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Inter-annual variation in prevalence of *Borrelia burgdorferi* sensu stricto and *Anaplasma phagocytophilum* in host-seeking *Ixodes scapularis* (Acari: Ixodidae) at long-term surveillance sites in the upper midwestern United States: Implications for public health practice

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Abstract

The geographic range of the blacklegged tick, *Ixodes scapularis*, and its associated human pathogens have expanded substantially over the past 20 years putting an increasing number of persons at risk for tick-borne diseases, particularly in the upper midwestern and northeastern

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None

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United States. Prevention and diagnosis of tick-borne diseases rely on an accurate understanding by the public and health care providers of when and where persons may be exposed to infected ticks. While tracking changes in the distribution of ticks and tick-borne pathogens provides fundamental information on risk for tick-borne diseases, metrics that incorporate prevalence of infection in ticks better characterize acarological risk. However, assessments of infection prevalence are more labor intensive and costly than simple measurements of tick or pathogen presence. Our objective was to examine whether data derived from repeated sampling at longitudinal sites substantially influences public health recommendations for Lyme disease and anaplasmosis prevention, or if more constrained sampling is sufficient. Here, we summarize inter-annual variability in prevalence of the agents of Lyme disease (*Borrelia burgdorferi* s.s.) and anaplasmosis (*Anaplasma phagocytophilum*) in host-seeking *I. scapularis* nymphs and adults at 28 longitudinal sampling sites in the Upper Midwestern US (Michigan, Minnesota, and Wisconsin). Infection prevalence was highly variable among sites and among years within sites. We conclude that monitoring infection prevalence in ticks aids in describing coarse acarological risk trends, but setting a fixed prevalence threshold for prevention or diagnostic decisions is not feasible given the observed variability and lack of temporal trends. Reducing repeated sampling of the same sites had minimal impact on regional (Upper Midwest) estimates of average infection prevalence; this information should be useful in allocating scarce public health resources for tick and tick-borne pathogen surveillance, prevention, and control activities.

Keywords

Tick surveillance; Tick-borne disease; *Ixodes scapularis* ; *Borrelia burgdorferi* ; *Anaplasma phagocytophilum*

Introduction

Tick-borne diseases are an increasing public health burden in the United States. Of the nearly 650,000 cases of vector-borne diseases reported in the United States from 2004 to 2016, more than 75% were tick-borne and a majority of those were associated with the blacklegged tick, *Ixodes scapularis* (Rosenberg et al., 2018). In addition to transmitting *Borrelia burgdorferi* sensu stricto, the primary causative agent of Lyme disease, which is the most commonly reported vector-borne disease in the United States, the tick also transmits a less common agent of Lyme disease (*Borrelia mayonii*) and agents of anaplasmosis (*Anaplasma phagocytophilum*), babesiosis (*Babesia microti*), hard tick relapsing fever (*Borrelia miyamotoi),* ehrlichiosis (*Ehrlichia muris eauclairensis*), and a viral neuroinvasive disease (Powassan virus) (Eisen and Eisen 2018). Although the reported geographic range of each pathogen varies across the tick's range, all have been identified in host-seeking *I. scapularis* in the upper midwestern United States (Johnson et al., 2018). In the past two decades, the geographic range of *I. scapularis* and its associated human pathogens have expanded dramatically, resulting in an increase in reported tick-borne disease cases, most notably in the Upper Midwest, Northeast, and Mid-Atlantic regions (Kugeler et al., 2015; Eisen et al., 2016, 2017; Eisen and Paddock, 2021).

Prevention and diagnosis of tick-borne diseases rely on an accurate understanding by the public and health care providers of when and where persons are at risk for exposure to human-biting ticks and their associated pathogens. However, national maps showing the distribution and abundance of medically important ticks and their associated pathogens are often incomplete, not current, or lack data entirely (Eisen and Paddock 2021). Efforts to generate data to inform such maps have been hampered by a lack of standardized routine tick-based surveillance. A recent survey of vector-borne disease professionals in the U.S. revealed that fewer than half of respondents were engaged in routine active tick surveillance. Most of those engaged in tick surveillance were focused on describing the distribution of ticks, with fewer aiming to describe pathogen presence or prevalence within the targeted tick populations. Cited barriers to conducting tick surveillance and pathogen testing included a lack of guidance and funding constraints (Mader et al., 2021).

In 2018, the U.S. Centers for Disease Control and Prevention (CDC) issued guidance aimed at standardizing tick and tick-borne pathogen surveillance and increased support to public health partners to conduct tick surveillance (CDC, 2018; Eisen and Paddock 2021). The recommendations describe a set of objectives that progressively increase the amount of data available to support assessments of human risk of exposure to ticks and tick-borne pathogens. Objectives range from describing the distribution of medically important ticks to identifying the presence of human pathogens in ticks, and progress to quantifying tick densities and the prevalence of pathogens in host-seeking ticks. While the utility of the data increases with each escalating objective, the resources required to conduct tick surveillance also intensify with those requiring pathogen detection being among the most costly and time-consuming.

Tick and tick-borne pathogen surveillance data are commonly used to explain epidemiological trends (primarily at coarse spatial scales), to guide tick bite prevention recommendations and to establish a prior probability of exposure when diagnosing a tick-borne disease (Pepin et al., 2012; Stromdahl and Hickling 2012; Dahlgren et al., 2016; Moore et al., 2016; Bisanzio et al., 2020; Kugeler and Eisen 2020; O'Connor et al., 2021; Eisen and Paddock 2021; Lantos et al., 2021). Recognizing resources are limited for conducting tick and tick-borne pathogen surveillance, we sought to assess the feasibility of scaling back tick testing without seriously compromising data used in public health practice. Here, we describe spatial and temporal variation in the prevalence of the two most common pathogens (*B. burgdorferi* s.s. and *A. phagocytophilum*) in host-seeking *I. scapularis* nymphs and adults in the Upper Midwest (for the purposes of this study, the Upper Midwest is defined as a region including Michigan, Minnesota, and Wisconsin). Additionally, we sought to determine if a less intensive approach yielded comparable regional (Michigan, Minnesota, and Wisconsin) estimates of infection prevalence in host-seeking ticks compared with multiple-year sampling of the same sites.

Specifically, in this study we analyzed historic *I. scapularis* nymphal and adult surveillance records among sites in the Upper Midwest with multiple years of collections and pathogen testing. We summarized inter-annual variability in infection prevalence of each pathogen in host-seeking *I. scapularis* nymphs and adults at sites that were sampled at least three years. We also assessed whether pathogen prevalence in one year is predictive of future years within the same site and whether pathogen prevalence changes significantly over time. We further estimated regional and state averages and ranges in infection prevalence of each pathogen by tick life stage and created random subsets of the data to assess the impacts of a reduced sampling regime for estimating regional averages in infection prevalence.

Methods

Collection sites

Retrospective tick collection and pathogen testing records from three states in the Upper Midwest were provided by state public health agencies or their academic partner institutions. These data were used originally for public health tick surveillance or research, and in many instances have been published in part or fully (Hamer et al., 2010, 2012, 2014; Pritt et al., 2016; Bjork and Schiffman 2020), but not previously as a combined data set. From 2000 through 2019 host-seeking *I. scapularis* nymphs and adults were collected by dragging at 34 forested sites, including edge habitat, in areas considered by the collectors to be of public health concern. Drag sampling is recommended in areas where *I. scapularis* is endemic or emerging, as the method reliably quantifies tick density and yields a highly correlated measure of the human risk of contact with infected host-seeking ticks (Falco and Fish 1992; Mather et al., 1996). Sites included novel areas of potential human exposure to *I. scapularis*; areas where *I. scapularis* is newly established; areas where incidence of *I. scapularis*-borne illnesses have changed over time; heavily used recreational areas; areas where novel pathogens are suspected to be circulating; and representative habitat types in areas where *I. scapularis*-borne infections are prevalent. Sites were sampled one or more times per year during peak nymphal and/or adult activity periods. When sampling was conducted more than once per year, the highest observed density per life stage was considered the peak value.

