Annals of Internal Medicine ORIGINAL RESEARCH

Borrelia miyamotoi Disease in the Northeastern United States

A Case Series

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Background: The first recognized cases of *Borrelia miyamotoi* disease (BMD) in North America were reported in the northeastern United States in 2013.

Objective: To further describe the clinical spectrum and laboratory findings for BMD.

Design: Case series.

Setting: Patients presenting to primary care offices, emergency departments, or urgent care clinics in 2013 and 2014.

Participants: Acutely febrile patients from the northeastern United States in whom the treating health care providers suspected and ordered testing for tick-transmitted infections.

Measurements: Whole-blood polymerase chain reaction (PCR) testing was performed for the presence of specific DNA sequences of common tickborne infections (including BMD). Serologic testing for *B. miyamotoi* was performed using a recombinant glycerophosphodiester phosphodiesterase (rGlpQ) protein. Clinical records were analyzed to identify the major features of acute disease.

Results: Among 11 515 patients tested, 97 BMD cases were identified by PCR. Most of the 51 case patients on whom clinical histories were reviewed presented with high fever, chills, marked headache, and myalgia or arthralgia. Twenty-four percent were hospitalized. Elevated liver enzyme levels, neutropenia, and thrombocytopenia were common. At presentation, 16% of patients with BMD were seropositive for IgG and/or IgM antibody to *B. miyamotoi* rGlpQ. Most (78%) had seropositive convalescent specimens. Symptoms resolved after treatment with doxycycline, and no chronic sequelae or symptoms were observed.

Limitation: Findings were based on specimens submitted for testing to a reference laboratory, and medical records of only 51 of the 97 case patients with BMD were reviewed.

Conclusion: Patients with BMD presented with nonspecific symptoms, including fever, headache, chills, myalgia, and arthralgia. Laboratory confirmation of BMD was possible by PCR on blood from acutely symptomatic patients who were seronegative at presentation. *Borrelia miyamotoi* disease may be an emerging tickborne infection in the northeastern United States.

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The public health burden of infectious agents transmitted by the deer tick (*Ixodes dammini*, also known as the blacklegged tick or *I. scapularis*), comprising *Borrelia burgdorferi*, *Babesia microti*, *Anaplasma phagocytophilum*, and deer tick virus (Powassan virus lineage II), seems to be increasing in the northeastern United States (1–4). The first human cases of infection with *Borrelia miyamotoi*, previously considered nonpathogenic, were identified in Russia and described in 2011 (5, 6). In 2013, the American index case of *B. miyamotoi* disease (BMD) was described (7), and 2 other case patients (8) were reported with a clinical illness that was initially diagnosed as human anaplasmosis. Cases have subsequently been identified in New England (9), the Netherlands (10), and Japan (11). The prevalence of *B. miyamotoi* in host-seeking deer ticks (about 1% to 5% [12–14]) is such that human exposure is likely; indeed, a serosurvey suggests that 10% of tick-exposed New England residents may have been exposed to *B. miyamotoi* (15). It is possible that zoonotic transmission has been common but not discriminated from other deer tick–transmitted zoonoses because the acute clinical presentation of BMD cases reported to date is nonspecific.

To better define the clinical spectrum of this newly recognized zoonosis, we identified definitive cases of acute BMD by polymerase chain reaction (PCR) analysis of patient blood samples sent to a reference laboratory by clinical practices. We summarized the clinical features of BMD from 51 case patients out of 97 whom we identified as having active *B. miyamotoi* infection. In addition, we describe relevant associated laboratory findings and compare the observed frequency of BMD with that of human anaplasmosis and babesiosis, thereby providing a preliminary assessment of its relative public health burden.

METHODS

Blood Samples

Emergency departments, urgent care clinics, and primary care offices in Massachusetts, Rhode Island, New Jersey, and New York sent whole blood samples (EDTA-anticoagulated) to IMUGEN (Norwood, Massachusetts) as part of the clinical management of patients who were acutely symptomatic with features or laboratory findings suggestive of possible tickborne infection (typically fever, myalgia, flu-like illness, headache, or rash). An active detection protocol was adopted to identify samples that contained evidence of *B. miyamo-*

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Context

The first known cases of *Borrelia miyamotoi* infection in North America were reported in 2013. The incidence, prevalence, and clinical spectrum of this infection are currently undefined.

