

Two Cases of *Borrelia miyamotoi* Meningitis, Sweden, 2018

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We report 2 human cases of *Borrelia miyamotoi* disease diagnosed in Sweden, including 1 case of meningitis in an apparently immunocompetent patient. The diagnoses were confirmed by 3 different independent PCR assays and DNA sequencing from cerebrospinal fluid, supplemented by serologic analyses.

Borrelia miyamotoi is the cause of an emerging disease in the Northern Hemisphere, transmitted by hard (*Ixodes*) ticks. The bacterial species, described in Japan in 1995 (1), is genetically related to the relapsing fever borreliae and may be divided into Siberian, European, and American genotypes (2). *B. miyamotoi* disease (BMD), described in case series from Russia (3) and the United States (4,5), is a systemic illness causing relapsing fever, headache, myalgia, arthralgia, elevated liver enzymes, neutropenia, and thrombocytopenia. In addition, 3 cases of meningoencephalitis caused by *B. miyamotoi* have been reported worldwide, 2 from Europe and 1 from the United States, all in highly immunocompromised patients (6–8). We report 2 human cases of BMD diagnosed in Sweden, including 1 case of meningitis in an apparently immunocompetent patient.

The Patients

On July 29, 2018, a 53-year-old woman (patient A) sought care at a hospital for headache, neck stiffness, and high-grade fever that had progressively worsened during the preceding week (Figure 1, panel A). Her medical history included previous cholecystectomy and gastric bypass surgery. Her sole medication was oxycodone (an opioid drug used to manage

pain), which she was taking because of a recent elbow fracture. She had not been abroad during the preceding months but she had removed an attached tick while staying in Stockholm County 6 weeks earlier. No subsequent erythema appeared around the bite site. At admission, we found no neurologic deficits or signs of impaired consciousness. Cerebrospinal fluid (CSF) analysis showed total leukocyte count 321 cells/ μ L (reference ≤ 5 cells/ μ L), mononuclear cells 276 cells/ μ L (reference ≤ 5 cells/ μ L), and albumin 1,270 mg/L (reference < 420 mg/L). Bacterial culture; anti-*Borrelia* antibody testing; and PCR for herpes simplex virus, varicella zoster virus, and enterovirus were negative in CSF. Serologic test results for tickborne encephalitis were negative. Viral meningitis was suspected.

The next day, clinical improvement occurred, and the patient was discharged. However, the patient's condition then worsened, with more pronounced headache and neck pain, and she was readmitted on August 6. Blood cell and platelet counts and C-reactive protein levels were normal. CSF analysis showed total leukocyte count 517 cells/ μ L (reference ≤ 5 cells/ μ L), mononuclear cells 354 cells/ μ L (reference ≤ 5 cells/ μ L), and CXCL13 327 pg/mL (reference < 190 pg/mL). We initiated intravenous treatment with ampicillin to cover *Listeria* meningitis; the fever resolved within 1 day. The CSF *Borrelia* antibody index came back weakly positive for IgM (Table) and, under the diagnosis of (atypical) Lyme neuroborreliosis (LNB), oral doxycycline was initiated (200 mg 2 \times /d for 14 d). Panbacterial *16S* rRNA gene sequencing (9) of the CSF sample suggested *B. miyamotoi*, a finding that later was confirmed by specific PCR, sequencing, and serologic testing (Table).

At follow-up on August 24, the patient showed continued improvement without any fever relapses. Complementary tests for immunodeficiency showed normal serum levels of immunoglobulins. We analyzed convalescent serum using several commercially available tests for laboratory diagnosis of Lyme borreliosis (Table); all results were negative.