Data elements included site location, year of collection, peak number of nymphal and adult *I. scapularis* collected per area sampled, number of nymphal and adult *I. scapularis* tested for *B. burgdorferi* s.s. and *A. phagocytophilum*, and number of nymphal and adult *I. scapularis* positive for *B. burgdorferi* s.s. and *A. phagocytophilum* by site and year. For inclusion of records in this study, site selection, tick collection and pathogen identification methods had to conform to *I. scapularis* surveillance guidance published by the CDC (CDC, 2018). Data were screened to exclude sites with less than three years of repeated sampling within a sequential five-year period. One additional site in which sampling was conducted for three consecutive years was excluded because sample sizes were extremely low (n= one, two and five ticks tested per year), yielding consistently unreliable estimates of infection prevalence. After screening, 28 sampling sites met the criteria for inclusion in the study for one or more pathogen and life stage combinations. The geographic range of sites meeting all data inclusion criteria is shown in Fig. 1. Within included sites, years where only one tick or no ticks were tested were excluded from analyses. The inclusion of years where low numbers of ticks were collected was done to ensure that sites with emerging tick or pathogen populations were not excluded from our data set.

Pathogen detection

Pathogen detection methods varied by state and entity performing the testing but met minimum criteria for acceptability according to CDC *I. scapularis* surveillance guidance (CDC 2018). Briefly, collected nymphal and adult ticks were tested individually using molecular assays specific to *B. burgdorferi* s.s. or *A. phagocytophilum.* Assays were demonstrated to be species-specific by testing against genetically similar species or designed according to previously published assays meeting the same criteria. While all assays specifically targeted *B. burgdorferi* sensu stricto, *A. phagocytophilum* assays did not discriminate human-active (ha) variant or variant 1 (v1). Specific pathogen detection methods used are listed in Supplemental Table 1.

Statistical analysis

To generate descriptive statistics, pathogen infection prevalence was calculated for *B. burgdorferi* s.s. and *A. phagocytophilum* by *I. scapularis* life stage, sampling site, and year. Site specific 95% confidence intervals (95% C.I.) were calculated as Wilson score intervals, which are applied to binomial data including small sample sizes, or point estimates close to one or zero (Wilson 1927). State and regional (all states combined) averages were based on these site-specific point estimates of infection prevalence and 95% confidence intervals were derived assuming a t-distribution to account for small sample sizes (< 30 sites).

The resulting annual site-level point estimates were used in mixed effects models to determine if infection prevalence increased or decreased over time. Only sites with at least five years of continuous pathogen testing data were included. First, qualifying sites were classified as ‘emerging,’ or ‘established’ where an ‘emerging’ site was defined as any site where the prevalence point estimates for the first three years of sampling were below the lower 95% CI for the Upper Midwest region. Data were analyzed separately for each of the four pathogen and life stage combinations, and each of these groups were split into ‘emerging’ and ‘established’ analyses for a total of eight models. Each model included ‘year’ as a fixed effect and ‘site’ as a random effect, if more than one site was included in the analysis. Recognizing that pathogen detection methods varied among sites and over time, we included pathogen testing method as a second random effect. However, it did not significantly improve Akaike information criterion (AIC) scores, indicating testing method did not explain observed differences, and the variable was not included in the final models.

In addition to general linear trends that evaluated consistent increases or decreases in infection prevalence over time, we applied an autocorrelation function (ACF) to determine if the annual prevalence of pathogens was generally temporally autocorrelated within each site which would indicate that infection prevalence in one year is predictive of observed prevalence the following year.

We aimed to determine if limiting observations on each site to a single year significantly affected estimates of regional infection prevalence compared against estimates that were generated using the full dataset. We subsampled the full data set ten times. In these subsamples, each site was limited to a single year that was selected randomly with replacement from those available. For nymphs, the full dataset contained 25 sites that were sampled over multiple years (156 total yearly prevalence point estimates). Each nymph subset contained all 25 sites which included approximately 16% of the observations present in the full dataset. These subsamples were compared against estimates generated using all 156 prevalence point estimates (i.e. the full dataset). For adults, the full dataset contained 14 sites that were sampled over multiple years (117 total yearly prevalence point estimates). Each adult subset contained all 14 sites which included approximately 12% of the observations present in the full dataset. These subsamples were compared against estimates generated using all 117 prevalence point estimates. Differences between the regional point estimates of the subsamples and full dataset were analyzed using analysis of variance (ANOVA) and pairwise comparisons were made using post-hoc Tukey tests. These analyses were only conducted with data for *B. burgdorferi* infected nymphs and adults as fewer sites were sampled for *A. phagocytophilum* infected ticks.

All data analyses were conducted in R version 4.0.3 (R Foundation for Statistical Computing, Vienna, Austria) or JMP v. 13.2.1 (SAS Institute, Cary, N.C.). Mixed effects models were constructed using the lme4 package.

Results

Prevalence of B. burgdorferi s.s. in I. scapularis nymphs

From 2004 to 2019 a total of 12,594 host-seeking *I. scapularis* nymphs were collected from 25 sites across three states in the Upper Midwest. Sampling was conducted from three to 12 years (median: six years) per site with two to 817 nymphs tested per site per year (median: 69.5 nymphs tested per site per year). The mean site-specific prevalence of *B. burgdorferi* s.s. was as low as 1.40% (95% CI: 0.60–3.23%) at the Fenner Nature Center in Michigan and as high 28.18% (95% CI: 23.57–32.80%) at Tower Hill State Park in Wisconsin. State specific mean prevalence of *B. burgdorferi* s.s. ranged from 13.63% (95% CI: 5.72–21.54%) in Michigan to 18.54% (95% CI: 14.32–22.76%) in Wisconsin. The regional mean prevalence of *B. burgdorferi* s.s. in host-seeking *I. scapularis* nymphs across all sampling sites and years was 16.97% (95% CI: 13.96–19.98%) (Table 1, Supplemental Table 2). Overall, 80% (20 of 25) and 60% (15 of 25) of site estimates were statistically similar to state-specific and regional averages, respectively (Table 1).

Among 10 established sites for which we had at least five years of contiguous data, the mixed effects model for *B. burgdorferi* s.s. infected nymphs showed no statistically significant temporal trend ($t = -1.7$, df = 84, $p = 0.10$) in infection prevalence indicating that infection prevalence was not consistently increasing or decreasing over time. Only Fenner Nature Center in MI met our criteria for an emerging site and we detected no statistically significant temporal trend ($t = 0.34$, df = 3, $p = 0.76$), although data were limited for this analysis with only five years of observations (Fig. 2). The ACF plots revealed no temporal autocorrelation between sampling years for any site, meaning that prevalence in one year was not predictive of prevalence in the next (Supplemental Figure 1).

There was no statistically significant difference when comparing the regional point estimates of *B. burgdorferi* s.s infection prevalence in nymphs generated using the full data set (16.97% [95% CI: 14.12–19.83%]) to the subsets where each site was limited to a single year of data (d.f. = 10,264; $F = 1.26$; $p = 0.253$). In pairwise comparisons, none of the regional point estimates from the ten subsamples differed significantly ($p > 0.05$) from the regional point estimate derived from the full data set (Supplemental Figure 5).

Prevalence of B. burgdorferi s.s. in I. scapularis adults

From 2000 to 2019, a total of 8262 host-seeking *I. scapularis* adults were collected from 14 sites in three states. In Minnesota, all sites sampled for nymphs were also sampled for adults, but in Michigan two additional sites with limited nymph data were included, and in Wisconsin 14 sites were sampled for nymphs and an independent site (Stevens Point) was sampled only for adults. Sampling years for adults ranged from three to 20 years (median 12 years) with two to 232 adults tested for *B. burgdorferi* s.s. per site per year (median: 92.5 adults tested per site per year). Across all sampling sites and years, the regional mean prevalence of *B. burgdorferi* s.s. in host-seeking *I. scapularis* adults was 29.53% (95% CI: 22.08–36.98%). Ionia Recreation Area in Michigan yielded the lowest infection prevalence in adult ticks (3.57% [95% CI: 0.18–17.71%]), while Richard J. Dorer Memorial Hardwood State Forest in Minnesota yielded the highest prevalence (45.07% [95% CI: 42.30–47.88%]) (Table 2, Supplemental Table 3). In total 85% (11 of 13 sites) and 64% (9 of 14 sites) of site-specific estimates were statistically similar to state and regional estimates, respectively (Table 2).

