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SHORT COMMUNICATION

Borrelia miyamotoi DNA in a patient suspected of Lyme borreliosis

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Abstract

Introduction and Objective. Manifestations of infection caused by *Borrelia miyamotoi* can mimic highly variable symptoms of Lyme borreliosis. The aim of the study was to detect DNA from *B. miyamotoi* samples from patients with suspected neuroborreliosis.

Materials and Method. Samples of blood serum and cerebrospinal fluid (CSF) were collected from 133 patients. Diagnosis was established by the detection of specific antibodies to *Borrelia burgdorferi* sensu lato (s.l.) with ELISA and immunoblot. All Borrelia-positive samples were tested by nested PCR for the B. miyamotoi and B. burgdorferi s.l. DNA.

Results. B. miyamotoi DNA was detected in the CSF of one (0.8%) patient. DNA of *B. burgdorferi* s.l. was not found in any samples.

Conclusions. Detection of the *B. miyamotoi* in patients with central nervous system infections expand the development of knowledge on infections caused by Borrelia spirochetes.

Key words

BMD, neuroborreliosis, *Borrelia miyamotoi*, Borrelia miyamotoi disease, central nervous system infections, relapsing fever group

INTRODUCTION

The spirochetes of *Borrelia miyamotoi* belong to the group of bacteria that cause relapsing fever (Borrelia Relapsing Fever Group). These bacteria were first isolated from *Ixodes persulcatus* in Japan in 1995 [1, 2]. Subsequently, *B. miyamotoi* DNA has been found in *Ixodes* ticks in Asia, North America and Europe [3, 4]. They were considered non-pathogenic bacteria until the first human cases of *B. miyamotoi* disease were diagnosed in Russia in 2009 [2, 5]. The status of *B. miyamotoi* as a pathogen was established only recently; subsequently, cases have been described in the United States, Europe and Asia. There have been 561 total diagnosed cases: 367 in Russia, 101 in the United States, 57 in France, 30 elsewhere in Asia, and 6 elsewhere in Europe [2, 6].

B. miyamotoi seroprevalence averages from 1–3% of the human population, compared with 15–20% for *B. burgdorferi* sensu lato (s.l.) [5]. In Poland *B. miyamotoi* was detected in 0.3%-3.5% of ticks [7, 8, 9].

Both in the USA and in Europe, it has been reported that BMD in people causes flu-like symptoms and neurological abnormalities [2, 6]. Routine serological test C6 ELISA in the confirmation of *B. burgdorferi* s.l. can detect *B. miyamotoi* antibodies in 50–80% of samples [10].

Recent serological studies on reactivity to GlpQ and Vmp proteins ('in-house' test) have revealed maximum sensitivities of 79% for IgM and 86.7% for IgG and a specificity of 100% for IgM antibodies, and 98.3% for IgG. [11]. Molecular tests (PCR or RT-PCR) are currently more appropriate and reliable methods for routine diagnostics [12]. The genes detected most frequently in the molecular diagnostics of *B. miyamotoi* are *glpQ*, *p66*, and *fla* genes [2]. The *glpQ* gene is present in relapsing fever *Borrelia* but not in *B. burgdorferi* s.l. and therefore can discriminate between the two types [13].

OBJECTIVE

The aim of the study is to detect DNA from *B. miyamotoi* samples from patients with suspected neuroborreliosis.

MATERIALS AND METHOD

Only patients with meningitis, neck stiffness, facial nerve palsy, and cerebrovascular diseases were included in the study. All had a tick bite history within the last six months. No information was available on the history of erythema migrans in the study subjects. Blood and cerebrospinal fluid (CSF) samples from 133 patients (72 women and 61 men) were taken from the patients within one or two days after the onset of symptoms.

A total of 266 samples (serum and CSF from each patient) were tested with *Borrelia burgdorferi* s.l. ELISA (DRG MedTec, Germany) and immunoblot (Euroimmun,

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Germany) [14]. DNA was extracted from CSF and serum samples with a Syngen Tissue DNA kit (Syngen Biotech). The presence of *B. miyamotoi* in DNA isolates was detected by the amplification of three loci: the glycerophosphodiester phosphodiesterase (*glpQ*) gene, the *p66* gene, and the flagellin (*fla*) gene. Nested PCR targeting a fragment of *glpQ* gene and *p66* gene was performed with Gold Hot Start PCR MIX LOAD (Syngen Biotech, Poland) [13]. Two sets of primers were used to amplify a fragment of *fla* gen [13, 15].

A positive sample was analyzed with primers targeting a 723 bp fragment of the *glpQ* gene [16]. Each run of the PCR included positive (*B. miyamotoi* DNA, concentration 5×10^4 organisms/mL) and negative controls (water). The PCR products of *glpQ* (425 bp and 700 bp), *p66* (569 bp), and *fla* (411 bp) genes were sequenced and identified using BLAST software.

