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Spatiotemporal distribution of *Borrelia miyamotoi* (Spirochaetales: Spirochaetaceae) and coinfection with other tick-borne pathogens in host-seeking *Ixodes scapularis* (Acari: Ixodidae) from New York State, USA

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Blacklegged ticks (*Ixodes scapularis* Say, Acari: Ixodidae) were collected from 432 locations across New York State (NYS) during the summer and autumn of 2015–2020 to determine the prevalence and geographic distribution of *Borrelia miyamotoi* (Spirochaetales: Spirochaetaceae) and coinfections with other tick-borne pathogens. A total of 48,386 *I. scapularis* were individually analyzed using a multiplex real-time polymerase chain reaction assay to simultaneously detect the presence of *Bo. miyamotoi, Borrelia burgdorferi* (Spirochaetales: Spirochaetaceae), *Anaplasma phagocytophilum* (Rickettsiales: Anaplasmataceae), and *Babesia microti* (Piroplasmida: Babesiidae). Overall prevalence of *Bo. miyamotoi* in host-seeking nymphs and adults varied geographically and temporally at the regional level. The rate of polymicrobial infection in *Bo. miyamotoi*-infected ticks varied by developmental stage, with certain co-infections occurring more frequently than expected by chance. Entomological risk of exposure to *Bo. miyamotoi*-infected nymphal and adult ticks (entomological risk index [ERI]) across NYS regions in relation to human cases of *Bo. miyamotoi* disease identified during the study period demonstrated spatial and temporal variation. The relationship between select environmental factors and *Bo. miyamotoi* ERI was explored using generalized linear mixed effects models, resulting in different factors significantly impacting ERI for nymphs and adult ticks. These results can inform estimates of *Bo. miyamotoi* disease risk and further our understanding of *Bo. miyamotoi* ecological dynamics in regions where this pathogen is known to occur.

Key words: tick, entomological risk index, *Borrelia miyamotoi* disease, polymicrobial infection, co-infection

Borrelia miyamotoi is a spirochete belonging to the relapsing fever borreliae group. It was first isolated from *Ixodes persulcatus* (Schulze, Acari: Ixodidae) ticks and small mammals in Japan (Fukunaga et al. 1995), and later detected in Connecticut, United States from *Ixodes scapularis* (Say, Acari: Ixodidae) (Scoles et al. 2001). This pathogen now has a broad distribution throughout the northern hemisphere (Platonov

et al. 2011, Gellar et al. 2012, Jahfari et al. 2014, Sato et al. 2014, Krause et al. 2015) and is considered the etiological agent of a relapsing fever illness, *Borrelia miyamotoi* disease (BMD), in humans (Platonov et al. 2011, Chowdri et al. 2013, Krause et al. 2015, Wroblewski et al. 2017). Common symptoms of BMD include fatigue, headache, fever >40 °C, chills, arthralgia, nausea, and myalgia (Dworkin et al. 2002, Chowdri et al. 2013, Krause et al. 2013, Wagemakers et al. 2015). Compatible clinical presentation accompanied by travel history to a region where Lyme disease is endemic help to identify suspect BMD cases warranting further evaluation. Laboratory tests, including polymerase chain reaction (PCR) of whole blood and antibody determination, are used to confirm infection with *Bo. miyamotoi* (Ullmann et al., 2005, Chowdri et al., 2013, Molloy et al., 2015, Wroblewski et al. 2017). Most patients experience uncomplicated recovery following treatment with doxycycline (Platonov et al. 2011, Chowdri et al. 2013), though potential complications in immunocompromised individuals may include meningoencephalitis and central nervous system involvement (Gugliotta et al. 2013, Molloy et al. 2015). Polymicrobial infection of *Bo. miyamotoi* with *Borrelia burgdorferi* sensu stricto (Spirochaetales: Spirochaetaceae) the causative agent of Lyme disease in the northeastern United States, *Anaplasma phagocytophilum* (Rickettsiales: Anaplasmataceae) the causative agent of anaplasmosis or *Babesia microti* (Piroplasmida: Babesiidae) the causative agent of babesiosis, has been reported in vector ticks (DiBernardo et al. 2014, Takano et al. 2014, Johnson et al. 2018, Lehane et al. 2021) and the occurrence of tick-borne coinfections of may complicate or delay patient diagnosis and treatment (Magnarelli et al. 1987, Krause et al. 1996, Belongia 2002).

In the northeastern United States and Canada, *Bo. miyamotoi* is primarily transmitted by *I. scapularis*, and infection is maintained in the vector both transstadially and transovarially (Scoles et al. 2001, Rollend et al. 2013). *Ixodes pacificus* (Cooley and Kohls, Acari: Ixodidae) and *I. ricinus* (L., Acari: Ixodidae) serve as vectors of *Bo. miyamotoi* in the northwestern United States and Europe, respectively. In previous studies, prevalence of *Bo. miyamotoi* in host-seeking ticks from the northeastern United States and New England ranged from 0 to 10% (Scoles et al. 2001, Tokarz et al. 2010, Krause et al. 2015, Keesing et al. 2021, Lehane et al. 2021). *Bo. miyamotoi* has been detected in North America in the white-footed mouse *Peromyscus leucopus* (Rafinesque, Rodentia: Cricetidae) wild turkeys *Meleagris gallopavo* (L., Galliformes: Phasianidae) and in several species of passerine birds, particularly northern cardinals *Cardinalis cardinalis* (L., Passeriformes: Cardinalidae) (Scoles et al. 2001, Hamer et al. 2012). Additionally, the existence of a cryptic enzootic maintenance cycle involving the rabbit tick, *Ixodes dentatus* (Marx, Acari: Ixodidae) and reservoir competent bird species has been suggested (Hamer et al. 2012).

Both nationally and in New York State (NYS), *Bo. miyamotoi* human infections are not currently classified as a reportable disease under public health law. As such, physicians and clinical laboratories are not legally obligated to report diagnosed BMD cases or positive clinical laboratory test results detecting *Bo. miyamotoi* to the NYS Department of Health (NYSDOH) or Centers for Disease Control and Prevention, and there is limited knowledge of the full epidemiologic and geographic distribution of *Bo. miyamotoi* infection in NYS residents and nationally. However, more than 224 human cases of BMD have been identified in residents of the northeastern United States (Gugliotta et al. 2013, Krause et al. 2013, 2014, Molloy et al. 2015, Fiorito et al. 2017, Marcos et al. 2020), including NYS (Wroblewski et al. 2017). A statewide pilot study conducted in NYS identified 8 patients out of 1,162 clinical samples (0.7%) who tested positive for *Bo. miyamotoi* after testing negative for suspected anaplasmosis infection (Wroblewski et al. 2017). The same study also documented the prevalence of *Bo. miyamotoi* and other tick-borne pathogens in *I. scapularis* adults obtained from hunter-killed white-tailed deer, *Odocoileus virginianus* (Zimmerman, Artiodactyl a: Cervidae) harvested from 13 counties in the Hudson Valley and Capital District regions of NYS in 2013 and 2014; finding an overall prevalence of 1.5, 20, and 27% for *Bo. miyamotoi*, *Bo. burgdorferi*, and *A. phagocytophilum*, respectively. An earlier study on the polymicrobial infection of host-seeking adult ticks in 4 southeastern NYS counties found a prevalence of 2, 20, 20, and 64% for *Bo. miyamotoi*, *A. phagocytophilum*, *Ba. microti* and *Bo. burgdorferi*, respectively (Tokarz et al. 2010) and a substantial overall coinfection rate of 30%, with *Bo. miyamotoi* coinfection occurring in only 0.6% of the ticks tested. More recently, *Bo. miyamotoi* was detected in 1.2% of host-seeking ticks collected from >100 locations across Dutchess County, NY, with a maximum site-level prevalence of 9.1% and no temporal trend in infection prevalence noted over 8 yrs (Keesing et al. 2021). However, it is important to note that these previous NYS studies were limited in their scope, both geographically and with respect to sample sizes.

