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Surveillance for and Discovery of *Borrelia* Species in US Patients Suspected of Tickborne Illness

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Abstract

Background—Tick-transmitted *Borrelia* species fall into two heterogeneous bacterial complexes comprised of multiple species, the relapsing fever (RF) group and the *Borrelia burgdorferi sensu lato* group, which are the causative agents of Lyme borreliosis (LB), the most common tickborne disease in the northern hemisphere. Geographic expansion of human LB in the United States and discovery of emerging *Borrelia* pathogens underscores the importance of surveillance for disease causing *Borrelia*.

Methods—De-identified clinical specimens, submitted by providers throughout the United States, for patients suspected of LB, anaplasmosis, ehrlichiosis, or babesiosis, were screened using a *Borrelia* genus level TaqMan PCR. *Borrelia* species and sequence types (STs) were characterized by multi-locus sequence typing (MLST) utilizing next generation sequencing.

Results—Among the 7,292 tested specimens tested, five different *Borrelia* species were identified: two causing LB, *B. burgdorferi* (n=25) and *B. mayonii* (n=9), and three RF borreliae, *B. hermsii* (n=1), *B. miyamotoi* (n=8), and *Candidatus B. johnsonii* (n=1), a species previously detected only in the bat tick, *Carios kelleyi*. ST diversity was greatest for *B. burgdorferi* positive specimens, with new STs identified primarily among synovial fluids.

Conclusion—These results demonstrate broad PCR screening followed by MLST is a powerful surveillance tool for uncovering the spectrum of *Borrelia* species causing human disease, improving understanding of their geographic distribution, and investigating the correlation between *B. burgdorferi* STs and joint involvement. Detection of *Candidatus B. johnsonii* in a

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Potential conflicts of interest

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patient with suspected tickborne disease suggests this species may be a previously undetected cause of illness in humans with exposure to bat ticks.

Keywords

Borrelia; Lyme disease; Relapsing fever; Molecular surveillance; Amplicon sequencing

Introduction

The *Borrelia* genus encompasses at least 50 known species of spirochetes, only a subset of which are known to cause human illness [1, 2]. Species are generally divided into two major complexes, *Borrelia burgdorferi sensu lato* (Bbsl) and relapsing fever (RF), comprised of 21 and 29 different species, respectively. The Bbsl complex includes the causative agents of Lyme borreliosis (LB), the most common tickborne illness in the northern hemisphere, which are transmitted through the bite of infected *Ixodes* spp. hard ticks [3, 4]. The second complex includes the etiologic agents of tickborne and louseborne RF [1, 5]. Tickborne RF is most often associated with transmission by infected soft (argasid) ticks; however, the emerging pathogen, *Borrelia miyamotoi*, is transmitted by *Ixodes* ticks.

In the United States, the geographic distribution of human cases of LB and RF is complex and linked to the range of key tick vectors and varied ecological factors [2, 6]. For example, in the Upper Midwest, three human disease causing *Borrelia* are transmitted by *Ixodes scapularis*. These include the LB causing spirochetes, *B. burgdorferi sensu stricto (*hereafter called *B. burgdorferi)* and *Borrelia mayonii* as well as the RF spirochete, *B. miyamotoi* [7, 8]. In the Northeast and mid-Atlantic, *B. burgdorferi* and *B. miyamotoi*, but not *B. mayonii*, also cause human illness [9]. In mountainous regions of western states, the RF spirochete, *Borrelia hermsii*, transmitted by the bite of the soft tick *Ornithodoros hermsi*, causes human disease [6]. Adding to this complexity, ticks can carry multiple *Borrelia* species that have not been associated with human illness [10].

Clinical presentations of LB and RF include a range of overlapping symptoms early in infection with more distinct symptoms at later stages of disease [3, 6, 8, 11]. Early LB is typically characterized by localized skin infection resulting in an erythema migrans (EM) rash, frequently accompanied by fatigue, fever, and headache. Without early treatment, spirochetes can disseminate to secondary sites causing neurologic effects, cardiac abnormalities or arthritis. Arthritis is more commonly associated with *B. burgdorferi* infection, whereas LB caused by *B. mayonii* has been associated with higher spirochetemia [8, 12]. Manifestations of RF consist of fever, fatigue, headache, chills, myalgia, arthralgia, nausea, with rash rarely reported. Recurring febrile episodes can occur as untreated illness advances, particularly for *B. hermsii*, but appears to be less frequent in *B. miyamotoi* infection [6, 11, 13].