A 66-year-old woman (patient B) living in Stockholm County was referred in August 2018 for 6 weeks of intermittent high-grade fever and 9 months of various other symptoms (Figure 1, panel B). She had rheumatoid arthritis, treated with methotrexate together with rituximab twice a year since 2011, but had been physically very active. Her symptoms began with headache and increasing fatigue in November 2017, a few days after returning from a 2-week

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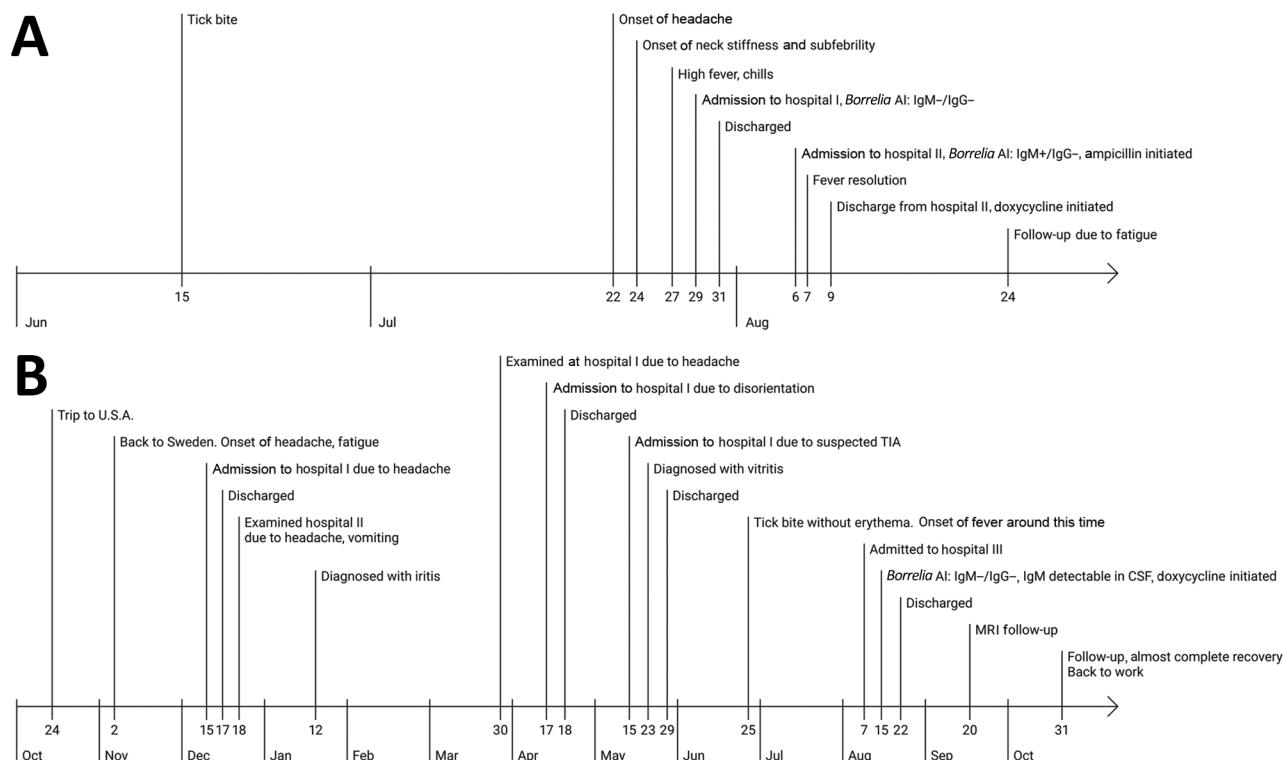


Figure 1. Time course of *Borrelia miyamotoi* meningitis in 2 patients, Sweden, 2018. A) Patient A, a 53-year-old immunocompetent woman; B) patient B, a 66-year-old immunocompromised woman. AI, antibody index; CSF, cerebrospinal fluid; MRI, magnetic resonance imaging; TIA, transient ischemic attack.

trip to California and Nevada, USA. She had not noticed any tick bites during the trip but had had several tick bites in Sweden during the summer of 2017. She subsequently started experiencing progressing difficulties with concentration and memory and had relapsing febrile episodes. In January 2018, she received diagnoses of uveitis and iritis; vitritis of unknown cause was later diagnosed. She also had progressive hearing loss, and hearing aids were prescribed.

In addition, she had loss of appetite and weight (15 kg within 6 months). In May she had a short episode of left-sided weakness, and transient ischemic attack was suspected. All this resulted in her quitting her work as an accountant and hardly being able to leave her house.