The NYSDOH has conducted statewide active surveillance of pathogens in host-seeking ticks since 2008, including *Bo. burgdorferi*, *A. phagocytophilum*, and *Ba. microti*. In 2015, *Bo. miyamotoi* was added to the NYSDOH routine tick surveillance testing panel to identify presence and prevalence in tick populations on public lands across NYS. In this study, we examined spatial and temporal trends in *Bo. miyamotoi* and co-infection data from 48,386 individual host-seeking *I. scapularis* systematically collected and tested over a 6-yr period from across NYS. In addition, we calculated entomological risk indices to determine the timing and location of potential high-risk encounters with *Bo. miyamotoi*-infected *I. scapularis* nymphs and adults. We used generalized linear mixed effects models to examine the potential impact of certain environmental factors on *Bo. miyamotoi* entomological risk index (ERI) and evaluated polymicrobial infection rates in *Bo. miyamotoi*-infected host-seeking ticks to further explore pathogen ecology. Our study is the first to evaluate the spatiotemporal dynamics of *Bo. miyamotoi*-infected host-seeking *I. scapularis* nymph and adult tick populations in relation to human BMD cases across the entirety of NYS; our results can inform estimates of BMD risk to humans and further our understanding of *Bo. miyamotoi* ecological dynamics in regions where this pathogen is emerging or established.

Materials and Methods

BMD Human Case Data

Clinical samples submitted to the NYSDOH Wadsworth Center Bacteriology Laboratory from 2014 to 2020 for anaplasmosis testing that subsequently screened negative for *A. phagocytophilum* and positive for the presence of *Bo. miyamotoi* by previously described methods (Wroblewski et al. 2017) were classified as BMD cases and included in this study. Epidemiological factors associated with BMD cases, including the NYS county and United States Postal Service (USPS) ZIP code of patient residence, the month and year of symptom onset, age, sex, race, and ethnicity, were recorded with *Bo. miyamotoi* test results in the NYSDOH Electronic Clinical Laboratory Reporting System.

Tick Collections

Host-seeking nymphal and adult *I. scapularis* were collected from publicly accessible forested lands across NYS (Figs. 1 and 2) during May-August and October–December of 2015–2020, respectively,

using a 1 m^2 piece of white fabric during standardized dragging and flagging surveys as previously described (Prusinski et al. 2014). Ambient air temperature, relative humidity, and general weather and site conditions were recorded at the time of sampling. A total of 559 collection sites were selected across NYS, excluding New York City, based on established criteria for habitat suitability for *I. scapularis*, associated vertebrate hosts, and for human exposure potential. Ticks were preserved in the field in 99.5% ethanol, returned to the laboratory, and maintained at 4°C until identified to species under a dissecting microscope (Model SMZ1000, Nikon, Tokyo, Japan) using dichotomous keys (Cooley and Kohls 1944, Keirans and Clifford 1978, Keirans and Litwak 1989, Durden and Keirans 1996, Coley 2015, Egizi et al. 2019). Tick specimens were accessioned, placed into individually blind-labeled sterile 1.5 ml Eppendorf tubes containing 99.5% ethanol, and stored at −20°C until nucleic acid extraction.

Nucleic Acid Extraction

Briefly, individual ticks were double rinsed with nuclease-free distilled water and homogenized as previously described (Prusinski et al. 2014). Tick DNA was extracted on the QIAcube HT automated platform using the QIAamp 96 kit (Qiagen, Valencia, CA) and a manufacturer generated protocol with final elution volumes of 100 µl and 200 µl for nymphs and adult ticks, respectively, and stored at −20°C until polymerase chain reaction (PCR) testing. Each 96-well extraction plate consisted of 92 samples and 4 randomly distributed negative extraction control wells, with 200 µl Buffer AE (Qiagen, Valencia, CA.) substituted for sample. Extraction of tick DNA was conducted under BSL2 conditions in a class 2 biological safety cabinet with laminar airflow (SterilGARDIII Advance, Baker Co., Sanford, ME) designated exclusively for DNA extraction, and sterile aerosol-barrier tips were used during all procedures to prevent cross contamination.

Real-Time Polymerase Chain Reaction

Individual *I. scapularis* nymphs and adults were tested for *Bo. burgdorferi*, *Ba. microti*, *A. phagocytophilum*, and *Bo. miyamotoi* by real-time multiplex PCR assay as detailed elsewhere (Piedmonte et al. 2018). All fluorogenic probes and primers were synthesized by Integrated DNA Technologies (Coralville, IA) and Applied Biosystems (Foster City, CA). Reactions were prepared in a 1:1 mixture of 5× PerfeCta MultiPlex qPCR ToughMix, Low ROX and 2× PerfeCta MultiPlex qPCR SuperMix, Low ROX (Quanta Biosciences, Gaithersburg, MD) such that each reaction contained 5 µl of ToughMix/SuperMix with primer and probe concentrations as described (Piedmonte et al. 2018), to which 9 µl of template was added, yielding a 25 µl final reaction volume. A negative control with nuclease-free water substituted for template DNA, and a positive control consisting of 7.8 µl purified pathogen-free *I. scapularis*

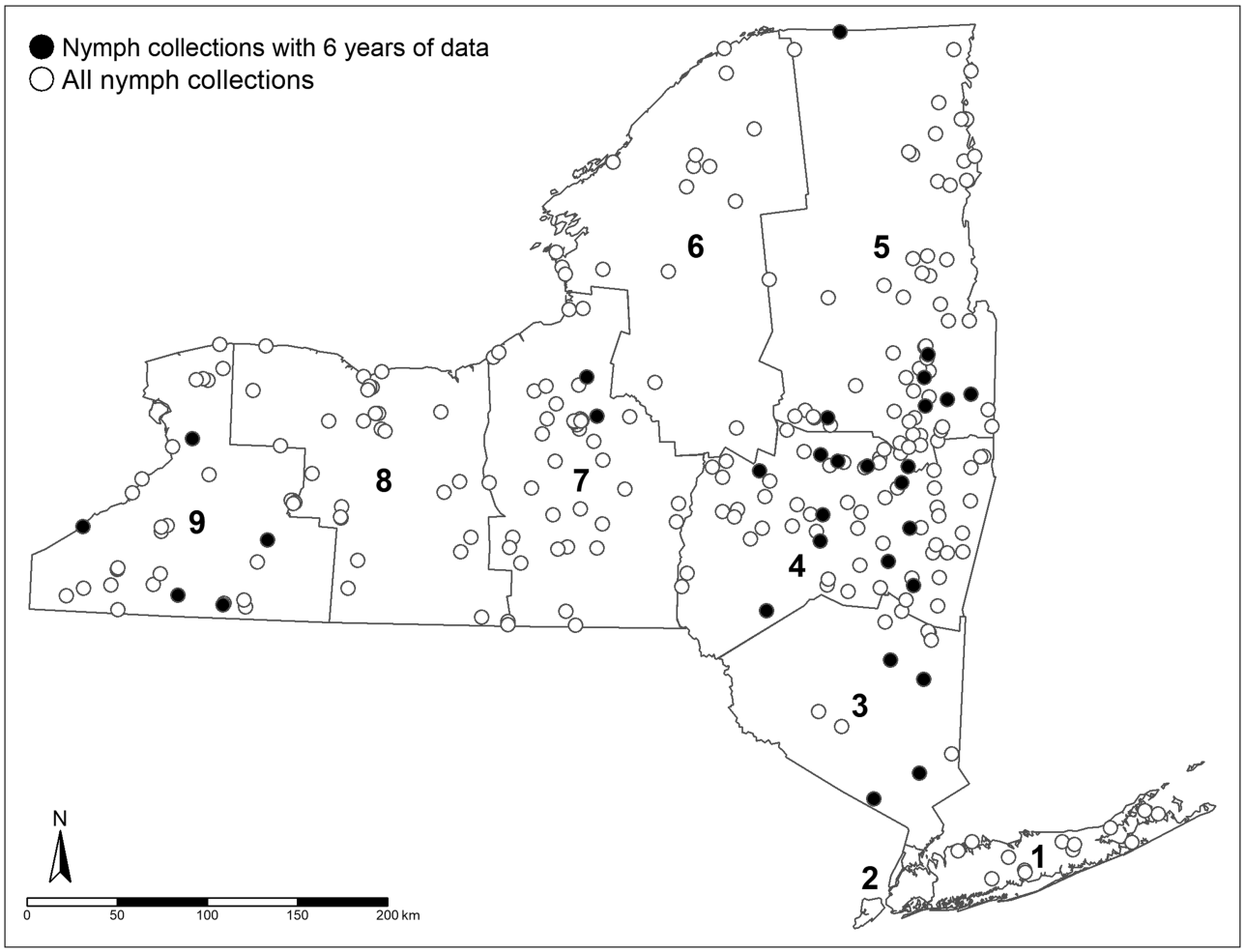

Fig. 1. Spatial distribution of nymphal *Ixodes scapularis* collections conducted by the New York State Department of Health in New York during 2015–2020.

