



# Frequency and Geographic Distribution of *Borrelia miyamotoi*, *Borrelia burgdorferi*, and *Babesia microti* Infections in New England Residents

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**Background.** *Borrelia miyamotoi* is a relapsing fever spirochete that relatively recently has been reported to infect humans. It causes an acute undifferentiated febrile illness that can include meningoencephalitis and relapsing fever. Like *Borrelia burgdorferi*, it is transmitted by *Ixodes scapularis* ticks in the northeastern United States and by *Ixodes pacificus* ticks in the western United States. Despite reports of clinical cases from North America, Europe, and Asia, the prevalence, geographic range, and pattern of expansion of human *B. miyamotoi* infection are uncertain. To better understand these characteristics of *B. miyamotoi* in relation to other tickborne infections, we carried out a cross-sectional seroprevalence study across New England that surveyed *B. miyamotoi*, *B. burgdorferi*, and *Babesia microti* infections.

**Methods.** We measured specific antibodies against *B. miyamotoi*, *B. burgdorferi*, and *B. microti* among individuals living in 5 New England states in 2018.

**Results.** Analysis of 1153 serum samples collected at 11 catchment sites showed that the average seroprevalence for *B. miyamotoi* was 2.8% (range, 0.6%–5.2%), which was less than that of *B. burgdorferi* (11.0%; range, 6.8%–15.6%) and *B. microti* (10.0%; range, 6.5%–13.6%). Antibody screening within county residence in New England showed varying levels of seroprevalence for these pathogens but did not reveal a vectoral geographical pattern of distribution.

**Conclusions.** Human infections caused by *B. miyamotoi*, *B. burgdorferi*, and *B. microti* are widespread with varying prevalence throughout New England.

**Keywords.** *Borrelia miyamotoi*; *Borrelia burgdorferi*; *Babesia microti*; seroprevalence; New England.

*Borrelia miyamotoi*, a spirochete that was first reported to infect humans in 2011, is related to relapsing fever spirochetes (eg, *Borrelia hermsii*) and to Lyme disease spirochetes (eg, *Borrelia burgdorferi*) [1–4]. *B. miyamotoi* infection is associated with an acute undifferentiated febrile illness but also can cause relapsing fever and meningoencephalitis. Like *B. burgdorferi*, it is transmitted by *Ixodes scapularis* ticks in the northeastern United States and by *Ixodes pacificus* ticks in the western United States [2]. An increase in the number of *I. scapularis* and their geographic expansion is thought to be the major cause of emergence of *B. burgdorferi* and *Babesia microti* (the primary cause of human babesiosis) in the Northeast. From their initial location

in southeastern New England, both Lyme disease and babesiosis have expanded to much of the northeast, from Maine to Virginia, over the past 3 decades [5–7]. In concert with this expansion, the number of confirmed Lyme disease cases reported to the Centers for Disease Control and Prevention doubled from 12 801 in 1997 to 23 558 in 2018, while *B. microti* cases doubled over the past decade from 1126 in 2011 to 2161 in 2018 [8, 9].

Despite case reports/case series in North America, Europe, and Asia and several focal serosurveys conducted in the United States and Europe, the prevalence, geographic range, and pattern of expansion of human *B. miyamotoi* infection are not well characterized [10–16]. We hypothesized that human *B. miyamotoi* infection is less common and has dispersed less widely than human *B. burgdorferi* or *B. microti* infections. To test this hypothesis, we carried out a comparative assessment of the frequency and geographic range of human infection due to *B. miyamotoi*, *B. burgdorferi*, and *B. microti* infections among residents living in the New England region using a cross-sectional serosurvey approach.