Among the five established sites with ≥ five years of contiguous sampling there was no statistically significant temporal trend ($t = 0.66$, df = 55, $p = 0.51$) in *B. burgdorferi* s.s. infection prevalence, indicating infection prevalence was stable over time. However, the model that included the three emerging sites showed a statistically significant positive temporal trend ($t = 3.1$, df = 30, $p = 0.004$) or consistent increase in infection prevalence over time (Fig. 3). The autocorrelation function plots revealed no statistically significant temporal autocorrelation between sampling years for any sites regardless of its status as an emerging or established site (Supplemental Figure 2).

There was no statistically significant difference when comparing the regional point estimates of *B. burgdorferi* s.s infection prevalence in adults generated using the full data set (29.53% [95% CI: 22.77–36.29%]) to the subsets where each site was limited to a single year of data (df = 10,143; $F = 0.383$, $p = 0.952$). In pairwise comparisons, none of the regional point estimates from the ten subsamples differed significantly ($p > 0.05$) from the regional point estimate derived from the full data set (Supplemental Figure 5).

Prevalence of A. phagocytophilum in I. scapularis nymphs

From 2005 to 2019, 7562 nymphs collected from 10 sites in Minnesota and Wisconsin were tested for *A. phagocytophilum*. Among sites included in estimates of *A. phagocytophilum* prevalence, the number of years included per site ranged from four to 12 (median: eight years). From each site and year, the number of nymphs tested ranged from six to 738 (median: 84.25 nymphs tested). The regional mean prevalence of *A. phagocytophilum* in host-seeking *I. scapularis* nymphs across all sampling sites and years was 6.57% (95% CI: 4.47–8.66%) and was as low as 2.67% (95% CI: 1.23–5.69%) at McCaslin Brook in Wisconsin, and as high as 9.98% (95% CI: 8.18–12.12%) at Camp Ripley in Minnesota

(Table 3, Supplemental Table 4). Site-specific estimates were statistically similar to state specific averages for 90% of sites (9 of 10) and 80% (8 of 10 sites) were statistically similar to the regional average (Table 3).

Among the five established sites for which ≥five years of contiguous data were available, results of the mixed effect model for *A. phagocytophilum* infected nymphs showed no statistically significant temporal trend ($t = -0.05$, df = 44, $p = 0.96$) in infection prevalence, indicating that infection prevalence was not increasing or decreasing consistently over time. Only American Legion Northern Highland in Wisconsin met our criteria for an emerging site and we detected no statistically significant temporal trend ($t = 1.03$, df = 3, $p = 0.38$), although data were limited for this analysis with only five years of observations (Fig. 4). Autocorrelation function plots revealed no significant temporal autocorrelation between sampling years by site (Supplemental Figure 3).

Prevalence of A. phagocytophilum in I. scapularis adults

From 2005 to 2019, 6381 adult ticks were collected from five sites in Minnesota and Wisconsin. The number of years sampled per site ranged from 10 to 20 (median 16 years per site) and the number of adults tested per site per year ranged from eight to 232 (median 93.7 adults tested per year). The regional mean prevalence of *A. phagocytophilum* in host-seeking *I. scapularis* adults across all sampling sites and years was 8.59% (95% CI: 5.01–12.17) (Table 4, Supplemental Table 5). All site-specific prevalence estimates were statistically similar to the state and regional averages (Table 4).

Results of the mixed effect model for four established sites showed a marginally statistically significant positive temporal trend ($t = 1.9$, df = 42, $p = 0.06$) in infection prevalence. Only Stevens Point in Wisconsin was classified as ‘emerging’ and data were analyzed in a linear model which detected a statistically significant positive trend ($t = 3.1$, df = 18, $p = 0.007$) in infection prevalence (Fig. 5). Autocorrelation function plots revealed no significant temporal autocorrelation between sampling years by site (Supplemental Figure 4).

Discussion

Consistent with previous studies from other endemic regions, the prevalence of *B. burgdorferi* s.s. and *A. phagocytophilum* were both highly variable in ticks among sites and among years within individual sites in the upper Midwest (Piesman et al., 1999; Eisen et al., 2004; Diuk-Wasser et al., 2012; Keesing et al., 2014; Prusinski et al., 2014; Feldman et al., 2015; Johnson et al., 2018). At sites considered “established,” prevalence of *B. burgdorferi* s.s. exhibited high interannual variability, but there were no discernable increasing or decreasing trends over time. Prevalence of *A. phagocytophilum* in host-seeking nymphs remained stable over time at ‘established’ sites, but a slight marginally significant increase in infection prevalence was noted across sites where host-seeking adults were tested. Similarly, no temporal trends for either *B. burgdorferi* or *A. phagocytophilum* infection prevalence were detected at the ‘emerging’ nymphal sites, although only a limited number of observations were analyzed. However, a significant positive temporal trend in infection prevalence was detected in adults for both pathogens in sites classified as ‘emerging’. At all sites regardless of pathogen or tick life stage, infection prevalence in one year was not predictive of the next, according to ACF analysis.

In addition to sharing a common vector, *B. burgdorferi* and *A. phagocytophilum* share a common primary reservoir host, the white-footed mouse (*Peromyscus leucopus*). Compared with *B. burgdorferi* the infectious period for *A. phagocytophilum* in white-footed mice is transient (Telford et al., 1996; Stafford et al., 1999; Levin and Ross, 2004). This contributes to explaining why prevalence of *A. phagocytophilum* is generally lower than *B. burgdorferi* in host-seeking nymphs and adults. Neither pathogen is transmitted transovarially (Piesman, 1989; Teglas and Foley, 2006). Thus, acquisition is limited to single blood feeding events per life stage, with adults having two opportunities to acquire infection and nymphs only one. As a result, prevalence of infection is typically higher in adults. With higher prevalence of infection in adults, we were more likely to detect significant trends in adults than nymphs. However, in most cases due to differences in contiguous yearly sampling data, both life stages were not assessed for temporal trends at the same sites. Therefore, it is not clear if observed positive temporal trends observed in adults reflects the higher prevalence of infection, or differences in sites included in the nymphal compared with adult tick mixed-effect models.

The high degree of spatial and temporal variability in pathogen prevalence in ticks suggests that identifying and adhering to a fixed and precise prevalence threshold for prevention or diagnostic decisions is not feasible. However, coarse level estimates of pathogen prevalence (e. g., state or regional estimates) provide sufficient data for most public health purposes. We showed that sampling the included sites for as little as a single year yielded similar regional estimates of infection prevalence to multi-year sampling of the same sites. This implies that Upper Midwest regional estimates based on reduced sampling effort (i.e., as little as a single year of sampling per site) are comparable with more extensive longitudinal sampling of sites. Resampling sites with low infection prevalence may provide useful information regarding an emerging site but is unlikely to strongly impact regional estimates or public health messaging at larger scales. This suggests that tick sampling and testing efforts can be scaled to optimize scarce public health resources.

In addition to providing valuable data explaining ecological drivers of variation in acarological risk indices (e.g., host-seeking tick densities, infection prevalence, densities of infected host-seeking ticks) (Schulze and Jordan 1996; Jones and Kitron 2000; Ostfeld et al., 2001, 2006; Ginsberg et al., 2004; Elias et al., 2011; Ogden et al., 2018; Larson et al., 2021), longitudinal sampling of ticks and tick-borne pathogens from fixed sites provides insights into the complexity of characterizing acarological risk. Our long-term sampling data show that at any given location, the peak abundances of nymphs or adults is highly variable, as is the prevalence of infection in host-seeking ticks. Specifically, within a single site, we observed up to a 160-fold difference among years in the density of host-seeking nymphs and up to a 6.9-fold difference among years in the density of host-seeking adults. Site-specific point estimates of the prevalence of *B. burgdorferi* s.s. in host-seeking nymphs varied as much as 6.9-fold among years within a single site.