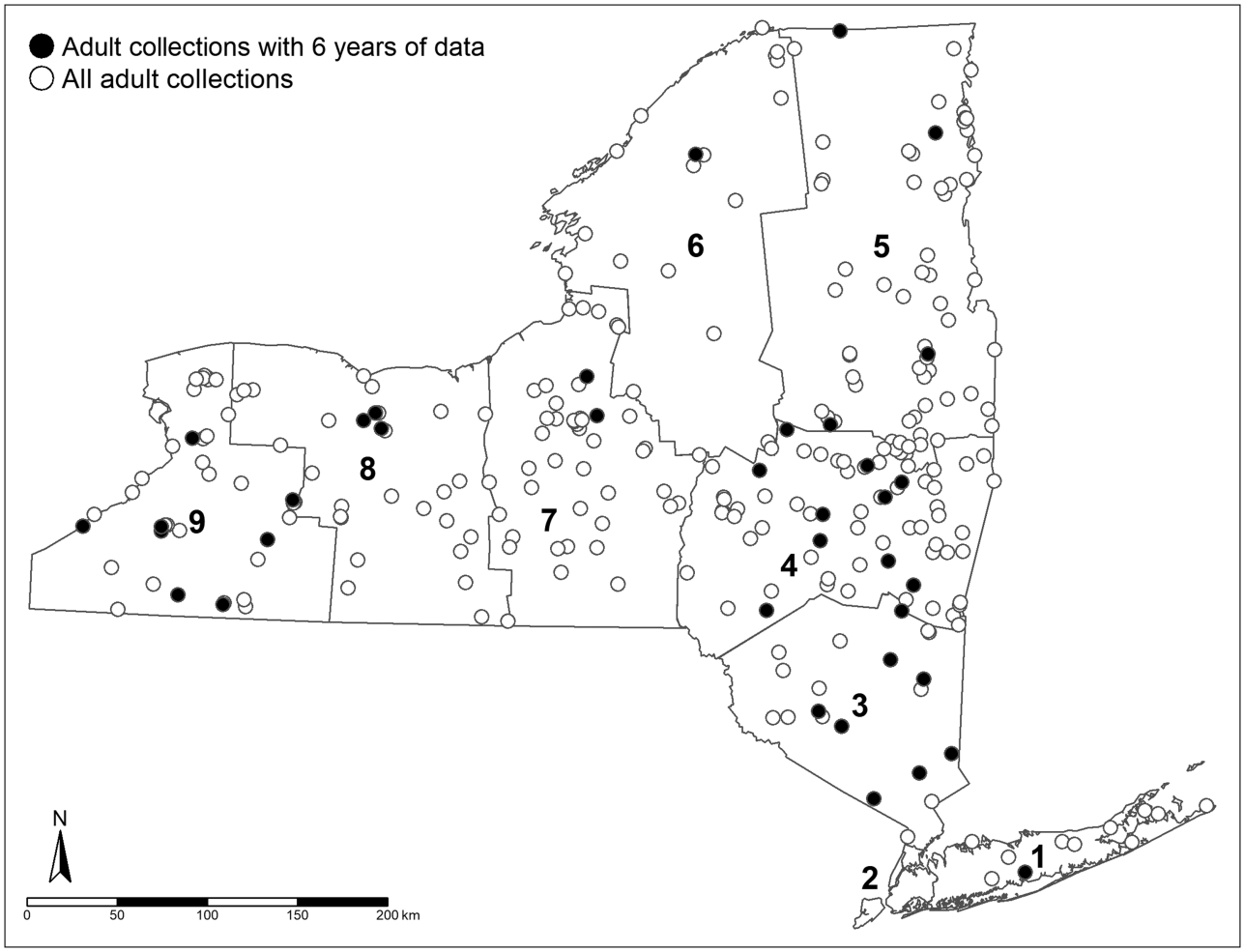

Fig. 2. Spatial distribution of adult *Ixodes scapularis* collections conducted by the New York State Department of Health in New York during 2015–2020.

DNA in nuclease-free water with 0.3 µl each of the following: DNA extracted from whole blood of a *Ba. microti*-infected C3H/HeN mouse (10% parasitemia), low-passage *Bo. burgdorferi* B31 lysate (Stony Brook, NY), previously isolated *A. phagocytophilum* genomic DNA (CDC, Atlanta, GA), and *B. hermsii* DNA purified from culture (DSMZ) were included with each run. Amplification was carried out on an ABI 7500 Real-time PCR System (Applied Biosystems, Foster City, CA) using white 96-well semi-skirted plates with optically clear adhesive seal (Thermo Scientific, USA). Thermocycling conditions consisted of 50°C for 2 min and initial denaturation and activation of AccuStart Taq DNA polymerase at 95°C for 10 min, followed by 45 cycles of amplification with denaturation at 95°C for 15 sec and annealing at 63°C for 1 min. All PCR was performed in an isolated location separate from DNA extraction, in a class 2 biological safety cabinet with laminar airflow designated exclusively for PCR, using sterile aerosol-barrier tips, designated micropipettors, and strict sterile technique to prevent cross contamination of samples. All real-time mulitplex PCR data were interpreted with a cut-off of cycle 40 and analyzed using the Applied Biosystems 7500 SDS Software Version 1.4 (Applied Biosystems, Foster City, CA) as previously described (Piedmonte et al. 2018). Samples testing positive for *A. phagocytophilum* by multiplex PCR were further tested using a custom TaqMan SNP genotyping PCR assay to differentiate between the Ap-ha and Ap-V1 variants of *A. phagocytophilum* as described elsewhere (Krakowetz et al. 2014, Prusinski et al. 2023).

Data Analysis

Data were imported into R (RStudio Team, 2022) (version 4.1.2). Collection locations were assigned to 1 of 8 NYS Department of Environmental Conservation (NYSDEC) regions (Figs. 1 and 2). An ERI was calculated for nymphs and adults separately for each collection event as the product of *I. scapularis* nymphs or adults per 1,000 m^2 sampled and the proportion of ticks infected with *Bo. miyamotoi*. In instances where a location was visited on multiple occasions within a given sampling season, the infection prevalence and tick density measures were averaged using the R package "dplyr" (Wickham et al. 2021), and the resulting averages were used to generate a single ERI value for each site and year. There was no control for the effect of phenology due to the limited ticks collected per location and lack of repeated sampling events per collection season at most locations. The site-level data for *I. scapularis* nymphs and adults, separately, were used to generate risk maps for each developmental stage using the tmap package in R (Tennekes 2018) where individual data points represented a collection site, the color indicated detection of *Bo. miyamotoi*, and size of each data point represented the degree of risk (ERI).

A Tweedie distribution generalized linear mixed effects model (GLMM) (Foster and Bravington 2013) was generated using site-level data from locations with consistent collections across all 6 study years for nymphs (177 sampling events, 30 sites) and adults (221 sampling events, 37 sites) separately in R studio using the

"glmmTMB" package (Brooks et al. 2017), to examine the significance of the following predictors on ERI: year, region, elevation (30 km resolution), average maximum temperature and average cm of rainfall from weather stations during the collection season for each life stage (DeGaetano et al. 2014), and average relative humidity at the time of field collection. A Tweedie distribution was chosen for this model to accommodate the zero-inflated semi-continuous ERI outcome variable. These ecological variables were scaled using the base R "scale" function, where each value was normalized by subtracting the mean and dividing by the standard deviation, to account for large variation in values among variables (Becker et al. 1988). To determine model fit, backwards selection was conducted with the variables of interest. The model with the lowest Akaike's information criterion (AIC) value (Burnham and Anderson 2004) was chosen. Year and region were treated as categorical variables and their significance was determined using ANOVA within the R package "car" (Fox and Weisberg 2019). The packages "lmer4" and "simr" were used to conduct a post-hoc power analysis following a simplified version of the Tweedie GLMM models in R studio (Bates et al. 2015, Green and MacLeod 2016).

To analyze spatial and temporal trends at the region and year level, the R package "dplyr" was used to aggregate *I. scapularis* density, *Bo. miyamotoi* prevalence, and ERI. These aggregated values were plotted in a bar chart to visualize overall variation in risk among regions and years. *Post hoc* analysis of the model was conducted to determine which regions and years were different from one another using pairwise comparisons in the R package "emmeans" (Lenth 2022); significant values ($\alpha \leq 0.05$). The R package "emmeans" was also used to visualize estimated marginal means among the significant interactions. In addition to ERI, polymicrobial tick infection rates were determined for *Bo. miyamotoi*-infected *I. scapularis*. Chi-square tests were performed with the R package "stats" (R Core Team 2021) and were used to determine if *Bo. miyamotoi* coinfection with any other pathogens occurred more frequently than expected by chance.

