Gmail

christine: massey <cmssyc@gmail.com>

FOI to CDC re: studies showing bacteria cause "Lyme disease"

christine: massey <cmssyc@gmail.com> Sat, Aug 26, 2023 at 7:30 PM
To: "FOIA Requests (CDC)" <FOIARequests@cdc.gov>

August 26, 2023

To:
Roger Andoh, Freedom of Information Officer
1600 Clifton Rd NE MS T-01
Atlanta, Georgia [30333]
Email: FOIARequests@cdc.gov
Phone: 770-488-6277
Fax: 770-488-6200

Dear Roger,

I require access to general records*;*

Description of Required Records:

All studies - **authored by anyone, anywhere, ever,** in the possession, custody or control of the Centers for Disease Control and Prevention (CDC) and/or the Agency for Toxic Substances and Disease Registry (ATSDR) and/or the people who are responsible for the **CDC's claim** that bacteria cause "Lyme disease", that:

used purified bacterial cultures (i.e. Borrelia burgdorferi, B. mayonii, B. bissettiae, B. garinii, B. afzelii, B. spielmanii, or B. lusitaniae) **to scientifically prove or provide evidence of causation of "Lyme disease".**

I am not seeking records that describe attempts to prove causation using unscientific methods; recall that opinions, declarations, speculation, review papers and descriptive papers are not scientific proof/evidence. Scientific proof/evidence requires use of the scientific method to test falsifiable hypotheses through valid, repeatable controlled experiments where only 1 variable differs between the experimental and control groups.

If any records match the above description and are currently available elsewhere, please provide enough information about each one so that I may identify and access them with certainty: title, author(s), date, journal, location, URLs where possible;

Format:
Pdf documents sent to me via email; I do not want anything shipped to me;

Thank you and best wishes;
by: christine massey

Gmail

christine: massey <cmssyc@gmail.com>

Your CDC FOIA Request #23-01704-FOIA

uls9@cdc.gov <uls9@cdc.gov> Tue, Aug 29, 2023 at 8:51 AM
To: cmssyc@gmail.com

August 29, 2023

Request Number: 23-01704-FOIA

Dear Ms. Massey:

This is regarding your Freedom of Information Act (FOIA) request of August 28, 2023, for I require access to general records; Description of Required Records: All studies - authored by anyone, anywhere, ever, in the possession, custody or control of the Centers for Disease Control and Prevention (CDC) and/or the Agency for Toxic Substances and Disease Registry (ATSDR) and/or the people who are responsible for the CDC's claim that bacteria cause "Lyme disease", that: used purified bacterial cultures (i.e. Borrelia burgdorferi, B. mayonii, B. bissettiae, B. garinii, B. afzelii, B. spielmanii, or B. lusitaniae) to scientifically prove or provide evidence of causation of "Lyme disease". I am not seeking records that describe attempts to prove causation using unscientific methods; recall that opinions, declarations, speculation, review papers and descriptive papers are not scientific proof/evidence. Scientific proof/evidence requires use of the scientific method to test falsifiable hypotheses through valid, repeatable controlled experiments where only 1 variable differs between the experimental and control groups. If any records match the above description and are currently available elsewhere, please provide enough information about each one so that I may identify and access them with certainty: title, author(s), date, journal, location, URLs where possible.

Please see the attached letter.

Sincerely,
CDC/ATSDR FOIA Office
770-488-6399

Acknowledgement (Complex) Within 30 days.pdf
122K

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Public Health Service

Centers for Disease Control
and Prevention (CDC)
Atlanta GA 30333

August 29, 2023

Ms. Christine Massey
[redacted]
Via email: cmssyc@gmail.com

Dear Ms. Massey:

The Centers for Disease Control and Prevention and Agency for Toxic Substances and Disease Registry (CDC/ATSDR) received your Freedom of Information Act (FOIA) request dated August 28, 2023. Your request assigned number is 23-01704-FOIA, and it has been placed in our complex processing queue.

Extension of Time

In unusual circumstances, an agency can extend the twenty-working-day limit to respond to a FOIA request.

We will require an additional ten-working-days to respond to your request because:

X We reasonably expect to receive and review voluminous records in response to your request.

To process your request promptly, please consider narrowing the scope of your request to limit the number of responsive records. If you have any questions or wish to discuss reformulation or an alternative time frame for the processing of your request, you may contact the analyst handling your request Ryan Hohl at 404-639-5033 or our FOIA Public Liaison, Roger Andoh at 770-488-6277. Additionally, you may contact the Office of Government Services (OGIS) to inquire about the FOIA mediation services they offer. The contact information for OGIS is as follows: Office of Government Information Services; National Archives and Records Administration; 8601 Adelphi Road-OGIS; College Park, Maryland 20740-6001; e-mail at ogis@nara.gov; telephone at 202-741-5770; toll free at 1-877-684-6448; or facsimile at 202-741-5769.

