, and noted the presence of fences, compost piles, log/brush piles, bird and squirrel feeders, and signs of deer or rodents.

2.5. Pathogen detection in ticks

We homogenized individual *I. scapularis* nymphs in a lysis mix comprised of buffer ATL, proteinase K, and DX anti-foaming reagent (Qiagen, Valencia, CA, USA), and we extracted DNA from each homogenate using the QIAcube HT automated nucleic acid system and the *cador* Pathogen 96 QIAcube HT Kit (Qiagen) as described elsewhere (Graham et al., In Review). We then tested each sample for five *Ixodes scapularis*-borne pathogens using the testing algorithm detailed in Graham et al. (in press). Briefly, we employed a pair of multiplex, probe-based real-time polymerase chain reaction (PCR) assays to detect *A. phagocytophilum*, *Ba. microti*, and *Borrelia*. The paired assays included two targets for each pathogen and a tick actin target to verify the integrity of each DNA sample. We subsequently tested all *Borrelia*-positive extracts using three additional probe-based real-time PCR assays comprising species-specific targets to detect and differentiate *B. burgdorferi* sensu stricto, *B. mayonii* and *B. miyamotoi*.

2.6. Statistical analysis

2.6.1. Deriving tick density

We calculated the density of each tick species and life stage by both site and by land use by dividing the number of ticks collected by the area sampled in each residential and recreational site or each land use in a site. We present results as ticks collected per 100 m^2. Tick density values were log transformed to achieve normality. All statistical analyses were conducted using SAS 9.3 (SAS Institute Inc., Cary, NC).

2.6.2. Association between land use type and density of I. scapularis nymphs within residential properties

Mixed effects analysis of variance (ANOVA) was conducted to compare tick densities between land uses within residential sites. A random effect for residential site was included to group the observed tick densities across land uses within a residential site and because we wanted to make inferences beyond the sites sampled in this study. Pairwise comparisons were conducted using Tukey post-hoc analyses and p-values less than 0.05 were considered statistically significant.

Table 1
Total density of *I. scapularis* (ticks/100 m^2) by life stage and land use in recreational sites (4500 m^2 land area sampled at each recreational site over two visits; 2250 m^2 per visit per site).

Recreational Site	Nymphs			Adults		
	Trail	Next to Trail	Off Trail	Trail	Next to Trail	Off Trail
LEPR[a]	0.1	0.2	0.7	0.3	0.1	0.1
KAP[a]	0.3	0	0.3	0.1	0	0.4
WOSP[a]	0.1	0	0.1	0.4	0.1	1.2
SCBRP[a]	0.7	0.1	0.4	0	0	0.1
Median	0.2	0.1	0.4	0.2	0.1	0.3

[a] LEPR = Lake Elmo Park Reserve; KAP = Katherine Abbott Park; WOSP = William O'Brien State Park; SCBRP = St. Croix Bluffs Regional Park.

2.6.3. Calculating yard-level variables and landscape metrics for residential properties

We created indicator variables for the presence of yard-level factors that may affect tick density from the data collected on the observational surveys at residential sites. Land cover data were obtained from the USGS National Land Cover dataset (Homer et al., 2015). We used the MetroGIS Regional Parcel Dataset (MetroGIS, 2016) to calculate the percent forest and forest edge density within 500 m and 1 km of the property boundary using ArcGIS 10.3 (ESRI, Redlands, CA) and the Patch Analyst extension.

2.6.4. Association between environmental risk factors and the density of host-seeking I. scapularis nymphs on residential properties

We used a Poisson model in a generalized estimating equation (GEE) framework to assess associations between *I. scapularis* nymphal abundance and the presence of yard-level variables and landscape metrics in and around each residential property. We included an offset for the number of drags completed on a residential property to standardize for sampling effort. We also included a variable to indicate the neighborhood of each residential property (e.g. near either LEPR or WOSP recreational area) using an exchangeable correlation structure to account for covariance within neighborhoods.

