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***Borrelia mayonii* sp. nov., a member of the *Borrelia burgdorferi sensu lato* complex, detected in patients and ticks in the upper midwestern United States**

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Abstract

Lyme borreliosis (LB) is a multisystem disease caused by spirochetes in the *Borrelia burgdorferi sensu lato* (Bbsl) genospecies complex. We previously described a novel Bbsl genospecies (type strain MN14-1420^T) that causes LB among patients with exposures to ticks in the upper midwestern USA. Patients infected with the novel Bbsl genospecies demonstrated higher levels of spirochetemia and somewhat differing clinical symptoms as compared with those infected with other Bbsl genospecies. The organism was detected from human specimens using PCR, microscopy, serology and culture. The taxonomic status was determined using an eight-housekeeping-gene (*uvrA*, *rplB*, *recG*, *pyrG*, *pepX*, *clpX*, *clpA* and *nifS*) multi-locus sequence analysis (MLSA) and comparison of 16S rRNA gene, *flaB*, *rrf-rrl*, *ospC* and *oppA2* nucleotide sequences. Using a system threshold of 98.3% similarity for delineation of Bbsl genospecies by MLSA, we demonstrated that the novel species is a member of the Bbsl genospecies complex, most closely related to *B. burgdorferi sensu stricto* (94.7–94.9% similarity). This same species was identified in *Ixodes scapularis* ticks collected in Minnesota and Wisconsin. This novel species, *Borrelia mayonii* sp. nov, is formally described here. The type strain, MN14-1420, is available

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The GenBank/EMBL/DDBJ accession number for *Borrelia mayonii* type strain MN14-1420^T are KM877342 (*uvrA*), KM877343 (*rplB*), KM877344 (*recG*), KM877345 (*pyrG*), KM877346 (*pepX*), KM877347 (*clpX*), KM877348 (*clpA*), KP972469 (*nifS*), KP972468 (16S rRNA), KR154295 (*flaB*), KR154297 (*ospC*), KR154296 (*rrf-rrl*).

A set of supplementary methods and two supplementary tables are available with the online Supplementary Material.

through the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSM 102811) and the American Type Culture Collection (ATCC BAA-2743).

Lyme borreliosis (LB) is a multisystem disease caused by spirochetes of the *Borrelia burgdorferi sensu lato* (Bbsl) complex (Steere, 2001; Stanek *et al.*, 2012). It is the most common vector-borne disease in temperate regions of the northern hemisphere, causing an estimated 300 000 annual cases in the United States and 85 000 annual cases in Europe (Lindgren & Jaenson, 2006; Hinckley *et al.*, 2014). To date, 20 Bbsl genospecies have been described, with a subset of these considered pathogenic for humans. Whereas *Borrelia garinii*, *Borrelia afzelii* and *Borrelia bavariensis* cause the majority of LB cases in Eurasia, *B. burgdorferi sensu stricto* is the predominant cause of LB in the USA (Margos *et al.*, 2013; Schotthoefer & Frost, 2015; Stanek *et al.*, 2012). Among humans, LB is characterized by tissue localization and low levels of spirochetemia (van Dam *et al.*, 1993; Agüero-Rosenfeld *et al.*, 2005; Liveris *et al.*, 2012; Babady *et al.*, 2008). This is in contrast to infection by relapsing fever borreliæ in which spirochete loads range from 10^5 to $>10^6$ ml⁻¹ of blood (Dworkin *et al.*, 2008).

We recently reported the identification of a novel Bbsl genospecies in six patient specimens (five blood and one synovial fluid) submitted to the Mayo Clinic laboratory (Rochester, MN, USA) for LB testing using a real-time PCR assay that uses hybridization probes and targets the oligopeptide permease A2 gene (*oppA2*) (Pritt *et al.*, 2016). This PCR assay detects and differentiates Bbsl genospecies by melting temperature (T_m) analysis (Babady *et al.*, 2008; Pritt *et al.*, 2016). The patients were all residents of the upper midwestern USA (Minnesota, Wisconsin and North Dakota) and reported tick exposure in Minnesota and Wisconsin during 2012–2014. Patients presented with somewhat differing clinical presentations when compared with patients infected with *B. burgdorferi sensu stricto* including diffuse macular rash not typical of erythema migrans (EM), nausea or vomiting and symptoms potentially consistent with neurological involvement. By *oppA2* quantitative PCR and dark-field microscopy, the genome and spirochete load, in blood of acutely ill patients, was estimated to be approximately 10^5 ml⁻¹, which is higher than previously reported among patients infected with other Bbsl genospecies (Bil-Lula *et al.*, 2015; Liveris *et al.*, 2012). Motile spirochetes were cultured from blood of two patients under microaerophilic conditions at 34 °C in modified Barbour–Stoenner–Kelly (BSK) medium. After freezing at –80 °C, the isolates, MN14-1420 and MN14-1539, were transferred to BSK-II (without gelatin) (Zückert, 2007) and grown to densities of over 10^7 spirochetes ml⁻¹.

