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Insights into *Borrelia miyamotoi* infection from an untreated case demonstrating relapsing fever, monocytosis and a positive C6 Lyme serology

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ABSTRACT

We describe a patient from the United States with PCR- and serology-confirmed *Borrelia miyamotoi* infection who recovered without antibiotics. Our findings suggest that *B. miyamotoi* infection may cause relapsing fever, blood monocytosis and antibody reactivity to the C6 peptide. Further studies are required to better define the spectrum of clinical and laboratory findings for this emerging tick-transmitted infection.

Borrelia miyamotoi is a relapsing fever spirochete that may be found in all *Ixodes* spp. ticks that potentially transmit *Borrelia burgdorferi* sensu lato (Fukunaga et al., 1995; Krause et al., 2015; Crowder et al., 2014). However, in total less than 100 patients with this hard-tick transmitted infection have been reported on in the United States and for fewer than 10 of these patients were any of the specific clinical and laboratory data presented (Gugliotta et al., 2013; Chowdri et al., 2013; Molloy et al., 2015; Telford et al., 2015). Cases have also been reported from Russia, the Netherlands and Japan (Platonov et al., 2011; Hovius et al., 2013; Sato et al., 2014). In this report, we describe an adult patient from suburban New York City, in the Lower Hudson Valley region of New York State, with *B. miyamotoi* infection. This patient was not treated with antibiotics and experienced a relapsing febrile illness, providing insights into the natural history of this infection.

1. Case summary

A 44-year-old previously well Caucasian man from Westchester County, NY, who had no recent travel except to Connecticut and no history of Lyme disease or an immunocompromising condition, was evaluated on August 24, 2015, because of a 4-week history of fatigue and generalized weakness (Table 1). He had had 2 bouts of fevers with rigors and myalgias; the first at the end of July lasting 4–5 days, and the second occurred 3 weeks later. The patient was febrile on August 22 and August 23, 2015, but had no fever on August 24, 2015 and thereafter. He recalled at least 6 tick bites within the month preceding the onset of his illness. He had not seen a physician regarding his complaints. Physical examination revealed a temperature of 36.3 degrees centigrade, a blood pressure of 120/80 millimeters of mercury, and a pulse rate of 77 beats per minute. The rest of the examination was within normal limits.

Laboratory testing revealed normal values for the hemoglobin level (15.6 g/dL), the white blood cell count (6200 cells/mm^3), the absolute neutrophil count (2288 cells/mm^3), and the lymphocyte count (2492 cells/mm^3). However, the patient had an elevated monocyte count of 1073 cells/mm^3 (normal range ≤500 cells/mm^3 (Berliner, 2016)) and mild thrombocytopenia of 140,000 cells/mm^3 (normal range 150,000–450,000 cells/mm^3); creatinine, liver enzymes and bilirubin were within normal limits. Testing for *Anaplasma phagocytophilum* and for *Babesia microti* by blood smear and polymerase chain reaction were negative. Serologic testing for antibodies to *Borrelia burgdorferi* was positive by an enzyme-linked immunosorbent assay (ELISA) using a whole cell sonicate (WCS) of *B. burgdorferi* as

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Table 1
Chronology of illness and of selected laboratory test results.

Case	
Chronology	Year 2015, except where indicated
Date of tick bite	July 4–July 20, 6 tick bites
Date of onset of fever	July 28
Duration of fever	4–5 days
Date of onset of recurrent fever	August 22
Duration of fever	2 days
Date of initial clinical evaluation	August 24
Date of follow-up evaluation	September 9
Date of final evaluations	November 4 (in person) and May 3, 2016 (by telephone)
Total duration of symptoms	4 weeks
Lab testing	
Borrelia miyamotoi test results	August 24: Positive PCR on blood November 4: Positive GlpQ antibody (see Table 2); Negative PCR on blood
Borrelia burgdorferi serology*	August 24: Positive WCS ELISA (value 1.72); Negative immunoblots (IgM 41 kDa band, IgG 41 kDa band); C6 ELISA not done; November 4: Positive WCS ELISA (value 1.79); Negative immunoblots (IgM 41 kDa band, IgG no bands); Positive C6 ELISA (value 7.48)

* Only bands that are used to judge seropositivity by the Centers for Disease Control and Prevention criteria are listed (Centers for Disease Control and Prevention (CDC), 1995).

antigen, but supplemental IgM and IgG immunoblot testing was negative (Centers for Disease Control and Prevention (CDC), 1995) (Table 1). PCR on blood for *B. burgdorferi* DNA was negative.