We began with univariate models and plots of *I. scapularis* nymphal density and the environmental variables to look for outliers. Next, pairwise correlations of continuous environmental variables found to be significantly associated with the density of nymphs on a property in univariate models were assessed and only variables with a Pearson correlation coefficient < 0.80 were included in the same multivariate model. For the multivariate analysis, we used backwards stepwise variable selection, dropping predictors with a p-value > 0.05. The logarithm of the area of a property was retained in all models to control for confounding that may occur if larger properties tend to have more forested area and are associated with tick density due to another factor, such as having more unmaintained area on the property that is conducive for wildlife.

3. Results

3.1. Residential and recreational site enrollment

We mailed 1607 letters to eligible households in Washington County. We received responses from 224 (response rate: 14%) individuals: 100 from residences within 5 km of LEPR, 44 from residences within 5 km of WOSP, 53 from residences within 5 km of KAP, and 27 from residences within 5 km of SCBRP. Based on the number of responses per location, we selected LEPR as the suburban recreational site and WOSP as the rural recreational site. The majority of responses (71%) were received within two weeks of mailing the recruitment letter, and we stopped taking responses after a three week recruitment period.

3.2. Recreational site results

Over two sampling sessions, we sampled 4500 m^2 of land area (2250 m^2 each visit) in each recreational site and collected a total of 45 nymphal, 44 adult, and 1 larval *I. scapularis*. We collected *I. scapularis* nymphs (median: 11.5, range: 3–19 nymphs per site over two visits) and adults (median: 8, range: 2–26 adults per site over two visits) at all four recreational sites, and only 1 larva from one site (LEPR). On average, the peak density observed (at either site visit) of each *I. scapularis* life stage was 0.4 nymphs/100 m^2 (range: 0.1–0.6), 0.4 adults/100 m^2 (range: 0–0.8), and 0 larvae/100 m^2. On average across recreational sites over two sampling sessions, the highest densities of *I. scapularis* nymphs and adults were observed in wooded areas off trail; however, we collected *I. scapularis* nymphs and adults in all three land use types that were sampled, including on trails and immediately adjacent to trails (Table 1). Assessing sites individually, it is evident that some sites had similar or higher densities of *I. scapularis* nymphs or adults on the trail in comparison to the wooded areas > 10 m off trail. Across recreational sites, the lowest densities of *I. scapularis* nymphs and adults were encountered consistently in the area next to the trail.

We found a median of 14.5 (range: 1–30) adult *D. variabilis* across recreational sites. On average, the peak density observed (at either site visit) of *D. variabilis* adults was 0.5 ticks/100 m^2 (range: 0–1.2). In contrast to *I. scapularis*, we found the lowest adult *D. variabilis* densities in the forested areas off trail (median: 0.1, range: 0–0.2 ticks/100 m^2) and the highest density on the trail (median: 0.5, range: 0.1–1.1 ticks/100 m^2).

3.3. Residential site results

We collected a total of 209 nymphal, 74 adult, and 61 larval *I. scapularis* on residential properties. The mean density of each *I. scapularis* life stage across residential properties was 0.5 nymphs/100 m^2 (range: 0–4.4), 0.2 adults/100 m^2 (range: 0–1.2), and 0.1 larvae/100 m^2 (range: 0–1.8). The mean density of *D. variabilis* adults was 0.1 ticks/100 m^2 (range: 0-0.4).

3.4. Association between land use type and density of I. scapularis nymphs within residential properties

The mean density of host-seeking *I. scapularis* nymphs was significantly higher in the ecotone (0.8 ticks/100 m^2) compared to the lawn (0.1 ticks/100 m^2) or ornamental land cover (0.1 ticks/100 m^2) ($F(3,91) = 7.31$, $p < 0.001$). The mean nymphal density was not significantly different in the forest (0.6 ticks/100 m^2) compared to the ecotone, lawn, or ornamental land uses, and there were no significant differences in density of *I. scapularis* adults or larvae across residential land uses. The mean density of adult *D. variabilis* was significantly higher in the forest (0.2 ticks/100 m^2) compared to the ecotone (0 ticks/100 m^2), lawn (0 ticks/100 m^2), or ornamental areas (0 ticks/100 m^2) ($F(3,91) = 6.92$, $p < 0.001$).