Factors that influence variation in estimates of host-seeking tick density derived from drag or flag sampling at a single site include (1) seasonal and diel timing of tick collections (Schulze and Jordan 1996, 2003; Diuk-Wasser et al., 2006; Thomas et al., 2020), (2) number of sampling occasions that are used to estimate the seasonal peak (Dobson et al., 2014), (3) host composition (Daniels et al., 1993; VanBuskirk and Ostfeld 1995; Ostfeld et al., 2001, 2006; Ginsberg et al., 2020), and (4) weather conditions at the time of sampling and preceding sampling (Eisen, Eisen, Ogden and Beard, 2016). Infection prevalence estimates should be less sensitive to error introduced by timing or frequency of tick sampling compared with tick density estimates because the cohort of nymphs or adults being examined was infected over a long duration (months) when the previous life stage (larvae or nymphs, respectively) was active. Therefore, the absolute proportion of nymphs or adults infected with *B. burgdorferi* or *A. phagocytophilum* is expected to be constant during the sampling season; interannual variability in infection prevalence is explained mainly by host composition when the prior life stage was active (Ostfeld et al., 2001; Vuong et al., 2017). While the product of host-seeking tick density and infection prevalence is believed to be a more accurate correlate of human risk of exposure to infected ticks than either measure alone (Mather et al., 1996; Pepin et al., 2012), in this study, we focused primarily on assessing variability in infection prevalence because this is the costliest measure to assess. Our intent was to evaluate if less intensive testing to support tick surveillance activities could yield useful data for public health action.

Tick surveillance data are typically used to 1) explain epidemiological trends (Pepin et al., 2012; Stromdahl and Hickling 2012; Dahlgren et al., 2016; Bisanzio et al., 2020; Kugeler and Eisen 2020; O'Connor et al., 2021), 2) inform public health messaging for tick-bite prevention by identifying areas posing a risk for exposure to infected host-seeking ticks (Eisen and Paddock 2021), and 3) assess a likelihood of human exposure to pathogens following a tick bite (Lantos et al., 2021). Several studies have demonstrated a positive association between the density of *B. burgdorferi*-infected host-seeking nymphs and occurrence of Lyme disease (Mather et al., 1996; Stafford et al., 1998; Connally et al., 2006; Pepin et al., 2012). Although some of these analyses have focused on county or sub-county spatial scales, owing in part to the high degree of variability in both acarological and epidemiological data, these reported trends are generally more consistent when comparing between rather than within regions. Variation in pathogen prevalence between regions influences the epidemiology of tick-borne diseases. This is evident in the contrasting risk of acquiring Lyme disease in the southeastern U.S. versus other regions where *I. scapularis* is currently established. Despite presence of *I. scapularis* in southern states, the prevalence of *B. burgdorferi* s.s. in host-seeking ticks is significantly lower than the Northeast, Mid-Atlantic, and Upper Midwest where Lyme disease incidence is significantly higher than in southeastern states (Diuk-Wasser et al., 2012; Schwartz et al., 2017; Lehane et al., 2021). Therefore, determining prevalence of tick-borne pathogens provides greater insights into regional risk of acquiring tick-borne disease than tick presence or density alone.

Prevention of tick-borne diseases, including Lyme disease and anaplasmosis, relies primarily on education promoting the use of personal protection measures. In general, persons who perceive their risk of encounters with infected ticks or of acquiring a tick-borne disease to be higher are more likely to take precautions against tick bites or pathogen exposure (e.g., wearing repellents, checking for and removing ticks) than those with lower perceived risks (Herrington et al., 1997; Niesobecki et al., 2019). Tick surveillance data aid in raising awareness of locations where risk of exposure to infected ticks is elevated. However, public health education or personal protection strategies are not likely to differ based on data suggesting a moderately low (e.g., Fenner Nature Center in Michigan) compared with a moderately high prevalence of infection in ticks (e.g., Tower Hill State Park in Wisconsin). Therefore, coarse (state or regional scale) data-driven estimates of infection prevalence in host-seeking ticks by life stage are generally adequate for public health messaging. While some have advocated for prevention strategies (use of antibiotic prophylaxis to prevent Lyme disease) based on a high likelihood of exposure to *B. burgdorferi*-infected *I. scapularis* where highly endemic areas are generally defined as > 20% *B. burgdorferi* prevalence in host-seeking *I. scapularis* nymphs (Wormser et al., 2006; Lantos et al., 2021), our data indicate oscillation above or below that 20% prevalence threshold across years. Such variation within single sites was observed both in states considered high incidence for Lyme disease (Wisconsin and Minnesota) or not (Michigan). The high degree of spatio-temporal variation in our data set demonstrates the difficulty of gaging such precise estimates across localities for public health action.

Nonetheless, we show that site-specific estimates of *B. burgdorferi* infection prevalence in host-seeking nymphs or adults were statistically similar to state averages for ≥80% of sites, and statistically similar to regional averages for ≥ 60% of sites. Although fewer sites were included, site specific estimates of *A. phagocytophilum* prevalence in host-seeking nymphs or adults was statistically similar to state or regional averages for ≥ 90% or ≥ 80% of sites, respectively. Where site estimates of *B. burgdorferi* s.s. prevalence differed significantly from state or regional averages, in most instances site estimates were lower than state or regional averages. Some of the lower-than-average estimates may have arisen because site specific estimates included a period of introduction or emergence of *B. burgdoferi* s.s. Significant increases in *B. burgdorferi* s.s. prevalence over time were observed more commonly in longitudinal sampling sites classified as emerging compared with those classified as established, suggesting that if lower than expected prevalence is observed, resampling is indicated. However, in some cases at established sites, specifically Saugatuck Dunes State Park in Michigan where *I. scapularis* has been present since 2004, prevalence of *B. burgdorferi* s.s. remained stable at low prevalence. This could be explained by host composition (a factor not examined in this study) contributing to a stable low prevalence of infection, or perhaps other site-level factors slowing the establishment of the *I. scapularis* population at this site.

The data presented here demonstrate the high degree of variability in estimates of infection prevalence at fine spatial and temporal scales. However, they also demonstrate that, in general, after *B. burgdorferi* s.s. or *A. phagocytophilum* become established in an area, their prevalence of infection in *I. scapularis* nymphs and adults typically reaches stable and predictable levels as noted elsewhere (Hamer et al., 2014; Keesing et al., 2014; Prusinski et al., 2014; Feldman et al., 2015). Here, we estimate that in the Upper Midwest, regional infection prevalence of *B. burgdorferi* s.s. and *A. phagocytophilum* in nymphal *I. scapularis*, the most epidemiologically important life stage, averaged 16.97% (95% CI: 13.96–19.98%) and 6.57% (95% CI: 4.47–8.66%) respectively. This is consistent with estimates from a separate data set presented recently by Lehane et al. (2021) which found similar rates of *B. burgdorferi* s.s. in *I. scapularis* nymphs (17.99% [16.82–19.22%]) in the Midwest (IN, MI, MN, WI). However, the estimate of *A. phagocytophilum* in *I. scapularis* nymphs (4.03% [3.46–4.69%]) was slightly but not significantly lower than shown in our study, but differences are not likely to impact public health action and might be attributable to inclusion of more sites in the Lehane et al. (2021) study along the leading edge of *A. phagocytophilum* expansion. Similarly, in New York, Prusinski et al. (2014) presented a regional prevalence of *B. burgdorferi* s.s. infection in nymphs as 14.4%, again consistent with state estimates derived from an independent surveillance data set (Lehane et al., 2021). Although there are relatively fewer studies focused on *A. phagocytophilum*, Keesing et al. (2014) showed an 8.3% (±0.6% SEM) infection prevalence in questing *I. scapularis* nymphs in Dutchess County, NY, (an estimate similar to the New York estimate presented by Lehane et al. (2021)) and demonstrated stability of infection prevalence with no discernable temporal trends.

Although our data represent many years of repeated, systematic sampling of *I. scapularis* at sites in the Upper Midwest, there are some significant time breaks at select sites in the data. We accounted for this in our analysis by only running the mixed effect model and ACF on those sites with ≥ five years of contiguous sampling which limited the data included in the analyses, and therefore, our ability to draw broader conclusions.