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## METHODS

### Study Population

We obtained 100 to 200 serum samples from outpatients who underwent medical testing at each of 11 medical laboratory catchment sites in New England between May 2018 and August 2018 (Table 1). Study sites were located in Connecticut, New Hampshire, Maine, Massachusetts, and Rhode Island. They were arranged along longitudinal and latitudinal lines to determine if there was evidence of expanding human *B. miyamotoi* infection from southeastern New England to the west and north, as previously demonstrated for human *B. burgdorferi* and *B. microti* infections [5–7]. The sera were obtained in a random manner from residual samples of blood after clinical tests had been completed and would otherwise have been discarded. Samples that were tested for tickborne diseases were excluded. Sample collection was performed in a deidentified manner so that we obtained residence zip codes, age, and gender for each sample without personal identifying information.

We determined seroprevalence to *B. burgdorferi*, *B. microti*, and *B. miyamotoi*, defined as the percentage of people in our study population who had antibody in their blood against 1 or more of these pathogens. We calculated seroprevalence at each catchment site using the number of seropositive samples divided by the total number of samples obtained in the catchment area at each site. Individual serum samples were then categorized by the zip code where the study participant resided to determine the residential county of each participant. We determined the seroprevalence for each pathogen in individual counties using the same methods described above for the catchment site analysis. The average seroprevalence of all study participants was described using total raw rate values (sum of the seropositive samples across all sites divided by the total number of samples across all sites) and range (minimum and maximum site seroprevalence).

### Antibody Assays

#### *Borrelia miyamotoi* Luminex assay

The presence of *B. miyamotoi* immunoglobulin G (IgG) antibody was screened using the Multiplex Luminex assay at L2 Diagnostics in New Haven, Connecticut [17]. Test antigens included GlpQ protein (glycerophosphodiester phosphodiesterase), a protein found in *B. miyamotoi* but not in *B. burgdorferi*, and 4 variable proteins (vlp1, vlp5, vlp15/16, and vlp18) [1, 13, 17, 18]. Briefly, serum samples were diluted 1:400 with phosphate-buffered saline (PBS) and mixed with antigen-coupled beads. Fluorescently labeled anti-human IgG phycoerythrin (PE) conjugated secondary antibody was mixed with the antigen/serum antibody-coupled beads before reading the medium fluorescent intensity using an LX200 instrument (Luminex). Determination of a positive reaction was performed as previously described [17]. We included 2 *B. miyamotoi* seropositive control samples and 5 *B. miyamotoi* seronegative controls on each plate.

#### *Borrelia burgdorferi* Enzyme-Linked Immunosorbent Assay

Serum samples were tested for the presence of *B. burgdorferi* IgG antibody using the US Food and Drug Administration-cleared ZEUS VlsE1/pepC10 enzyme-linked immunosorbent assay kit based on VlsE1 and pepC10 antigens (ZEUS Scientific, Inc.). The assay was performed using the manufacturer's instructions and as previously described [19–22]. There is no standard Lyme disease antibody algorithm for use in epidemiological studies. We used a single-tier VlsE1/pepC10 assay rather than a 2-tier test assay (used for clinical diagnosis) to maximize sensitivity without significant loss in specificity for this epidemiologic study [21].

#### *Babesia microti* Immunofluorescence Assay

The presence of *B. microti* antibody was determined using a modification of the standard *B. microti* immunofluorescence assay (IFA) [23]. *Babesia microti*-infected red blood cells (iRBCs) grown in BALB/cJ mice were used as the antigen source. Slides were prepared using a diluted suspension of  $5 \times 10^6$  *B. microti* iRBCs/10 $\mu$ L, dried overnight, and stored at -70°C until use. Test sera were then diluted to 1:64 and 1:128 in 1 $\times$  PBS/bovine serum albumin and added in 20- $\mu$ L increments into each well on a 12-well slide. Two positive and 1 negative control sera were included for each test. Slides were processed as previously described [23]. After review by a trained microscopist, any ambiguous samples were reviewed by a second microscopist who made the final decision after discussion with the first microscopist. We compared *B. microti* seropositivity using the standard *B. microti*-positive cutoff level of  $\geq 1:64$  serum dilution with the next higher cutoff dilution of  $\geq 1:128$  in our study population because the higher cutoff has been used in previous studies [24]. In this study, we used a serum dilution of  $\geq 1:64$  to define seropositivity to *B. microti* unless stated otherwise.