Fee Category

Because you are considered an "Other requester" you are entitled to two hours of free search time, and up to 100 pages of duplication (or the cost equivalent of other media) without charge, and you will not be charged for review time. We may charge for search time beyond the first two hours and for duplication beyond the first 100 pages. (10 cents/page).

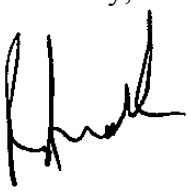
Cut-off-date

If you don't provide us with a date range for your request, the cut-off date for your request will be the date the search for responsive records starts.

You may check on the status of your case on our FOIA webpage https://foia.cdc.gov/app/Home.aspx and entering your assigned request number. If you have any questions regarding your request, please contact Ryan Hohl at uls9@cdc.gov.

We reasonably anticipate that you should receive documents by October 11, 2023. Please know that this date roughly estimates how long it will take the agency to close requests ahead of your request in the queue and complete work on your request. The actual date of completion might be before or after this estimated date.

Sincerely,



Roger Andoh
CDC/ATSDR FOIA Officer
Office of the Chief Operating Officer
(770) 488-6399
Fax: (404) 235-1852

23-01704-FOIA

Gmail

christine: massey <cmssyc@gmail.com>

Your CDC FOIA Request #23-01704-FOIA

uls9@cdc.gov <uls9@cdc.gov> Mon, Oct 2, 2023 at 1:18 PM
To: cmssyc@gmail.com

October 2, 2023

Request Number: 23-01704-FOIA

Dear Ms. Massey:

This is regarding your Freedom of Information Act (FOIA) request of August 28, 2023, for I require access to general records; Description of Required Records: All studies - authored by anyone, anywhere, ever, in the possession, custody or control of the Centers for Disease Control and Prevention (CDC) and/or the Agency for Toxic Substances and Disease Registry (ATSDR) and/or the people who are responsible for the CDC's claim that bacteria cause "Lyme disease", that: used purified bacterial cultures (i.e. Borrelia burgdorferi, B. mayonii, B. bissettiae, B. garinii, B. afzelii, B. spielmanii, or B. lusitaniae) to scientifically prove or provide evidence of causation of "Lyme disease". I am not seeking records that describe attempts to prove causation using unscientific methods; recall that opinions, declarations, speculation, review papers and descriptive papers are not scientific proof/evidence. Scientific proof/evidence requires use of the scientific method to test falsifiable hypotheses through valid, repeatable controlled experiments where only 1 variable differs between the experimental and control groups. If any records match the above description and are currently available elsewhere, please provide enough information about each one so that I may identify and access them with certainty: title, author(s), date, journal, location, URLs where possible.

Please see the attached letter.

Sincerely,
CDC/ATSDR FOIA Office
770-488-6399

2 attachments

23-01704.pdf
6048K

Final Response Full Grant.pdf
110K

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Public Health Service

Centers for Disease Control
and Prevention (CDC)
Atlanta GA 30333

October 2, 2023

Ms. Christine Massey
[redacted]
Via email: cmssyc@gmail.com

Dear Ms. Massey:

This letter is our final response to your Centers for Disease Control and Prevention and Agency for Toxic Substances and Disease Registry (CDC/ATSDR) Freedom of Information Act (FOIA) request of August 28, 2023, assigned #23-01704-FOIA, for:

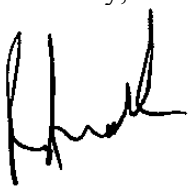
> All studies - authored by anyone, anywhere, ever, in the possession, custody or control of the Centers for Disease Control and Prevention (CDC) and/or the Agency for Toxic Substances and Disease Registry (ATSDR) and/or the people who are responsible for the CDC's claim that bacteria cause "Lyme disease", that:
>
> used purified bacterial cultures (i.e. Borrelia burgdorferi, B. mayonii, B. bissettiae, B. garinii, B. afzelii, B. spielmanii, or B. lusitaniae) to scientifically prove or provide evidence of causation of "Lyme disease".
>
> I am not seeking records that describe attempts to prove causation using unscientific methods; recall that opinions, declarations, speculation, review papers and descriptive papers are not scientific proof/evidence. Scientific proof/evidence requires use of the scientific method to test falsifiable hypotheses through valid, repeatable controlled experiments where only 1 variable differs between the experimental and control groups.
>
> If any records match the above description and are currently available elsewhere, please provide enough information about each one so that I may identify and access them with certainty: title, author(s), date, journal, location, URLs where possible.