3.5. Association between environmental risk factors and the density of I. scapularis nymphs between residential properties

There was considerable variability in the total *I. scapularis* nymphal density across residential sites (Fig. 2). We collected at least one *I. scapularis* nymph at 20 of 24 sites (83%). The median density of *I. scapularis* nymphs on residential sites was 0.3 nymphs/100 m^2. There were 6 sites with between 0.5 and 1.1 nymphs/100 m^2, and one site with 4.4 nymphs/100 m^2.

The predominant forest type on the majority of residential sites was deciduous forest (n = 16, 67%), but the forest on 7 (30%) residential sites was predominately mixed deciduous/conifer, and the forest on one site had only coniferous trees (Table 2). Residential properties contained approximately 49% forest cover, on average, and ranged from 11

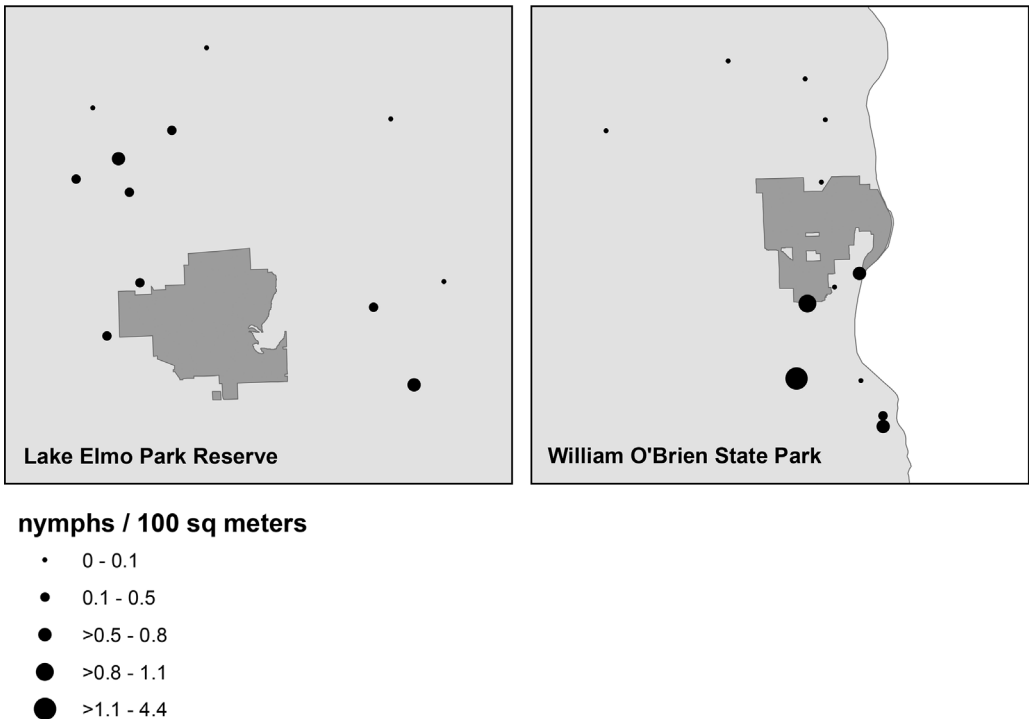

Fig. 2. Geographic variation in the density of host-seeking *I. scapularis* nymphs (per 100 m^2) found in the 24 residential properties around Lake Elmo Park Reserve (suburban) and William O'Brien State Park (rural). Dark grey polygons show the park boundaries.a.

Table 2
Environmental characteristics of the sampled residential properties.