In patients infected with *B. burgdorferi*, the most common cause of LB in the United States, the number of spirochetes/genomic copies in blood is low, estimated at only $0.1/10^2$-10^3 per ml [14, 15], limiting sensitivity of direct detection methods. Laboratory diagnosis therefore relies primarily on serologic assays to detect the patient's immune response to the infection [16], which is useful for diagnosis, but precludes surveillance for Bbsl genospecies and

strain types causing human illness. In the case of the two emerging *Borrelia* species, *B. mayonii* and *B. miyamotoi*, higher numbers of spirochetes and/or genomic copies have been reported in infected patients, at 10^5-10^6 and 10^4-10^5 per ml of blood, respectively [8, 9]. Concentrations of *B. hermsii* spirochetes in patient blood are estimated at >10^6 per ml [6]. As spirochete levels for many *Borrelia* infections are directly detectable, a broad molecular approach (genus level PCR followed by multi-locus next generation sequencing typing) was utilized to screen clinical specimens from patients suspected of tickborne illness in order to expand knowledge of *Borrelia* species and strain types associated with human disease in the United States and the geographic regions where these infections occur.

Materials and Methods

Specimen Collection

Residual clinical specimens [whole blood (EDTA), synovial fluid, cerebrospinal fluid (CSF), and tissue] submitted to Mayo Clinic from health care providers nationwide for patients suspected of having a tickborne illness [*i.e.* testing by either Tick Borne Pathogen PCR Panel (*Babesia, Ehrlichia/Anaplasma*) or Lyme (*Borrelia burgdorferi sensu lato*) PCR] and the accompanying nucleic acid extract were stored at 4°C or −70°C, de-identified and shipped to the Minnesota Department of Health (MDH). Synovial fluid, CSF, and tissue specimens were originally submitted for Lyme PCR, whereas blood specimens were submitted for either Tick Borne Pathogen PCR Panel or Lyme PCR. Aliquots of each clinical specimen were prepared, frozen at −70°C, and shipped to the Centers for Disease Control and Prevention, Fort Collins, CO. Associated patient information included specimen type, originating state of the ordering provider, patient age, and sex. As travel history of patients was not available, the state of the ordering provider does not necessarily correlate to the patient's state of residence or exposure. Analysis of de-identified specimens was approved by the Institutional Review Board at Mayo Clinic (Protocol ID: 14-001148). Review at MDH and CDC determined the protocol to be non-human subjects research.

PCR

DNA was extracted at Mayo Clinic using the MagNA Pure 2.0 Instrument (Roche Diagnostics, Indianapolis, IN). Residual DNA (2.5 μl) was tested at MDH using a 16S rRNA pan-*Borrelia* TaqMan™ PCR assay. At CDC, 16S rRNA pan-*Borrelia* results were confirmed by testing DNA independently extracted from aliquots of the *Borrelia* positive specimens using the MagNA Pure 96 (Roche).

The pan-*Borrelia* TaqMan assay was modified from Parola *et al.* [17]. Degenerate nucleotides were incorporated into the forward primer and probe sequences, based on alignment of 19 Bbsl and RF *Borrelia* species. Primer and probe sequences are as follows. Forward: 5′-AGCYTTTAAAGCTTCGCTTGTAG-3′, Reverse: 5′-GCCTCCCGTAGGAGTCTG G-3′, Probe: 5′-FAM-CCGGCCTGAGAGGGTGAWCGG-BHQ-3. Optimized final PCR concentrations included 1X PerfeCTa™ FastMixII or qPCR ToughMix™ (Quanta Biosciences, Beverly, MA), 600 nM of each primer and 200 nM of probe. Real-time PCR [ViiA™ 7 (Thermo Fisher) or 7500 Fast (Applied Biosystems)] cycling conditions were 95°C for 5 minutes, followed by 40-45 cycles at 95°C for 15

seconds, and 60°C for 30 seconds. Sensitivity and specificity were analyzed as described (Supplemental Materials).