Contribution

A review of the clinical records of 51 of 97 patients with acute *B. miyamotoi* infection diagnosed by polymerase chain reaction testing at a reference laboratory provides preliminary data on the clinical presentation, course, and response to antibiotics of this infection.

Caution

The study was not prospective or population-based.

Implication

Borrelia miyamotoi disease may be an emerging tick-borne infection of clinical significance in the northeastern United States.

toi DNA during the 2013 and 2014 transmission seasons (1 April to 30 November). A positive *B. miyamotoi* finding on PCR prompted a request for chart review, and we focused on patients under our care with a goal of obtaining detailed clinical histories on at least 50 patients. The study was approved by the New England Institutional Review Board, and written, informed consent was obtained for clinical and laboratory follow-up of patients with positive PCR findings for *Borrelia* species.

Molecular Detection of Infection

Specimen receipt and handling, DNA extraction, and PCR setup were performed with enhanced contamination control practices and precautions at IMUGEN, which operates under good laboratory practice standards, Clinical Laboratory Improvement Amendments and College of American Pathologists certification, and New York State approval (Clinical Laboratory Evaluation Program). The laboratory has environmentally separate specimen processing, archive, and molecular extraction and setup areas. Extracted DNA from whole blood was tested in triplicate for the presence of *Borrelia* species by real-time PCR (**Appendix**, available at www.annals.org) (16). Real-time PCR targeting the *msp2* gene of *A. phagocytophilum* and the 18S ribosomal RNA gene of *B. microti* was performed from whole blood DNA extractions as previously described (17–20). Representative samples were independently analyzed by PCR sequencing at the Telford laboratory of Tufts University (**Appendix**).

B. burgdorferi Serology

Sera were tested at IMUGEN by antibody capture enzyme immunoassay (EIA) for IgM, IgA, and IgG isotypes to *B. burgdorferi* sensu stricto strain 49736 and by immunoblot for IgM and IgG isotypes to *B. burgdorferi* sensu stricto strain G39/40 (21–23). Immunoblot findings were interpreted according to the 2-tiered protocol (24).

Recombinant *B. miyamotoi* GlpQ Enzyme-Linked Immunosorbent Assay

The glycerophosphodiester phosphodiesterase (GlpQ) antigen has been found to be useful in the serodiagnosis of relapsing fever (25, 26). Given the genetic relatedness of *B. miyamotoi* to relapsing fever spirochetes, we analyzed the utility of GlpQ for confirmation of a BMD diagnosis. The *GlpQ* gene sequence from *B. miyamotoi* (GenBank accession number AY368276) was used as the basis for cloning and expression as a 38-kDa recombinant protein (rGlpQ), which was used in an indirect EIA for detection of antibody to *B. miyamotoi* (**Appendix**).

Case Definitions

A positive blood sample on 2 independent PCR assays (genus- and species-specific) was considered definitive evidence of active *B. burgdorferi* or *B. miyamotoi* infection. A blood sample that was repeatedly positive (reextracted and retested from the original sample) for *B. microti* or *A. phagocytophilum* on PCR was considered definitive evidence of active infection.

Role of the Funding Source

Internal funding from IMUGEN enabled the development and validation of test methods, the study design and execution, the clinical analysis, and the laboratory studies. The decision to prepare a manuscript for publication was not predicated on the availability of funding or the funding source.

RESULTS

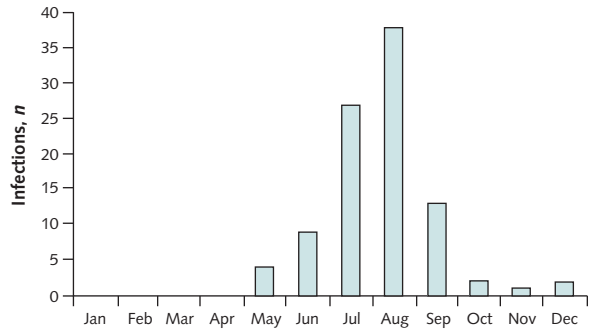
Confirmation of *B. miyamotoi* as the Infecting Agent

We attempted to sequence portions of the *GlpQ* and flagellin genes, as previously described (7), from the 23 BMD cases from 2013 for which we had adequate remaining blood samples for DNA extraction. The organism was confirmed to be *B. miyamotoi* (GenBank accession numbers KP754938 to KP754960) in 17 samples (*GlpQ*, *fla*, or both sequences were obtained from the sample). The other 6 samples contained few spirochetes; those with high cycle threshold values in the real-time *GlpQ* PCR did not provide sequence (Spearman rank correlation coefficient, −0.67 [$P < 0.001$]).