Optimizing effort and resource allocation for tick surveillance is important because public health resources are limited. Designing optimal sampling strategies depends on local factors and goals of public health agencies. For sites where prevalence of *B. burgdorferi* s.s. and/or *A. phagocytophilum* are consistent with regional averages in local *I. scapularis* populations, our study suggests extending the interval between sampling events is likely sufficient to maintain up-to-date estimates of infection prevalence for the public and health care providers. Moreover, our subset analyses where reduced sampling (infection prevalence estimates based on as little as a single year per site) yielded similar infection prevalence results to multiple-year sampling estimates at a regional level, suggests single year sampling across a broad spatial area yields estimates of infection prevalence that are similar to more labor-intensive and costly longitudinal sampling efforts. However, because the data are a convenience sample of previous tick surveillance activities and not a designed study, we are unable to make evidence-based recommendations regarding the optimal number of sampling sites or site placement. Future efforts to refine tick surveillance to improve efficiency and cost-effectiveness should focus on optimal placement of sampling locations, and the minimum number of sites required to generate reliable risk estimates. Our study was limited in scope to assessing estimates of infection prevalence. However, we recognize a need for similar assessments that address other surveillance metrics, including tick densities and describing host-seeking phenology.

Given the observed variability, lack of temporal trends, and consistency of site-specific estimates with regional estimates of *B. burgdorferi* and *A. phagocytophilum* prevalence, we conclude that monitoring infection prevalence in ticks aids in describing coarse acarological risk trends, but setting a fixed prevalence threshold for prevention or diagnostic decisions is not feasible. Additionally, we show that reducing repeated sampling of the same sites has minimal impact on calculation of regional estimates of average infection prevalence, information that might be useful in allocating scarce public health resources for tick and tick-borne disease surveillance and control activities.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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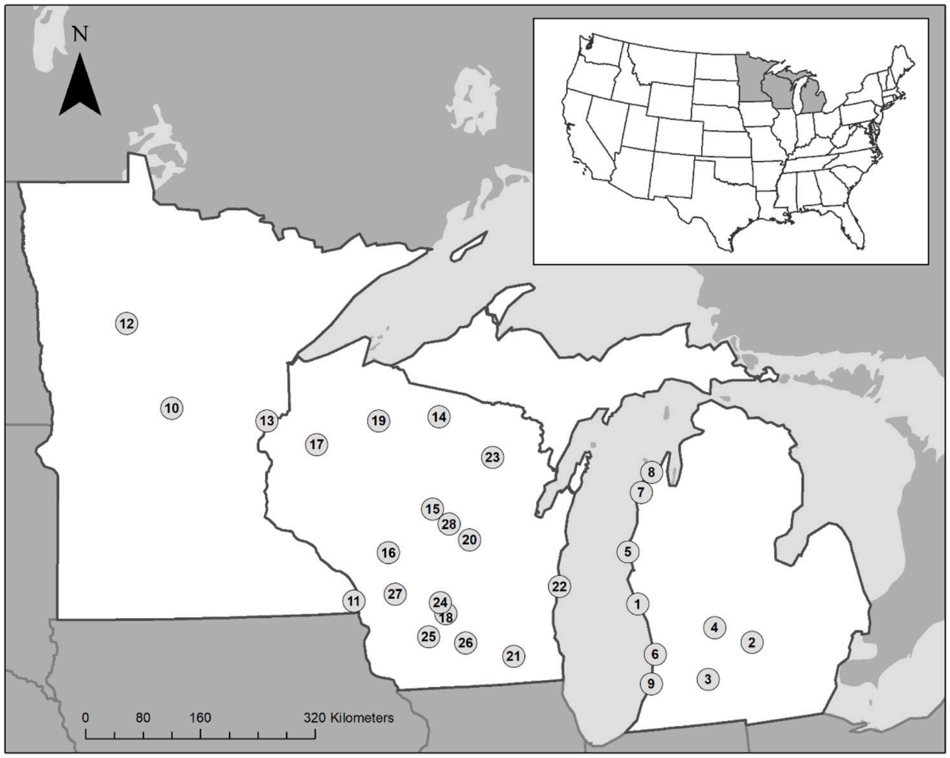

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Fig. 1.

Geographic locations of tick surveillance sites in Michigan ($N = 9$), Minnesota ($N = 4$), and Wisconsin ($N = 15$), meeting study inclusion criteria. Numbered labels correspond to site identification numbers referenced in subsequent tables and figures.

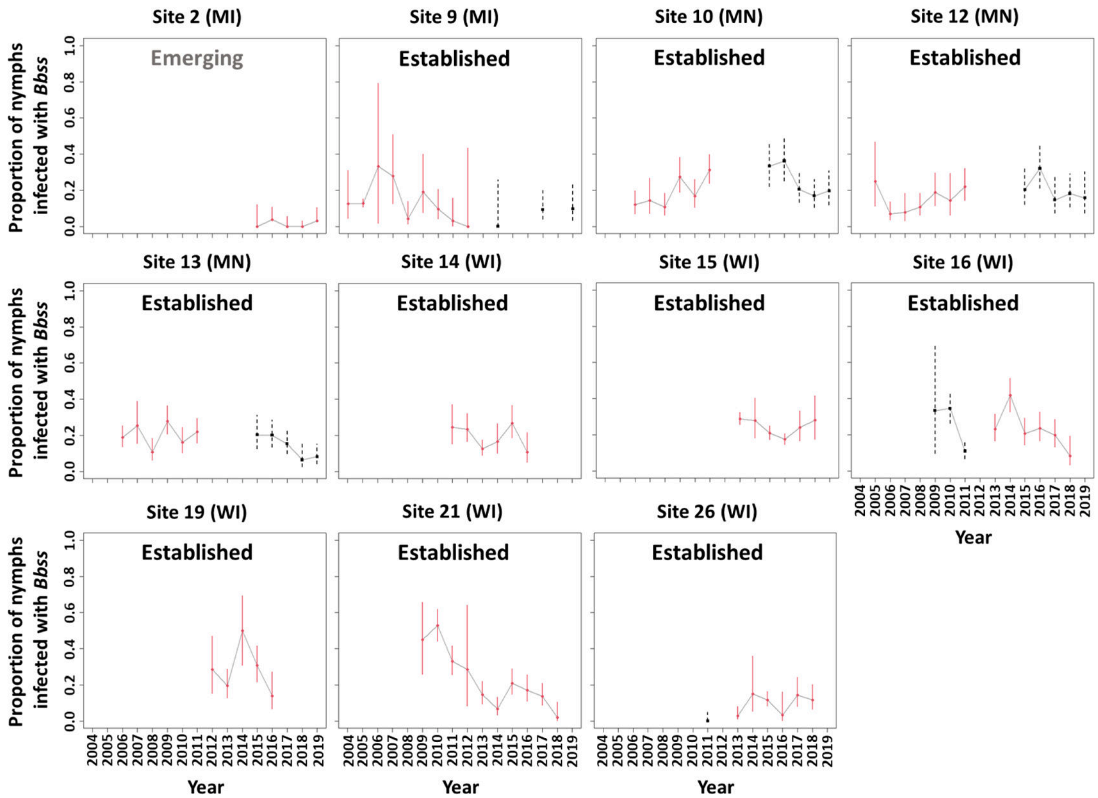

Fig. 2.

Point estimates with bars showing 95% confidence intervals for the annual proportion of *I. scapularis* nymphs infected with *B. burgdorferi* s.s. at sites with ≥ 5 contiguous years of data. Breaks in the lines connecting dots represent years where data were not collected. For 10 established sites, the mixed effects model for *B. burgdorferi* s.s. infected nymphs showed no significant temporal trend ($t = -1.7$, df = 84, $p = 0.10$) in infection prevalence. Additionally, at a single site classified as emerging, no significant temporal trend in infection prevalence was detected ($t = 0.34$, df = 3, $p = 0.76$). Points with solid 95% CI lines were included in the autocorrelation function (ACF) plots (Supplemental Figure 1).