We located 31 pages of responsive records. After a careful review of these pages, no information was withheld from release. These pages were responsive to the terms "Borrelia burgdorferi, B. mayonii, B. bissettiae, B. garinii, B. afzelii, B. spielmanii, B. lusitaniae, Lyme disease."

For the other genospecies listed, the National Center for Emerging and Zoonotic Infectious Diseases provided this additional information: "The principle science has been driven by scientists outside of CDC and therefore no responsive documents were found. Dr. Willy Burgdorfer at the National Institutes of Health first identified Borrelia burgdorferi spirochetes as the causative agent of Lyme disease. Subject matter experts recommend that you search the existing medical literature or consult a librarian to find additional records on such topics.

If you need any further assistance or would like to discuss any aspect of the records provided please contact either our FOIA Requester Service Center at 770-488-6399 or our FOIA Public Liaison at 770-488-6246.

Sincerely,



Roger Andoh
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23-01704-FOIA

Published in final edited form as:

Int J Syst Evol Microbiol. 2016 November ; 66(11): 4878–4880. doi:10.1099/ijsem.0.001445.

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

Bobbi S. Pritt[1], **Laurel B. Respicio-Kingry**[2], **Lynne M. Sloan**[1], **Martin E. Schriefer**[2], **Adam J. Replogle**[2], **Jenna Bjork**[3], **Gongping Liu**[3], **Luke C. Kingry**[2], **Paul S. Mead**[2], **David F. Neitzel**[3], **Elizabeth Schiffman**[3], **Diep K. Hoang Johnson**[4], **Jeffrey P. Davis**[4], **Susan M. Paskewitz**[5], **David Boxrud**[3], **Alecia Deedon**[4], **Xia Lee**[5], **Tracy K. Miller**[6], **Michelle A. Feist**[6], **Christopher R. Steward**[4], **Elitza S. Theel**[1], **Robin Patel**[1], **Cole L. Irish**[1], and **Jeannine M. Petersen**[2]

[1]Department of Clinical Microbiology, Mayo Clinic, Rochester, Minnesota, USA

[2]Division of Vector Borne Diseases, Centers for Disease Control and Prevention, Fort Collins, CO, USA

[3]Minnesota Department of Health, St. Paul, MN, USA

[4]Wisconsin Department of Health Services, Madison, WI, USA

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[6]North Dakota Department of Health, Bismarck, ND, USA

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

Correspondence Jeannine M. Petersen, nzp0@cdc.gov.

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 Zelkulturen 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; Aguero-Rosenfeld *et al.,* 2005; Liveris *et al.,* 2012; Babady *et al.,* 2008). This is in contrast to infection by relapsing fever borreliae 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	*Borrelia burgdorferi sensu lato*
CDC	Centers for Disease Control and Prevention
LB	Lyme borreliosis

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Author manuscript

Lancet Infect Dis. Author manuscript; available in PMC 2016 November 01.

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Identification of a novel pathogenic *Borrelia* species causing Lyme borreliosis with unusually high spirochaetaemia: a descriptive study

Bobbi S Pritt, **Paul S Mead**, **Diep K Hoang Johnson**, **David F Neitzel**, **Laurel B Respicio-Kingry**, **Jeffrey P Davis**, **Elizabeth Schiffman**, **Lynne M Sloan**, **Martin E Schriefer**, **Adam J Replogle**, **Susan M Paskewitz**, **Julie A Ray**, **Jenna Bjork**, **Christopher R Steward**, **Alecia Deedon**, **Xia Lee**, **Luke C Kingry**, **Tracy K Miller**, **Michelle A Feist**, **Elitza S Theel**, **Robin Patel**, **Cole L Irish**, and **Jeannine M Petersen**

Mayo Clinic, Rochester, MN, USA (B S Pritt MD, L M Sloan MT, E S Theel PhD, Prof R Patel MD, C L Irish BS, J A Ray MPH); Division of Vector Borne Diseases, US Centers for Disease Control and Prevention, Fort Collins, CO, USA (P S Mead MD, L B Respicio-Kingry MS, L C Kingry PhD, M E Schriefer PhD, A J Replogle BS, J M Petersen PhD); Wisconsin Department of Health Services, Madison, WI, USA (D K Hoang Johnson BS, Prof J P Davis MD, C R Steward MPH, A Deedon BS); Minnesota Department of Health, St Paul, MN, USA (D F Neitzel MS, E Schiffman MPH, J A Ray, J Bjork DVM); University of Wisconsin–Madison, Madison, WI, USA (Prof S M Paskewitz PhD, X Lee MS); and North Dakota Department of Health, Bismarck, ND, USA (T K Miller PhD, M A Feist BS)

Summary

Background— Lyme borreliosis is the most common tick-borne disease in the northern hemisphere. It is a multisystem disease caused by *Borrelia burgdorferi* sensu lato genospecies and characterised by tissue localisation and low spirochaetaemia. In this study we aimed to describe a novel *Borrelia* species causing Lyme borreliosis in the USA.