Amplicon Sequencing

Portions of the 16S rRNA, *flaB, glpQ, uvrA, rplB, recG, pyrG, pepX, clpX, nifS*, and *clpA* genes were amplified using previously described primers and cycling conditions [18-20] (http://pubmlst.org/borrelia) and Premix Ex Taq hot start master mix (Takara Bio USA, Inc, Mountain View, CA) (Supplemental Materials). DNA concentration of PCR products was measured using the Qubit fluorimeter (ThermoFisher, Grand Island, NY) and normalized to 0.2 ng/μl. Multiplexed libraries were prepared using the Nextera XT DNA library preparation kit (Illumina, Inc. San Diego, CA) per the manufacturer's protocol with unique Nextera indexes (Illumina) added to all amplicons from the same specimen. Sequencing was performed on the MiSeq platform (Illumina) using the V2 300 cycle reagent kit (Illumina).

Sequence Analysis

Amplicon sequence reads were de-multiplexed and adapter sequences removed, followed by import into CLC Genomics Workbench 8.0 (Qiagen, Valencia, CA). Sequence reads were mapped to 8 reference housekeeping genes, *clpA, clpX, nifS, pepX, pyrG, recG, rplB*, and *uvrA* from *B. burgdorferi* B31, *B. mayonii* MN14-1420, and *B. miyamotoi* CT13-2396 using default parameters [21-23]. Sequence reads for 16S rDNA, *flaB*, and *glpQ* from sample 15-3581 were de-novo assembled.

Consensus sequences for the 8 housekeeping genes were trimmed to lengths present in the *Borrelia* PubMLST database (https://pubmlst.org/borrelia/), concatenated in frame in the order *clpA, clpX, nifS, pepX, pyrG, recG, rplB, and uvrA* using Lasergene 12 (DNASTAR, Inc, Madison, WI). Consensus sequences for *glpQ*, *flaB*, and 16S rDNA were concatenated. Concatenated sequences were imported into MEGA 6, aligned (ClustalW), and phylogenetic trees constructed by maximum likelihood analysis using the generalized time-reversible nucleotide substitution model with gamma distribution (four categories) followed by bootstrap analysis (1000 replicates). Pairwise genetic distances were calculated using the Kimura-2 model and Bbsl species identified using the threshold (98.3% similarity, genetic distance 0.017). Sequences for *Borrelia* species included in analyses were obtained from http://pubmlst.org/borrelia/ or NCBI. iTOL v3 was for circularization of phylogenetic trees. Alleles and sequence types (STs) were assigned using http://pubmlst.org/borrelia/.

Results

PCR detection of *Borrelia* species in specimens from patients suspected of tickborne illness

Residual extracted DNA and originating specimens for 7,292 de-identified clinical specimens from patients suspected of LB, anaplasmosis, ehrlichiosis, or babesiosis were available for testing. Specimens were submitted by providers in 47 states and the District of Columbia (Figure 1; Supplemental Table 1) and were collected between 5/1/2014 - 11/15/2014 and 5/1/2015 – 12/11/2015. The majority (84%) of specimens were blood, followed by CSF (11.8%), synovial fluid (4.1%), and tissue (0.1%).

Of the 7,292 residual DNA specimens tested by *Borrelia* genus TaqMan PCR at the MDH, 44 were positive (31 bloods, 12 synovial fluids and 1 CSF). Independent DNA extraction and PCR testing at CDC confirmed all 44 specimens as *Borrelia* positive.

Multi-locus sequence typing (MLST) of *Borrelia* species in clinical specimens

Eight housekeeping genes (*clpA, clpX, nifS, pepX, pyrG, recG, rplB*, and *uvrA*) were amplified from 43 of the 44 *Borrelia* PCR positive specimens. For one specimen, amplicons were produced for 6 of the 8 housekeeping genes, likely due to a low level of spirochetal DNA. Sequence analysis of the 6 genes amplified confirmed the species as *B. burgdorferi*. Analysis of 8 concatenated housekeeping gene sequences (4,791 nucleotides) for the remaining 43 *Borrelia* positive specimens, identified five species falling into the two groups, LB or RF (Figure 2). These included two LB pathogens, *B. burgdorferi* (n=24; >99.2% identity to *B. burgdorferi* B31) and *B. mayonii* (n=9; >99.97% identity to *B. mayonii* MN14-1420) and three different RF *Borrelia*, *B. miyamotoi* (n=8; 100% identity to *B. miyamotoi* LB-2001), *B. hermsii* (n=1; 100% identity to *B. hermsii* YOR), and a *Borrelia* species (n=1) displaying highest sequence identity to *B. parkeri* (97.5%).