Environmental variables	Mean ± SD	Range
Continuous variables		
Percent forest within residential parcel	49 ± 26	11–100
Percent forest within 500 m of residential parcel	32 ± 13	9–59
Percent forest within 1 km of residential parcel	27 ± 13	8–55
Forest edge density within 500 m of residential parcel (m/hectare)	105 ± 22	57–140
Forest edge density within 1 km of residential parcel (m/hectare)	85 ± 21	44–119
Total property area (m^2)	11,222 ± 5725	4076–26,019

Dichotomous variables	Percent of residences (n)
Signs of deer on property	88 (21)
Presence of fence on property	21 (5)
Compost pile on property	50 (12)
Log pile on property	92 (22)
Bird/squirrel feeder on property	67 (16)
Dominant forest type	
Deciduous	67 (16)
Coniferous	4 (1)
Mixed deciduous/conifer	30 (7)

up to nearly 100% forest cover. Within a 1 km buffer of the sampled properties, forests accounted for approximately 27% (range: 8–55%) of the land cover, on average. The mean edge density of the landscapes in the same area was 85 m/hectare (range: 44–119 m/hectare). We noted obvious signs of deer (e.g. game trails, bedding areas) at 88% (n = 21) of residences, but only 21% (n = 5) had a fence on their property. Half of residences (n = 12) had a compost pile on their property, and 92% (n = 22) of residences had log piles. Bird or squirrel feeders were present on 67% (n = 16) of properties.

The residential site with the highest nymphal density and the residential site with exclusively coniferous forest were identified as outliers that may affect the nymphal density models. Removing the site with the coniferous forest did not substantially change the results of the univariate models; however, the relationships between the environmental risk factors and the density of *I. scapularis* nymphs were substantially different after removing the site with the highest nymphal density (Table 3). The percent forest cover within 500 m and 1 km of a property and forest edge density within 500 m and 1 km of a property were highly correlated so only the percent forest cover within 1 km of a property was used in further analyses. Signs of deer on a property and presence of a log pile were associated with having a higher density of *I. scapularis* nymphs on a property in univariate models.

Based on the results of the univariate models which showed that the site with high nymphal density substantially affected the statistical relationship between several environmental risk factors and *I. scapularis* nymphal density, the multivariate model was built without this outlier. Overall, our multivariable model (Table 4) showed a 4-fold increase in the risk of encountering a host-seeking *I. scapularis* nymph on properties where there were evident signs of deer compared to those properties where there were no signs of deer ($p < 0.0001$). Similarly, the presence of a log pile on a property increased the risk of encountering a host-seeking *I. scapularis* nymph by 12-fold compared to properties without log piles ($p < 0.0001$). For every 10% increase in the amount of forested area on a property, the risk of encountering a host-seeking *I. scapularis* nymph increased 1.5-fold after controlling for the size of a property.

3.6. Pathogen testing in I. scapularis

In order to increase the sample size for pathogen detection, in addition to the 254 *I. scapularis* nymphs and 118 adults collected from transects on residential and recreational properties, an additional 49 nymphs and 8 adults were collected from these sites after the final transect sampling was completed. Among the 303 *I. scapularis* nymphs tested from all sites combined, 42 (13.9%) were infected with *B. burgdorferi* sensu stricto, 14 (4.6%) were infected with *A. phagocytophilum*, and 2 (0.6%) were infected with *B. miyamotoi*. Of the 126 *I. scapularis* adults tested, 40 (31.7%) were infected with *B. burgdorferi* sensu stricto, 7 (5.6%) were infected with *A. phagocytophilum*, and 3 (2.4%) were infected with *B. miyamotoi*. Regardless of life stage, we did not detect *B. mayonii* or *Ba. microti* in any ticks. *Ixodes scapularis* infected with *B. burgdorferi* sensu stricto were found on all four recreational sites and 13 out of 24 (54%) residential properties sampled. Likewise, ticks that

Table 3
Association between environmental predictors and the density of *I. scapularis* nymphs on residential properties in univariate Poisson models using all observations and after removing the two outlier residential sites[a] one at a time to test the sensitivity of model[b].