The presence of *B. burgdorferi* s.l. DNA was investigated with PCR in CSF and serum samples to rule out the cases of co-infection [17].

RESULTS

Borrelia burgdorferi s.l. antibodies were detected in both sera and CSF of 45 (33.8%) patients, including 12 (9.0%) patients with both classes of antibodies in serum and CSF. Eighty-eight (66.2%) patients had developed *B. burgdorferi* s.l. antibodies in serum only, including 33 (24.8%) patients with IgM class antibodies, 27 (20.3%) patients with IgG class antibodies, and 28 (21%) patients with both classes of antibodies (Tab.1).

Table 1. ELISA test results of patients with clinical symptoms of Lyme borreliosis

No. of patients	Sera test results		CSF test results	
	IgM	IgG	IgM	IgG
12	positive	positive	positive	positive
17	positive	positive	negative	positive
14	negative	positive	negative	positive
2	positive	negative	positive	negative
33	positive	negative	negative	negative
27	negative	positive	negative	negative
28	positive	positive	negative	negative

Positive results in ELISA were confirmed by Western blot in 80% patients and the results in IgM class antibodies were as follows: the *OspC* band was detected in 80 patients (60%), the *p39* band in 13 (10%), the *p41* band in 66 (50%) and *VlsE* 13 patients (10%). In IgG class antibodies the *p83* band was detected in 40 patients (30%), the *p18* band in 53 (40%), the *VlsE* band in 93 (70%), the *p58* band in 13 (10%), the *p41* band in 93 (70%), *OspC* in 106 (80%), and *p39* in 27 patients (20%).

DNA of *B. miyamotoi* (*glpQ*, *p66* and *fla* genes) was detected in the CSF of one patient. DNA of *B. burgdorferi* s.l. was not found in any samples.

CASE STUDY

A 47-year-old alcoholic male from the Warsaw area was admitted to hospital in 2011 after suffering for three months from blurred vision in the left eye. No history of fever, recurrent fever, erythema migrans (EM) was found in the medical interview. Ophthalmoscopy examination revealed a mildly oedematous optic disc. The vessels, macula lutea, and retina were normal. Extraocular optic neuritis of the left eye was recognized. Routine laboratory investigations did not show abnormalities. Magnetic resonance imaging (MRI) revealed abnormalities in hyperintense signal in the white matter of the brain hemispheres (FLAIR-T2 images). The optic nerve was thinned and obliterated, which was indicative of fibrosis of the nerve and its sheath. In addition, some demyelinating changes were found in both hemispheres. The parameters of the CSF were as follows: elevated total protein 107 mg/dL (ref. value 0–40 mg/dL), glucose 102 mg/dL (ref. value 50–80 mg/dL), cells 8/μL (ref. value 0–5/ μL). Specific IgM and IgG antibodies to *B. burgdorferi* s.l. were detected in serum and only IgG antibodies were detected in CSF. Specificity was confirmed with the immunoblot test (positive reactions with *OspC*, *p41* (int.) and *VlsE* in IgG), according to European Federation of Neurological Societies (EFNS) criteria [14, 17, 18]. A retrospective PCR test for *B. miyamotoi* infection was performed. The 425 bp *glpQ* gene fragment (Acc. No. MK674170) revealed 100% homology to the sequences: LC164098, KU749386, and KJ950108. The 723 bp fragment of the *glpQ* gene (Acc. No. MK674171) revealed 100% homology to *B. miyamotoi* sequences*:* AP024399, CP036914, and CP037471 from *Ixodes persulcatus* or human blood (Fig. 1). The *omp66* gene fragment (Acc. No. OP946656) revealed 100% homology to *B. miyamotoi* sequences*:* MN689815, AP024396, and CP024351 from *I. persulcatus* and human blood. The results are summarized in Figure 2.

The *fla* gene fragment (Acc. No OP946657) revealed 100% homology to *B. miyamotoi* sequences*:* CP037471, CP037215, and KU749379 from *I. persulcatus* and human blood (Fig. 3). All sequences obtained were identical to those of *B. miyamotoi* obtained from Asia.

Nucleotide sequence Accession Nos. Detected sequences were submitted to GenBank under Acc. Nos: MK674170, OP946656, OP946657.