Frequency of BMD

Between 1 April 2013 and 31 October 2014, we engaged in active case detection using specimens submitted to IMUGEN and identified 97 patients whose samples contained *B. miyamotoi* DNA. Of these, we obtained clinical information on 51 who had not been previously reported (7, 8) from selected practices in Massachusetts, Rhode Island, New Jersey, and New York. The months of onset or diagnosis of these BMD cases (**Figure 1**) overlapped with those for the other deer

Figure 1. Seasonal distribution of acute *Borrelia miyamotoi* incident infections.



The months of acute blood collection are presented for 97 samples submitted in 2013–2014 that were determined by active case detection or retrospectively to contain *B. miyamotoi* DNA.

tick–transmitted infections, although most cases had onset in July and August.

To provide a crude estimate of the incidence of BMD, we compared the frequency of PCR-confirmed findings of *B. burgdorferi*, *B. miyamotoi*, *B. microti*, and *A. phagocytophilum* in the patient samples submitted during the peak transmission months (May through October) in 2013 and 2014. Of 11 515 unique samples that were submitted and tested for all 4 agents, 3.1% (exact binomial 95% CI, 2.7% to 3.4%) contained *B. microti* DNA, 1.4% (CI, 1.2% to 1.6%) contained *A. phagocytophilum* DNA, and 2.5% (CI, 2.2% to 2.8%) contained *Borrelia* species DNA. *Borrelia miyamotoi* DNA was detected in 0.8% (CI, 0.6% to 1.0%) of all samples; hence, 1.7% (CI, 1.5% to 1.9%) of all samples contained *B. burgdorferi* or cognate sequences that were not assigned to species.

Clinical Spectrum of BMD

Basic clinical data were available for 51 case patients with definitive BMD selected out of 97 total. The clinical spectrum of disease was variable, but presenting symptoms were often suggestive of an undifferentiated flu-like illness. Patients presented with acute headache, fever, and chills and were often found to have leukopenia, thrombocytopenia, and elevated aminotransferase levels, mimicking human anaplasmosis infection (Tables 1 and 2). Patients were commonly described as appearing "toxic"; more than 50% were suspected of having sepsis, and 24% required hospitalization. The headaches were most commonly described as severe, resulting in head computed tomography scans and spinal taps in 5 patients. Two patients presented with recurrent fever, and 1 of them, who was not treated initially, yielded blood samples drawn a month apart that were positive on PCR.

Treatment regimens were typical for suspected tickborne disease in our referral area practices. Of the 51 case patients, 40 received a 2- to 4-week course of oral doxycycline. Seven of 51 received oral amoxicillin, and 3 of these had received 1 or 2 doses of ceftriaxone beforehand. One of 51 received levofloxacin for 10 days. One case patient first received atovaquone and azithromycin for a laboratory-confirmed diagnosis of babesiosis and later received ceftriaxone after the results of laboratory assays were reported. Of the 42 patients for whom information on outcome was available, 40 had prompt resolution of signs and symptoms 2 days to 1 week after beginning specific treatment; 2 reported residual fatigue or other symptoms for the entire course of therapy, and 1 reported fatigue for 2 months after completion of antimicrobial therapy. None reported chronic, nonspecific signs or symptoms before their recent onset of symptoms (the 2 with recurrent courses notwithstanding).

Co-infections

Co-infections with other *Ixodes*-transmitted pathogens were observed, primarily with the agent of Lyme disease. Three of the 51 case patients yielded acute blood samples that contained *B. burgdorferi* DNA (2 of whom were also considered to have serologic evidence of exposure to *B. burgdorferi*), and 2 additional case patients demonstrated seroconversion to *B. burgdorferi* antigens (1 of whom had physician-diagnosed erythema migrans). Thus, 14% of the case patients with BMD were co-infected with *B. burgdorferi*. A blood sample from 1 additional case patient contained DNA of *B. burgdorferi*, *B. miyamotoi*, and *B. microti*; none of the other case patients seemed to be concurrently infected with *B. microti* or *A. phagocytophilum*.