**Table 1. Demographic Characteristics of 1153 Study Participants**

Characteristic	n (% of total samples)
Catchment area	
Bridgeport, Connecticut	146 (12.7)
Danbury, Connecticut	77 (6.7)
Greenwich, Connecticut	88 (7.6)
Guilford, Connecticut	115 (10.0)
Hanover, New Hampshire	54 (4.7)
New Haven, Connecticut	87 (7.5)
New London, Connecticut	105 (9.1)
North Haven, Connecticut	118 (10.2)
Northampton, Massachusetts	107 (9.3)
Portland, Maine	97 (8.4)
Westerly, Rhode Island	159 (13.8)
Gender	
Female	633 (54.9)
Male	466 (40.4)
Unknown	54 (4.7)

## Statistical Analyses

Associations between seropositivity and gender were investigated using logistic regressions for each pathogen antibody, where status (positive or negative) was the outcome and gender was the predictor. A generalized estimating equation model (geepack) with Poisson distribution was used to detect differences in risk between pathogen antibody, with pathogen antibody as the predictor, clinic as a repeated variable, and offset of total sample from each clinic [25]. Logistic regressions were used to determine if seropositivity to one pathogen antibody type increased the risk of seropositivity to a second pathogen antibody type. Finally, to determine if there was a difference in seroprevalence for each pathogen antibody between catchment sites, additional logistic regressions were calculated, with each pathogen antibody as the outcome and the catchment site at which the sample was collected as the predictor. A global F-test was used to indicate a site effect. A  $P$  value  $< .05$  was considered statistically significant. Statistical models and tests were performed in R (Version 4.0.3, 2020, RStudio, Inc).

Visualization of the distributional range and mapping of cluster densities were performed using ArcGIS Pro 2.4.19948 (Copyright (c) 2021, Esri Inc) with administrative boundaries taken from the US Census Bureau.[26]

## RESULTS

### Study Population

A total of 1153 human sera were collected in 2018 at 11 clinic catchment sites in New England states for testing of *B. miyamotoi*, *B. burgdorferi*, and *B. microti* antibody. The number of sera obtained from each catchment site (represented by the town in which the clinic is located) and the gender of study participants are shown in Table 1. Almost all (99%) sera collected were from adults aged  $\geq 18$  years. The mean age of the participants in our sample population was 58 years.

### Seroprevalence of *B. miyamotoi*, *B. burgdorferi*, and *B. microti*

The average seroprevalence of *B. miyamotoi* was 2.8% (range, 0.6%–5.2%) from all catchment sites compared with 11.0% (range, 6.8%–15.6%) for *B. burgdorferi* and 10.0% (range, 6.5%–13.6%) for *B. microti* (Table 2). *Babesia microti* seropositivity was lower using the 1:128 assay cutoff than with the 1:64 cutoff (8.2% and 10.0%, respectively), but the difference was not statistically significant.

We did not find any associations between seropositivity and gender among the 3 pathogens (data not shown). Overall, *B. miyamotoi* seropositivity was found to be 0.28 times the risk than that of *B. microti* ( $P < .001$ ; 95% confidence interval [CI]: .197–.385) and 0.24 times the risk than that of *B. burgdorferi* ( $P < .001$ ; 95% CI: .161–.365). There was no difference between *B. burgdorferi* and *B. microti* seropositivity (ratio = 0.88;  $P = .29$ ; 95% CI: .696–1.115). There was variability but no significant difference in seroprevalence between catchment sites for *B. miyamotoi* ( $P = .59$ ), *B. burgdorferi* ( $P = .31$ ), or *B. microti* ( $P = .09$ ; Figure 1).