	Unit of increase	Using all observations (n = 24)		Removing conifer property (n = 23)		Removing high density *I. scapularis* nymph property (n = 23)	
Environmental variable		RR[c]	p-value	RR	p-value	RR	p-value
Percent forest within residential parcel	10%	1.2	< 0.001[d]	1.2	< 0.001[d]	1.0	0.67
Percent forest within 500 m of residential parcel	10%	1.5	< 0.0001[d]	1.5	< 0.0001[d]	0.9	< 0.0001[d]
Percent forest within 1 km of residential parcel	10%	1.4	< 0.0001[d]	1.4	< 0.0001[d]	0.9	< 0.0001[d]
Forest edge density within 500 m of residential parcel	10 m/m^2	1.2	< 0.0001[d]	1.2	< 0.001[d]	0.9	< 0.001[d]
Forest edge density within 1 km of residential parcel	10 m/m^2	1.2	< 0.0001[d]	1.2	< 0.0001[d]	0.9	< 0.001[d]
Signs of deer on property	Presence	3.3	0.12	3.8	0.09	6.3	< 0.001[d]
Presence of fence on property	Presence	1.4	0.01	1.4	0.06	2.0	0.22
Compost pile on property	Presence	0.3	< 0.0001[d]	0.3	< 0.0001[d]	0.9	0.08
Log pile on property	Presence	4.2	0.10	4.8	0.08	14.3	< 0.001[d]
Bird feeder on property	Presence	0.4	0.29	0.4	0.27	0.8	0.02
Forest type (versus deciduous forest)							
Coniferous forest	Presence	1.0	0.98	–	–	1.8	0.35
Mixed deciduous/coniferous forest	Presence	3.9	< 0.01[d]	4.2	< 0.0001[d]	0.8	0.42

[a] Two outlier residential sites that were tested are: (1) property with coniferous forest and 2) property with high *I. scapularis* nymphal density.
[b] Offset variable included for number of drags completed on a residential property in order to account for sampling effort. Log (area of the property in km^2) included as a fixed effect to account for differences in property size. Variable to indicate whether a residential property was located near Lake Elmo Park Reserve or William O'Brien State Park was included to account for correlation among households within a neighborhood.
[c] RR = Risk Ratio.
[d] Significant at α = 0.05.

Table 4
Association between environmental predictors and the density of *I. scapularis* nymphs on residential properties in a multivariate Poisson model[a].

Environmental variable	Unit of increase	RR[b]	95% CI	p-value
Percent forest on property	10%	1.5	(1.3, 1.7)	< 0.0001[c]
Signs of deer on property	Presence	4.2	(2.7, 6.8)	< 0.0001[c]
Log pile on property	Presence	12.0	(7.3, 19.7)	< 0.0001[c]

[a] Offset variable included for number of drags completed on a residential property in order to account for sampling effort. Log (area of the property in km^2) included as a fixed effect to account for differences in property size. Variable to indicate whether a residential property was located near Lake Elmo Park Reserve or William O'Brien State Park was included to account for correlation among households within a neighborhood.
[b] RR = Risk Ratio.
[c] Significant at α = 0.05.

tested positive for *B. miyamotoi* and *A. phagocytophilum* were found on both residential and recreational sites. Accounting for numbers of ticks tested by using maximum likelihood, we estimate that 20.8% (95% CI: 16.9–25.2) of all *I. scapularis* tested positive for *B. burgdorferi* sensu stricto, whereas 15.1% (95% CI: 11.1–19.9) of the *I. scapularis* nymphs and 33.1% (95% CI: 25.0–41.9) of the *I. scapularis* adults were *B. burgdorferi* sensu stricto positive. Approximately 24.4% (95% CI: 13.6–38.4) of nymphs and 47.7% (95% CI: 33.4–62.3) of adults tested from recreational sites were infected with *B. burgdorferi* sensu stricto (Tables 5 and 6). The prevalence of *B. burgdorferi* sensu stricto in nymphs and adults collected in residential sites was approximately half the prevalence in recreational sites, at 13.0% (95% CI: 9.0–18.1) and 24.3% (95% CI: 15.6–35.0), respectively (Tables 5 and 6).