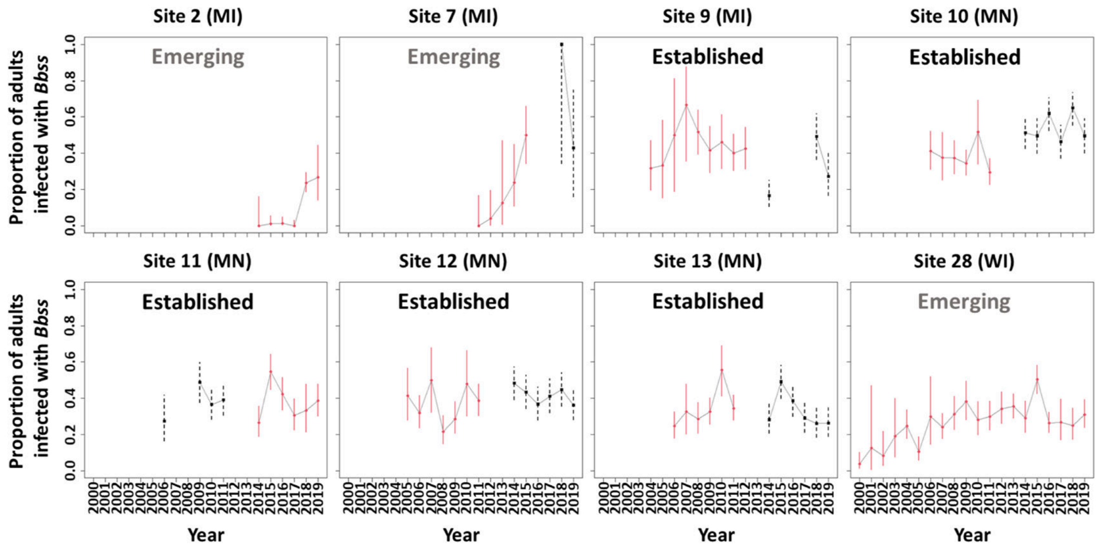

Fig. 3.

Point estimates with bars showing 95% confidence intervals for the annual proportion of *I. scapularis* adults infected with *B. burgdorferi* ss at sites with ≥ 5 contiguous years of data. Breaks in the lines connecting dots represent years where data were not collected. For 5 established sites, the mixed effects model for *B. burgdorferi* s.s. infected adults showed no significant temporal trend (t = 0.66, df = 55, p = 0.51) in infection prevalence. For 3 emerging sites, a significant positive temporal trend in infection prevalence was detected (t = 3.1, df = 30, p = 0.004). Points with solid 95% CI lines were included in the autocorrelation function (ACF) plots (Supplemental Figure 2).

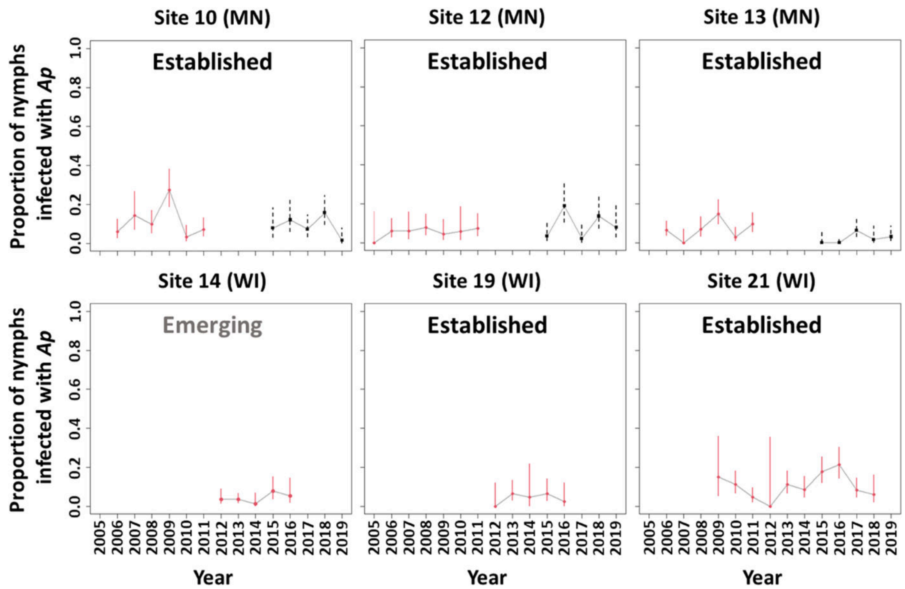

Fig. 4.

Point estimates with bars showing 95% confidence intervals for the annual proportion of *I. scapularis* nymphs infected with *A. phagocytophilum* at sites with ≥ 5 contiguous years of data. Breaks in the lines connecting dots represent years where data were not collected. For 5 established sites, the mixed effects model for *A. phagocytophilum.* infected nymphs showed no significant temporal trend ($t = -0.05$, df = 44, $p = 0.96$) in infection prevalence. Additionally, at a single site classified as emerging, no significant temporal trend in infection prevalence was detected ($t = 1.03$, df = 3, $p = 0.38$). Points with solid 95% CI lines were included in the autocorrelation function (ACF) plots (Supplemental Figure 3).

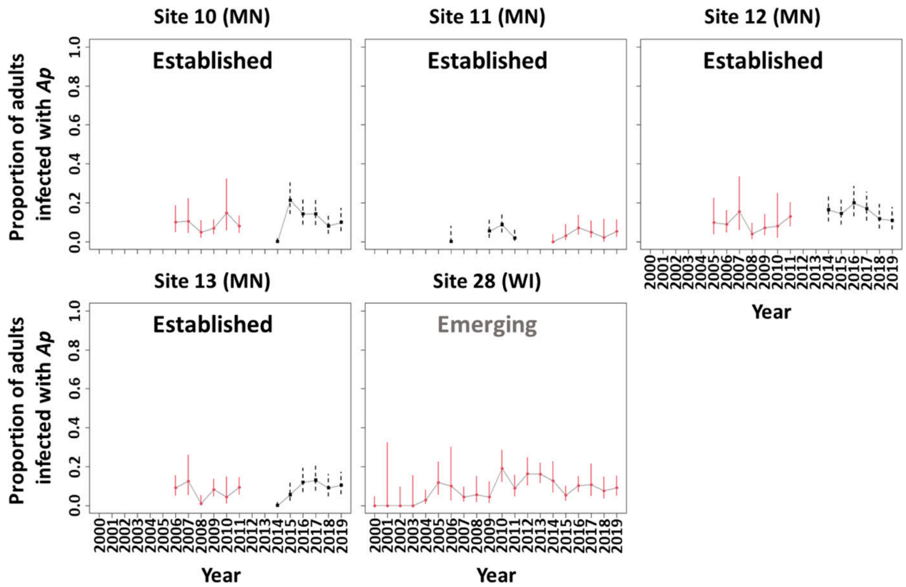

Fig. 5.

Point estimates with bars showing 95% confidence intervals for the annual proportion of *I. scapularis* adults infected with *A. phagocytophilum* at sites with ≥ 5 contiguous years of data. Breaks in the lines connecting dots represent years where data were not collected. For 4 established sites, the mixed effects model for *A. phagocytophilum* infected adults showed no significant temporal trend ($t = 1.9$, df = 42, $p = 0.06$) in infection prevalence. At a single site classified as emerging, a significant positive temporal trend in infection prevalence was detected ($t = 3.1$, df = 18, $p = 0.007$). Points with solid 95% CI lines were included in the autocorrelation function (ACF) plots (Supplemental Figure 4).

Table 1

Abundance and prevalence estimates for *B. burgdorferi* s.s. in host-seeking nymphal *I. scapularis* at 25 sites, surveyed multiple years, in the Upper Midwestern United States.

