

Borrelia miyamotoi DNA in a patient suspected of Lyme borreliosis

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Abstract

Background Clinical manifestations in infection caused by *B. miyamotoi* can mimick highly variable symptoms of Lyme disease. The aim of our studies was to detect DNA of *B. miyamotoi* spirochetes in clinical materials from patients suspected of neuroborreliosis(retrospectively).Methods Samples of blood serum and cerebrospinal fluid were collected from 133 patients with clinical manifestations of neuroborreliosis. Diagnosis was established by detection of IgM and / or IgG specific antibodies to *B. burgdorferi* with ELISA in both sera and CSF. Specificity of positive ELISA results in sera were confirmed with Western-blot test. Bacterial DNA from the collected material was extracted, amplified and sequenced.Results Among 133 patients with clinical manifestations of neuroborreliosis recognized in the years 2010-2018., DNA of *B. miyamotoi* was detected in CSF from 1 (0.8%) patient with extraocular optic neuritis of the left eye (GenBank accession No. MK674170 and MK674171).Conclusion Detection of *B. miyamotoi* in patients with central nervous system infections, will allow a better understanding of the epidemiology of infections caused by *Borrelia* sp. spirochetes. Patients with neurological symptoms and questionable serological findings are a serious diagnostic problem, due to failure to meet the criteria for neuroborreliosis. This indicates the need for further studies of patients with signs of CNS infection.

Background

Borrelia miyamotoi spirochetes 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. Subsequently, DNA of *B. miyamotoi* have been found in *Ixodes* ticks in Asia (China, Korea, Japan and Russia), North America (United States and Canada) and Europe (France, Czech Republic, Norway, Poland, Belgium, England, Denmark, Estonia, Sweden, Switzerland, Germany and Hungary, Netherlands and Spain) [1, 2, 3, 4, 5]. They had been considered to be non-pathogenic bacteria for humans until the first cases of infected people were diagnosed in Russia in 2009 [6]. *Borrelia miyamotoi* status as a pathogen was established only recently. Subsequently, cases have been described in the USA, Europe and Japan and China but the incidence rate of the disease in humans is not known [1, 7, 8, 9].

In Poland, *B. miyamotoi* studies were carried out on ticks. In 2153 ticks collected in central and eastern Poland, *B. miyamotoi* was detected in 7 ticks (0.3%) [10]. Among 325 *I. ricinus* ticks collected at two forest sites in north-western Poland *B. miyamotoi* and *B. afzelii* was detected in 2 nymphs (5.1%) [11]. In ticks from Olsztyn of north-eastern Poland (an endemic region of tick-borne diseases) *B. miyamotoi* was identified in 3.5% of them [12]. There is no any publications on *B. miyamotoi* infections in human in Poland.

Clinical manifestations in infections caused by *B. miyamotoi* can mimic highly variable symptoms (some cases of meningitis, neck stiffness, facial nerve palsy and cerebrovascular diseases have been reported) of Lyme disease. Both in the USA and in Europe, it has been reported that *Borrelia miyamotoi* disease in people causes fever (up to 40°C), pain and dizziness, chills, sweating, nausea, vomiting, increased thirst and muscle and joints pains. [5, 6, 13, 14, 15].

Routine serological tests used in the confirmation of *B. burgdorferi* infections have insufficient sensitivity and can detect *B. miyamotoi* antibodies in 50–80% of samples but cannot discriminate antibodies to *B. miyamotoi* and *B. burgdorferi* [16]. So far, infections with *B. miyamotoi* have been detected only occasionally. The diagnostic kits used for determination of *B. miyamotoi* antibodies are produced in some laboratories (“in-house” tests) and they are not standardized or subjected to quality control by independent laboratories. Recent serological studies on reactivity to proteins glpQ and Vmp have revealed maximum sensitivities of 79% for IgM and 86.7% for IgG and a specificity of 100% for IgM class of antibodies, and 98.3% for IgG “in-house” tests. This test will be used in clinical practice in the future and it will improve serologic examination in clinical studies [17]. Therefore, molecular tests (PCR or real time PCR) are more appropriate and reliable methods for routine diagnosis at this moment [6, 18].

Some reports highlight that *B. miyamotoi* has a neurotropism whose physiopathology remains unknown [14]. The aim of our studies was to detect DNA of *B. miyamotoi* spirochetes in clinical materials from patients suspected of neuroborreliosis (retrospectively).