Methods—At the Mayo clinic, from 2003 to 2014, we tested routine clinical diagnostic specimens from patients in the USA with PCR targeting the *oppA1* gene of *B burgdorferi* sensu lato. We identified positive specimens with an atypical PCR result (melting temperature outside of the expected range) by sequencing, microscopy, or culture. We collected *Ixodes scapularis* ticks from regions of suspected patient tick exposure and tested them by *oppA1* PCR.

Findings—100 545 specimens were submitted by physicians for routine PCR from Jan 1, 2003 to Sept 30, 2014. From these samples, six clinical specimens (five blood, one synovial fluid)

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Contributors

BSP and JMP did the literature search, created tables and figures, participated in study design, data collection, analysis and interpretation, co-drafted the manuscript, and edited and approved the final report. PSM, LMS, MES, LBR-K, and AJR contributed to the literature search, helped to create figures, participated in study design, data collection, analysis and interpretation, and culture edited and approved the final manuscript. DKHJ, JPD, DFN, and ES contributed to literature search, helped to create figures, participated in data collection, analysis and interpretation, and edited and approved the final manuscript. SMP, JAR, JB, CRS, AD, XL, TKM, MAF, EST, RP, LCK, and CLI participated in data collection, analysis and interpretation, and edited and approved the final manuscript.

yielded an atypical *oppA1* PCR product, but no atypical results were detected before 2012. Five of the six patients with atypical PCR results had presented with fever, four had diffuse or focal rash, three had symptoms suggestive of neurological inclusion, and two were admitted to hospital. The sixth patient presented with knee pain and swelling. Motile spirochaetes were seen in blood samples from one patient and cultured from blood samples from two patients. Among the five blood specimens, the median *oppA1* copy number was 180 times higher than that in 13 specimens that tested positive for *B burgdorferi* sensu stricto during the same time period. Multigene sequencing identified the spirochaete as a novel *B burgdorferi* sensu lato genospecies. This same genospecies was detected in ticks collected at a probable patient exposure site.

Interpretation—We describe a new pathogenic *Borrelia burgdorferi* sensu lato genospecies (candidatus *Borrelia mayonii*) in the upper midwestern USA, which causes Lyme borreliosis with unusually high spirochaetaemia. Clinicians should be aware of this new *B burgdorferi* sensu lato genospecies, its distinct clinical features, and the usefulness of *oppA1* PCR for diagnosis.

Introduction

Lyme borreliosis is a spirochaetal tick-borne disease caused by some genospecies of the *Borrelia burgdorferi* sensu lato complex.[1–4] With 85 000 cases estimated annually in Europe and 300 000 cases estimated annually in the USA, it is the most common tick-borne disease in the northern hemi sphere.[5,6] Nearly all human infections are caused by three *B burgdorferi* sensu lato genospecies: *Borrelia garinii, Borrelia afzelii*, and *B burgdorferi* sensu stricto.[1] All three species cause Lyme borreliosis in Europe, whereas only *B burgdorferi* sensu stricto causes Lyme borreliosis in the USA.[7]

The clinical features of Lyme borreliosis are broad and seem to be associated with distinct tissue tropisms of specific *B burgdorferi* sensu lato genospecies.[8] Early localised infection typically results in erythema migrans rash, after which spirochaetes can disseminate to the nervous system, joints, and other organs. *B burgdorferi* sensu stricto is often associated with arthritis, *B garinii* with neurological effects, and *B afzelii* with acrodermatitis chronicum atrophicans.[8]

Lyme borreliosis is characterised by a low level of spirochaetaemia.[9] Spirochaetes are detectable by PCR in the peripheral blood of less than 50% of patients with erythema migrans, with average estimation of about 2330 genome copies per mL,[9,10] whereas the mean number of spirochaetes detected by culture of peripheral blood is only 0·1 spirochaetes per mL.[9] As expected, microscopic detection of *B burgdorferi* sensu lato spirochaetes has never been reported in peripheral blood, by marked contrast with relapsing fever borreliae, which have loads ranging from 10^5 spirochaetes per mL to more than 10^6 spirochaetes per mL, and are readily seen in peripheral blood.[11] We describe a new *B burgdorferi* sensu lato genospecies causing Lyme borreliosis with substantially elevated spirochaetaemia in acutely ill patients.