Serologic Studies of BMD

Sensitivity of rGlpQ EIA for Confirmation of BMD

A detectable immune response to rGlpQ was evident in only 8 of 51 (16%) case patients in the acute sample. Convalescent sera (≥5 days) were available for 36 case patients. Of these, 29 were seronegative at baseline; 25 of the 29 (86%) demonstrated seroconversion (Table 3 and Figure 2).

Specificity of rGlpQ EIA for B. miyamotoi *Infection*

To assess the specificity of the *B. miyamotoi* serologic assay, we tested samples from patients with PCR-

Table 1. Clinical Features of the 51 Case Patients With BMD

Feature	Value*
Mean age (range), *y*	55 (12–82)
Male	29 (57)
Fever/chills	49 (96)
Headache†	49 (96)
Myalgia	42 (84)
Arthralgia	39 (76)
Malaise/fatigue	42 (82)
Rash	4 (8)
Gastrointestinal symptoms‡	3 (6)
Cardiac/respiratory symptoms§	3 (6)
Neurologic symptoms‖	4 (8)

BMD = *Borrelia miyamotoi* disease.
* Number (percentage) unless otherwise indicated.
† Severe in most patients.
‡ Nausea, abdominal pain, diarrhea, and anorexia.
§ Dyspnea.
‖ Dizziness, confusion, and vertigo.

Table 2. Laboratory Findings for the 51 Case Patients With BMD*

Variable	Normal			Abnormal		
	Patients, *n*	Range	Mean (95% CI)	Patients, *n*	Range	Mean (95% CI)
Leukocyte count, × 10^9 cells/L†	21	4.800–8.000	6.329 (5.928–6.729)	22 (low)	1.200–4.400	3.318 (2.988–3.648)
Platelet count, × 10^9 cells/L‡	17	159–326	195 (175–215)	26 (low)	18–139	104 (93–116)
Aspartate aminotransferase level, U/L§	9	17–40	29 (22–36)	27 (high)	41–314	107 (85–128)
Alanine aminotransferase level, U/L‖	9	21–42	31 (25–36)	27 (high)	47–219	102 (85–119)

BMD = *Borrelia miyamotoi* disease.
* Complete information was not available for all 51 patients.
† Normal range, 4.800–11.200 × 10^9 cells/L.
‡ Normal range, 140–400 × 10^9 cells/L.
§ Normal range, 0–40 U/L.
‖ Normal range, 0–45 U/L.

confirmed, late-stage Lyme disease as well as previously identified babesiosis and human granulocytic anaplasmosis. Only 1 of 39 patients with detectable hematogenous *B. burgdorferi* DNA was found to be reactive for *B. miyamotoi* IgM, and none was found to be reactive for *B. miyamotoi* IgG in acute or convalescent samples. For 8 patients with Lyme arthritis whose synovial fluid tested positive for *B. burgdorferi* by PCR, sera were positive for *B. burgdorferi* antigens by EIA and immunoblot but antibody-negative for *B. miyamotoi* rGlpQ. Similarly, acute and convalescent sera from 8 patients with early neuroborreliosis (cerebrospinal fluid positive for *B. burgdorferi* by PCR) were tested. All were positive for *B. burgdorferi* and negative for *B. miyamotoi* rGlpQ.

Of 24 serum samples from patients with PCR-confirmed *B. microti*, 17% had IgM reactivity to rGlpQ and none had IgG reactivity. We tested an additional 13 convalescent sera from patients who were negative for *B. microti* by PCR (IgG titer of 64 to >1024 on an indirect fluorescent antibody test [IFAT]). One had low IgM seroreactivity to rGlpQ, and 1 had low IgG seroreactivity. Sera from 12 of 16 patients who were seropositive for *A. phagocytophilum* (IgG titer of 64 to 128 on IFAT) were negative for reactivity to rGlpQ; 3 had low IgM reactivity, and 1 had low IgG reactivity. Sera from 10 of 11 patients who were seropositive for *Ehrlichia chaffeensis* (IgG titer of 64 to 128 on IFAT) were negative for reactivity to rGlpQ, but 1 had low IgG reactivity to rGlpQ.

To determine whether rGlpQ reactivity is less likely to be observed in geographic areas where Lyme disease is not endemic, sera from 250 healthy blood donors from west of the Mississippi River were tested for reactivity to rGlpQ. None demonstrated IgM seroreactivity, but 9 had IgG reactivity.