Methods

The main criterion for inclusion to the studies was the detection of neurological symptoms resembling neuroborreliosis (meningitis, neck stiffness, facial nerve palsy and cerebrovascular diseases).

All patients came from central Poland. They all had tick bite history. All patients included were diagnosed with neuroborreliosis. These patients were not part of the previous study. We don't have information about erythema migrans history in the population patients. Samples were taken from patients within 1-2 days of the onset of neurological symptoms. All patients had antibiotic therapy (samples were taken in 80% of patients before antibiotic therapy, 20% after treatment initiation) Blood serum and cerebrospinal fluid (CSF) samples from 133 (72 women and 61 men) patients with clinical suspicion of neuroborreliosis were investigated. All patients agreed to the use of their material for scientific research. A total of 266 samples (two samples per patient: one serum and one CSF) were collected between 2010 and 2018 at the Laboratory of Rickettsiae, Chlamydiae and Spirochetes, NIPH-NIH. Diagnosis was established by detection of IgM and/or IgG specific antibodies to *Borrelia burgdorferi* with ELISA (DRG MedTec, Germany) in sera and/or CSF. The specificity of positive ELISA results in sera was confirmed with the Western-blot test (DRG MedTec, Germany).

As positive results in: Western-blot IgM class antibodies was accepted the presence of specific antibodies reacting with at least two of the following fractions: p18; p39, OspC, p41 and VlsE or strong reaction with the OspC antigen; Western-blot IgG class antibodies was accepted the presence of specific antibodies reacting with at least two of the following fractions: p83, p58, p41(int.), p39, OspC, p18 i VlsE.

The status of all *Borrelia*-positive samples was confirmed by nested PCR for the glycerophosphodiester phosphodiesterase (*glpQ*) *B. miyamotoi* gene using the protocol described by Fomenko et al. [19], with modifications. Bacterial DNA from the collected materials (266 samples; 133 samples of CSF and 133 blood samples) was extracted with a SYNGEN Tissue DNA kit (SYNGEN BIOTECH).

Nested PCR reactions were performed with Gold Hot Start PCR MIX LOAD (Syngen Biotech, Poland) using forward and reverse primers under the following conditions: 15 min at 95°C, then 35 cycles of 30 s 95°C, 60 s 59°C, 30 s 72°C and finishing with 10 min at 72°C for external primer pair (Q1: 5'-CACCATTGATCATAGCTCACAG-3' and Q2: 5'-CTGTTGGTGCCTCATTCCAGTC-3') and internal primer pair (Q3: 5'-GCTAGTGGGTATCTCCAGAAC-3' and Q4: 5'-CTTGGTGTATGCCAGAAGGGT-3'): 15 min at 95°C; 35 cycles of 30 s 95°C, 60 s 52°C, 30 s 72°C and finishing with 10 min at 72°C. Each run of the PCR test included positive (*B. miyamotoi*) DNA obtained during annual participation in proficiency tests, INSTAND Germany, DNA concentration 5×10⁴ organisms/mL) and negative controls (H₂O). Amplified amplicons were analyzed using electrophoresis with 2% agarose gel (Basica LE, Prona) stained with Midori Green Advance DNA Stain (Nippon Genetics Europe GmbH). The nested PCR products (425 bp fragments) were sequenced and identified using BLAST software by comparison with sequences available in GenBank.

The nested-PCR-positive sample was additionally analyzed with primers targeting a 723 bp fragment of *glpQ* gene. The PCR was performed with Gold Hot Start PCR MIX LOAD (Syngen Biotech, Poland) using forward 5'-ATGGGTTCAAACAAAAAGTCACC-3' and reverse primers 5'-CCAGGGTCCAATTCCATCAGAATATTGTGCAAC-3' under the following conditions: 15 min 95°C, then 40 cycles of 30 s 95°C, 30 s 65°C, 90 s 72°C and finishing with 10 min at 72°C (protocol described by Wagemakers et al. 2017, with modifications) [9]. The melting temperatures of the used primers varied by 15°C PCR with gradient temperature (from 53°C to 68°C) was performed and the most appropriate temperature (65°C) was chosen. Amplicons were sequenced and identified using BLAST software by comparison with sequences available in GenBank.