Methods

Patients

Mayo Medical Laboratories provides diagnostic PCR testing for Lyme borreliosis.[12] From Nov 1, 2003, to Sept 30, 2014, physicians throughout the USA submitted 100 545 specimens (synovial fluid, cerebrospinal fluid, EDTA [edetic acid]-anticoagulated whole blood, or fresh tissue) to our laboratory for routine clinical PCR testing. We interviewed patients with specimens yielding a PCR result that differed from that expected for *B burgdorferi* sensu stricto, *B garinii*, or *B afzelii*, to obtain clinical and epidemiological information. We reviewed medical records and requested additional samples. Patient follow-up and DNA sequencing of clinical specimens was approved by the Mayo Clinic institutional review board. Patients were interviewed by state public health officials as part of routine surveillance for a reportable condition.

Real-time PCR and DNA sequencing

We extracted DNA from diagnostic specimens using the MagNA Pure Instrument (Roche) and tested for *B burgdorferi* sensu stricto, *B afzelii*, and *B garinii* with a diagnostic real-time PCR assay that uses hybridisation probes and targets the chromosomal *oppA1* gene.[12,13] This assay is specific for *B burgdorferi* sensu lato and does not detect relapsing fever borreliae. We subjected PCR products to melting temperature analysis to differentiate *B burgdorferi* sensu lato genospecies (appendix). We established the number of *oppA1* copies with standard curves that were prepared with genomic DNA from *B burgdorferi* sensu stricto B31 and the MN14-1420 isolate; *oppA1* is present on the chromosome in a single copy in both genospecies. We used the Wilcoxon rank-sum test (two-sided) for *oppA1* PCR crossing point comparison.

We amplified and sequenced portions of the 16S rDNA, *ospC, flaB, rrf-rrl, oppA1, uvrA, rplB, recG, pyrG, pepX, clpX, nifS*, and *clpA* genes using previously described primers.[12,14–17] We analysed, assembled, and trimmed sequences in Lasergene v9·0 (DNASTAR). Using BLAST, 16S rDNA, *ospC, flaB*, and *rrf-rrl* sequences were compared with *B burgdorferi* sensu lato and relapsing fever borreliae sequences in GenBank. For construction of phylogenetic trees, we obtained homologous *B burgdorferi* sensu lato and relapsing fever borreliae sequences from GenBank and PubMLST (appendix). We used MEGA 5 (ClustalW) to align sequences and trees constructed by maximum likelihood analysis using the generalised time-reversible nucleotide substitution model with gamma distribution (four categories) followed by bootstrap analysis (1000 replicates).[18] Housekeeping genes were concatenated in frame in the order *uvrA, rplB, recG, pyrG, pepX, clpX*, and *clpA*, with or without *nifS* and exported into MEGA 5 to calculate pairwise genetic distances using the Kimura-2 model.

Microscopy and culture

Two clinical blood specimens with an atypical *oppA1* PCR melting temperature were available for microscopy and culture after storage at 4°C for either 5 days (one sample) or 39 days (one sample) (appendix). Other specimens were unavailable or previously frozen and

not amenable to microscopy and culture. We examined wet mounts from patient samples and cultures with dark-field microscopy at 400 times magnification (appendix).

Serological testing

We tested serum samples and plasma samples for antibodies reacting to *B burgdorferi* sensu stricto using FDA-cleared commercially available kits, following the recommended two-tiered algorithm (appendix).[19]

Tick collection and processing

We collected *Ixodes scapularis* ticks at approximate sites of possible patient exposure in Barron County, WI, USA, during 2013 and 2014 (appendix), and processed the ticks for PCR using a modification of a published protocol.[20] We also tested archived DNA from *I scapularis* collected in Eau Claire County, WI, USA, during 2009–10.

Role of the funding source

The funders of the study, Mayo Clinic and the US Centers for Disease Control and Prevention, had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

From Jan 1, 2012, to Sept 30, 2014, 9197 clinical specimens from residents of Minnesota, Wisconsin, and North Dakota were submitted to Mayo Clinic, Rochester, MN, USA, for routine diagnostic *B burgdorferi* sensu lato *oppA1* PCR testing. 3127 (34%) of 9197 specimens tested were blood samples, 1196 (13%) were synovial fluid, 4782 (52%) were cerebrospinal fluid, and 92 (1%) were tissue. 102 specimens were positive for *B burgdorferi*, including 13 blood, 81 synovial fluid, three cerebrospinal fluid, and five tissue samples. Six specimens (five blood, one synovial fluid) had positive *oppA1* PCR results with an atypical melting temperature (60·38–61·24°C), falling between the expected melting temperature for *B burgdorferi* sensu stricto (61·7–66·7°C) and *B afzelii* or *B garinii* (51·7–56·7°C) (table 1, figure 1A, figure 2, appendix).[12] No atypical melting temperatures were identified among 24 786 clinical specimens tested from 44 other states during the same time period (Fisher-Exact, p=0·00039), or among more than 66 562 clinical specimens from all states tested by the same method during 2003–11 (0 of 66 562 *vs* six of 33 983, p=0·00149). The five positive blood specimens with atypical melting temperatures were collected 1–4 days after onset of illness; the synovial fluid specimen was obtained 34 days after onset of illness (table 1). The median *oppA1* PCR crossing point was significantly lower (median 29·82, IQR 28·89–30·75) for the five blood specimens with atypical *oppA1* melting temperature compared with the 13 blood specimens that tested positive for *B burgdorferi* sensu stricto (median 34·51, IQR 33·73–35·55; p=0·0016; figure 3).