Table 3. Serologic Findings With rGlpQ EIA in Case Patients With BMD

Specimen	Patients, *n*	Detectable Immune Response, *n* (%)		
		IgM	IgG	IgM or IgG
Acute only	51	5 (10)	5 (10)	8 (16)
Acute	36	4 (11)	4 (11)	5 (14)
Convalescent	36	23 (64)	22 (61)	28 (78)

BMD = *Borrelia miyamotoi* disease; EIA = enzyme immunoassay; rGlpQ = recombinant glycerophosphodiester phosphodiesterase.

Serologic Reactivity to *B. burgdorferi*

Sera from all 51 case patients with BMD were analyzed for antibodies to *B. burgdorferi* with EIA and immunoblot using whole cell antigen preparations. We excluded 2 patients with a known documented history of Lyme disease and 5 who were concomitantly infected with Lyme disease (4 whose index specimen was positive for *B. burgdorferi* by PCR and 1 with erythema migrans fulfilling serologic diagnostic criteria). Of the 44 patients with BMD who had no history of Lyme disease, 10 (23%) showed EIA reactivity for IgM isotype to *B. burgdorferi* on the acute specimen, 4 (9%) showed reactivity for IgA, and 1 (2%) showed reactivity for IgG. Of the 30 patients with convalescent specimens, 27 (90%) had EIA reactivity for IgM isotype to *B. burgdorferi*, 6 (20%) had reactivity for IgA, and 6 (20%) had reactivity for IgG; 28 (93%) were seropositive if we considered any reactive isotype. However, only 10% of convalescent specimens were positive for IgM or IgG on immunoblot (Table 4).

Quantification of Spirochetemia

Calculated spirochetal concentrations for acute blood samples ranged from 5 to 53 081 (mean, 7787 [CI, 3978 to 11 597]) organisms/mL. The blood samples from the 12 hospitalized case patients, who may have had more severe symptoms, tended to contain more spirochetes (median, 3064 [interquartile range, 368 to 34 034]) than those from the 39 who were not hospitalized (median, 747 [interquartile range, 93 to 6764]), although this tendency did not achieve statistical significance (Mann–Whitney $U = 157$; $P = 0.109$). Spirochetemia was not correlated with elevated liver enzyme levels ($P = 0.44$ for aspartate aminotransferase and 0.64 for alanine aminotransferase [Spearman rank correlation]); indeed, we failed to demonstrate significant associations between spirochetemia and specific signs or symptoms.

Sensitivity of PCR for Confirmation of BMD

We used rGlpQ EIA to analyze sera from 814 patients whose whole blood samples were negative for *B. miyamotoi* DNA by PCR. We identified 16 (2%) that contained IgG against rGlpQ (12 with low titer and 4 with high titer); 8 patients were reactive for IgM against

rGlpQ, including those determined among *B. microti* samples as described earlier. We found 2 patients who demonstrated seroconversion and thus may have been diagnosed by serology only.