Of the 25 *B. burgdorferi* positive specimens, one was CSF and the remainder comprised equally of blood and synovial fluid. The remaining non-*B. burgdorferi* positive specimens were all blood (Table 1). The *B. burgdorferi* positive specimens originated from 8 states in the Midwest, mid-Atlantic and Northeast (IA, MD, MN, MO, NJ, NY, PA, VA, and WI), whereas all 9 *B. mayonii* positives were submitted only from MN or WI. The *B. burgdorferi* positivity rate for synovial fluid samples differed between the Upper Midwest [MN (2/75; 2.7%] and four Northeastern and mid-Atlantic states [MD, VA, PA, and NY (9/99; 9%)]. The *B. miyamotoi* positives came from states in the Upper Midwest and the Northeast (MN, NJ, and WI). The single *B. hermsii* positive specimen originated from Montana.

ST diversity was greatest among the *B. burgdorferi* positive specimens (Figure 2). Eighteen different MLST sequence types (STs) were identified, including 10 known STs (ST1, ST3, ST7, ST8, ST19, ST29, ST32, ST48, ST56, ST530) and 8 new STs (ST754, ST755, ST756, ST757, ST758, ST759, ST760, ST761) comprised of either known loci in previously unobserved combinations or newly observed loci (Table 1). New *B. burgdorferi* STs were predominately associated with synovial fluid specimens (6/8; 75%). The 6 synovial fluid specimens assigned new STs were submitted by providers in Northeast and mid-Atlantic states (Table 1).

In contrast to the *B. burgdorferi* positive specimens, limited or no sequence diversity across the 8 housekeeping genes was observed among the *B. mayonii* or *B. miyamotoi* positive specimens, respectively (Table 1). For the 9 *B. mayonii* positive specimens, three different MLST STs were identified, which included the known STs, ST674, ST675, and a new ST (ST762), which has a single nucleotide variant in *pepX* as compared to ST674 (Supplemental Table 3).

Identification of *Candidatus Borrelia johnsonii* in a patient specimen

To further characterize the RF *Borrelia* spp. (15-3581) most closely related to *B. parkeri*, sequencing and phylogenetic analysis of the 16S rRNA, *flaB* and *glpQ* genes was

performed. As these genes have historically been utilized for characterization of RF *Borrelia*, more sequences are publicly available as compared to the housekeeping genes more commonly used for Bbsl species. Analysis of the concatenated *glpQ, flaB* and 16S rDNA sequence (3,048 nucleotides) demonstrated 100% identity between 15-3581 and *Candidatus Borrelia johnsonii* strain IA-1, a *Borrelia* species previously identified only in bat ticks (*Carios kelleyi*) (Figure 3) [24]. The *Candidatus Borrelia johnsonii* positive blood specimen was submitted from a provider in Wisconsin.

Discussion

A better understanding of the scope and geographic distribution of *Borrelia* species infecting humans is essential for improving clinical recognition, laboratory diagnosis, and prevention. Here, we demonstrated a broad molecular surveillance approach successfully detected both known pathogens and a novel *Borrelia* species in specimens from US patients suspected of tickborne illness. The five *Borrelia* species identified included the 2 Bbsl genospecies known to cause LB, *B. burgdorferi* and *B. mayonii*, the 2 known RF pathogens, *B. hermsii* and *B. miyamotoi*, and a third RF species, *Candidatus B. johnsonii*, not previously associated with human illness.

Prior description of *Candidatus B. johnsonii* is limited to molecular and microscopic detection in bat ticks, *Carios kelleyi*, collected from an Iowa farmhouse [24, 25]. *C. kelleyi* ticks are widely distributed in the Americas and can be found in houses and buildings infested with bats. Although this tick prefers to feed on bats, it will also feed on humans and persons presumably bitten by *C. kelleyi* ticks have reported expanding, erythematous skin lesions, lymphadenopathy, fever, weight loss, malaise, and fatigue [26]. Whether these symptoms were due to infection with *Candidatus B. johnsonii* is unknown. The clinical presentation for the *Candidatus B. johnsonii* infected patient identified here is also unknown. Nonetheless, we can infer that the physician suspected a tickborne illness, given that the blood specimen was originally submitted for either Lyme or Tickborne Pathogen PCR Panel.