B. burgdorferi DNA was searched in CSF samples for rule out coinfection case.

The PCR was performed also with Gold Hot Start PCR MIX LOAD (Syngen Biotech, Poland) using forward 5'-ATGGGTTCAAACAAAAAGTCACC-3' and reverse primers 5'- OA 149 (sequence 5'-TTA TGA AAA AAT ATT TAT TGG GAAT-3 ') and OA 319 (sequence 5' CTT TAA GCT CAA GCT TGT CTA CTGT-3 ') complementary to the fragment of the *OspA* gene for *B. burgdorferi* (product 170bp) under the following conditions: 15 min 95°C, then 40 cycles of 60 s 95°C, 60 s 55°C, 60 s 72°C and finishing with 3 min at 72°C [20].

Results

Borrelia burgdorferi antibodies were detected in both sera and CSFs of 45 (33.8%) patients, including 12 (9.0%) patients with both classes of antibodies in serum and CSF. Eighty-six (64.7%) patients had developed *B. burgdorferi* antibodies in serum only, including 33 (24.8%) patients with IgM class antibodies, 27 (20.3%) patients with IgG class antibodies and 26 (19.5%) patients with both classes of antibodies.

Western-blot test results for *B. burgdorferi* are following: in IgM class antibodies: OspC band was detected in 80 patients (60%), p39 band was detected in 13 patients (10%), p41 band was detected in 66 patients (50%) and VlsE 13 patients (10%); in IgG class antibodies : p100 band was detected in 40

patients (30%), p18 band was detected in 53 patients (40%), VlsE band was detected in 93 patients (70%), p58 band was detected in 13 patients (10%), p41 band was detected in 93 patients (70%), OspC in 106 persons (80%) and p39 in 27 patients (20%).

DNA of *B. miyamotoi* was detected in CSF from 1 patient. DNA of *B. burgdorferi* was not found in all CSF samples. This 47-year-old patient (with alcohol abuse) was admitted to the hospital in February of 2011 suffering from blurred vision in the left eye from 3 months. It was a man from surroundings of Warsaw. No history of fever, recurrent fever, tick-bite, erythema migrans (EM) was found in the medical interview. On ophthalmoscopy examination, the optic disc was bright pink with clear boundaries. Vessels, macula lutea and retina were normal. Extraocular optic neuritis of the left eye was recognized. Routine laboratory investigations showed no abnormalities. Brain magnetic resonance imaging (MRI) revealed hyperintense signal abnormalities 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. CSF parameters were as follows: elevated total protein 107 mg/dL (reference 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* were detected in serum and IgG antibodies only in CSF. Specificity was confirmed with the Immuno-blot test. Positive reactions of IgG antibodies were detected with 3 bands corresponding to the proteins OspC, p41 (int.), and VlsE, and IgM antibodies reacted with bands OspC and p41, according to EFNS criteria [21]. The patient refused further diagnosis and treatment in the Neurological Clinic without giving any reasons. A PCR test for *B. miyamotoi* infection was performed retrospectively. The 425 bp glpQ gene fragment (sequence deposited in the GenBank acc. No. MK674170) revealed 100% homology to sequences LC164098.1; KU749386.1; KJ950108.1. The second sequence 723 bp glpQ gene fragment (deposited in the GenBank acc. No. MK674171) revealed 99% homology to sequences: LC164098.1; [AB900798.1](#); [LC164124.1](#).

The sequence MK674171 (current studies) at position 765 (AB900798.1) has cytosine (C) instead of adenine (A), which causes a change in the amino acid sequence of the coding protein: glutamine (Q) is substituted for lysine (K), (assembling by ClustalX 2.1 program). In our studies, the influence of the detected transversion within the glpQ *B. miyamotoi* gene for function and changes in the structure of the encoded protein was not determined. These results have been confirmed by independent labs (Genomed, Poland and Oligo.pl, Poland).

Discussion

The serological and molecular results indicated that etiological agent of infection was detected. In this case probably antibodies to *B. miyamotoi* infection cross-reacted in *B. burgdorferi* serological test.

However, from obtained results certainly we cannot exclude co-infection with *B. burgdorferi* and *B. miyamotoi* due to the low sensitivity of PCR [22]. *Borrelia miyamotoi* spirochetes often coexist with *B. burgdorferi* sensu lato in ticks [19, 23]. In southern Poland, 2% ticks tested have been found to be infected with *B. miyamotoi*. Mixed infections with *B. afzelii*, *B. burgdorferi* s. s. or *B. garinii* have also been

recognized [24]. This indicates the possibility of mixed infections of this etiology in humans. In the co-infections, *B. burgdorferi* is responsible for erythema migrans development, whereas *B. miyamotoi* is able to cause meningoencephalitis mainly in immuno-compromised persons including alcoholics [13, 14, 25].