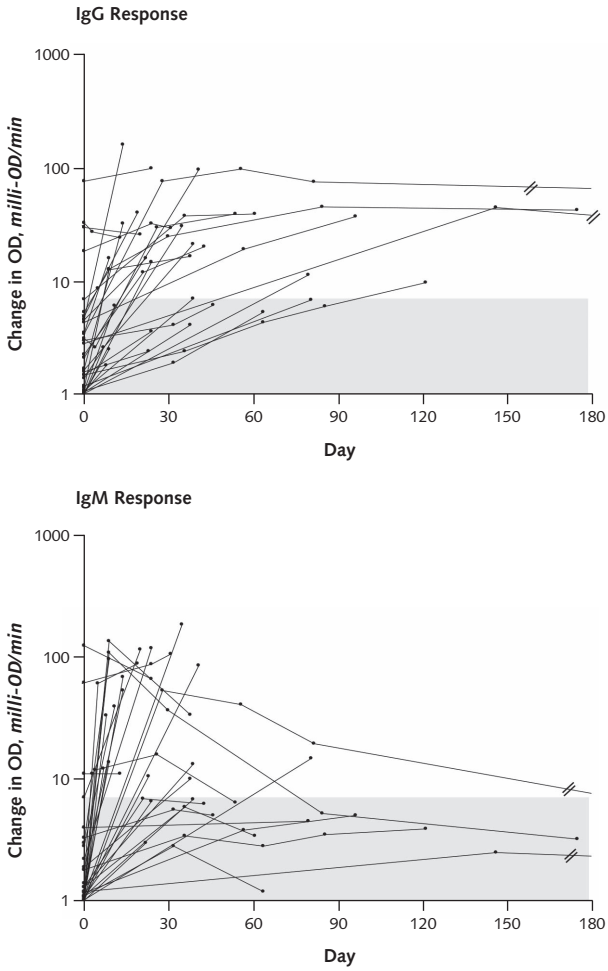
DISCUSSION

This BMD case series, definitively diagnosed by PCR in acutely symptomatic patients, expands our knowledge of the clinical spectrum of this disease. Early Lyme disease without erythema migrans, babesiosis, and human anaplasmosis may manifest nonspecifically with fever, chills, fatigue, myalgia, and arthralgia (27). Our case patients with BMD presented with similar nonspecific acute symptoms, leading the treating physicians to suspect a tickborne illness. These patients also frequently had severe headache; 60% had low platelet counts, and 82% had elevated aminotransferase levels. The treating physicians were struck by how sick and septic these patients frequently seemed; 24% of the patients were hospitalized. The index case of *B. miyamotoi* infection in the United States was in an immunocompromised patient (7) with clinical meningitis and spirochetes visualized in the cerebrospinal fluid, whereas the prominent headaches in our patients were associated with normal cerebrospinal fluid in cases where a spinal tap was performed (data not shown) and thus likely do not reflect neuroborreliosis. Treatment with doxycycline, amoxicillin, or ceftriaxone seemed effective.

Although BMD was confirmed less frequently than babesiosis or human granulocytic anaplasmosis in our sample of acutely symptomatic patients, our findings suggest that it may not be a rare infection in the northeastern United States. The months during which cases were identified are consistent with transmission of *B. miyamotoi* by deer ticks. However, unlike acute Lyme disease, most cases occurred in July and August, perhaps suggesting transmission by larval ticks, which have their peak activity during these months. Nymphal deer ticks are most abundant in June and early July, and 67% of all acute Lyme disease cases occur during these months (28). *Borrelia miyamotoi* is known to undergo transovarial transmission and has been experimentally transmitted by larvae (12). These considerations should prompt enhanced public education about the need to continue personal protection measures during the late summer, which is usually believed to be a less risky period because the agents of Lyme disease, babesiosis, and human anaplasmosis are not transmitted by larval deer ticks.

We analyzed the performance characteristics of the rGlpQ EIA, using only samples from definitively diagnosed (positive by PCR), symptomatic case patients. Se-

Figure 2. Acute and convalescent antibody responses to rGlpQ in sera from *Borrelia miyamotoi* incident infections, expressed as kinetic OD ratios.



The shaded area indicates nonreactivity. OD = optical density; rGlpQ = recombinant glycerophosphodiester phosphodiesterase.

Table 4. Reactivity of Sera on *Borrelia burgdorferi* EIA and Immunoblot Among Case Patients With BMD*

Specimen	Patients, *n*	Reactivity, *n* (%)						
		EIA				Immunoblot†		
		IgM	IgA	IgG	Any	IgM	IgG	IgM or IgG
Acute only	44	10 (23)	4 (9)	1 (2)	10 (3)	2 (5)	1 (2)	2 (5)
Acute	30	5 (17)	2 (7)	2 (7)	6 (20)	2 (7)	0 (0)	2 (7)
Convalescent	30	27 (90)	6 (20)	6 (20)	28 (93)	1 (3)	2 (7)	3 (10)

BMD = *Borrelia miyamotoi* disease; EIA = enzyme immunoassay.
* Excludes 2 with known past physician diagnosis of Lyme disease and 5 with evidence of concurrent *B. burgdorferi* infection.
† Interpreted as recommended by the Centers for Disease Control and Prevention (24).

rologic testing using the rGlpQ EIA seems insensitive in diagnosing acute BMD infection given that it was positive for IgG or IgM in only 16% of the case patient samples at the time of clinical presentation. Serology, therefore, has limited utility in diagnosis of acute BMD. Convalescent specimens were available from 36 case patients and were seroreactive in 28 (78%). As with *B. burgdorferi*, the lack of seroconversion in some case patients could be related to prompt antibiotic treatment (27).

The sensitivity of PCR for diagnosis of acute BMD is probably better than for serology. It is possible that some BMD cases were not detected because the magnitude of spirochetemia was below the limit of detection by PCR or the specimen was obtained outside the "window" of spirochetemia. Thus, if BMD is suspected and the index specimen does not demonstrate the presence of the organism (that is, is negative by PCR), seroconversion on a convalescent specimen may support the diagnosis; we identified 2 such cases. This approach is consistent with diagnostic protocols for diverse infectious diseases.