Upon referral, we performed a lumbar puncture, which showed total CSF leukocytes of 331 cells/ μ L (reference \leq 5 cells/ μ L), 273 cells/ μ L mononuclear (reference \leq 5 cells/

Table. Confirmatory analyses performed on cerebrospinal fluid and serum/plasma samples from 2 patients with diagnoses of meningitis caused by *Borrelia miyamotoi*, Sweden, 2018*

Analysis	Patient A, dates samples collected					Patient B, dates samples collected			
	Jul 30		Aug 6		Aug 24	Aug 9		Oct 31	
Sample	Serum	CSF	Serum	CSF	Serum	Serum	Plasma	CSF	Serum
BM-specific PCR	-	+	+	+	NA	NA	+	+	NA
BM-specific serologic testing	IgM+, (GlpQ, Vlp5), IgG-	NA	IgM+ (GlpQ, Vlp5), IgG+ (GlpQ)	NA	IgM+ (GlpQ, Vsp1), IgG+ (GlpQ, Vlp15/16)	IgM+ (GlpQ), IgG-	NA	NA	IgM+ (GlpQ), IgG-
LB serology†	-	-	-	IgM AI+	-‡	-	NA	IgM detectable, but AI-	-‡
Culture attempts	-	-	-	-	NA	NA	-	NA	NA

*Patient A: a 53-year-old immunocompetent woman; patient B: a 66-year-old immunocompromised woman. AI, antibody index; BM, *Borrelia miyamotoi*; CSF, cerebrospinal fluid; GlpQ, glycerophosphodiester-phosphodiesterase; LB, Lyme borreliosis; NA, not analyzed; Vlp, variable large protein; Vsp, variable small protein; +, positive; -, negative.

†LIAISON *Borrelia burgdorferi* (DiaSorin, <https://www.diasorin.com>).

‡In addition to LIAISON, the sample was analyzed by Enzygnost Lyme link VlsE IgG and Enzygnost Borreliosis IgM (DADE Behring, <https://www.siemens.com>), recomBead *Borrelia* IgM and IgG (Mikrogen GmbH, <https://www.mikrogen.de>), C6 Lyme ELISA Kit (Immunetics, <https://immunetics.com>), and Anti-Borrelia EUROLINE-RN-AT IgG and IgM (EUROIMMUN, <http://www.euroimmun.com>). All test results were negative.

μL), albumin 1,550 mg/L (reference <420 mg/L), lactate 4.2 mmol/L (reference 1.2–2.1 mmol/L), and CXCL13 >500 pg/mL (reference <250 pg/mL). Magnetic resonance imaging showed contrast enhancement in both oculomotor nerves and the left trigeminal nerve, as well as thickening of the pituitary stalk. CSF was PCR negative for herpes simplex virus, varicella zoster virus, enterovirus, *Mycoplasma*, and *Toxoplasma*. Bacterial, mycobacterial, and fungal CSF cultures were negative. Serologic results for tickborne encephalitis and Lyme borreliosis were negative, with the exception of detectable *B. burgdorferi* IgM in CSF (Table). The 16S rRNA gene sequencing (9) was positive for *B. miyamotoi*.

We treated the patient with doxycycline (200 mg 2×/d for 14 d); within 5 days, the patient regained her hearing, and the fever and headache disappeared. MRI 1 month later showed an almost complete regression of the contrast enhancement of the cranial nerves. By follow-up 2 months after finishing the treatment, the patient had resumed employment and felt almost completely recovered.

We performed *B. miyamotoi* quantitative PCR (qPCR) targeting the flagellin gene, slightly modified from Hovius et al. (6) (Appendix, <http://wwwnc.cdc.gov/EID/article/25/10/19-0416-App1.pdf>). The 2 successive CSF samples and 1 serum

sample from patient A were positive by qPCR, as were the CSF and plasma samples from patient B (Table).