Optic neuritis has not been reported among patients infected with *B. miyamotoi* so far and is described as a rare syndrome in the course of Lyme disease [26, 27, 28]. However, the results from a study conducted by Platonov et al. [29], show that *B. miyamotoi* infection may lead to pathological changes, including erythrocyte aggregates and obstructed sinuous capillaries. In 79% of *Borrelia miyamotoi* patients organ dysfunctions were found by microscopic examination of eye capillary blood flow.

Detection of DNA suggested infection with *B. miyamotoi* but serologic test results were positive for *B. burgdorferi* sensu lato. Literature data have shown the similarity of antigenic structures within the *Borrelia* genus. In consequence, specific antibodies to *B. miyamotoi* presented in the sera of one patient with this infection (confirmed by molecular studies) may also react with antigens of *B. burgdorferi* sensu lato (cross-reactions) [8, 30]. Patients with neurological symptoms and questionable serological findings present a serious diagnostic problem, due to the failure to meet the criteria for neuroborreliosis. In Poland, 12,773 cases of Lyme borreliosis have been registered [31], including 1,267 (10%) cases of neuroborreliosis in 2013. Nevertheless, only 175 cases (14% of all cases of neuroborreliosis) fulfilled the criteria of neuroborreliosis recognition, such as central nervous system abnormalities and the presence of CSF antibodies. Detection of this new bacterium in patients with central nervous system infections will allow a better understanding of the epidemiology of infections caused by *Borrelia* sp. spirochetes, expand the development of knowledge on neuroborreliosis, advance diagnostic procedures in severe neurological cases of infections caused by the spirochetes and reduce the time for appropriate patient treatment.

Nucleotide sequence accession numbers. Sequences for the *B. miyamotoi* glpQ gene: 425 bp and 723 bp were submitted to GenBank under accession No. MK674170 and MK674171, respectively.

Conclusions

Patients with neurological symptoms and questionable serological findings are a serious diagnostic problem, due to failure to meet the criteria for neuroborreliosis. This indicates the need for further studies of patients with signs of CNS infection. In this study, *Borrelia miyamotoi* was first time detected in Poland, in a patient with extraocular optic neuritis of the left eye. The study results were confirmed by sequencing the obtained products (MK674171), which additionally allowed to detect a mutation within position 765 (AB900798.1). The influence of the detected transversion within the glpQ *B. miyamotoi* gene for function and changes in the structure of the encoded protein was not determined and further research in this direction is necessary.

This research is a commentary on the question of whether patients with specific *B. burgdorferi* antibodies in blood serum alone (CSF-negative result, below Cut-off index) may also be regarded as confirmed cases of neuroborreliosis and whether EFNS criteria (neurological symptoms, cerebrospinal fluid (CSF)

pleocytosis, *Borrelia burgdorferi sensu lato* specific antibodies produced intrathecally) should be modified (Guidelines on the diagnosis and management of European Lyme neuroborreliosis) [21].

Declarations

Declarations

Ethics approval and consent to participate

The study was pursued following the approval of the Institutional Review Board (IRB) at the National Institute of Public Health – National Institute of Hygiene, issued on 28.06.2012, Approval No: 4/2012.

Consent for publication

All patients agreed to the use of their material for scientific research

Availability of data and materials

Representative sequences generated in this study were deposited in the GenBank database under the accession numbers No. MK674170 and MK674171.

Competing interest

The authors declare that they have no conflict of interests. All co-authors contributed to writing the manuscript. All authors read and approved the final manuscript.

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Authors' Contributions

Authors' Contributions

Designed the study: BF, GL, UR, WRB, STW and TC; performed the experiments: BF, GL, UR; analyzed the data: BF, WRB, TC.; wrote the paper: BF, WRB, TC. All authors read and approved the final version of the manuscript.