4. Discussion

We found all three life stages of *I. scapularis* on residential properties as well as public recreational land in Washington County, Minnesota. Ticks infected with *B. burgdorferi* sensu stricto, *B. miyamotoi*, and *A. phagocytophilum* were found on both residential and recreational sites. Our study provides data to support prevention messages that emphasize the importance of personal protection against tick bites when spending time outdoors in forested recreational and residential settings when ticks are actively seeking hosts. Our modeling results suggest that clearing out log piles or other brush that may provide habitat for tick hosts and preventing deer from entering residential properties may decrease the abundance of ticks in yards.

Although we found *I. scapularis* nymphs in all of the land use types that we sampled on residential properties, assuming equal time spent among land use classes, the risk of encountering an *I. scapularis* nymph was highest in the ecotone between forested areas and the lawn. Our results are consistent with studies of *I. scapularis* in the northeastern United States where others have reported a higher abundance of *I. scapularis* nymphs in lawns adjacent to woods than in lawns adjacent to other lawns (Carroll et al., 1992), decreasing nymphal abundance in

Table 5
Infection prevalence of bacterial pathogens in field-collected *I. scapularis* nymphs in recreational and residential sites in Washington County, Minnesota shown as percent prevalence (95% confidence interval) based on the maximum likelihood estimator.

Sampling site (Number of ticks tested)	*Borrelia burgdorferi* sensu stricto	*B. mayonii*	*B. miyamotoi*	*Anaplasma phagocytophilum*	*Babesia microti*
RECREATIONAL (45)	24.4 (13.6–38.4)	0 (0–7.9)	2.2 (0.1–10.2)	2.2 (0.1–10.2)	0 (0–7.9)
RESIDENTIAL (207)	13.0 (9.0–18.1)	0 (0–1.8)	0.5 (0–2.3)	5.8 (3.2–9.6)	0 (0–1.8)
LEPR[a] (64)	15.6 (8.3–26.0)	0 (0–5.7)	0.0 (0.0–5.7)	1.6 (0.1–7.3)	0 (0–5.7)
WOSP[a] (143)	11.9 (7.3–18.0)	0 (0–2.6)	0.7 (0–3.3)	7.7 (4.1–12.9)	0 (0–2.6)
OVERALL (252)	15.1 (11.1–19.9)	0 (0–1.5)	0.8 (0.1–2.6)	5.2 (2.9–8.4)	0 (0–1.5)

[a] LEPR = Residential sites located within 5 km of Lake Elmo Park Reserve; WOSP = Residential sites located within 5 km of William O'Brien State Park.

Table 6
Infection prevalence of bacterial pathogens in field-collected *I. scapularis* adults in recreational and residential sites in Washington County, Minnesota shown as percent prevalence (95% confidence interval) based on the maximum likelihood estimator.

Sampling site (Number of ticks tested)	*Borrelia burgdorferi* sensu stricto	*B. mayonii*	*B. miyamotoi*	*Anaplasma phagocytophilum*	*Babesia microti*
RECREATIONAL (44)	47.7 (33.4–62.3)	0 (0–8.0)	6.8 (1.8–17.3)	9.1 (3.0–20.3)	0 (0–8.0)
RESIDENTIAL (74)	24.3 (15.6–35.0)	0 (0–4.9)	0 (0–4.9)	4.1 (1.1–10.5)	0 (0–4.9)
LEPR[a] (12)	16.7 (3.1–44.3)	0 (0–24.2)	0 (0–24.2)	8.3 (0.5–33.8)	0 (0–24.2)
WOSP[a] (62)	25.8 (16.1–37.7)	0 (0–5.8)	0 (0–5.8)	3.2 (0.6–10.1)	0 (0–5.8)
OVERALL (118)	33.1 (25.0–41.9)	0 (0–3.2)	2.6 (0.7–6.7)	5.9 (2.7–11.3)	0 (0–3.2)

[a] LEPR = Residential sites located within 5 km of Lake Elmo Park Reserve; WOSP = Residential sites located within 5 km of William O'Brien State Park.

lawns as the distance to woods increased (Carroll et al., 1992), or higher densities of *I. scapularis* in ecotone or forest habitat compared to lawn and ornamental vegetation (Dister et al., 1997; Frank et al., 1998; Maupin et al., 1991; Stafford and Magnarelli, 1993). Ticks are likely to be abundant in forest and forest edge habitat due to the movement of animal hosts as well as an increased likelihood of survival after falling off their hosts due to the availability of shade and leaf litter that can provide protection from desiccation (Schulze et al., 1995).