Notably, ten infections due to three RF *Borrelia* species, *B. miyamotoi* (n=8), *B. hermsii* (n=1) and *Candidatus B. johnsonii* (n=1), associated with three different tick species, *I. scapularis, O. hermsi* and *C. kelleyi*, respectively, were detected in patient samples submitted for tickborne diseases other than RF. As *B. miyamotoi* is transmitted by *I. scapularis*, the same tick which transmits *B. burgdorferi, B. mayonii, Anaplasma phagocytophilum*, *Ehrlichia muris* ssp. *eauclairensis* and *Babesia microti*, its detection in this sample set is not unexpected and has been reported previously [9, 27, 28]. All 8 *B. miyamotoi* positive samples originated from patients in the Northeast and Upper Midwest where human cases or *B. miyamotoi* infected *I. scapularis* have been previously described [7, 9, 27]. Detection of *B. hermsii* in these samples was unexpected. The specimen originated from Montana where cases of RF are reported due to the bite of infected *O. hermsi* and locally acquired cases of LB, anaplasmosis, ehrlichiosis and babesiosis are not known to occur.

Of the 11 *Bbsl* genospecies identified in ticks or animals in the United States [2, 5, 29], only *B. burgdorferi* and *B. mayonii* were detected in samples from over 7000 US patients. Consistent with the initial description of *B. mayonii* as a cause of LB in the Upper Midwest

[8], all 9 *B. mayonii* positive specimens described here were submitted by providers in either Minnesota or Wisconsin. Given that 3 of the identified *B. mayonii* positives were from 2014, the same time period in which the initial 6 *B. mayonii* infected patients were detected by Lyme PCR [9], it appears likely the providers suspected these patients of having a tickborne illness other than LB. All 9 *B. mayonii* positive specimens were blood, as compared to *B. burgdorferi* positive specimens, which were an equal distribution of synovial fluid and blood. The *B. burgdorferi* positive specimens originated from Lyme endemic regions in the Northeast, mid-Atlantic and Upper Midwest, with the exception of a synovial fluid specimen from Missouri. Due to lack of patient travel information, exposure to ticks in a Lyme endemic region for this patient cannot be excluded and appears likely, given the ST of the infecting strain, ST19, has only been observed previously in the Northeast. No Bbsl positives were identified among specimens originating from 10 southeast or southcentral states (FL, GA, SC, AL, TN, NC, LA, AR, OK, and TX), where *I. scapularis* is also present, and which accounted for 10% (739/7292) of the samples tested.

ST diversity among *B. burgdorferi* positive specimens contrasted with what was observed for *B. mayonii* and *B. miyamotoi* positive specimens. Only a single MLST ST, ST634, was observed among *B. miyamotoi* positive specimens from the upper midwestern and northeastern United States, in agreement with previous studies demonstrating low genetic variability [30]. ST diversity among *B. mayonii* positive specimens was also limited, with 7 of the 9 identified as ST674. The three identified *B. mayonii* ST's differ by only 1 or 2 loci, and thus appear to belong to the same clonal complex. In contrast, among the *B. burgdorferi* positive samples, 18 STs were identified. The most common *B. burgdorferi* ST in this study, ST530, appears to have a localized geographic distribution in the Upper Midwest (MN, WI). Consistent with this, the only previous reported detection of this ST is from *I. scapularis* collected from Manitoba, Canada (http://pubmlst.org/borrelia/).

Newly observed *B. burgdorferi* MLST STs were most abundant in synovial fluids (71%), a specimen type for which 8 housekeeping MLST data is publicly lacking [18, 19, 31-33]. Four of the STs identified in synovial fluid specimens submitted by providers in the Northeast and mid-Atlantic regions, grouped with MLST ST3 (node support 0.89), a ST previously associated with disseminated LB [32]. Whether MLST STs may be predictive of arthritic outcome requires further study.