DISCUSSION

Molecular results confirmed the presence of an etiological agent of BMD in the CSF of one patient. In serological results, antibodies to *B. miyamotoi* infection probably cross-reacted with *B. burgdorferi* antigens in the serological tests for *B. burgdorferi.*

However, from obtained results, it cannot be unequivocally excluded that co-infection with *B. burgdorferi* s.l. and *B. miyamotoi* due to the low sensitivity of *B. burgdorferi* s.l. PCR – *B. miyamotoi* spirochetes often co-exist with *B. burgdorferi* s.l. in ticks [13, 19]. Mixed infections with *B. afzelii*, *B. burgdorferi* sensu stricto or *B. garinii* have also been recognized. This indicates the possibility of mixed infections of this etiology in humans. In the co-infections, *B. burgdorferi* s.l. is responsible for the development of erythema migrans, while *B. miyamotoi* can cause meningoencephalitis, mainly in immunosuppressed persons, including alcoholics [2, 20].

Optic neuritis has not been reported in patients infected with *B. miyamotoi* so far [21]. *B. miyamotoi* infection may

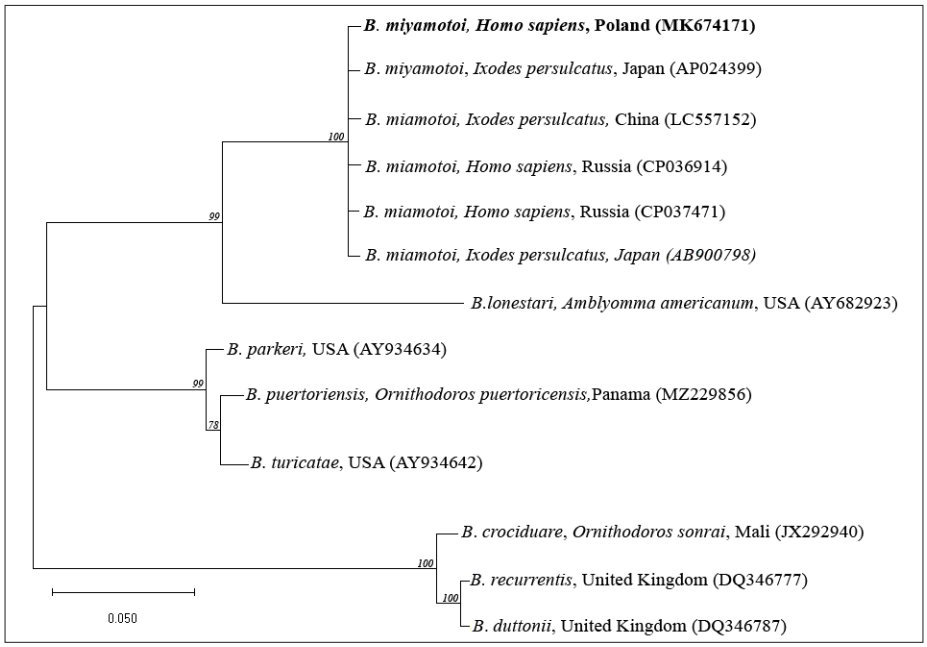

Figure 1. Phylogenetic tree of glpQ gene of Borrelia spp., constructed by MEGA (ME) analysis using MEGA version 11. For ME analysis (nucmodel=codon), the T93+G model was chosen based on jModelTest version 2.1.4 [24, 25] using Akaike Information Criterion. Hosts, country and GenBank accession numbers of origin are shown. Nodal support is indicated as MEGA posterior probabilities. Sequences generated are show in bold.