**Table 2. Seroprevalence of *Borrelia miyamotoi*, *Borrelia burgdorferi*, and *Babesia microti* in Serum Samples from 2018 by Catchment Area**

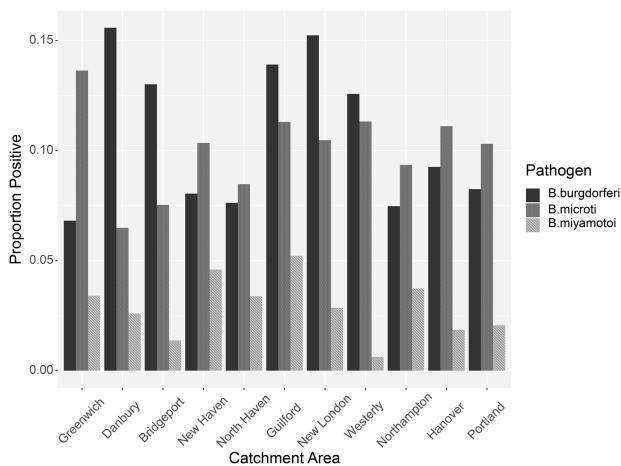
Study Site	Borrelia miyamotoi	Borrelia burgdorferi		Babesia microti
		Number seropositive/number tested (%)	Number seropositive/number tested (%)	
Westerly, Rhode Island	1/159 (0.6)	20/159 (12.6)	18/159 (11.3)	
New London, Connecticut	3/105 (2.7)	16/105 (15.2)	11/105 (10.5)	
Guilford, Connecticut	6/115 (5.2)	16/115 (13.9)	13/115 (11.3)	
New Haven, Connecticut	4/87 (3.4)	7/87 (8.0)	9/87 (10.3)	
North Haven, Connecticut	4/118 (3.4)	9/118 (7.6)	10/118 (8.5)	
Bridgeport, Connecticut	2/146 (1.4)	19/146 (13.0)	11/146 (7.5)	
Danbury, Connecticut	2/77 (2.6)	12/77 (15.6)	5/77 (6.5)	
Greenwich, Connecticut	3/88 (3.4)	6/88 (6.8)	12/88 (13.6)	
Hanover, New Hampshire	1/54 (1.9)	5/54 (9.3)	6/54 (11.1)	
Northampton, Massachusetts	4/107 (3.7)	8/107 (7.5)	10/107 (9.4)	
Portland, Maine	2/97 (2.0)	8/97 (8.3)	10/97 (10.3)	
Total	32/1153 (2.8)	126/1153 (11.0)	115/1153 (10.0)	

Catchment areas are listed by the town where the clinic laboratories were located. The corresponding number of seropositive serum for each site is listed over the total number of samples collected at each site.

We also analyzed data for evidence of multiple infections to these pathogens in New England residents. A total of 2.3% (27 of 1153) of the study participants were seropositive to 2 pathogens. More individuals were seropositive for both *B. burgdorferi* and *B. miyamotoi* (18 of 1153, 1.6%) compared with *B. burgdorferi* and *B. microti* (5 of 1153, 0.4%) or *B. microti* and *B. miyamotoi* (4 of 1153, 0.3%; Table 3). Using logistic regression, we determined that a participant with antibody to *B. miyamotoi* had no greater chance of having additional antibody to *B. burgdorferi* or *B. microti*.

### Geographic Range and Expansion Pattern of *B. miyamotoi*, *B. burgdorferi*, and *B. microti*

Next, we determined seroprevalence by county of residence. Sera were obtained from residents of 59 counties in New England. For our analysis, we excluded counties with low



**Figure 1.** The proportion seropositive for *Borrelia miyamotoi*, *Borrelia burgdorferi*, and *Babesia microti* at catchment areas. The raw proportion seropositive (N positive/total tested) is shown.