There are similarities and differences between the patients described here and those reported previously (5). The patients in the previous study presented with fever and headache and were described as exhibiting more systemic features and higher temperatures than the comparative patients with Lyme disease. Leukocyte and platelet counts were low but remained in the normal range, in contrast to our case patients with BMD. Some relapsing clinical courses were described, but this may have been related to a failure to promptly treat febrile patients with antibiotics.

Of interest is the question of cross-reactivity to *B. burgdorferi* antigens in sera from patients with BMD. Most convalescent sera from our case patients had IgM reactivity to *B. burgdorferi* antigens by antibody capture whole cell EIA. For serologic support of the diagnosis of BMD, the Lyme disease EIA performed better than the rGlpQ EIA for the detection of IgM (90% vs. 64%). However, sera were found to be reactive by *B. burgdorferi* immunoblot in only 10% of patients when the generally accepted 2-tiered protocol was followed (24). Thus, sera from patients with acute BMD will frequently exhibit reactivity to *B. burgdorferi* in the EIA test used in this study, but this reactivity would rarely be confirmed by immunoblot. This finding is clinically relevant because a patient presenting with an acute "summer fever" and no rash and testing positive by whole cell antigen serologic test for Lyme disease could actually be infected with *B. burgdorferi*, *B. miyamotoi*, or both. However, sera from patients with BMD generally do not react to *B. burgdorferi* antigens in IgG or IgA tests, which is partly consistent with what has been previously reported for a small number of case patients with BMD (15, 29).

Among the acute blood samples, a mean of 7787 (CI, 3978 to 11 597) spirochetes/mL was estimated by real-time PCR. In contrast, a mean of 1718 (CI, 805 to 2631) spirochetes/mL was estimated for 184 PCR-confirmed cases of *B. burgdorferi* evaluated in our laboratory during the same period (2013 to 2014) using the same extraction and amplification technology (data not shown). Thus, *B. miyamotoi* attains a denser spirochetemia than *B. burgdorferi*. Although there are few studies of the relationship between clinical severity or outcome and the bloodstream density of true relapsing fever spirochetes (transmitted by body lice or argasid ticks), spirochetemia is not believed to be predictive of disease severity (30), other than being associated with liver function test abnormalities. *Borrelia miyamotoi* spirochetemia, however, did not seem to be associated with elevated liver enzyme levels, and other than a tendency for spirochetemia to be greater in hospitalized case patients, we identified no correlations with serologic response or any other sign or symptom.

Our study is unique due to the large number of definitively diagnosed case patients identified by active laboratory surveillance using PCR analyses of acute blood samples. Sequencing of the flagellin and *GlpQ* genes was performed for a representative sample of the templates derived from the PCR assays and confirmed the specificity of those assays. We were able to follow a large proportion of these case patients and review clinical and supporting laboratory findings. We attempted to rule out the presence of other confounding tick-transmitted infections, other than for the few co-infected patients. Accordingly, our study differs from previous published studies on BMD, most of which were based on retrospective analyses of archived sera or included small numbers of definitively diagnosed case patients.

The potential weaknesses of our study include the unknown duration of spirochetemia in BMD, which has implications for case detection and estimation of the true incidence. If spirochetemia is brief during the acute phase, analysis of a single specimen collected at clinical presentation may influence case detection. Also, our case patient sample may have been biased to those with a more clinically severe presentation. Samples from patients with mild infections probably would not have been sent for testing. Other potential weaknesses are the structured case-finding process, which relied on decisions by health care providers on when to order tickborne disease testing for a given patient presentation (perhaps influencing the recording of detailed clinical information), and inclusion of only 51 of 97 patients with BMD chosen from selected practices, which could have introduced bias. Furthermore, our study was not designed to measure the absolute incidence of BMD. In particular, the relative frequency of Lyme disease was probably underestimated because blood samples would not necessarily be drawn for a typical erythema migrans presentation of Lyme disease. However, even with these caveats, we suggest that BMD is almost as common as human anaplasmosis among tick-exposed febrile residents presenting at our referring clinics.

We conclude that *B. miyamotoi* infection may not be a rare infection in the northeastern United States. The primary signs and symptoms include fever, headache (often severe), myalgia, fatigue, and arthralgia.