Results

BMD Human Cases

Between the years of 2014 and 2020, there were 5 human cases of BMD identified by the NYSDOH Wadsworth Center Bacteriology Laboratory of 942 clinical specimens tested for *Bo. miyamotoi* (0.5%). Demographic data for BMD cases are presented in Table 1. Race and ethnicity fields were incomplete for all BMD cases and were subsequently excluded from our analyses. Information on travel history was also incomplete for all 5 identified cases. BMD cases were residents of 4 different NYS geographic regions. Spatially, in Region 1, 1 of 147 (0.6% positive) was identified in 2014; in Region 4, 2 of 266 (0.8%) patients tested positive in 2015; in Region 3, 1 of 172 (0.6%) patients tested positive in 2017; and in Region 2, 1 of 88 (1.1%) tested positive in 2018. There were no detected cases reported in 2016 (n = 118 patients tested), 2019 (n = 87), and 2020 (n = 64) (Figs. 3 and 4).

Tick Collections

A total of 151,065 ticks were collected during 2,463 sampling attempts at 559 locations across all 57 NYS counties (excluding New York City) between 15 April 2015 and 27 November 2020. Of these, 111,437 *I. scapularis* (23,399 larvae [L], 27,553 nymphs [N], 60,485 adults [A]) were obtained from 432 locations in 57 counties and comprised 73.8% of the total ticks collected. Other tick species encountered (26%) included: *Amblyomma americanum* (L., Acari: Ixodidae) (10,682 N, 3,525 A), *Dermacentor albipictus* (Packard, Acari: Ixodidae) (125 L), *Dermacentor variabilis* (Say, Acari: Ixodidae) (27 L, 2 N, 1,453 A), *Haemaphysalis leporispalustris* (Packard, Acari: Ixodidae) (261 L, 32 N), *Haemaphysalis longicornis* (Neumann, Acari: Ixodidae) (23,290 L, 67 N, 95 A), *Ixodes angustus* (Neumann, Acari: Ixodidae) (2 L), *Ixodes dentatus* (Marx, Acari: Ixodidae) (2 L, 36 N), *Ixodes cookei* (Packard, Acari: Ixodidae) (5 L, 11 N, 4 A), *Ixodes marxi* (Banks, Acari: Ixodidae) (1 L, 5 N, 2 A), and *Ixodes muris* (Bishopp, Acari: Ixodidae) (1 A). Average tick densities (ticks per 1,000 m^2 ± SE) for host-seeking *I. scapularis* nymphs and adults, respectively, were 22.5 ± 1.3 and 47.3 ± 2.2, with annual variation and regional differences in average density (Table 2).

Pathogen Prevalence

A total of 48,386 *I. scapularis* (18,235 N, 30,151 A) were tested for *Bo. burgdorferi*, *Ba. microti*, *A. phagocytophilum*, and *Bo. miyamotoi* by real-time multiplex PCR assay, of which 581 were positive for the presence of *Bo. miyamotoi*. Overall prevalence of these pathogens for nymphs and adults, respectively, was 25% (n = 4,502) and 54% (n = 16,117) for *Bo. burgdorferi*, 5.3% (n = 964) and 8.4% (n = 2,522) for *A. phagocytophilum*, 4.6% (n = 834) and 5.1% (n = 1,535) for *Ba. microti*, and 1.1% (n = 194) and 1.3% (n = 387) for *Bo. miyamotoi*. *Borrelia miyamotoi* was detected in 34 of 57 NYS counties (60%) where *I. scapularis* nymphs were obtained, and from 47 of 57 NYS counties (83%) across all geographic regions where host-seeking adult *I. scapularis* populations were found (Figs. 3 and 4). Regional mean prevalence of *Bo. miyamotoi* in nymphs and adults, respectively, ranged from 0.1% and 0.6% to 3.1% and 2.8% (Table 2). Annual site-level *Bo. miyamotoi* prevalence ranged from 1.6 to 20% for nymphs from 67 of 288 locations (23.3%) and from 0.8 to 33% in adult ticks from 131 of 379 locations (34.6%). At locations with sufficient sample size to accurately assess pathogen prevalence ($n \geq 50$ ticks tested), *Bo. miyamotoi* site level infection rates in host-seeking *I. scapularis* ranged from 1.6 to 16.0% for nymphs and 0.8–12.0% for adults. The overall mean prevalence of *Bo. miyamotoi* in

Table 1. Positive cases of laboratory-confirmed *Borrelia miyamotoi* infection in New York State from 2014 to 2020.

Characteristic	*Bo. miyamotoi*-positive cases (%)
Age (yr)	
10–19	1 (20%)
40–49	2 (40%)
50–59	1 (20%)
60–69	1 (20%)
Age (yr)	
Mean (SD)	43.4 (19.23)
Median (max, min)	46 (13, 64)
Sex	
Male	2 (40%)
Female	2 (40%)
Missing	1 (20%)
Month of symptom onset	
May	1 (20%)
Jun	1 (20%)
Aug	2 (40%)
Sep	1 (20%)

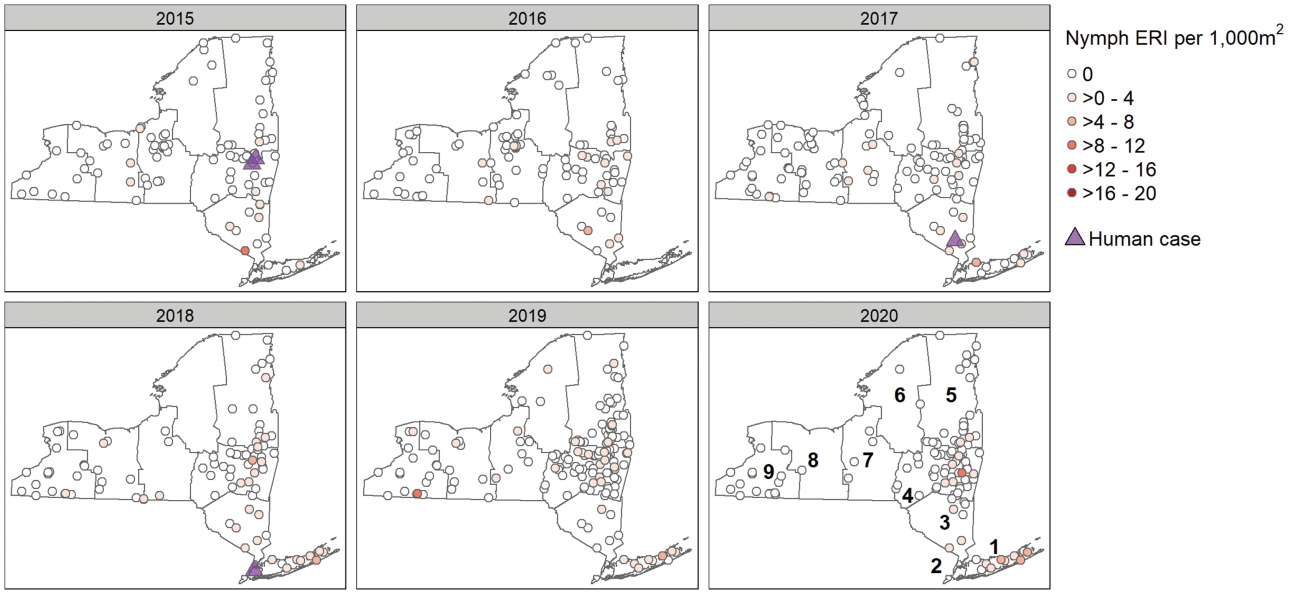

Fig. 3. Risk of encountering *Borrelia miyamotoi*-infected *Ixodes scapularis* nymphs during 2015–2020 across New York State regions and years in relation to BMD human cases.