**Table 3. Human Antibody to More Than 1 Pathogen in the Study Participants**

Pathogen	Number of Seropositive Participants
<i>Borrelia burgdorferi</i> , <i>Babesia microti</i>	18 (1.6%)
<i>B. burgdorferi</i> , <i>Borrelia miyamotoi</i>	5 (0.4%)
<i>B. miyamotoi</i> , <i>B. microti</i>	4 (0.3%)
<i>B. burgdorferi</i> , <i>B. miyamotoi</i> , <i>B. microti</i>	0 (0.0%)

Evidence for coinfection or sequential infection is demonstrated through the detection of multiple antibodies against more than 1 pathogen in a single serum sample. Seropositivity is shown as the number of human sera reacting against 2 or 3 of the different pathogens and the percent seropositive out of the 1153 serum samples tested.

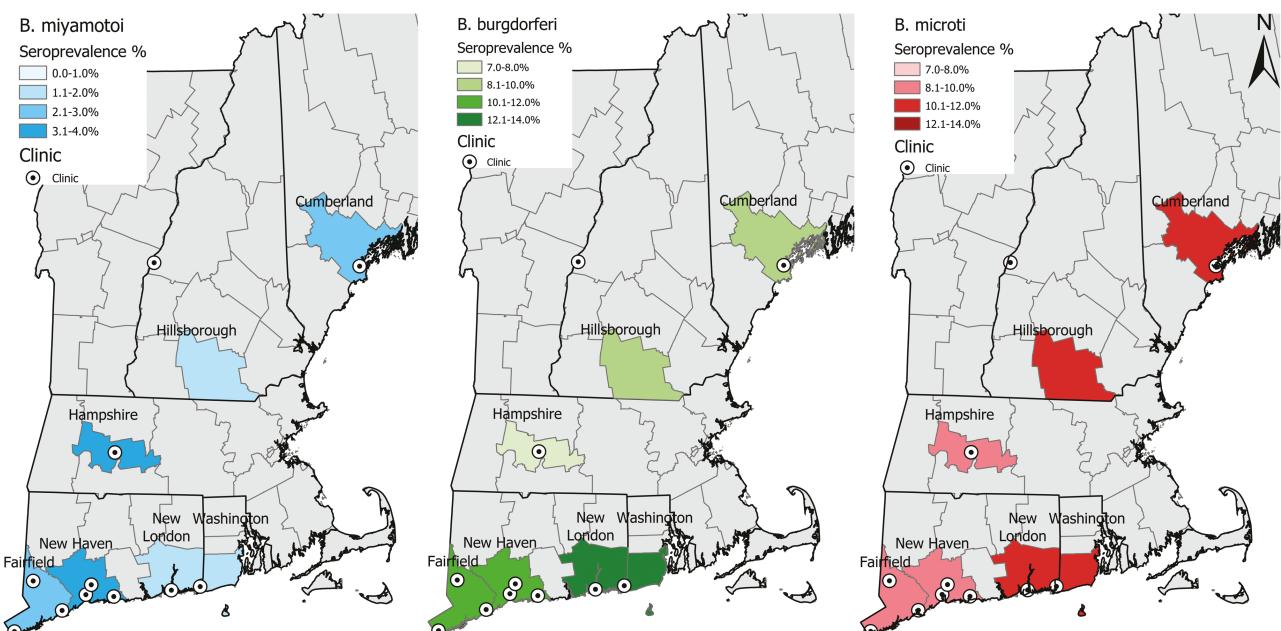
sample collection (fewer than 50 samples), leaving 7 counties where seroprevalence could be described. Evidence of previous *B. miyamotoi*, *B. burgdorferi*, and *B. microti* infection was found in all counties. Seroprevalence across counties ranged from 1.3% to 3.9% for *B. miyamotoi*, 7.5% to 13.9% for *B. burgdorferi*, and 9.0% to 11.3% for *B. microti* (Figure 2).