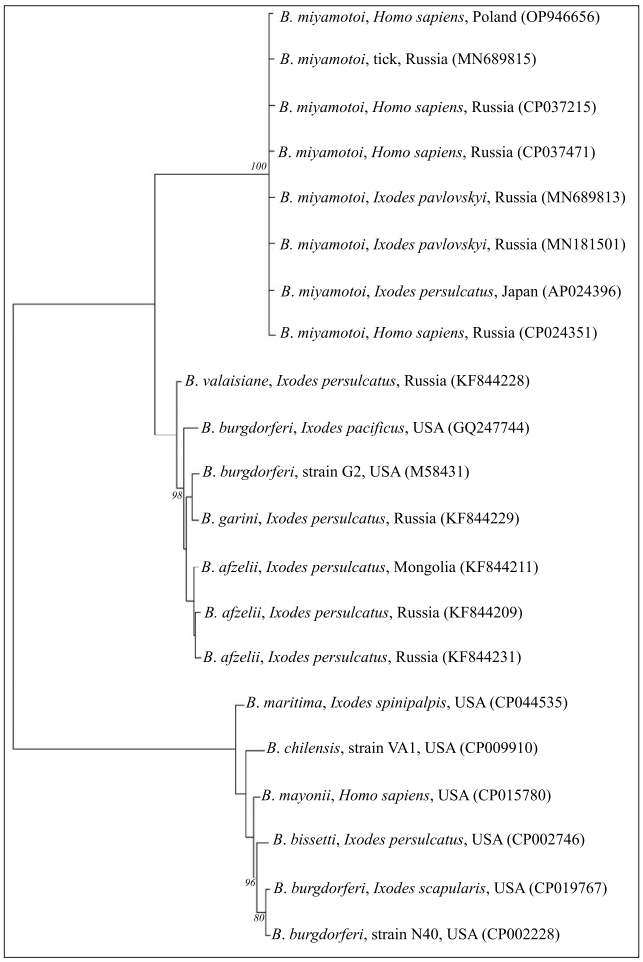

Figure 2. Phylogenetic tree of omp66 gene of Borrelia spp., constructed by MEGA (ME) analysis using MEGA version 11. For ME analysis (nucmodel=codon), the T92+G+I model was chosen based on jModelTest version 2.1.4 [24, 25] using Akaike Information Criterion. Hosts, country and GenBank Accession Numbers of origin are shown. Nodal support is indicated as MEGA posterior probabilities. Sequences generated are show in bold

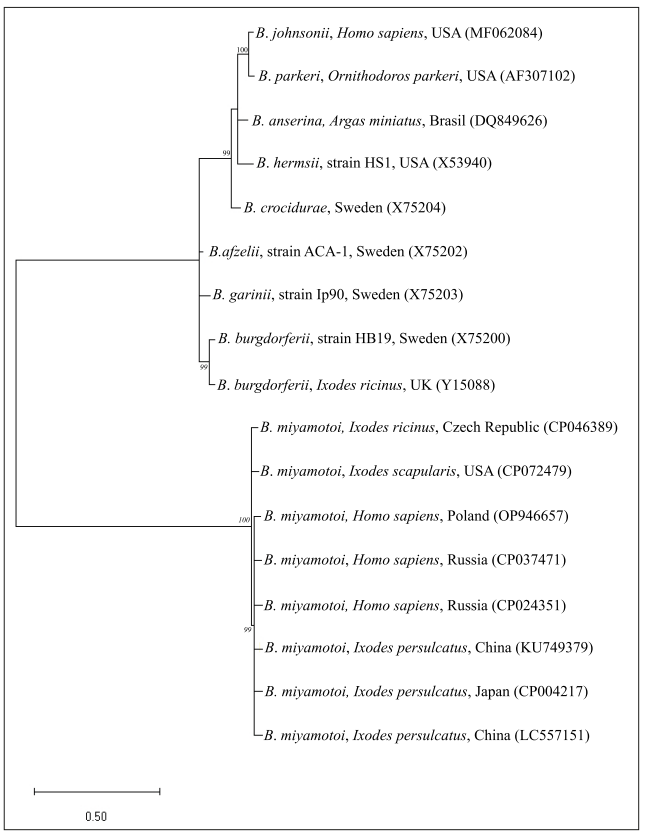

Figure 3. Phylogenetic tree of flaB gene of Borrelia spp., constructed by MEGA (ME) analysis using MEGA version 11. For ME analysis (nucmodel=codon), the T92+G model was chosen based on jModelTest version 2.1.4 [24, 25] using Akaike Information Criterion. Hosts, country and GenBank accession numbers of origin are shown. Nodal support is indicated as MEGA posterior probabilities. Sequences generated are show in bold.

cause pathological changes, including erythrocyte aggregates and obstructed sinuous capillaries [22]. In 79% of patients with *B. miyamotoi*, organ dysfunctions were found by microscopic examination of the eye capillary blood flow. Patients with neurological symptoms and questionable serological findings pose a serious diagnostic problem due to the failure to meet the criteria for neuroboreliosis. In Poland, 25,293 cases of Lyme borreliosis have been registered, including 471 (1.9%) cases of neuroborreliosis in 2023 [23]. Detection of this bacterium in patients with central nervous system infections expand the development of knowledge on infections caused by *Borrelia* spirochetes, allow diagnosis in severe neurological cases of infections caused by spirochetes, and reduce the time to initiate treatment.

CONCLUSIONS

Patients with neurological symptoms and questionable serological findings are a serious diagnostic problem due to failure to meet the criteria for neuroboreliosis. This indicates the need for further studies in patients with signs of the central nervous system (CSN CNS) infection. In the current study, *B. miyamotoi* infection in a patient with extraocular optic neuritis was confirmed by sequencing the amplified products of PCR (fragments of *fla*, *omp66* and *glpQ* genes). The influence of detected transversion within the *qlpQ B. miyamotoi* gene on function and changes in the structure of the encoded protein was not determined, and further research is necessary.

This study is a commentary on the question of whether patients with specific *B. burgdorferi* s.l. antibodies in blood serum only (negative CSF result) can also be regarded confirmed cases of neuroborreliosis, and whether the criteria of EFNS (neurological symptoms, cerebrospinal fluid pleocytosis, specific antibodies of *B. burgdorferi* s.l. produced intrathecally), should be modified [18].

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