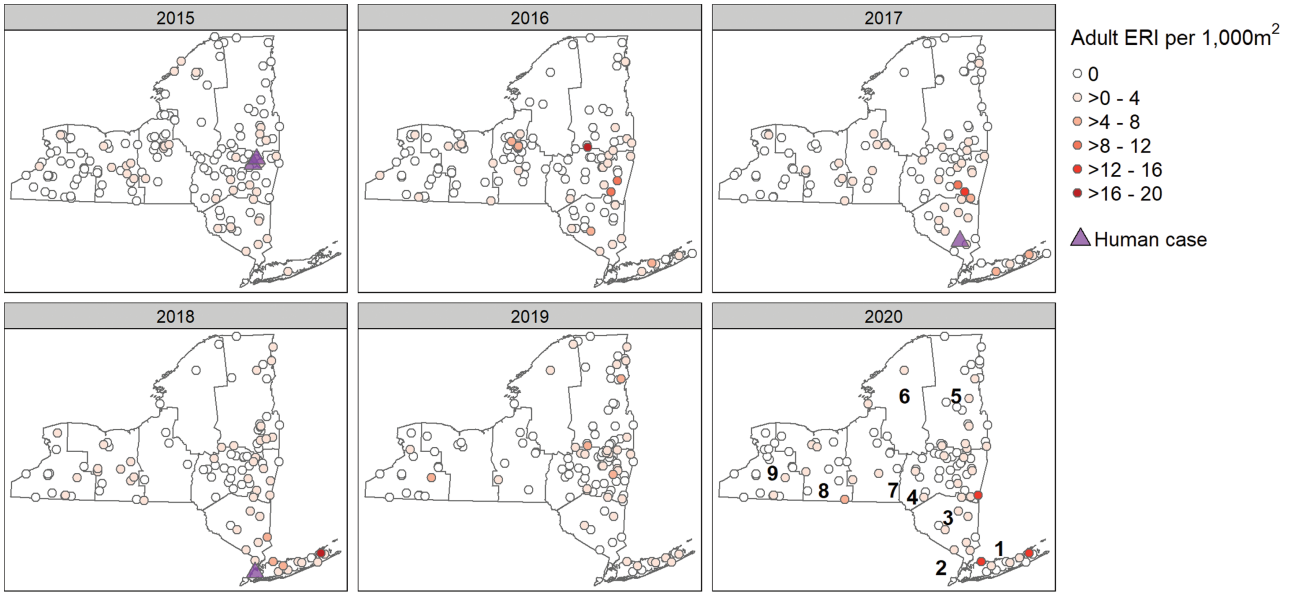

Fig. 4. Risk of encountering *Borrelia miyamotoi*-infected *Ixodes scapularis* adults during 2015–2020 across New York State regions and years in relation to BMD human cases.

nymphs was lowest in 2015 and 2017 (0.4%) and highest in 2018 and 2020 (1.0%) and ranged from 0.8% in 2016 to 1.6% in 2018 for adult *I. scapularis* (Table 2).

Polymicrobial Infections

Of the 581 ticks (194 N, 387 A) testing positive for *Bo. miyamotoi*; 299 (124 N, 175 A) were single infections (52%), 228 (55 N, 173 A) were dual infections (39%), and 50 (15 N, 35 A) were triple infections (8.6%). Four adult ticks (0.7%) were infected with all 4 pathogens (Table 3). The overall rate of polymicrobial infection was 36% for *Bo. miyamotoi*-infected nymphs and 55% for adults. Adult ticks accounted for 75% of *Bo. miyamotoi* co-infections. The most frequently detected pathogens found together in the same tick were *Bo. miyamotoi* and *Bo. burgdorferi* (n = 202, 35%). Of pairwise combinations, *Bo. miyamotoi* and *Ba. microti* co-occurred together (n = 47, 8.1%) more often than expected by chance (χ^2 = 10.27, df = 1, P-value = 0.001). Ticks simultaneously infected with 2 pathogens were found in all NYS geographic regions; as were ticks co-infected with 3 pathogens, with the exception of Region 6 (Fig. 5). Three of the four quadruple infected adult ticks were collected in Region 1 and 1 was from Region 3, both located in the southeastern portion of NYS (Fig. 5).

Borrelia miyamotoi Entomological Risk Index

Overall, the average *Bo. miyamotoi* ERI for adults and nymphs, respectively was 0.65 (SE ± 0.07) and 0.27 (SE ± 0.04). Mean nymphal

Table 2. Percent *Borrelia miyamotoi* positive collections, mean tick density and mean *Bo. miyamotoi* Entomological Risk Index for *Ixodes scapularis* nymphs and adults across years and New York State regions from 2015 to 2020.

	% Positive collections (positive collections/total collections)	Mean tick density per 1,000 m² (±SE)	Mean prevalence (±SE)	Mean ERI per 1,000 m² (±SE)
Nymph	17.60% (116/659)	22.50 (1.32)	0.007 (0.001)	0.27 (0.04)
Year				
2015	11.11% (10/90)	20.67 (3.51)	0.004 (0.002)	0.16 (0.11)
2016	15.79% (15/95)	16.32 (2.98)	0.006 (0.003)	0.14 (0.06)
2017	14.05% (17/121)	24.19 (2.89)	0.004 (0.001)	0.20 (0.07)
2018	27.96% (26/93)	22.66 (3.92)	0.010 (0.002)	0.44 (0.11)
2019	19.21% (29/151)	31.39 (3.37)	0.006 (0.001)	0.29 (0.09)
2020	17.43% (19/109)	15.08 (1.95)	0.010 (0.002)	0.36 (0.13)
Region				
Region 1	69.23% (27/39)	45.78 (6.64)	0.031 (0.005)	1.67 (0.34)
Region 3	45.45% (20/44)	65.79 (8.91)	0.017 (0.005)	0.84 (0.26)
Region 4	16.41% (32/195)	23.38 (2.40)	0.007 (0.002)	0.21 (0.05)
Region 5	10.49% (15/143)	10.68 (1.44)	0.003 (0.001)	0.06 (0.02)
Region 6	5.26% (1/19)	11.77 (2.65)	0.001 (0.001)	0.04 (0.04)
Region 7	15.71% (11/70)	12.48 (1.23)	0.004 (0.001)	0.06 (0.02)
Region 8	9.52% (6/63)	22.60 (4.58)	0.004 (0.002)	0.10 (0.05)
Region 9	4.65% (4/86)	17.94 (2.71)	0.002 (0.001)	0.15 (0.13)
Adult	30.91% (230/744)	47.27 (2.21)	0.012 (0.001)	0.65 (0.07)
Year				
2015	23.72% (37/156)	29.51 (3.10)	0.012 (0.003)	0.32 (0.06)
2016	29.41% (40/136)	56.85 (7.31)	0.008 (0.001)	0.77 (0.18)
2017	31.53% (35/111)	53.66 (5.22)	0.015 (0.005)	0.81 (0.20)
2018	43.27% (45/104)	36.21 (3.22)	0.016 (0.003)	0.78 (0.190
2019	29.51% (36/122)	56.23 (5.10)	0.009 (0.001)	0.52 (0.11)
2020	32.17% (37/115)	54.38 (6.62)	0.013 (0.002)	0.82 (0.21)
Region				
Region 1	66.67% (30/45)	73.37 (10.55)	0.028 (0.004)	2.33 (0.58)
Region 3	52.31% (34/65)	52.79 (4.11)	0.017 (0.003)	0.87 (0.13)
Region 4	32.62% (61/187)	55.90 (6.04)	0.012 (0.002)	0.79 (0.15)
Region 5	25.52% (37/145)	37.92 (4.21)	0.009 (0.002)	0.49 (0.14)
Region 6	23.33% (7/30)	37.07 (5.09)	0.008 (0.003)	0.35 (0.14)
Region 7	29.49% (23/78)	37.82 (6.34)	0.013 (0.006)	0.43 (0.13)
Region 8	27.17% (25/92)	49.65 (5.47)	0.009 (0.002)	0.34 (0.08)
Region 9	12.75% (13/102)	38.27 (4.32)	0.006 (0.003)	0.26 (0.09)

and adult *Bo. miyamotoi* ERI values across study years and NYS regions are shown in Table 2. *Borrelia miyamotoi* ERI for nymphal ticks was lowest in Region 6 (0.04) and highest in Region 1 (1.67). Nymphal *Bo. miyamotoi* ERI was lowest in 2016 (0.14) and highest in 2018 (0.44). *Borrelia miyamotoi* ERI for adult ticks was lowest in Region 9 (0.26) and highest in Region 1 (2.33). Risk of encountering *Bo. miyamotoi*-infected adult *I. scapularis* was lowest in 2015 (0.32) and highest in 2020 (0.82). The geographic distribution of *Bo. miyamotoi* ERI values at sampling sites was similar throughout all study years for both nymphal and adult tick collections. Specifically, the highest ERI values were located in regions 1, 3, and 4 which were also the regions containing identified cases of BMD (Figs. 3 and 4.) Notably, the risk of encountering *Bo. miyamotoi*-infected adults was higher than the risk of encountering infected nymphs across all regions and years ($t = 5.05$, df = 1198, P-value ≤ 0.001).