It is important to clarify the 0.6% (44/7,292) *Borrelia* positivity rate in this sample set does not reflect disease incidence or PCR positivity rates for a given *Borrelia* species. To enhance the likelihood of uncovering the spectrum of *Borrelia* species causing human disease in US patients, specimens from persons suspected of a number of tickborne diseases (LB, anaplasmosis, ehrlichiosis or babesiosis) were tested. The percentage of samples submitted specifically for Lyme as opposed to Tickborne Pathogen PCR Panel (*Babesia, Ehrlichia/ Anaplasma*) was unknown. Second, the ability to detect *Borrelia* DNA by PCR is dependent on a number of factors, including when the specimen is collected in relation to illness onset, whether the sample is taken pre-treatment, and the level of spirochetemia resulting from the infecting *Borrelia* species. These factors could lead to over and under-representation of specific *Borrelia* species or even MLST STs. This is particularly relevant to *B. burgdorferi*,

where the number of spirochetes/genomic copies in blood is low, often below the limit of PCR detection [14, 15].

Our results demonstrate that broad PCR followed by MLST utilizing next generation sequencing is a powerful surveillance tool for uncovering *Borrelia* species causing human disease, understanding the geographic distribution of disease-causing *Borrelia* and improving understanding of the pathogenic properties of *B. burgdorferi* STs. Ongoing surveillance for *Borrelia* pathogens in US patients suspected of tickborne illness is in progress. In ill patients with potential exposure to bat ticks,, *Candidatus B. johnsonii* may be considered.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Summary

Broad molecular surveillance for *Borrelia* species in patients suspected of tickborne illness followed by next-generation sequence typing provides insight into the different *Borrelia* species causing human illness and geographical distribution of *Borrelia* infections and sequence types throughout the United States.

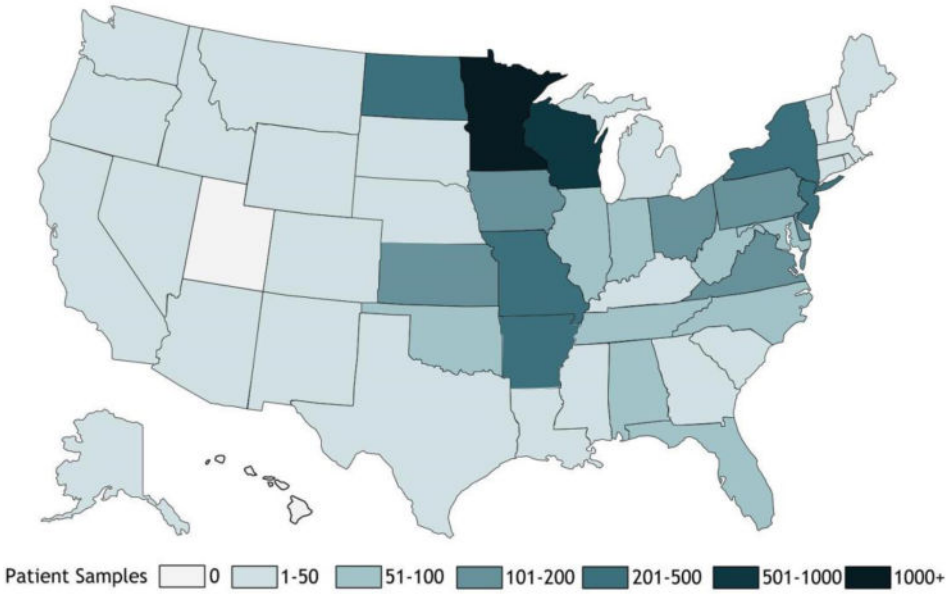

Figure 1. Origin of clinical specimens from patients suspected of tickborne illness

Origin of 7,292 clinical specimens tested in this study. Differential shading indicates the number of patient specimens originating from each state.