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References

1. Jiang BG, Jia N, Jiang JF, Zheng YC, Chu YL, Jiang RR et al (2018) *Borrelia miyamotoi* infections in humans and ticks, Northeastern China. *Emerg Infect Dis.* 24:236-241. doi: 10.3201/eid2402.160378.
2. Cutler S, Vayssier-Taussat M, Estrada-Peña A, Potkonjak A, Mihalca AD, Zeller H et al (2019) A new *Borrelia* on the block: *Borrelia miyamotoi* - a human health risk? *Euro Surveill.* 24:18. doi:10.2807/1560-7917.ES.2019.24.18.1800170.
3. Palomar AM, Portillo A, Santibáñez P, Santibáñez S, Oteo JA (2018) *Borrelia miyamotoi*: Should this pathogen be considered for the diagnosis of tick-borne infectious diseases in Spain? *Enferm Infecc Microbiol Clin.* 36:568-571. doi: 10.1016/j.eimc.2017.10.020.
4. Remesar S, Díaz P, Venzal JM, Prieto A, Estrada-Peña A, López CM et al (2019) Longitudinal Study of Infection with *Borrelia* spp. in Questing Ticks from North-Western Spain. *Vector Borne Zoonotic Dis.* 9:785-792. doi: 10.1089/vbz.2019.2442.
5. Krause PJ, Fish D, Narasimhan S, Barbour AG (2015) *Borrelia miyamotoi* infection in nature and in humans. *Clin Microbiol Infect.* 21:631–639. <https://doi.org/10.1016/j.cmi.2015.02.006>
6. Platonov AE, Karan LS, Kolyasnikova NM et al (2011) Humans infected with relapsing fever spirochete *Borrelia miyamotoi*, Russia. *Emerg Infect Dis.* 17:1816–1823.
<http://dx.doi.org/10.3201/eid1710.101474>
7. Molloy PJ, Telford SR 3rd, Chowdri HR et al (2015) *Borrelia miyamotoi* Disease in the Northeastern United States: A Case Series. *Ann Intern Med.* 163:91–98. doi: 10.7326/M15-0333
8. Wagemakers A, Staarink PJ, Sprong H, Hovius JW (2015) *Borrelia miyamotoi*: a widespread tick-borne relapsing fever spirochete. *Trends Parasitol.* 31:260–269.
<https://doi.org/10.1016/j.pt.2015.03.008>
9. Wagemakers A, Jahfari SB, de Wever L et al (2017) *Borrelia miyamotoi* in vectors and hosts in The Netherlands. *Ticks Tick Borne Dis.* 8:370–374. <https://doi.org/10.1016/j.ttbdis.2016.12.012>
10. Sytykiewicz H, Karbowiak G, Chorostowska-Wynimko J, Szpechciński A, Supergan-Marwicz M, Horbowicz M et al (2015) Coexistence of *Borrelia burgdorferi* s.l. genospecies within *Ixodes ricinus* ticks from central and eastern Poland. *Acta Parasitol.* 60:654-661.doi:10.1515/ap-2015-0093.
11. Wodecka B, Skotarczak B (2016) Identification of host blood-meal sources and *Borrelia* in field-collected *Ixodes ricinus* ticks in north-western Poland. *Ann Agric Environ Med.* 23:59-63.
doi:10.5604/12321966.1196853.