Overall, the average *Bo. miyamotoi* ERI for nymphal ticks across sites that were sampled consistently over all 6 study years was 0.28 (SE ± 0.07), with the highest risk in Region 3 (Fig. 6A). All other regions were significantly lower than Region 3 based on pairwise comparisons. In addition, Region 5 was significantly lower than Region 4. The overall average *Bo. miyamotoi* ERI for adult ticks across sites with consistent sampling data across all 6 yr of our study was 0.88 (SE ± 0.13), with the highest risk in Region 1 (Fig. 6B). There were no significant differences among regions in adult tick *Bo. miyamotoi* ERI. The risk of encountering *Bo. miyamotoi*-infected nymphs was highest in 2018 across sites that were sampled during all 6 study years with 0.46 (SE ± 0.20). However, based on pairwise comparisons, there was no significant difference among years (Fig. 7A). The risk of encountering *Bo. miyamotoi*-infected adults was highest in 2017 across sites with 6 years of sampling data with 1.39 (SE ± 0.52) infected adults encountered every 1,000 m². However, pairwise comparisons show that risk in 2017 was significantly higher than 2015 and no other years (Fig. 7B).

Borrelia miyamotoi ERI Environmental Models

Elevation influenced risk of encountering *Bo. miyamotoi*-infected nymphs as determined by ERI (Table 4). Elevation was negatively associated with ERI ($\beta = -1.37$, $P < 0.001$) in the analyses of nymphal *I. scapularis*. ANOVA of the final nymphal model revealed NYS region had a significant impact on ERI ($\chi^2 = 39.38$, df = 4, P-value ≤ 0.001) but year did not. Due to the lack of consistent nymphal sampling and environmental data collection at the time of tick sampling at sites on Long Island (Region 1) during the study period, it was excluded from our analyses of nymphal *I. scapularis*.

Mean rainfall, relative humidity, maximum temperature, and the interaction between relative humidity and maximum temperature influenced adult tick *Bo. miyamotoi* ERI when comparing collection

Table 3. Co-infections in *Borrelia miyamotoi*-infected *Ixodes scapularis* collected in New York State from 2015 to 2020.

Pathogens	No. ticks (n = 581) (%)	Life stage	*A. phagocytophilum* variant
Bmiy/Bb/Bm/Ap	4 (0.7%)	F = 3 M = 1 N = 0	ha = 3 v1 = 1 ha/v1 = 0 undetermined = 0
Bmiy/Bb/Bm	25 (4.3%)	F = 8 M = 5 N = 12	–
Bmiy/Bb/Ap	23 (4.0%)	F = 8 M = 13 N = 2	ha = 18 v1 = 1 ha/v1 = 0 undetermined = 4
Bmiy/Bm/Ap	2 (0.3%)	F = 1 M = 0 N = 1	ha = 2 v1 = 0 ha/v1 = 0 undetermined = 0
Bmiy/Bb	196 (33.7%)	F = 87 M = 65 N = 44	–
Bmiy/Bm	16 (2.8%)	F = 6 M = 1 N = 9	–
Bmiy/Ap	16 (2.8%)	F = 9 M = 5 N = 2	ha = 11 v1 = 5 ha/v1 = 0 undetermined=0
Bmiy only	299 (51.5%)	F = 87 M = 88 N = 124	–

Bmiy = *Borrelia miyamotoi*, *Bb* = *Borrelia burgdorferi*, *Bm* = *Babesia microti*, *Ap* = *Anaplasma phagocytophilum*, ha = human active, v1 = variant 1.

data for sites with consistent adult tick sampling across all 6 study years (Table 5). Mean relative humidity was positively associated with ERI (β = 0.25, *P*-value = 0.048); mean maximum temperature was positively associated with ERI (β = 1.11, *P*-value < 0.001); mean rainfall was negatively associated with ERI (β = −0.42, *P*-value = 0.018); and the interaction between mean relative humidity was negatively associated with ERI (β = −0.31, *P*-value = 0.018). ANOVA analysis of the final adult model revealed year was significantly associated with adult tick *Bo. miyamotoi* ERI (χ^2 = 20.25, df = 5, *P*-value = 0.001) but region was not.

Discussion

Global *Bo. miyamotoi* infection rates reported in ticks range from 0.02% to 6.4% (Cutler et al. 2019) and the prevalence of *Bo. miyamotoi* in host-seeking *I. scapularis* from NYS was similar at the site level to those reported previously (Scoles et al. 2001, Tokarz et al. 2010, Krause et al. 2015, Keesing et al. 2021). We detected *Bo. miyamotoi* in 3.1% of nymphs and 2.8% of *I. scapularis* adults from Suffolk County (Region 1), consistent with the 2.6% and 3.4%, respectively, reported previously (Tokarz et al. 2017). A study recently conducted in Dutchess County, NY (Region 3), had an overall *Bo. miyamotoi* prevalence of 1.2% out of 3,647 nymphal *I. scapularis* (Keesing et al. 2021). Yuan et al. (2020) also reported *Bo. miyamotoi* infection rates in adult ticks from Region 3 (Westchester County) to be 2.2% (1/45) and 14% (1/7) in 2017 and 2018, respectively. In Region 3, the average *Bo. miyamotoi* prevalence for both nymphal and adult ticks was 1.7%. Targeted sampling of ticks from the southeastern portion of this NYS region, encompassing Westchester County, should be considered to better define areas with elevated risk.

Across all study sites, average *Bo. miyamotoi* ERI was 2.4 times higher in adult ticks in comparison to nymphs (Table 2), and geographic regions with higher *Bo. miyamotoi* ERI in ticks overlapped with locations of human BMD cases reported during the study period in NYS. However, the paucity of cases detected by NYSDOH Wadsworth clinical BMD testing and the lack of patient travel histories reduced our confidence in the significance of spatial overlap with areas of elevated predicted *Bo. miyamotoi* risk. *Bo. miyamotoi* ERI in adult ticks was highest in 2016 and 2017, and significantly lower in 2015. In contrast, nymphal ERI was highest in 2015. However, not all sites were sampled for both adults and nymphs every year and further examination of locations that were sampled annually for both developmental stages is warranted.

Our GLMM model results indicated that nymphal *Bo. miyamotoi* ERI was statistically higher in the Hudson Valley in southeastern NYS (Region 3) compared to the other regions. Nymphal *Bo. miyamotoi* ERI in Region 4, an area known as the "Capital District Region", was also significantly higher than risk in Region 5, which includes