From 1 of the CSF samples from patient A and the CSF sample from patient B, we confirmed the presence of *B. miyamotoi* by nested PCR amplification and sequencing of the glycerophosphodiester-phosphodiesterase (*glpQ*) and *p66* genes (6), as well as a fragment of the intergenic spacer (IGS) between the 16S rRNA and 23S rRNA genes (10) (Appendix). The DNA sequences of the 16S-23S IGS (Figure 2), *glpQ*, and *p66* from patients A and B were identical to *B. miyamotoi* sequences derived from Europe but different from sequences derived from Asia and North America, indicating BMD contracted in Europe.

We tested for GlpQ and variable major proteins (Vmps) IgM and IgG by ELISA, as described previously (11,12) (Table). A clear seroconversion from IgM to IgG against GlpQ was demonstrated in patient A, whereas patient B merely demonstrated IgM reactivity against GlpQ. However, in patient A, but not in patient B (the immunosuppressed patient), we could demonstrate IgM and IgG against different Vmps over time.

Finally, we pursued culture attempts in MKP-F media on CSF and serum samples drawn before initiation of

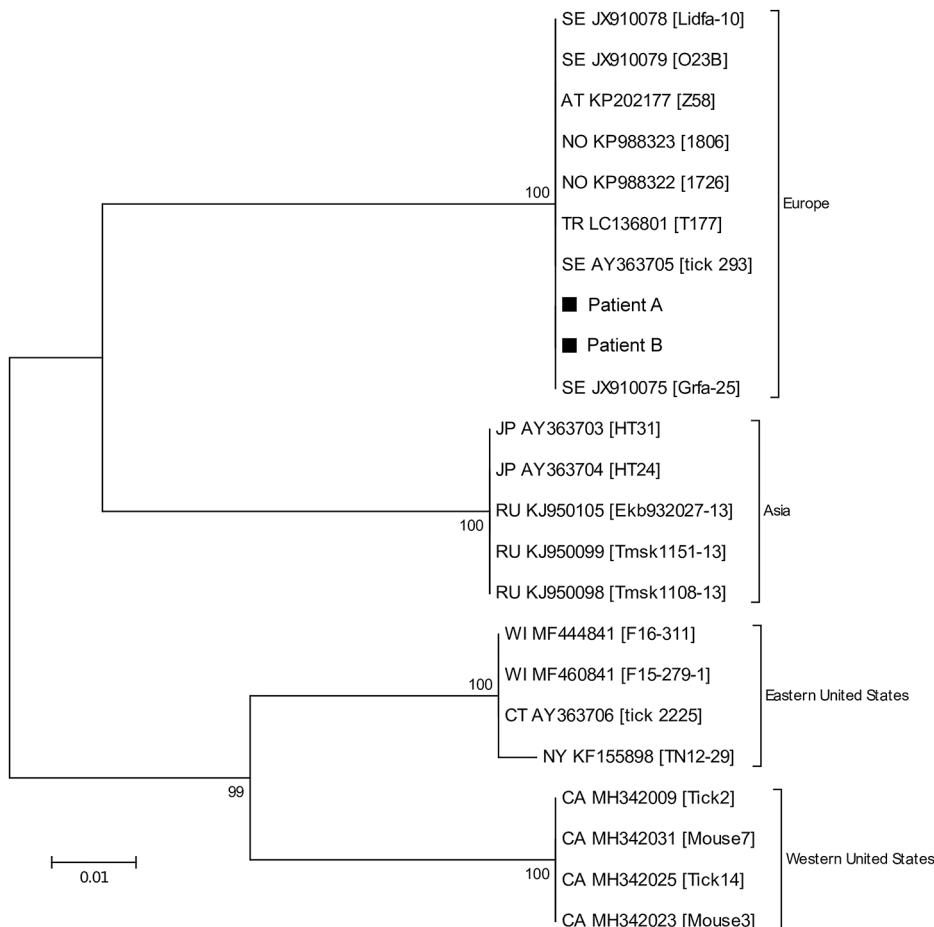


Figure 2. Phylogenetic tree based on 16S-23S intergenic spacer region sequences of *Borrelia miyamotoi* from 2 patients in Sweden, 2018 (patients A and B, black squares), and reference sequences. Tree constructed using the maximum-likelihood method based on the Tamura-Nei model and complete deletion. Sequences detected from patients in this study were deposited into GenBank under accession nos. MK458687 (patient A) and MK458688 (patient B). The source of each reference sequence is indicated by an accession number preceded by a state or country code: AT, Austria; CA, California; CT, Connecticut; JP, Japan; NO, Norway; NY, New York; RU, Russian Federation; SE, Sweden; TR, Turkey; WI, Wisconsin. The accession number is followed by the isolate name in brackets. The reliability of the tree was tested by 500 bootstrap replicate analyses; only values >50% are shown. The phylogenetic relationship between the *B. miyamotoi* strains detected in our patients was corroborated by the DNA sequences obtained from the *glpQ* and *p66* genes (data not shown). Scale bar indicates nucleotide substitutions per site.

antimicrobial drug treatment, retrieved from -80°C (Table), as described by Koetsveld et al. (13). After 2 months of culture, all samples remained negative.

Conclusions