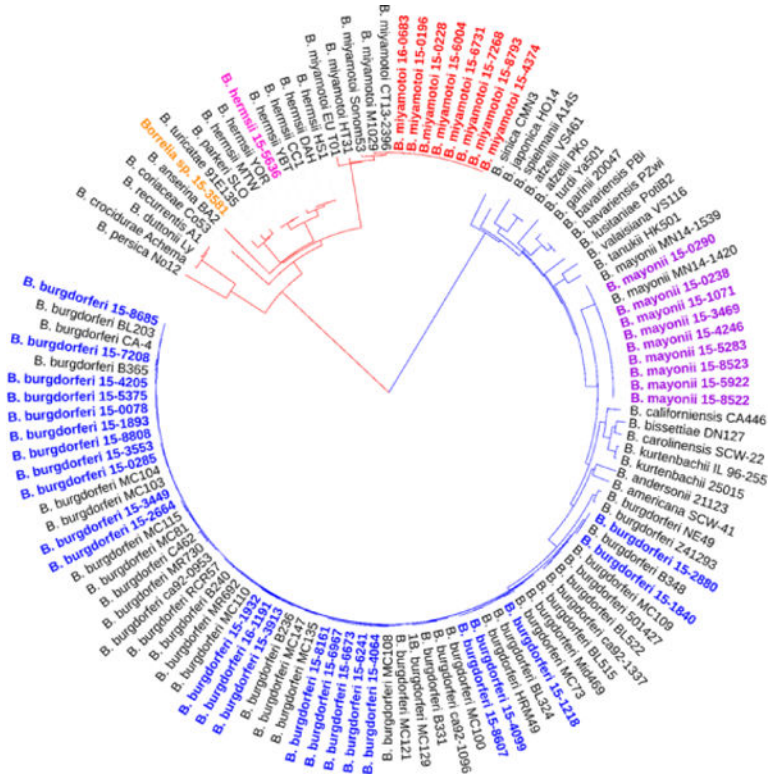

Figure 2. Phylogenetic relationships of identified *Borrelia* species

The phylogenetic tree is based on alignment of in frame concatenated DNA sequence fragments from 8 housekeeping genes (*clpA, clpX, nifS, pepX, pyrG, recG, rplB, and uvrA*) (n = 4,791 bp). Strains sequenced in this study are highlighted as follows: blue, *B. burgdorferi*; purple, *B. mayonii*; red, *B. miyamotoi*; pink, *B. hermsii*, and orange, *Candidatus B. johnsonii*. Publicly available sequences for 18 different Bbsl genospecies and 10 different RF borreliae (gray) were included for comparison. The scale bar corresponds to 0.1 substitutions per nucleotide position.

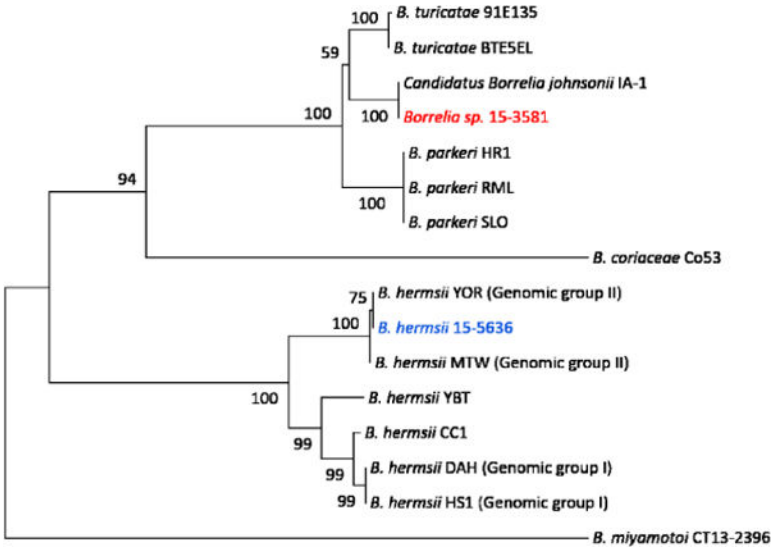

Figure 3. Identification of *Candidatus Borrelia johnsonii* in patient blood

Phylogenetic relationship of *Borrelia* spp. 15-3581 (red) based on analysis of concatenated *glpQ*, *flaB*, and 16S rDNA sequences (n = 3048 bp). Publicly available sequences for 6 different RF borreliae (gray) and *B. hermsii* 15-5636 from this study were included for comparison. Bootstrap support values greater than 50% are shown. The scale bar corresponds to 0.01 substitutions per nucleotide position.