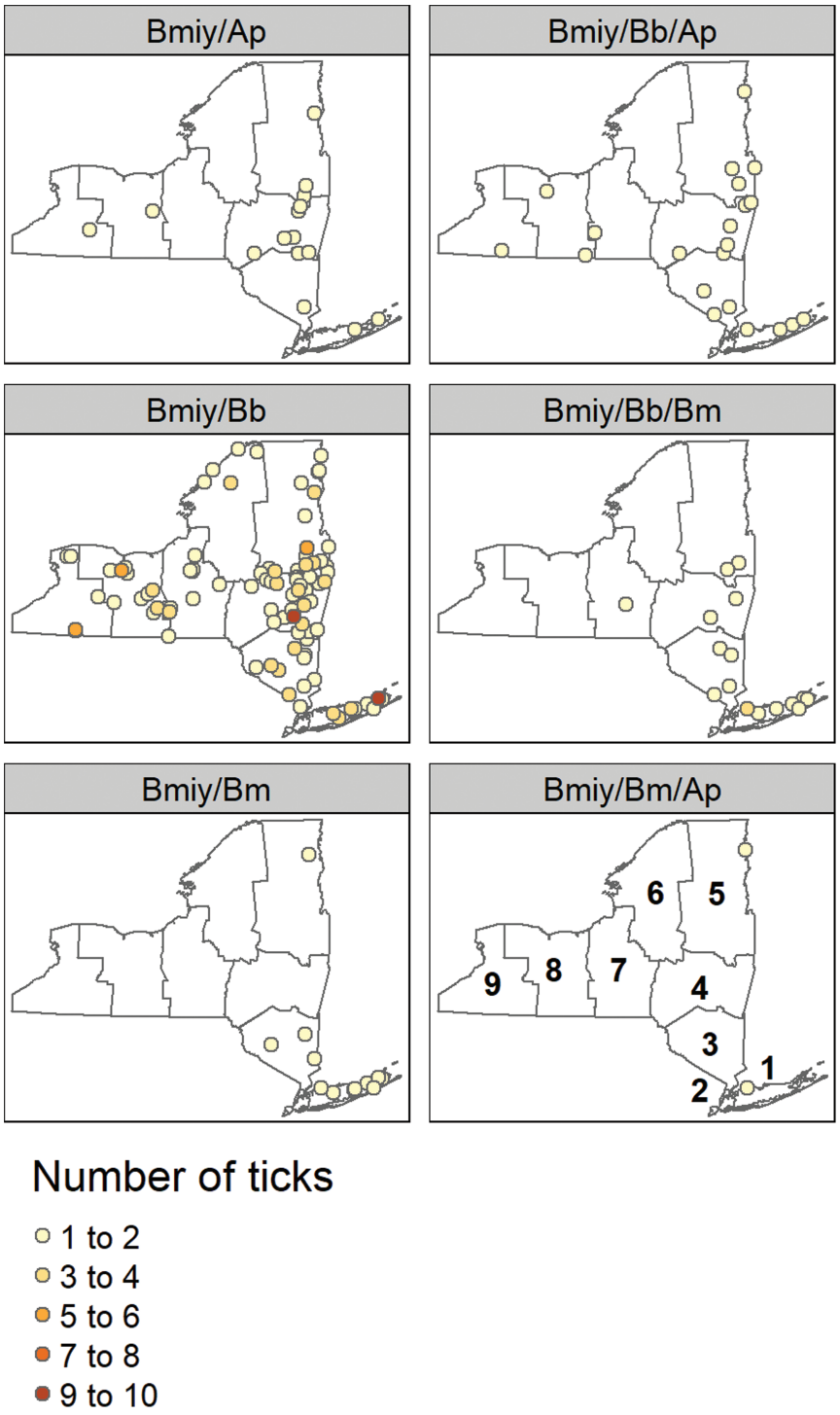

Fig. 5. Spatial distribution of co-infected *Ixodes scapularis* collected in New York State from 2015–2020. *Bmiy = *Borrelia miyamotoi,* Bb = *Borrelia burgdorferi,* Bm = *Babesia microti,* Ap = *Ananplasma phagocytophilum.*

the Adirondack Mountains. The relatively higher levels of risk in Regions 3 and 4 are not surprising, as these areas of NYS also have increased prevalence of other *I. scapularis*-associated pathogens, including *A. phagocytophilum* (Russell et al. 2021, Prusinski et al. 2023), *Bo. burgdorferi* (Prusinski et al. 2014, Lin et al. 2019) and *Ba. microti* (Kogut et al. 2005, Linden et al. 2018, O'Connor et al. 2021) with increased incidence of associated disease in humans. While Long Island (Region 1) was excluded from our nymphal analyses due to lack of consistent sampling at set locations during the entirety of the study period, almost 70% of the nymphal *I. scapularis* collection attempts in Suffolk County (Region 1) yielded *Bo. miyamotoi*-infected ticks (Table 3), with a county-level prevalence of 3.6%. Risk of encountering *Bo. miyamotoi*-infected adults was also highest in Region 1. However, these differences were not statistically significant in the adult tick model, likely due to the limited number of sites that were consistently sampled across all years in Region 1. The lack of

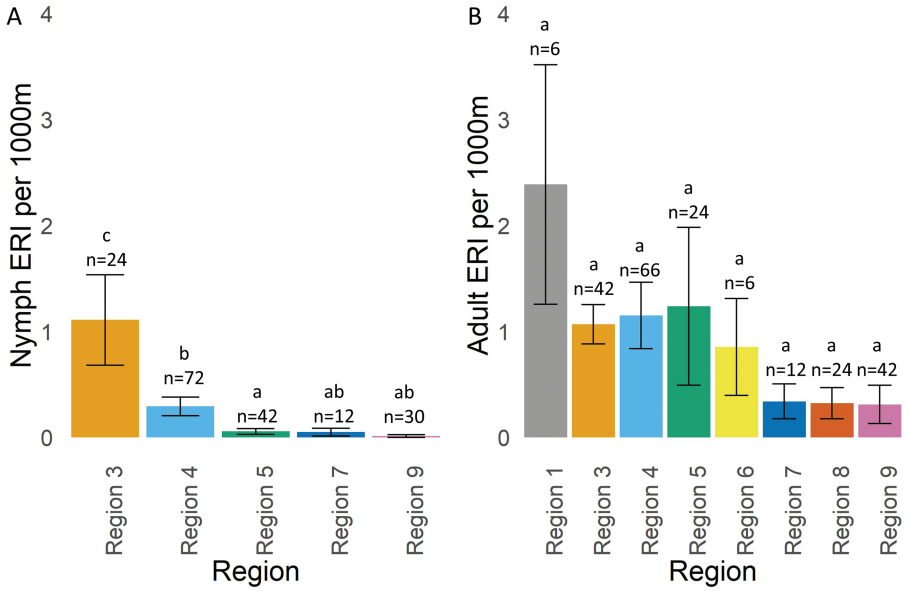

Fig. 6. Risk of exposure to *Borrelia miyamotoi*-infected *Ixodes scapularis* by New York State region, 2015–2020. Bars with different letters are significantly different from each other. Letters only signify statistical differences within that tick developmental stage and cannot be compared across developmental stages.

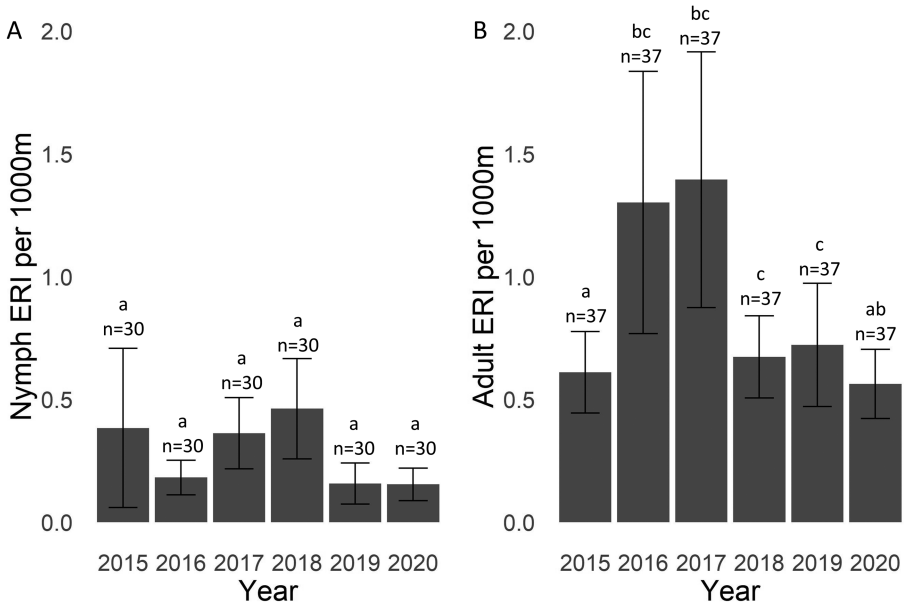

Fig. 7. Risk of exposure to *Borrelia. miyamotoi*-infected *Ixodes scapularis* by year of collection, 2015–2020 in New York State. Bars with different letters are significantly different from each other. The letters only signify statistical differences within that developmental stage and cannot be compared between both stages.

consistent sampling reduced our ability to identify true differences between Region 1 and other regions, particularly those with more consistent sampling during the study period. Future studies should consider consistent annual sampling of *I. scapularis* nymphs and adults at additional sites in Region 1 to enable higher resolution analyses of *Bo. miyamotoi* dynamics in this area of elevated risk.

Pathogen co-infection results for *I. scapularis* adults and nymphs infected with *Bo. miyamotoi* were similar. The most frequently detected pathogens found together in the same tick were *Bo. miyamotoi* and *Bo. burgdorferi* (Table 5). It is likely that these pathogens infect the same reservoir species and ticks ingest both pathogens while feeding on a single host. The white-footed mouse (*Peromyscus leucopus*) is a major reservoir of *Bo. burgdorferi* and has been found to be co-infected with *Bo. miyamotoi* in the northeastern United States (Barbour et al. 2009). Additionally, *Bo. burgdorferi* is the most prevalent and geographically dispersed tick-borne disease agent in NYS tick populations (Prusinski et al., 2014, Yuan et al. 2020). *Babesia microti* infection in ticks was significantly associated with *Bo. miyamotoi* infection. This result may also be attributed to *P. leucopus* serving as a common reservoir for both pathogens (Dunn et al. 2014) and is also likely related to the spatial distribution of *Ba. microti* in NYS, where the highest prevalence in host-seeking ticks occurred in the southeastern potion of NYS (O'Connor et al. 2021), including the Hudson Valley (Region 3) and

Table 4. Model parameters and significant predictors for human exposure to *Borrelia miyamotoi*-infected *Ixodes scapularis* nymphs in NYS. (AIC: 245.4)

Mean Entomological Risk Index per 1,000 m^2 ~ year + region + elevation + mean relative humidity + (1 | location)		
Number of sampling events: 177 Number of sites: 30		
Predictors	Coefficient estimates	*P*-values
Intercept (Region 3 2015)	–0.4291	0.3800
Elevation	–1.3671	<0.001[a]
Mean relative humidity	0.2960	0.094[a]

Mean *Bo. miyamotoi* ERI per 1,000 m^2 = average number of infected ticks encountered every 1,000 m^2, year = year collection occurred, region = region collection occurred, mean relative humidity = average on site relative humidity values for location that year, mean maximum temperature = average maximum temperature from May to August, elevation = elevation of collection site within 30 km, (1 | location) = random effects for location.