Nucleic acid of the novel Bbsl genospecies was also detected by *oppA2* PCR in *Ixodes scapularis* ticks collected near the approximate sites of possible patient exposures in Barron County, Wisconsin and Clearwater County, Minnesota during 2013–2015 and in *I. scapularis* ticks collected in three other counties (Eau Claire County, Wisconsin and Morrison and Pine Counties, Minnesota) during 2010–2015 (Table S1, available in the online Supplementary Material) (Pritt *et al.*, 2016). Overall, 29 (2.1 %, range 0%–5.2 %) out of 1381 adult and 6 (1.5 %, range 0%–3.7 %) out of 399 nymphal *I. scapularis* ticks tested were PCR-positive for the atypical Bbsl genospecies (Supplemental Methods, available in the online

Supplementary Material). Of the 35 total ticks testing positive for DNA of the novel Bbsl genospecies, 13 (37.1 %) were also positive for *B. burgdorferi sensu stricto* (Table S1).

Sequencing performed on the *oppA2* (149 bp) PCR products directly amplified from both patients and an *I. scapularis* tick collected in Wisconsin showed 89–95% similarity to genospecies of the Bbsl complex (Pritt *et al.*, 2016). The closest sequence identity for the 16S rRNA gene (1327 bp), *ospC* (561 bp), *flaB* (435 bp) and *rrf-rrl* (253 bp) sequences from the two patient isolates was to genospecies of the Bbsl complex at 99, 85, 97 and 95 %, respectively (Pritt *et al.*, 2016). The taxonomic relationship of the atypical Bbsl isolates, MN14-1420 and MN14-1539, was determined using an eight-housekeeping-gene multi-locus sequence analysis (MLSA) (*clpA*, *clpX*, *nifS*, *pepX*, *pyrG*, *recG*, *rplB* and *uvrA*) developed by Margos and colleagues for delineation of Bbsl genospecies (Margos *et al.*, 2009, 2010). A genetic-distance analysis using concatenated sequences of the eight MLSA loci (4785 nucleotides) demonstrated that the distance to *B. burgdorferi sensu stricto* (~0.051–0.053) and other species of the genus *Borrelia* (>0.053) exceeded the previously determined threshold for species determination (0.017) (Table S2). The MLST sequence types (STs) are ST674 for MN14-1420 and ST675 for MN14-1539. By seven-gene MLSA, the genospecies of the Bbsl complex detected in *I. scapularis* ticks was confirmed to be the same as that identified in patients (Pritt *et al.*, 2016). Taken together, these analyses reveal a distinct genospecies of the Bbsl complex among patients with LB and *I. scapularis* ticks from the upper midwestern USA. Further work is needed to determine the geographic distribution of infected humans and ticks. The novel species is named *Borrelia mayonii* sp. nov. in honor of William James Mayo and Charles Horace Mayo, founders of the Mayo Clinic, where this novel organism was first discovered.

Description of *Borrelia mayonii* sp. nov

Borrelia mayonii (ma.yo'ni.i. N.L. gen. n. after William James Mayo and Charles Horace Mayo, founders of the Mayo Clinic).

The morphology matches that of previously described species of the genus *Borrelia* (Barbour & Hayes, 1986). Spirochetes can be cultured *in vitro* under microaerophilic conditions (Johnson *et al.*, 1984) using BSK-II medium (without gelatin) (Zückert, 2007). *B. mayonii* can be distinguished from all other Lyme borreliosis-group spirochetes by MLSA of eight housekeeping loci (*clpA*, *clpX*, *nifS*, *pepX*, *pyrG*, *recG*, *rplB* and *uvrA*) (Margos *et al.*, 2010) and by 16S rRNA gene, *flaB*, *oppA2*, *ospC* and *rrf-rrl* nucleotide sequences. The MLST sequence types (STs) are ST674 for MN14-1420 and ST675 for MN14-1539.

The type strain, MN14=1420^T, was isolated from a patient exposed to infected ticks in the upper midwestern USA (Pritt *et al.*, 2016); it has been deposited in two microbial culture collections: ATCC, deposit number ATCC BAA-2743; DSMZ, deposit number DSM 10281. The GenBank/EMBL/DDBJ accession number for the type strain of *Borrelia mayonii* (MN14-1420^T) are KM877342 (*uvrA*), KM877343 (*rplB*), KM877344 (*recG*), KM877345 (*pyrG*), KM877346 (*pepX*), KM877347 (*clpX*), KM877348 (*clpA*), KP972469 (*nifS*), KP972468 (16S rRNA), KR154295 (*flaB*), KR154297 (*ospC*) and KR154296 (*rrf-rrl*). MN14-1539 has also been deposited at the ATCC, deposit number ATCC BAA-2744.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Abbreviations

Bbsl	<i>Borrelia burgdorferi sensu lato</i>
CDC	Centers for Disease Control and Prevention
LB	Lyme borreliosis

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