		Survey site information		*I. scapularis* nymphal abundance[a]	*I. scapularis* nymphs assayed for *B. burgdorferi* s.s.[b]		*B. burgdorferi* s.s. prevalence estimate (95% CI)[c]		
State	**Site ID**	**Site Name**	**Years Sampled (range)**	**Median peak abundance, ticks/100m^2 (range)**	**Total Ticks Tested**	**Median # Ticks Assayed / Year (range)**	**Mean**	**Lower**	**Upper**
MI	1	Duck Lake State Park	7	0.40 (0.03 – 1.03)	77	11 (3 – 22)	0.1429	0.0817	0.2380
	2	Fenner Nature Center	5	3.00 (1.00 – 8.12)	358	65 (28 – 122)	0.0140	0.0060	0.0323
	3	Fort Custer Recreation Area	5	0.40 (0.05 – 2.00)	82	8 (2 – 51)	0.2683	0.1844	0.3730
	5	Ludington State Park	3	0.70 (0.44 – 0.75)	62	14 (7 – 41)	0.1613	0.0900	0.2721
	6	Saugatuck Dunes State Park	6	0.88 (0.17 – 6.25)	96	11 (3 – 37)	0.0521	0.0224	0.1162
	7	SLBE Platte-Eldorado	6	0.44 (0.07 – 3.10)	133	13.5 (3 – 66)	0.1955	0.1370	0.2710
	9	Van Buren State Park	12	2.00 (0.17 – 5.00)	1116	27.5 (3 – 817)	0.1201	0.1023	0.1405
		MI Summary Data	*6 (3–12)*	*0.70 (0.40 – 3.00)*	*1924*	*13.5 (8 – 65)*	*0.1363*	*0.0572*	*0.2154*
MN	10	Camp Ripley	11	1.54 (0.38 – 12.50)	892	83 (49 – 125)	0.2119	0.1863	0.2399
	11	Richard J. Dorer Memorial Hardwood State Forest	8	0.46 (0.06 – 1.54)	310	22.5 (6 – 106)	0.1419	0.1075	0.1852
	12	Itasca State Park	12	0.83 (0.46 – 3.33)	723	56 (35 – 102)	0.1618	0.1368	0.1904
	13	St. Croix State Park	11	2.04 (0.59 – 12.00)	1132	105 (51 – 170)	0.1776	0.1564	0.2009
		MN Summary Data	*11 (8–12)*	*1.18 (0.46 – 2.04)*	*3057*	*69.5 (22.5 – 105)*	*0.1733*	*0.1262*	*0.2204*
WI	14	American Legion Northern Highland	6	2.50 (1.62 – 6.44)	624	84 (56 – 232)	0.1795	0.1514	0.2115
	15	Big Eau Pleine County Park	6	7.29 (0.41 – 65.70)	1960	273 (50 – 708)	0.2327	0.2145	0.2519
	16	Black River Falls State Forest	9	4.40 (2.10 – 12.10)	835	106 (6 – 128)	0.2359	0.2084	0.2659
	17	Camp Phillips	3	2.30 (2.00 – 7.90)	268	101 (50 – 117)	0.2239	0.1781	0.2775
	18	Devil's Lake State Park	3	0.22 (0.20 – 0.40)	146	45 (10 – 91)	0.0959	0.0580	0.1545
	19	Flambeau State Forest	5	1.79 (0.92 – 3.83)	263	43 (22 – 92)	0.2548	0.2059	0.3107
	20	Hartman Creek State Park	3	2.80 (0.50 – 17.80)	393	166 (50 – 177)	0.2697	0.2282	0.3157
	21	Kettle Moraine State Forest-Southern Unit	10	5.60 (0.33 – 12.40)	894	111 (7 – 130)	0.2248	0.1987	0.2533
	22	Kohler-Andrae State Park	4	11.45 (5.00 – 15.10)	333	82 (50 – 119)	0.2072	0.1671	0.2540

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State	Site ID	Survey site information		*I. scapularis* nymphal abundance[a]	*I. scapularis* nymphs assayed for *B. burgdorferi* s.s.[b]		*B. burgdorferi* s.s. prevalence estimate (95% CI)[c]		
		Site Name	**Years Sampled (range)**	**Median peak abundance, ticks/100m^2 (range)**	**Total Ticks Tested**	**Median # Ticks Assayed / Year (range)**	**Mean**	**Lower**	**Upper**
	23	McCaslin Brook	4	2.44 (0.96 – 2.80)	225	58.5 (23 – 85)	0.1244	0.0875	0.1740
	24	Mirror Lake State Park	3	3.23 (1.20 – 4.72)	358	77 (53 – 228)	0.0866	0.0617	0.1203
	25	Tower Hill State Park	4	3.40 (1.70 – 5.20)	369	99.5 (50 – 120)	0.2818	0.2357	0.3280
	26	UW-Arboretum	7	0.40 (0.06 – 0.81)	626	74 (20 – 239)	0.0879	0.0656	0.1101
	27	Wildcat Mt. State Park	3	3.60 (3.50 – 5.00)	319	101 (98 – 120)	0.0909	0.0640	0.1275
		WI Summary Data	*4 (3–10)*	*3.02 (0.22 – 11.45)*	*7613*	*91.75 (43 – 273)*	*0.1854*	*0.1432*	*0.2276*
		Regional Summary Data	*6 (4–11)*	*1.18 (0.70 – 3.02)*	*12,594*	*69.5 (13.5 – 91.75)*	*0.1697*	*0.1396*	*0.1998*

[a] Ticks were collected via drag cloth during peak nymphal activity periods. When sites were sampled multiple times per year, the highest value was denoted as the peak. Median tick abundance and range by state, and region calculated on site medians.

[b] To identify *B. burgdorferi* ss in *I. scapularis* nymphs, ticks were tested individually using species specific molecular assays which met the minimum criteria for acceptability according to the Centers for Disease Control and Prevention Guidelines: “Surveillance for *Ixodes scapularis* and pathogens found in this tick species in the United States” (2018). https://www.cdc.gov/ticks/resources/TickSurveillance_Iscapularis-P.pdf. Median ticks assayed and range by state, and region calculated on site medians.

[c] The proportion of ticks infected per site and Wilson score 95% confidence intervals are shown; state and regional averages were based on these site-specific point estimates and 95% confidence intervals were derived assuming a t-distribution to account for small sample sizes (<30).

Table 2

Prevalence estimates for *B. burgdorferi* s.s. in host-seeking adult *I. scapularis* at 14 sites, surveyed multiple years, in the Upper Midwestern United States.

Survey site information				***I. scapularis* adult abundance[a]**	***Ixodes scapularis* adults assayed for *B. burgdorferi* s.s.[b]**		***B. burgdorferi* s.s. prevalence estimate (95% CI)[c]**		
State	**Site ID**	**Site Name**	**Years Sampled (range)**	**Median peak abundance, ticks/100m^2 (range)**	**Total Ticks Tested**	**Median # Ticks Tested/ Year (range)**	**Mean**	**Lower**	**Upper**
MI	1	Duck Lake State Park	5	0.48 (0.11 – 3.06)	123	23 (3 – 49)	0.3008	0.2268	0.3869
	2	Fenner Nature Center	6	2.35 (0.50 – 4.29)	652	113 (20 – 229)	0.0997	0.0790	0.1251
	3	Fort Custer Recreation Area	5	1.64 (0.08 – 3.93)	136	24 (4 – 51)	0.5149	0.4311	0.5979
	4	Ionia Recreation Area	4	0.49 (0.22 – 3.93)	28	4 (4 – 16)	0.0357	0.0018	0.1771
	5	Ludington State Park	3	0.44 (0.31 – 0.70)	38	8 (8 – 22)	0.2368	0.1299	0.3921
	6	Saugatuck Dunes State Park	4	0.60 (0.33 – 0.75)	56	10.5 (2 – 18)	0.3036	0.1990	0.4334
	7	SLBE Platte-Eldorado	7	0.58 (0.40 – 0.77)	116	19 (2 – 34)	0.2500	0.1801	0.3360
	8	SLBE Pyramid Point	4	0.22 (0.10 – 0.37)	26	6.5 (2 – 11)	0.1923	0.0851	0.3788
	9	Van Buren State Park	12	1.50 (0.82 – 5.10)	574	50.5 (6 – 97)	0.3763	0.3376	0.4166
		MI Summary Data	*5 (3 – 12)*	*0.58 (0.22 – 2.35)*	*1749*	*19 (4 – 113)*	*0.2567*	*0.1469*	*0.3665*
MN	10	Camp Ripley	13	3.67 (1.00 – 7.21)	1145	101 (25 – 119)	0.3790	0.3514	0.4075
	11	Richard J. Dorer Memorial Hardwood State Forest	12	1.57 (0.70 – 3.92)	1218	101.5 (27 – 177)	0.4507	0.4230	0.4788
	12	Itasca State Park	10	1.73 (0.54 – 4.54)	912	105 (44 – 115)	0.3827	0.3517	0.4146
	13	St. Croix State Park	12	2.92 (0.75 – 5.58)	1291	108.5 (40 – 171)	0.3261	0.3011	0.3522
		MN Summary Data	*12 (10 – 13)*	*2.33 (1.57 – 3.67)*	*4566*	*103.25 (101 – 108.5)*	*0.3846*	*0.3034*	*0.4659*
WI	28	Stevens Point	20	NA	1947	92.5 (8 – 232)	0.2861	0.2664	0.3066
		WI Summary Data					*0.2861*	*NA*	*NA*
		Regional Summary Data	*12 (5 – 20)*	*1.46 (0.58 – 2.33)*	*8262*	*92.5 (19 – 103.25)*	*0.2953*	*0.2208*	*0.3698*