Sequence analysis of the atypical *oppA1* PCR products that were directly amplified from three patient specimens identified a *Borrelia* species with 89–95% similarity to *B burgdorferi* sensu lato genospecies (figure 4). Motile spirochaetes (two per 70 fields of

diluted blood) of the *B burgdorferi* sensu lato genospecies were microscopically recorded in blood from patient 6, obtained 1 day after illness onset and analysed 6 days later (figure 1B). The number of spirochaetes was estimated at around $8{\cdot}5 \times 10^4$/mL (appendix). No spirochaetes were seen in the haemolysed blood specimen from patient 5. Cultures of the *B burgdorferi* sensu lato genospecies (MN14-1420 and MN14-1539) were established from both available blood specimens (patients 5 and 6) after incubation for about 16 days. Spirochaetes were seen in primary and blind passaged cultures; sustained growth was achieved after cryopreservation and additional passage.

Based on the high quantity of spirochaetes that were detected microscopically, we estimated the number of *oppA1* copies per mL of blood for all 18 specimens that tested positive for *oppA1* PCR identified in Minnesota, Wisconsin, and North Dakota during 2012–14 by comparison of *oppA1* crossing point values to standard curves prepared with *B burgdorferi* sensu stricto B31 or MN14-1420 (appendix). For the five atypical *oppA1* positives, the median *oppA1* copy number was 180 times higher (median $8{\cdot}1 \times 10^5$, IQR $4{\cdot}6 \times 10^5$–$3{\cdot}6 \times 10^6$) when compared with the 13 *B burgdorferi* sensu stricto positives (median $4{\cdot}5 \times 10^3$, IQR $2{\cdot}3 \times 10^3$–$7{\cdot}5 \times 10^3$; figure 3).

Sequence analysis of 16S rRNA (1327 nucleotides), *ospC* (561 base pairs), *flaB* (435 base pairs), and *rrf-rrl* (253 base pairs) amplified from the two blood isolates substantiated that the *Borrelia* species was not identical to any other *Borrelia* species in GenBank (appendix). The closest sequence identity was to *B burgdorferi* sensu lato genospecies at 99% for 16S RNA, 85% for *ospC*, 97% for *flaB*, and 95% for *rrf-rrl*. Multilocus sequence analysis of seven genes, *uvrA, rplB, recG, pyrG, pepX, clpX*, and *clpA* genes (3774 nucleotides), showed that the spirochaetes isolated from patients 5 and 6 fell within the *B burgdorferi* sensu lato genospecies complex and were the same *B burgdorferi* sensu lato genospecies amplified from the blood of patients 1, 2, and 3. The blood isolates were further compared with 18 *B burgdorferi* sensu lato genospecies and three relapsing fever borreliae using an eight-gene multilocus sequence analysis (*uvrA, rplB, recG, pyrG, pepX, clpX, clpA*, and *nifS*; 4335 nucleotides) previously described for defining *B burgdorferi* sensu lato genospecies[21] (figure 4B). The highest pairwise similarity was to *B burgdorferi* sensu stricto (94·9–95·2% similarity, genetic distance 0·051–0·048), well above the threshold defined for separating genospecies (98·3% similarity, genetic distance 0·017),[21] substantiating that the organism detected in the six patients is a novel *B burgdorferi* sensu lato genospecies, and not a relapsing fever borrelia (eg, *B miyamotoi*). For comparison, the genetic distance recorded between the novel *B burgdorferi* sensu lato genospecies and *B burgdorferi* sensu stricto B31/Z41293 is greater than that seen between other formally recognised *B burgdorferi* sensu lato genospecies, including *B bissetti* and *B kurtenbachii* (0·035) and *B garinii* and *B bavariensis* (0·018).[14,21]

All patients were residents of the upper midwest (Minnesota, North Dakota, or Wisconsin) (figure 5). Median patient age was 36 years (range 10–67 years); four patients were male (table 1). Five presented with acute febrile illnesses, including four with rash. The sixth patient was afebrile but had a 1-month history of unilateral knee pain and swelling. Two patients were admitted to hospital, but none had a known immunocompromising disorder. Testing showed lymphopenia (four of five tested), mild thrombocytopenia (two of five), and

high hepatic transaminases (two of three). All patients reported onset of illness between May and July. Exposure to tick habitats in Minnesota or Wisconsin was reported by all patients and two recalled a tick bite less than 30 days before onset of illness. For one patient, the timing between tick bite and PCR sample acquisition was known to be 13 days.