12. Kubiak K, Dziekońska-Rynko J, Szymańska H, Kubiak D, Dmitryuk M, Dzika E et al (2019) Questing *Ixodes ricinus* ticks (Acari, Ixodidae) as a vector of *Borrelia burgdorferi* sensu lato and *Borrelia miyamotoi* in an urban area of north-eastern Poland. *Exp Appl Acarol.* 78:113-126. doi: 10.1007/s10493-019-00379-z.
13. Hoornstra D, Koetsveld J, Sprong H, Platonov AE, Hovius JW (2018) *Borrelia miyamotoi* disease in an immunocompetent patient, Western Europe. *Emerg Infect Dis.* 24:1770-1772.
<https://doi.org/10.3201/eid2409.180806>
14. Talagrand-Reboul E, Boyer PH, Bergström S, Vial L, Boulanger N (2018) Relapsing fevers: neglected tick-borne diseases. *Front Cell Infect Microbiol.* 8:98. doi: 10.3389/fcimb.2018.00098
15. Platonov AE, Toporkova MG, Kolyasnikova NM et al (2017) Clinical presentation of *Ixodes* tick-borne borreliosis caused by *Borrelia miyamotoi* in the context of an immune response to the pathogen. *Ter Arkh.* 89:35–43. doi: 10.17116/terarkh2017891135-43
16. Molloy PJ, Weeks KE, Todd B, Wormser GP (2018) Seroreactivity to the C6 peptide in *Borrelia miyamotoi* infections occurring in the Northeastern United States. *Clin Infect Dis.* 66:1407–1410
<https://doi.org/10.1093/cid/cix1023>
17. Koetsveld J, Kolyasnikova NM, Wagemakers A et al (2018) Serodiagnosis of *Borrelia miyamotoi* disease by measuring antibodies against GlpQ and variable major proteins. *Clin Microbiol Infect.* 24:1338.e1–1338.e7. <https://doi.org/10.1016/j.cmi.2018.03.009>
18. Karan L, Makenov M, Kolyasnikova N, Stukolova O, Toporkova M, Olenkova O (2018) Dynamics of spirochetemia and early PCR detection of *Borrelia miyamotoi*. *Emerg Infect Dis.* 24:860–867.
<https://doi.org/10.3201/eid2405.170829>
19. Fomenko NV, Livanova NN, Borgoiakov Vlu et al (2010) Detection of *Borrelia miyamotoi* in *Ixodes persulcatus* Ticks in Russia. *Entomological Review.* 90:1088–1094.
20. Nocton JJ, Dressler F, Rutledge BJ, Rys PN, Persing DH, Steere AC et al (1994) Detection of *Borrelia burgdorferi* DNA by polymerase chain reaction in synovial fluid from patients with Lyme arthritis. *N Engl J Med.* 330:229-234. doi: 1056/NEJM199401273300401
21. Mygland A, Ljostad U, Fingerle V, Rupprecht T, Schmutzhard E, Steiner I (2010) European Federation of Neurological Societies. EFNS guidelines on the diagnosis and management of European Lyme Neuroborreliosis. *Eur J Neurol.* 17:8–16. <https://doi.org/10.1111/j.1468-1331.2009.02862.x>
22. Barstad B, Quarsten H, Tveitnes D, Noraas S, Ask IS, Saeed M et al (2018) Direct Molecular Detection and Genotyping of *Borrelia burgdorferi* Sensu Lato in Cerebrospinal Fluid of Children with Lyme Neuroborreliosis. *J Clin Microbiol.* 56: e01868-17. doi:10.1128/JCM.01868-17.
23. Siński E, Welc-Fałęciak R, Zajkowska J (2016) *Borrelia miyamotoi*: A human tick-borne relapsing fever spirochete in Europe and its potential impact on public health. *Adv Med Sci.* 61:255–60.
<https://doi.org/10.1016/j.advms.2016.03.001>
24. Kiewra D, Stańczak J, Richter M (2014) *Ixodes ricinus* ticks (Acari, Ixodidae) as a vector of *Borrelia burgdorferi* sensu lato and *Borrelia miyamotoi* in Lower Silesia, Poland-preliminary study. *Ticks Tick Borne Dis.* 5:892–897. <https://doi.org/10.1016/j.ttbdis.2014.07.004>

25. Gugliotta JL, Goethert HK, Berardi VP, Telford SR 3rd (2013) Meningoencephalitis from *Borrelia miyamotoi* in an immunocompromised patient. *N Engl J Med.* 368:240–245. doi:10.1056/NEJMoa1209039
26. Scott U, Silva-Lepe A, Siatkowski RM (1997) Chiasmal optic neuritis in Lyme disease. *Am J Ophthalmol.* 123:136–138. [https://doi.org/10.1016/S0002-9394\(14\)71011-6](https://doi.org/10.1016/S0002-9394(14)71011-6)
27. Blanc F, Ballonzoli L, Marcel C, De Martino S, Jaulhac B, de Seze J (2010) Lyme optic neuritis. *J Neurol Sci.* 295:117–119. <https://doi.org/10.1016/j.jns.2010.05.009>
28. Burakgazi AZ, Henderson CS (2016) Unusual presentation of unilateral isolated probable Lyme optic neuritis. *Case Rep Neurol Med.* 7471842. doi:1155/2016/7471842
29. Platonov AE, Sarksyan DS, Karan LS et al (2015) The blood coagulation system and microcirculatory disorders in ixodid tick-borne borreliosis caused by *Borrelia miyamotoi*. *Ter Arkh.* 87:26–32. doi: 10.17116/terarkh2015871126-32
30. Krause PJ, Narasimhan S, Wormser GP et al (2014) Tick Borne Diseases Group. *Borrelia miyamotoi* sensu lato seroreactivity and seroprevalence in the Northeastern United States. *Emerg Infect Dis.* 20:1183–1190. <http://dx.doi.org/10.3201/eid2007.131587>
31. http://wwwold.pzh.gov.pl/oldpage/epimeld/index_p.html