[a]Ticks were collected via drag cloth during peak adult activity periods. Median tick abundance and range by state, and region calculated on site medians.

[b]To identify *B. burgdorferi* ss in *I. scapularis* adults, ticks were tested individually using species specific molecular assays which met the minimum criteria for acceptability according to the Centers for Disease Control and Prevention Guidelines: "Surveillance for *Ixodes scapularis* and pathogens found in this tick species in the United States" (2018). https://www.cdc.gov/ticks/resources/TickSurveillance_Iscapularis-P.pdf. Median tick abundance and range by state, and region calculated on site medians.

[c]The proportion of ticks infected per site and Wilson score 95% confidence intervals are shown; state and regional averages were based on these site-specific point estimates and 95% confidence intervals were derived assuming a t-distribution to account for small sample sizes (<30).

Table 3

Prevalence estimates for *A. phagocytophilum* in host-seeking nymphal *I. scapularis* at 10 sites, surveyed multiple years, in the Upper Midwestern United States.

Survey site information				*I. scapularis* nymphal abundance[a]	*I. scapularis* nymphs assayed for *A. phagocytophilum*[b]		*A. phagocytophilum* prevalence estimate (95% CI)[c]		
State	**Site ID**	**Site Name**	**Years Sampled (range)**	**Median peak abundance, ticks/100m^2 (range)**	**Total Ticks Tested**	**Median # Ticks Tested/ Year (range)**	**Mean**	**Lower**	**Upper**
MN	10	Camp Ripley	11	1.54 (0.38 – 12.50)	892	83 (49 – 125)	0.0998	0.0818	0.1212
	11	Richard J. Dorer Memorial Hardwood State Forest	8	0.46 (0.06 – 1.54)	310	22.5 (6 – 106)	0.0419	0.0247	0.0704
	12	Itasca State Park	12	0.83 (0.46 – 3.33)	723	56 (20 – 102)	0.0733	0.0565	0.0946
	13	St. Croix State Park	11	2.04 (0.59 – 12.00)	1132	105 (51 – 170)	0.0557	0.0437	0.0706
		MN Summary Data	*11 (8 – 12)*	*1.18 (0.46 – 2.04)*	*3057*	*69.5 (56 – 105)*	*0.0677*	*0.0279*	*0.1074*
WI	14	American Legion Northern Highland	5	2.50 (1.62 – 6.44)	567	90 (56 – 232)	0.0406	0.0272	0.0601
	15	Big Eau Pleine County Park	4	10.33 (4.72 – 65.70)	1827	519.5 (50 – 738)	0.0996	0.0867	0.1142
	16	Black River Falls State Forest	8	3.70 (3.30 – 12.10)	734	108 (6 – 128)	0.0572	0.0426	0.0764
	19	Flambeau State Forest	5	1.79 (0.92 – 3.83)	263	43 (22 – 92)	0.0494	0.0291	0.0827
	21	Kettle Moraine State Forest-Southern Unit	10	5.60 (0.33 – 12.40)	889	111 (7 – 130)	0.1125	0.0934	0.1349
	23	McCaslin Brook	4	2.44 (0.96 – 2.80)	225	58.5 (23 – 85)	0.0267	0.0123	0.0569
		WI Summary Data	*5 (4 – 10)*	*3.10 (1.79 – 10.33)*	*4505*	*99 (43 – 519.5)*	*0.0643*	*0.0285*	*0.1001*
		Regional Summary Data	*8 (5 – 11)*	*2.14 (1.18 – 3.10)*	*7562*	*84.25 (69.5 – 99)*	*0.0657*	*0.0447*	*0.0866*

[a] Ticks were collected via drag cloth during peak nymphal activity periods. Median tick abundance and range by state, and region calculated on site medians.

[b] To identify *A. phagocytophilum* in *I. scapularis* nymphs, ticks were tested individually using species specific molecular assays which met the minimum criteria for acceptability according to the Centers for Disease Control and Prevention Guidelines: "Surveillance for *Ixodes scapularis* and pathogens found in this tick species in the United States" (2018). https://www.cdc.gov/ticks/resources/TickSurveillance_Iscapularis-P.pdf. Median tick abundance and range by state, and region calculated on site medians.

[c] The proportion of ticks infected per site and Wilson score 95% confidence intervals are shown; state and regional averages were based on these site-specific point estimates and 95% confidence intervals were derived assuming a t-distribution to account for small sample sizes (<30).

Table 4

Prevalence estimates for *A. phagocytophilum* in host-seeking adult *Ixodes scapularis* at 5 sites, surveyed multiple years, in the Upper Midwestern United States.

Survey site information				*I. scapularis* adult abundance[a]	*I. scapularis* adults assayed for *A. phagocytophilum*[b]		*A. phagocytophilum* prevalence estimate (95% CI)[c]		
State	**Site ID**	**Site Name**	**Years Sampled**	**Median peak abundance, ticks/100m^2 (range)**	**Total Ticks Tested**	**Median # Ticks Tested/ Year (range)**	**Mean**	**Lower**	**Upper**
MN	10	Camp Ripley	12	3.67 (1.00 – 7.21)	1218	101.5 (27 – 177)	0.0944	0.0792	0.1121
	11	Richard J. Dorer Memorial Hardwood State Forest	10	1.57 (0.70 – 3.92)	912	103.5 (44 – 115)	0.0428	0.0314	0.0579
	12	Itasca State Park	13	1.73 (0.54 – 4.54)	1145	101 (25 – 119)	0.1223	0.1045	0.1425
	13	St. Croix State Park	12	2.92 (0.75 – 5.58)	1291	108.5 (40 – 171)	0.0790	0.0655	0.0950
		MN Summary Data	*12 (10 – 13)*	*2.33 (1.57 – 3.67)*	*4566*	*102.5 (101 – 108.5)*	*0.0846*	*0.0319*	*0.1374*
WI	28	Stevens Point	20	NA	1815	85 (8 – 232)	0.0909	0.0785	0.1050
		WI Summary Data					*0.0909*	*NA*	*NA*
		Regional Summary Data	*16 (12 – 20)*	*NA*	*6381*	*93.7 (85 – 102.5)*	*0.0859*	*0.0501*	*0.1217*

[a]Ticks were collected via drag cloth during peak adult activity periods. Median tick abundance and range by state, and region calculated on site medians.

[b]To identify *A. phagocytophilum* in *I. scapularis* adults, ticks were tested individually using species specific molecular assays which met the minimum criteria for acceptability according to the Centers for Disease Control and Prevention Guidelines: "Surveillance for *Ixodes scapularis* and pathogens found in this tick species in the United States" (2018). https://www.cdc.gov/ticks/resources/TickSurveillance_Iscapularis-P.pdf. Median tick abundance and range by state, and region calculated on site medians.

[c]The proportion of ticks infected per site and Wilson score 95% confidence intervals are shown; state and regional averages were based on these site-specific point estimates and 95% confidence intervals were derived assuming a t-distribution to account for small sample sizes (<30).