Despite the close proximity among residences sampled in this study, there was substantial variation in the *I. scapularis* density encountered among the properties. Although studies have shown spatial patterns in tick abundance at the regional and state scale (Bunnell et al., 2003; Kitron and Kazmierczak, 1997), it is evident that in many cases, there is considerable spatial variation in the abundance of *Ixodes* spp. nymphs at the community scale as well (Pardanani and Mather, 2004). In an effort to identify environmental risk factors that account for this variation in tick abundance, we found that indicators of deer presence (e.g. game trails, droppings, seeing a deer), presence of a log pile on the property, and a higher percentage of forest cover on a property were associated with elevated nymphal density. Others have found high nymphal abundance near stone walls on residential properties, particularly those near wooded habitats (Frank et al., 1998; Stafford and Magnarelli, 1993). In an assessment of high risk behaviors for exposure in a hardwood forest to *Ixodes pacificus*, a closely related species to *I. scapularis* that serves as the primary vector of Lyme disease spirochetes in the far-western United States, study subjects acquired the highest number of nymphs while sitting on a log compared to gathering wood, leaning on a tree, walking, or sitting on leaf litter (Lane et al., 2004). Like stone walls, a log pile on residential property may provide habitat for small mammals hosting ticks, particularly if it is an area that homeowners neglect to mow or maintain.

We found a slight increase in the density of host-seeking *I. scapularis* nymphs on a residential property as the percentage of forest on the property increased. Although the importance of forest cover for the survival of these woodland-associated ticks is well documented (Killilea et al., 2008), it is not surprising that we observed only a small increase in risk as the amount of forest cover on a property increased. One of the selection criteria for the residential properties included in this study was the presence of forest within the property boundaries; therefore, all of the properties sampled had substantial wooded areas that were dragged for ticks. As reported above, the highest density of nymphs was collected in the ecotone between the forest and lawn, regardless of the amount of forested area on the property, and we found nymphal ticks on the majority of properties. Mather et al. (1996) reported a lack of association between the amount of forest cover and reported cases of Lyme disease when conducting a study in six Rhode Island towns selected because of the high percentage of forest cover. Although our findings suggest that having a higher percentage of forested area on a property increases the risk of encountering an *I. scapularis* nymph, it is likely that simply having forested area on a property is a good predictor of *I. scapularis* presence in this region.

In recreational areas, *I. scapularis* nymphs were not only in forested areas, but also on mowed trails and on the edge of trails that were next to forested areas. Another study that included tick collections in the vegetation next to a dirt road and in the wooded areas nearby observed that immature life stages of *I. pacificus* tended to occur in higher numbers in leaf litter and wooded areas off the road while adults were found in higher numbers directly next to the road (Clover and Lane, 1995). This is most likely because sub-adult ticks are more susceptible to desiccation than adults and therefore avoid areas exposed to sunlight (Hayes and Piesman, 2003). In the present study, statistical comparisons of tick abundance by land use type in recreational areas was not possible because of the low numbers of ticks collected and because only four recreational sites were sampled. However, partial shade was available on most of the trails we sampled, and frequent rainstorms during the sampling period may have provided enough humidity to support nymphs in areas unprotected by leaf litter.