Epidemiologic surveillance of emerging tickborne pathogens is crucial to increase awareness of the diseases that can be contracted after tick bites. Previous studies have shown that *B. miyamotoi* is present in *Ixodes ricinus* ticks in Scandinavia (14,15), but no human cases of BMD have been reported, and public health importance has been uncertain. Until now, severe disease, including slowly progressive CNS symptoms (6,7), has been reported in immunocompromised patients, but our findings indicate that *B. miyamotoi* may also cause CNS infection in immunocompetent persons (patient A). The clinical presentation differs from that of LNB, and results of serologic tests that are routinely used for LNB diagnosis can be negative. Therefore, we need to raise awareness of BMD among healthcare providers and ensure that adequate diagnostic methods are available. BMD should be a differential diagnosis in cases of fever and CNS symptoms after a tick bite in both immunosuppressed and immunocompetent persons.

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Appendix

PCR Protocols

16S Next Generation Sequencing (NGS) Analysis Using Ion Torrent Platform

A ≈460 bp region of the 16S rRNA gene covering the hypervariable regions V3–V4 was amplified using PCR primers containing adaptor sequences and barcodes for downstream Ion Torrent sequencing. Ion Torrent sequencing was performed according to a modified version of the manufacturer's protocol using an Ion S5 XL instrument (<http://www.thermofisher.com>). The 16S sequences obtained were mapped to curated 16S databases (<https://www.arb-silva.de>). All sequencing reactions contained an internal DNA control, facilitating analysis of very low DNA contents and also allowing semiquantitative aspects of the samples. The analysis was performed at the Public Health Agency of Sweden.

Borrelia miyamotoi qPCR

A qPCR targeting the *B. miyamotoi* flagellin, as described by Hovius et al. (1), was modified and performed using the a BioRad CFX96 Real-Time PCR System (Bio-Rad Laboratories, <http://www.bio-rad.com>). Optimized conditions in a final volume of 20 µL were Maxima Probe qPCR Mix, 200 nM forward (5'-AGA AGG TGC TCA AGC AG-3') and reverse (5'-TCG ATC TTT GAA AGT GAC ATA T-3') primers each, 200 nM probe (5'-FAM-AGC ACA ACA GGA GGG AGT TCA AGC- BHQ1-3'), 5 µL of template DNA and RNase-free water up to 20 µL. Cycling conditions were: 10 min 95°C followed by 45 cycles of 95°C for 5 s and 60°C for 35 s, and finally 1 cycle of 37°C for 20 s. Samples with a positive signal were assumed to be positive for *Borrelia miyamotoi*. The specificity of primers and probe was evaluated earlier by Hovius et al. (1). As a positive control, a synthetic plasmid containing the target sequence of the assay was used.