Finally, we examined *B. miyamotoi*, *B. burgdorferi*, and *B. microti* seroprevalence along east–west and south–north gradients to determine if there was evidence of a differential dispersion pattern along these axes. Although there was variation between different study sites and counties, there was no clear gradation of an east to west or south to north seroprevalence gradient differential for any of the 3 study pathogens (Figures 1 and 2). Our dataset was insufficient, however, to clearly confirm or refute differential dispersion due to a lack of power within a greater distribution of sampled counties.

## DISCUSSION

We determined that human *B. miyamotoi*, *B. burgdorferi*, and *B. microti* infections are widely dispersed across New England and that the seroprevalence of *B. miyamotoi* is less than that of *B. burgdorferi* or *B. microti*. There is a patchy geographical seroprevalence distribution for each pathogen, and the frequency of antibody against each pathogen does not exist in fixed proportion with that of the other pathogens between study collection sites or counties. For example, areas with the highest *B. miyamotoi* seroprevalence do not necessarily have the highest *B. burgdorferi* or *B. microti* seroprevalence. Although widespread across New England, the overall frequency of human *B. miyamotoi* infection is significantly less than that of *B. burgdorferi* and *B. microti*.

*Borrelia miyamotoi* was the primary focus of our study because it more has recently been discovered to cause human disease compared with *B. burgdorferi* and *B. microti* and the amount of human exposure and geographic range of this pathogen are unclear. Cases have been reported in Massachusetts, Maine, New Jersey, New York, Rhode Island, and Wisconsin. Understanding the geographic range of human *B. miyamotoi* infection is important because healthcare workers and the general public need to be aware of infection risk in their region. *Borrelia miyamotoi* infection can cause meningoencephalitis or relapsing fever that can last for weeks or months if untreated [2, 10, 11]. *Borrelia miyamotoi* might also pose a risk to blood transfusion recipients, although there have been no reports of human transfusion transmission to date. *Borrelia miyamotoi* spirocheteemia is higher and of longer duration than that of



**Figure 2.** Map of *Borrelia miyamotoi*, *Borrelia burgdorferi*, and *Babesia microti* seroprevalence by New England county. These counties include Fairfield, New Haven, and New London (Connecticut), Hillsborough (New Hampshire), Cumberland (Maine), Hampshire (Massachusetts), and Washington (Rhode Island).

Lyme disease. Other relapsing fever borrelia have been shown to be transmitted through blood transfusion. Transfusion transmission of *B. miyamotoi* has been demonstrated in a mouse blood transfusion model, and *B. miyamotoi* persists in human blood when stored under blood banking conditions [27, 28].

Human *B. miyamotoi* disease was discovered more recently than babesiosis or Lyme disease, and we hypothesized that it might lag in geographic dispersion. Babesiosis and Lyme disease were first reported in southeastern New England and have spread throughout much of the East Coast, from Maine to Virginia [5–7]. Lyme disease has dispersed more widely and rapidly than babesiosis; there are many counties where Lyme disease alone is reported but none where babesiosis alone is reported [6]. We sought to determine whether *B. miyamotoi* infection might also be more limited geographically than Lyme disease, but found no evidence of this in New England.

The seroprevalence results for *B. burgdorferi*, *B. microti*, and *B. miyamotoi* in this study were similar to those of previous studies of healthy residents in the general population of New England [12, 13, 29–31]. In contrast, *B. microti* seroprevalence results were greater than those found in serosurveys of New England blood donor populations, which have ranged from 0.3% to 4.9% [24, 32–34]. Previous studies have shown large disparities between seroprevalence in the general population and blood donors living in the same area [14, 35]. These differences can be explained, in part, because there is a “healthy blood donor” sampling bias in blood donors who have both a heightened sense of health status and disease prevention [14, 35, 36]. Additionally, the average age of US blood donors is equivalent to the medium age of the general population at 39 years, whereas the mean age of our study population was 58 years. Therefore, our population is at higher risk of having experienced tickborne infection compared with blood donors [37].