We found *B. burgdorferi* sensu stricto infected *I. scapularis* nymphs, the life stage that presents the highest risk of transmitting Lyme disease bacteria to humans (Falco et al., 1996; Mead, 2015; Piesman et al., 1987; Spielman et al., 1985), at all four recreational sites and almost half of residential properties sampled. Overall, 15.1% (95% CI: 11.1–19.9) of *I. scapularis* nymphs tested positive for *B. burgdorferi* sensu stricto. In addition, 5.2% (95% CI: 2.9–8.4) of *I. scapularis* nymphs were infected with *A. phagocytophilum*. The median *I. scapularis* nymphal *B. burgdorferi* sensu stricto infection rate in a six city study in Rhode Island was 21.5% (range among cities: 0–35%) (Mather et al., 1996), and the overall *B. burgdorferi* sensu stricto infection rate from a 8-year longitudinal study in 10 residential backyards in Connecticut was 14.3% (range among years: 8.6–24.4%) (Stafford et al., 1998). From these studies we can see that the *B. burgdorferi* sensu stricto infection rate in the nymphs collected in this study was within the typical range for infection prevalence; however, infection prevalence can vary from year to year so these testing results represent a single-season snapshot of the potential risk for encountering infected black-legged ticks. Because of this variation and the small numbers of nymphs collected per property limiting precision in site-specific measures of infection prevalence, we focused primarily on the density of host-seeking nymphs, rather than the more conventional density of infected nymphs measure (Diuk-Wasser et al., 2012; Mather et al., 1996; Pepin et al., 2012), as an acarological risk measure. Nonetheless, by pooling infection data across the sampling region, we have demonstrated that *B. burgdorferi* sensu stricto, *A. phagocytophilum* and *Ba. microti* infect *I. scapularis* in Washington County, Minnesota.

Although the results of this study have identified areas within residential properties and recreational parks where people are more likely to encounter *I. scapularis* nymphs as well as environmental risk factors for having elevated nymphal density on a residential property, we need more detailed information on how people are spending time outdoors to estimate their true exposure risk. For example, although we collected the highest density of nymphs in the ecotone area, if people are spending the majority of their time outside in their lawn or working in their ornamental garden beds, even low densities of infected nymphs in these areas may present a disproportionate exposure risk. Future studies that incorporate time activity logs or GPS tracking of human movement within a backyard or in nearby recreational areas to create a time activity budget would add an important component to a more complete tick exposure risk assessment. While we collected ticks from residences

in both rural and suburban areas, the sample size for this pilot project was too small to draw conclusions regarding differences in nymphal density between the two neighborhoods. Future studies could use a similar sampling approach with a larger number of residences to assess the impact of human population density on tick density.

Although we selected the timing of our tick sampling to coincide with the expected peak host-seeking activity of *I. scapularis* nymphs based on previous phenology data from the Minnesota Department of Health, variation in environmental variables, particularly cumulative growing degree days (Moore et al., 2014) can shift this peak slightly from year to year. Although we sampled twice on recreational sites in order to account for this potential variation in peak density of host-seeking nymphs, it is possible that we would have encountered more or fewer ticks by shifting our sampling dates. It is unlikely that the distribution of the nymphs would have changed substantially, but we may have found more significant differences in the nymphal density among land use types. We did not observe any statistically significant differences in adult and larval density across land uses; however, both the timing and drag sampling methods used were optimized to collect *I. scapularis* nymphs. Adult *I. scapularis* are most active in Minnesota during the early spring and again during the fall (Minnesota Department of Health, 2003). Conducting tick sampling during these time periods would more than likely have produced higher numbers of adult ticks and therefore, more significant statistical findings with regard to their distribution within residential properties and recreational areas. Similarly, small mammal trapping would likely produce better estimates of larval density compared with drag sampling (Daniels and Fish, 1995).

To our knowledge, this was the first study to measure the density of *I. scapularis* on residential properties in the upper Midwest. As such, we chose to conduct a pilot study with a small sample size in order to test our recruitment methods, try our field sampling protocols, and collect enough data to test our hypotheses regarding community-scale tick distributions in one county in the north-central United States. The recruitment method using tax parcel data and mailed letters to community members was very successful, and we received a high response rate. We attribute much of this success to the partnership between federal, state, and local health departments that likely provided potential participants with both confidence and trust in the study.

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