Molecular Identification/Typing of *Borrelia miyamotoi*

The presence of *B. miyamotoi* was confirmed by nested PCR amplification and sequencing of a fragment of the intergenic spacer between *16S* rRNA gene and *23S* rRNA gene, as described earlier by Bunikis et al. (2). The first reaction mixture in a final volume of 50 µl were: 5X Phusion HF Buffer (Thermo Scientific, <http://www.thermofisher.com>), 200 µM dNTP, 500 nM forward (5'-GTA TGT TTA GTG AGG GGG GTG-3') and reverse (5'-GGA TCA TAG CTC AGG TGG TTA G-3') primers each, 0.5 U Phusion DNA Polymerase (Thermo Scientific), 5 µL of template DNA and RNase-free water up to 50 µL. Cycling conditions were 5 min at 98°C followed by 39 cycles of 94°C for 30 s, 58°C for 30 s, and 74°C for 60 s, and finally 1 cycle of 74°C for 7 min. Five µL of PCR product obtained from the first reaction was added to a second reaction mixture in a final volume of 50 µL consisting of 5X Phusion HF Buffer, 200 µM dNTP, 500 nM nested-forward (5'-AGG GGG GTG AAG TCG TAA CAA G-3') and nested-reverse (5'-GTC TGA TAA ACC TGA GGT CGG A-3') primers each, 0.5 U Phusion DNA polymerase, and RNase-free water up to 50 µL. Cycling conditions were 5 min at 98°C followed by 40 cycles of 94°C for 30 s, 60°C for 30 s and 74°C for 60 s, and finally 1 cycle of 74°C for 7 min.

PCR amplification of the *glpQ* gene was performed as earlier described by Hovius et al. (1). The reaction mixture in a final volume of 25 µL were 5X Phusion HF Buffer (Thermo Scientific), 200 µM dNTP, 500 nM forward (5'-ATG GGT TCA AAC AAA AAG TCA ML-3') and reverse (5'-CAT TAC TGT GTC AGT AAA ATC TGT AAA TAT ACC ATC TAC-3') primers each, 0.5 U Phusion DNA Polymerase (Thermo Scientific), 2.5 µL of template DNA, and RNase-free water up to 25 µL. Cycling conditions were 15 min at 94°C, then cycles of 20 s at 94°C, 30 s at 70°C, 30 s at 72°C lowering annealing temperature 1°C each cycle to 60°C, then 40 cycles at this annealing temperature and a final extension step of 7 min at 72°C.

The *p66* gene was amplified under identical conditions with different forward (5'-GAT ACT AAA TTA TTA AAT CCA AAA TCG-3') and reverse (5'-GGA AAT GAG TAC CTA CAT ATG G-3') primers, as described earlier by Hovius et al. (1). Nucleotide sequencing of the PCR products was performed by Macrogen Inc. (Amsterdam; <https://macrogenlab.com>). All sequences were confirmed by sequencing both strands. The obtained chromatograms were edited and analyzed using BioEdit software v7.0 (Tom Hall, Ibis Therapeutics,

<https://bioedit.software.informer.com>). Sequence alignment of the sequences acquired in this study and a representative selection of *B. miyamotoi* 16S-23S IGS, *glpQ*, and *p66* sequences (detected in different specimens; *I. ricinus*, *I. pacificus*, *I. scapularis*, *I. persulcatus* and human isolates in Europe, Asia, and North America) deposited in GenBank was performed using BioEdit. Phylogenetic analyses were conducted using MEGA version 7 (<https://www.megasoftware.net>), and the phylogenetic tree of 16S–23S IGS sequences was constructed by using the maximum likelihood method based on the Tamura-Nei model with complete deletion. The significance of the relationship was ascertained by bootstrap analysis (500 replicates). Sequences obtained in this investigation have been deposited in GenBank with accession numbers MK458687–MK458692.

Serologic Analyses

GlpQ and Vmps ELISA

The presence of anti-glycerophosphodiester-phosphodiesterase (GlpQ) and anti-Variable major proteins (Vmps) IgM and IgG antibodies was analyzed by ELISA, as described previously (3). Positive and negative controls were included in each run and the cutoff (optical density, OD) for reactivity was set at the median + 4 x SD of 8 healthy controls from a non-endemic area (northern Norway). We have recently shown that Vmps are highly immunogenic in patients with BMD and that the presence of antibodies against GlpQ combined with antibodies against Vmps had a 100% specificity for IgM and 98.3% for IgG (4).

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