The similarity in *B. burgdorferi* and *B. microti* seroprevalence might seem surprising, given the much greater number of cases reported nationally for Lyme disease compared with babesiosis. However, 3 previous studies have found a similar narrow disparity between the seroprevalence of these 2 pathogens [29–31]. Several factors may help explain these findings. Babesiosis is more restricted geographically than Lyme disease. Our current study was carried out in areas where both Lyme disease and babesiosis are endemic, whereas national incidence data include areas where Lyme disease is endemic but babesiosis is absent. Babesiosis lacks a distinctive and easily recognized sign, such as the erythema migrans rash of Lyme disease, and is therefore less readily diagnosed and reported. There are also a proportionally greater number of asymptomatic babesiosis cases than Lyme disease cases. Nationally, there is approximately 1 case of babesiosis for every 10 cases of Lyme disease [29]. In a carefully performed 10-year epidemiologic study on Block Island, Rhode Island, which is highly endemic for babesiosis and Lyme disease, we used both

case finding and serology to detect evidence of exposure to both pathogens [29]. We detected 7 cases of *B. microti* infection (including symptomatic and asymptomatic infections) for every 10 cases of *B. burgdorferi* infection, a ratio that is similar to the seroprevalence ratio we found in the current study. We also found that the incidence of babesiosis on the mainland in southeastern Connecticut was similar to that of Block Island [29]. Finally, differences in assay sensitivity may help account for the narrow difference in seropositivity. The standard *B. microti* IFA test that we used has a greater sensitivity for detection of a *Babesia* infection than the standard 2-tier *B. burgdorferi* antibody assay for detection of early acute and early convalescent Lyme disease (88%–96% and 39%–77%, respectively) and both have a comparable specificity (99% and 98%, respectively) [20, 23]. We chose the VlsE1 and pepC10 (ZEUS) *B. burgdorferi* single-tier assay because it has greater sensitivity compared with the standard 2-tier Lyme disease antibody assay for early acute and early convalescent Lyme disease (76% and 68%, respectively) but similar specificity (98% and 99%, respectively) [21].

Serosurveillance is an immunoepidemiological tool that has been used for more than 2 decades to assess the prevalence and geographic expansion of *B. microti* in New England [29, 38]. Seroprevalence studies help formulate current and future public health policy to minimize exposure to tickborne pathogens in endemic areas. Seroprevalence determination provides a measure of disease risk and exposure that supplements clinical case reporting [29, 38]. The incidence of Lyme disease through case reporting is thought to be underestimated by a factor of 3 to 12 and of babesiosis by a factor of 30, not counting those whose disease was undiagnosed [29, 39]. Such individuals are identified using serosurvey methods. Furthermore, case surveillance methods and case definitions often change over time. The case criteria for Lyme disease has been modified 4 times since its initial implementation in 1991 [40]. These changes can alter the interpretation of disease trends and disease incidence. Seroprevalence surveillance has its own limitations. As with case finding, a person could have acquired a tickborne infection in an area outside the zip code area where they provided a blood sample. Persistence of antibody beyond a year may lead to overestimation of infection, while a short antibody half-life may lead to underestimation of infection. Antibody persistence may vary in response to each pathogen [24, 41, 42]. There also may be over- or underestimation of the actual number of infections because of laboratory methodologic limitations, although we used standard antibody assays. Finally, our spatial analysis was constrained due to a limited number of study sites in northern New England and the need to aggregate residential zip code areas to coarser county units due to limited sample sizes in most zip codes.

In conclusion, our study demonstrates that human *B. miyamotoi* seroprevalence is more widespread in New England

than previously recognized, while seroprevalence is less than that of *B. burgdorferi* or *B. microti*. Residents of New England should be aware of their risk of infection from *B. burgdorferi*, *B. microti*, and *B. miyamotoi*. Additional studies are needed to better define the extent of human infection both within and outside the New England region, including additional seroprevalence studies, case finding, and mathematical modeling to predict future expansion of these emerging tickborne diseases.

## Notes

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