Presentation varied, but patients were frequently very ill. Abnormal aminotransferase levels, leukopenia, and thrombocytopenia were commonly observed. *Borrelia miyamotoi* disease may be clinically similar to or be confused with human anaplasmosis. Diagnosis in acutely ill patients may be established by PCR. Acute patient sera are typically not reactive in the rGlpQ EIA, but tests on convalescent specimens usually demonstrate seropositivity to rGlpQ. Infection with *B. miyamotoi* is the fifth recognized *Ixodes*-transmitted infection in the northeastern United States and should be part of the differential diagnosis of febrile patients from areas where deer tick–transmitted infections are endemic.

From IMUGEN, Norwood, Massachusetts; Cummings School of Veterinary Medicine, Tufts University, North Grafton, Massachusetts; Hawthorn Medical Associates and South Coast Hospital System, New Bedford, Massachusetts; Nantucket Cottage Hospital, Nantucket, Massachusetts; Hunterdon Medical Center, Flemington, New Jersey; and Robert Wood Johnson Medical School, New Brunswick, New Jersey.

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Appendix: Methods

Evidence of Active Borreliosis

Borrelia species DNA was detected by real-time PCR using primers targeting the 23S ribosomal RNA gene (*Bb23Sf/Bb23Sr* with probe *Bb23Sp-FAM*) as described previously (16). *Borrelia burgdorferi* DNA was identified in a second assay using primers targeting the *OspA* gene (*OspA333f*-TCCAAAGACAAGTCATCAA CAGAAG and *OspA412r*-CCGTCTGCTCTTGTTATTAT TTTTTCA with the labeled probe *OspA365p*-CAATGA AAAAGGTGAAGTAT), and *B. miyamotoi* DNA was identified using primers targeting the *GlpQ* gene (*BmyglpQ111-f*: AATGCACGACCCAGAAATTGA and *BmyglpQ174-r*: TCTAGCTCGATTGGGAAATAATTGT with labeled probe *BmyglpQ133-p* ACAACCACAAAT-GTTG).

Assay Sensitivity

The assays were determined to have a sensitivity of detection from whole blood DNA extractions of 20 organisms/mL for *B. miyamotoi*, 80 organisms/mL for *B. burgdorferi* sensu stricto (23S ribosomal DNA assay), 45 organisms/mL for *B. miyamotoi* (*GlpQ* gene), and 255 organisms/mL for *B. burgdorferi* sensu stricto (*OspA* gene) based on titrations of enumerated *B. miyamotoi* in infected severe combined immunodeficiency mouse blood (absolute numbers calculated from an erythrocyte count and number of spirochetes observed for 5000 erythrocytes in a Giemsa-stained blood smear) and culture-derived *B. burgdorferi* sensu stricto (enumerated by counting under dark field microscopy using a Petroff–Hausser chamber) diluted in human blood. The quantification of *B. miyamotoi* spirochetes in patient blood samples was performed by quantitative PCR, in which a standard curve was constructed from the PCR cycle threshold values obtained from dilutions of infected mouse blood containing enumerated *B. miyamotoi*, and cycle threshold values for patient samples were used for one variable of the equation for the calculation of the number of organisms per milliliter of whole blood.

PCR Sequencing for Confirmation of Specific Identity

For the case patient samples from 2013 with sufficient remaining volume for extraction, the *B. miyamotoi* flagellin and *GlpQ* genes were amplified and gel-purified at the Tufts University laboratory, and the amplicons were sequenced at the University of Maine DNA Sequencing Facility in Orono, Maine, as previously described (7). Resulting sequences were aligned and interpreted at Tufts.

EIA Procedure

Recombinant GlpQ antigen was used at 150 ng per test well, and patient sera were diluted at 1:100 in a typical indirect EIA using a peroxidase conjugated antihuman IgM or IgG secondary antibody and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) substrate. Optical densities (ODs [405 nm]) were recorded kinetically over 7 minutes. The assay positive control comprised 9 serial 2-fold dilutions of a standard assay positive starting at a 1:100 dilution; 3 negative control specimens were diluted at 1:100. A response curve of delta OD versus dilution of positive control was calculated and used for the determination of relative antibody concentration values. A positive/negative cutoff of 4 times the average delta OD of the 3 negative controls, selected on the basis of representative reactivity with a total control population, was established a priori to define seroreactivity.