[a]Statistically significant.

Table 5. Model parameters and significant predictors for human exposure to *Borrelia miyamotoi*-infected *Ixodes scapularis* adults in New York State. (AIC: 574.2)

Mean Entomological Risk Index per 1,000 m^2 ~ year + region + mean relative humidity + mean maximum temperature + mean rainfall cm + (1 | location)		
Number of sampling events: 221 Number of sites: 37		
Predictors	Coefficient estimates	*P*-values
Intercept (Region 1 2015)	–2.6115	0.0281[a]
Mean max. temperature (Oct–Dec)	1.1131	0.0003[a]
Mean rainfall (Oct–Dec)	–0.4179	0.0185[a]
Mean relative humidity (RH)	0.2544	0.0475[a]
Mean RH × mean max. temperature (Oct–Dec)	–0.3064	0.0179[a]

Mean *Bo. miyamotoi* ERI per 1,000 m^2 = average number of infected ticks encountered every 1,000 m^2, year = year collection occurred, region = region collection occurred, mean relative humidity = average on site relative humidity values for location that year, mean maximum temperature = average maximum temperature from October to December, mean rainfall cm = average rainfall in cm from October to December, (1 | location) = random effects for location.

[a]Statistically significant.

Long Island (Region 1), which also had the highest prevalence of *Bo. miyamotoi* in our study. Additional studies are necessary to better understand the transmission dynamics and microbial interactions in ticks co-infected with multiple pathogens, including *Bo. miyamotoi*.

The polymicrobial infection rates we detected have important clinical implications. Of the 581 ticks that were infected with *Bo. miyamotoi*, 35% were also infected with *Bo. burgdorferi*, the causative agent of Lyme disease in the northeastern United States. Since BMD is not currently a reportable condition in NYS under public health law, screening for *Bo. miyamotoi* in clinical samples is infrequent and significant misdiagnosis and underreporting of BMD likely occurs in NYS. Patients presenting with flu-like symptoms and history of tick bite in Lyme disease endemic areas are commonly tested for *Bo. burgdorferi* and subsequently treated with antibiotics, such as doxycycline, and may not be screened for other lesser-known emerging pathogens like *Bo. miyamotoi*. As both BMD and Lyme disease are successfully treated with doxycycline, the lack of specific clinical testing for *Bo. miyamotoi* infection likely leads to missed diagnoses of BMD in the event of Lyme disease co-infection, complicating our understanding of BMD epidemiology, burden, and risk. Additionally, babesiosis patients receiving appropriate treatment, who screen negative for concurrent Lyme disease or anaplasmosis infection but continue to exhibit symptoms consistent with these diseases should be screened for *Bo. miyamotoi* infection, as they would potentially benefit from proper diagnosis and prompt initiation of doxycycline treatment for BMD.

In our study, risk of exposure to *Bo. miyamotoi*-infected *I. scapularis* nymphs was influenced by elevation. Habitat suitability for *I. scapularis* has been shown to decrease as elevation increases (Hahn et al. 2016). In addition, fewer reproductive and reservoir hosts may be available at higher elevations (Mccain and Grytnes 2010). For example, Rand et al. (2003) reported that the number of *I. scapularis* collected from white-tailed deer decreased as elevation increased, potentially due to a lack of smaller mammal hosts and increased probability of adverse microclimatic conditions for the immature tick life stages. However, in contrast to nymphs, elevation was not shown to be a significant predictor of adult tick *Bo. miyamotoi* ERI, possibly indicating that elevation may have a larger impact on the survival of immature ticks in the larval stage than as engorged nymphs or adults.

Borrelia miyamotoi adult tick ERI was significantly influenced by mean seasonal temperature, mean seasonal rainfall, and relative humidity. Increases in temperature during the autumn questing period would lead to increased *Bo. miyamotoi* risk, as temperature has been positively correlated to questing activity in *I. scapularis* adults (Duffy and Campbell 1994). Rainfall led to a significant decrease in risk of encountering *Bo. miyamotoi* infected adults, as increased periods of rain may suppress questing activity and successful host acquisition over a season. Increases in relative humidity at the site level led to an

increased risk of encountering adults infected with *Bo. miyamotoi*. These results agree with previous results indicating that tick questing and abundance are positively related to relative humidity (Berger et al. 2014). Statistically significant interaction between relative humidity at the site level and mean seasonal temperature indicates that the risk of encountering a *Bo. miyamotoi*-infected adult *I. scapularis* varies across these 2 variables.

Our study is subject to several limitations that decreased our ability to robustly model BMD risk. Sample sizes of *Bo. miyamotoi*-infected host-seeking ticks were small in certain regions and years, due to limitations in available NYSDOH resources dedicated to surveillance sampling, making it difficult to make statistically significant comparisons across space and time. The collection data were analyzed with year as a categorical variable due to limited samples for each year, which limited our ability to determine temporal difference within years. It would be beneficial to analyze the data on a more granular scale with collection month included as a predictor, to potentially assess if these factors impact BMD risk seasonally within years and within the context of vector phenology. In addition to overall sample size, specific multi-year sampling at consistent locations was limited, further reducing statistical power to model risk. For instance, Region 1 had the highest reported *Bo. miyamotoi* risk in nymphal collections, but these values were excluded from the model due to lack of consistent nymphal sampling and/or recording of key environmental data at the time of sampling at some locations in the region. Results of our post hoc power analysis indicated that our sample size was sufficient to evaluate the effect of year, region, and maximum temperature on adult and nymphal ERI; however, our models lacked sufficient power to estimate effect sizes for other included covariates. Future annual sampling of both nymphal and adult ticks at set locations and complete recording of required site-level environmental data has now been implemented and will facilitate more precise risk estimates going forward. In addition, the data provided by the nearest weather stations were aggregated across the sampling season to provide an average temperature and rainfall value for each site across the target sampling season for each tick developmental stage. The generalized weather values may not accurately represent macro- and microhabitat conditions for the tick populations sampled. For future studies, measuring temperature, rainfall, and humidity at the microclimate level with data loggers or point measures at the time of sampling would provide more accurate information concerning the impact of these variables on tick questing and resulting human risk (Teel et al. 1982, Boehnke et al. 2017). Other measures such as saturation deficit could potentially represent more accurate predictors of risk and should be considered in future studies. Further investigations should be conducted to better understand the dynamic relationships among various climate and ecological variables on nymphal *Bo. miyamotoi* risk.

As a transovarially transmitted pathogen (Scoles et al. 2001, Breuner et al. 2018), sampling and testing of *I. scapularis* larvae for *Bo. miyamotoi* would contribute to estimates of BMD risk and may provide an early warning of emerging exposure risk to infected nymphs and adults. While a large number of *I. scapularis* larvae were collected during the course of our study, they were processed and tested for Powassan/Deer Tick virus (Amarillovirales: Flaviviridae) as part of ongoing enhanced NYSDOH arboviral surveillance efforts. Future prospective and retrospective screening of larval ticks for *Bo. miyamotoi* may better elucidate the role of larvae in pathogen transmission.

Our study is the first to evaluate the spatiotemporal dynamics of *Bo. miyamotoi*-infected host-seeking *I. scapularis* nymph and adult tick populations in relation to human BMD cases across the entirety of NYS. While *Bo. miyamotoi* prevalence in host-seeking ticks was low compared with other tick-borne pathogens and diagnosed cases of BMD are rare and likely vastly underreported due to lack of reporting mandates, risk of *Bo. miyamotoi* exposure was found to be present across all geographic regions of NYS. Our results highlighted areas of NYS where residents are potentially at higher risk of encountering *Bo. miyamotoi*-infected *I. scapularis*, and documented study years with the highest risk as determined by ERI. We identified factors that may influence *Bo. miyamotoi* risk and determined that abiotic factors influence tick risk differently by tick developmental stage. *Bo. miyamotoi* co-infections were common in our study, especially with *Bo. burgdorferi* and *Ba. microti*, and may have significant clinical implications. The association between *Bo. miyamotoi* and *Ba. microti* highlights the likelihood of overlapping reservoir hosts, spatial geography and resulting increased risk of exposure to both pathogens.

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