Descriptions of illness-associated rash varied from diffuse macular rashes involving the face, trunk, and upper extremities (figure 1A, patients 1 and 5) to a single 2 cm diameter erythematous leg lesion at the tick bite location (patient 6; table 1). Patient 3 presented with a single annular erythematous leg lesion with central punctum consistent with erythema migrans, and developed fever, leg and arm pain, and diffuse macular rash on the trunk, upper and lower extremities, and face within 8 h of receiving doxycycline. Differential diagnosis included Jarisch-Herxheimer reaction, drug eruption, and erythema multiforme. Doxycycline was discontinued after one dose. The patient improved without additional treatment, but 3 weeks later developed three erythema migrans lesions on the back and leg, which resolved after treatment with cefuroxime.

All six patients were given antibiotics (table 1). All five patients with febrile illnesses recovered; one with preexisting anaemia reported continuing fatigue. The patient with arthritis improved but reported persistent joint pain 6 months after treatment. Serum or plasma was available for five patients and was tested for reactivity to *B burgdorferi* sensu stricto antigens using the recommended two–tiered algorithm[19] (table 2). Patients 1, 3, and 4 were seropositive to *B burgdorferi* sensu stricto using this algorithm, including the 30 day cutoff for use of IgM immunoblot. Patient 5 had a positive EIA and IgM immunoblot in a sample obtained 32 days after onset of illness. All four seropositive patients had one or more samples positive using the first-tier C6 EIA; patients 3, 4, and 5 were positive using whole cell EIA. Two seropositive patients (3 and 5) had serial samples and sero converted from a negative to positive IgM immunoblot. The only specimen from patient 6 was obtained 1 day after illness onset and was seronegative.

Among archived and prospectively collected ticks *I scapularis*, 19 (2·9%, range 0–5·2%) of 658 were *oppA1* PCR positive for the novel *B burgdorferi* sensu lato genospecies and 195 (29·6%, range 9·8–33·3%) of 658 were positive for *B burgdorferi* sensu stricto; two were positive for both. Sequence analysis of *oppA1* for two ticks (EC10N1 and CP12150) and seven-gene multilocus sequence analysis for one tick (EC10N1) substantiated that the *B burgdorferi* sensu lato genospecies detected in *I scapularis* was the same identified in patients (figure 4A, appendix).

Discussion

We have identified a new *B burgdorferi* sensu lato genospecies (candidatus *Borrelia mayonii*) among patients and *I scapularis* ticks from the upper midwestern USA. A causal role in the patients' illnesses was suggested by the detection of DNA from this genospecies in patient specimens during acute illness, detection of motile spirochaetes in one blood specimen, culture of the novel *B burgdorferi* sensu lato genospecies from two patient specimens, development of a patient antibody response after illness onset, and clinical improvement after antimicrobial therapy active against other *B burgdorferi* sensu lato

genospecies. Failure to identify the organism in more than 90 000 clinical samples tested in previous years and from other states might suggest that this new species recently emerged in the upper midwestern USA.

Using an eight-gene multilocus sequence analysis and a published threshold for delineation of *B burgdorferi* sensu lato genospecies, we showed that the *Borrelia* species is a member of the *B burgdorferi* sensu lato group.

Spirochaetes were seen in the diluted blood of a patient who presented with a single erythema migrans lesion, estimated by microscopy at 10^5 genome copies per mL. The number of genomes in this specimen, based on the single-copy chromosomal gene *oppA1*, was estimated independently at 5×10^5 per mL. Importantly, the median *oppA1* copy number measured for the samples positive for *B burgdorferi* sensu stricto ($4{\cdot}5 \times 10^2$ per mL) agrees with that ($2{\cdot}3 \times 10^2$/mL) recorded previously using quantitative *flaB* PCR, thus supporting the use of the *oppA1* gene for *B burgdorferi* sensu lato quantitation.[10] For all five blood specimens from patients infected with the novel *B burgdorferi* sensu lato genospecies, the number of genomes was estimated to be 10^5–10^6 genome copies per mL. This number is similar to what has been reported for patients infected with relapsing fever borreliae and 50–8000 times higher than the blood specimens that tested positive for *B burgdorferi* sensu stricto during the same time period. The number of spirochaetes as estimated by both microscopy and PCR in blood from patients infected with the novel *B burgdorferi* sensu lato genospecies is greater than previously estimated for *Borrelia miyamotoi* (10^3–10^4 spirochaetes per mL of blood), a relapsing fever borreliae reported to cause human illness in the USA, Europe, and Russia.[22–25] Whether this high spirochaetaemia suggests a different tissue tropism for the new *B burgdorferi* sensu lato genospecies is an important question that needs to be further addressed; five of six novel *B burgdorferi* sensu lato genospecies PCR positives were blood specimens, whereas only 13 (13%) of the 102 *B burgdorferi* sensu stricto PCR positives detected during the same time period were blood, and 81 (79%) of 102 samples were synovial fluids.