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Table 1

B. burgdorferi											
Sample ID	**Originating state**	**Specimen type**	***clpA***	***clpX***	***nifS***	***pepX***	***pyrG***	***recG***	***rplB***	***uvrA***	**ST**
15-2664	MN	Blood	18	12	1	11	2	15	1	2	**29**
15-3449	MN	Blood	4	12	1	11	2	15	1	2	**756** *
15-4099	MN	Blood	8	1	1	1	4	16	1	7	**32**
15-6241	MN	Blood	14	1	5	2	2	1	1	10	**530**
15-6673	MN	Blood	14	1	5	2	2	1	1	10	**530**
15-6967	MN	Blood	14	1	5	2	2	1	1	10	**530**
15-8161	MN	Blood	14	1	5	2	2	1	1	10	**530**
15-3553	PA	Blood	5	5	4	5	5	5	1	6	**8**
15-0285	WI	Blood	8	1	1	1	4	16	1	7	**32**
15-3913	WI	Blood	128	12	1	8	1	6	1	10	**758** *
15-4064	WI	Blood	14	1	5	2	2	1	1	10	**530**
15-1932	IA	CSF	8	1	1	14	2	6	1	10	**48**
15-0078	MD	Synovial Fluid	4	1	1	1	224 *	6	1	217 *	**754** *
15-1218	MN	Synovial Fluid	24	14	4	18	11	19	1	12	**56**
16-1191	MN	Synovial Fluid	8	1	1	14	2	6	1	10	**48**
15-2880	MO	Synovial Fluid	4	4	3	3	3	3	3	3	**19**
15-5375	NY	Synovial Fluid	4	1	1	1	225 *	6	1	7	**759** *
15-7208	NY	Synovial Fluid	6	1	5	1	1	7	1	8	**7**
15-8685	NY	Synovial Fluid	1	1	1	1	1	1	1	1	**1**
15-4205	PA	Synovial Fluid	14	1	1	2	1	6	200 *	7	**758** *
15-8607	PA	Synovial Fluid	5	5	188 *	5	5	5	1	6	**760** *
15-1840	VA	Synovial Fluid	4	4	3	217 *	3	3	3	3	**755** *
15-1893	VA	Synovial Fluid	4	1	1	1	1	6	1	7	**3**
15-8808	VA	Synovial Fluid	4	1	189 *	1	1	6	1	7	**761** *
B. mayonii											

B. burgdorferi											
Sample ID	**Originating state**	**Specimen type**	***clpA***	***clpX***	***nifS***	***pepX***	***pyrG***	***recG***	***rplB***	***uvrA***	**ST**
			clpA	***clpX***	***nifS***	***pepX***	***pyrG***	***recG***	***rplB***	***uvrA***	**ST**
15-0238	MN	Blood	218	182	166	191	201	209	174	193	**674**
15-0290	MN	Blood	218	182	166	191	202	209	174	193	**675**
15-1071	MN	Blood	218	182	166	191	201	209	174	193	**674**
15-3469	WI	Blood	218	182	166	191	201	209	174	193	**674**
15-4246	MN	Blood	218	182	166	191	201	209	174	193	**674**
15-5283	MN	Blood	218	182	166	191	201	209	174	193	**674**
15-5922	MN	Blood	218	182	166	218*	201	209	174	193	**762***
15-8522	MN	Blood	218	182	166	191	201	209	174	193	**674**
15-8523	MN	Blood	218	182	166	191	201	209	174	193	**674**
B. miyamotoi											
			clpA	***clpX***	***nifS***	***pepX***	***pyrG***	***recG***	***rplB***	***uvrA***	**ST**
15-0196	WI	Blood	200	117	151	173	184	191	160	70	**634**
15-0228	MN	Blood	200	117	151	173	184	191	160	70	**634**
15-4374	NJ	Blood	200	117	151	173	184	191	160	70	**634**
15-6004	MN	Blood	200	117	151	173	184	191	160	70	**634**
15-6731	NJ	Blood	200	117	151	173	184	191	160	70	**634**
15-7268	NJ	Blood	200	117	151	173	184	191	160	70	**634**
15-8793	MN	Blood	200	117	151	173	184	191	160	70	**634**
16-0683	NJ	Blood	200	117	151	173	184	191	160	70	**634**
B. hermsii											
			clpA	***clpX***	***nifS***	***pepX***	***pyrG***	***recG***	***rplB***	***uvrA***	**ST**
15-5636	MT	Blood	246*	206*	190*	219*	226*	236*	201*	218*	**763***
Candidatus B. johnsonii											
			clpA	***clpX***	***nifS***	***pepX***	***pyrG***	***recG***	***rplB***	***uvrA***	**ST**
15-3581	WI	Blood	247*	207*	191*	220*	227*	237*	202*	219*	**764***

* Denotes allele or sequence type identified in this study