PCR on blood for *B. miyamotoi* DNA was positive, but because the test was in development when the patient presented, the original blood sample from August 24, 2015, was not able to be tested until approximately 1 month later. The patient was fully recovered without antibiotic therapy by August 26, 2015, and has remained well as of May 3, 2016. At about 14 weeks after the onset of illness his platelet count had risen to normal levels at 228,000 cells/mm^3. The absolute monocyte count had fallen to 673 cells/mm^3. At this time serologic testing for antibodies against the glycerophosphodiester phosphodiesterase (GlpQ) protein of *B. miyamotoi* was performed using both an ELISA and IgG and IgM immunoblots that was positive for IgM and IgG antibodies (Table 2). Repeat serologic testing for Lyme disease on this date revealed both a positive WCS ELISA and a positive C6 ELISA, however, both IgG and IgM immunoblot testing remained negative. PCR for *B. miyamotoi* was also negative.

2. Methods

2.1. Borrelia burgdorferi serologic testing

Testing for antibody to *B. burgdorferi* was done by an enzyme immunoassay using either a WCS of *B. burgdorferi* as the antigen by the Captia™ *B. burgdorferi* IgG/IgM assay (Trinity Biotech, Jamestown, NY), or by the C6 Lyme ELISA kit (Immunetics, Boston, MA). Separate IgM and IgG immunoblots were performed using the *B. burgdorferi* IgG and IgM MARBLOT strip test systems (Trinity Biotech, Jamestown, NY). All serologic testing was performed in accordance with the manufacturers' recommendations. Immunoblots were interpreted using the Centers for Disease Control and Prevention (CDC) (1995) guidelines.

2.2. Borrelia burgdorferi polymerase chain reaction testing

DNA from blood samples was extracted using the Qiagen Blood Mini Kit as per the instructions of the manufacturer. A real-time PCR assay, with a specific probe targeting the 16S rRNA gene of *B. burgdorferi,* was performed as previously described by Barbour et al. (2009).

2.3. Anaplasma phagocytophilum polymerase reaction testing

A real-time PCR assay targeting *A. phagocytophilum groE* [forward primer HS14 (5′-CATAGTCTTATGCTACGGTTG-3′), reverse primer HS197 (5′-AAGGCTTACTAATCGCTACAG-3′) and probe Apw1 (5′ 6FAM-TGCAGTTGGTTGTACTGCTGGTCCT-TAMRA 3′) was performed on an ABI 7500 instrument (Life Technologies, Foster City, CA) using the same protocol as previously described for a *B. microti* PCR assay (Wang et al., 2015).

2.4. Borrelia miyamotoi polymerase chain reaction and serologic testing

The presence of *B. miyamotoi* DNA in blood samples was initially analyzed using a real-time PCR assay with a relapsing fever-specific probe targeting the 16S rRNA gene as described by Barbour et al. (2009). A second real-time PCR assay targeting the *glpQ* gene of *B. miyamotoi* (Molloy et al., 2015) was subsequently performed for any positive samples. Sanger sequencing was used to verify that the amplicons were *B. miyamotoi.*

2.5. Borrelia miyamotoi and Borrelia hermsii serologic testing at CDC

B. miyamotoi and *B. hermsii* culture (CDC reference collection) sonicate protein was bound to 96 well plates (Immulon) at 1 μg/well for WCS EIAs. Blocked and washed wells were incubated with patient or control sera diluted 1:100. Alkaline phosphatase conjugated goat anti-human IgA + IgG + IgM (H&L) and pNPP substrate buffer (both reagents KPL) were used to detect bound antibody. EIA positive cutoffs were set at 3 standard deviations above the mean absorbance (405 nm) of 6 negative control sera from healthy subjects with no history of *Borrelia* spp. infection. For immunoblotting similar culture preparations were used to resolve proteins by polyacrylamide gel electrophoresis. Protein was transferred to nitrocellulose blots. The calculated culture protein per final blocked and washed 3×110 mm blot strip was 5 μg; 20 μL of patient or control sera was incubated with Western blot strips and bound antibody was incubated with alkaline phosphatase conjugated goat anti-human IgM or IgG and detected with BCIP/NBT phosphatase substrate (KPL).

The *glpQ* gene of *B. miyamotoi* was amplified from an *I. scapularis* tick collected in Connecticut 2012. The full length *glpQ* gene was amplified and cloned into plasmid pCR-Blunt (Invitrogen). Primers were designed to amplify the sequence of the mature peptide, removing the leading 20 codons and the stop codon and ligated with plasmid pQE-60 (Qiagen) using the BamHI site. Sequencing was done to verify gene identity and reading frame. The mature peptide with a C-terminal 6xHis tag was expressed in *Escherichia coli* and purified per the manufacturer's instructions. Purified protein, 1 μg/well, was coated on 96-well plates as

Table 2
Serologic testing for *Borrelia miyamotoi* (Bm) and *Borrelia hermsii* (Bh).

	Bm GlpQ IgG EIA	Bm WCS IgG EIA	Bm IgM immunoblot	Bm IgG immunoblot	Bh GlpQ IgG EIA	Bh WCS IgG EIA
CDC test site	+	+	+	+	−	+
Yale test site	+	ND	+	+	ND	ND

WCS = whole cell sonicate; EIA = enzyme immunoassay; CDC = Centers for Disease Control and Prevention; ND = Not done.