Patients infected with the novel *B burgdorferi* sensu lato genospecies presented with differing clinical presentations when compared with patients infected with *B burgdorferi* sensu stricto. At least two patients presented with diffuse macular rash not typical of erythema migrans, including one rash that might have involved the palms and soles. Four patients presented with nausea or vomiting and two with fever over 39°C, symptoms not usually reported for Lyme borreliosis[7,26–28] but often reported among patients infected with relapsing fever borreliae.[29] Similarly, three patients had symptoms potentially consistent with neurological effects (confused speech, profound somnolence, visual difficulties) and two were admitted to hospital.

An important issue raised by identification of the novel *B burgdorferi* sensu lato genospecies is whether existing Lyme borreliosis diagnostic tests can detect infection with this organism. The six patients described here were fortuitously detected during routine clinical testing, because the diagnostic *oppA1* PCR used at Mayo Clinic detects and differentiates *B burgdorferi* sensu lato genospecies by melting temperature analysis. However, it is unknown if diagnostic PCR assays specific for *B burgdorferi* sensu stricto will detect the novel

genospecies. Regarding serology, the *B burgdorferi* sensu stricto C6 EIA was positive in all four patients with specimens obtained 3 days or more after onset of illness and *B burgdorferi* sensu stricto IgM immunoblots of specimens obtained 6 to 32 days after onset were positive for three patients. The *B burgdorferi* sensu stricto IgG immunoblot, however, was positive only for the patient with more than 30 days of untreated illness. *B burgdorferi* sensu stricto serology was negative for three specimens drawn 1–3 days after onset of illness.

The patients' infections were probably acquired by the bite of *I scapularis*, which transmits *B burgdorferi* sensu stricto in the USA. Two patients recalled a tick bite before illness and *I scapularis* that tested positive for the new *B burgdorferi* sensu lato genospecies were collected at two Wisconsin locations, including one visited by two patients. Prevalence of the novel species in tested *I scapularis* ranged from 0·6–4·9%. Non-detection of the new *B burgdorferi* sensu lato genospecies in *I scapularis* collected in the midwestern USA during 2004–07 further suggests that it might have recently emerged in this region.[30,31]

The identification of a novel *B burgdorferi* sensu lato genospecies causing Lyme borreliosis with substantially elevated spirochaetaemia and clinical features distinct from other recognised *B burgdorferi* sensu lato genospecies has important implications for accurate diagnosis, treatment and disease reporting. In view of the differing clinical manifestations for patients infected with the novel *B burgdorferi* sensu lato genospecies, it is likely that Lyme borreliosis is not being considered—and therefore not diagnosed—in some patients with this infection. The clinical range of illness must be better defined in additional patients to ensure that physicians can recognise the infection and distinguish it from other tick-borne infections. Many tick-borne pathogens have global distribution, therefore studies are needed to establish the geographic distribution of human beings and ticks infected with the novel *B burgdorferi* sensu lato genopecies. Finally, clinicians should be aware of the potential role of *oppA I* PCR for diagnosing infection with this novel pathogen.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Declaration of interests

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Research in context

Evidence before this study

Lyme borreliosis is a multisystem tick-borne disease of wide public health significance. It is the most frequently reported vector-borne disease in the temperate northern hemisphere and is caused by spirochaetes in the *Borrelia burgdorferi* sensu lato genospecies complex. There have been no previous descriptions of the pathogenic *Borrelia* species reported in this study in either ticks or human beings, or reports of Lyme borreliosis with high spirochaetaemia.

Added value of this study

The identification of a new *B burgdorferi* sensu lato genospecies causing Lyme borreliosis with substantially elevated spirochaetaemia and clinical features distinct from other *B burgdorferi* sensu lato genospecies has important implications for accurate diagnosis and treatment. In view of the differing clinical manifestations for patients infected with the novel *B burgdorferi* sensu lato genospecies, it is likely that Lyme borreliosis is not being considered in some patients with this infection. The medical and health-care community need to be aware of this new pathogen to recognise the infection and to treat patients appropriately.

Implications of all the available evidence

The discovery of a novel *B burgdorferi* sensu lato genospecies was attributable to the use of a diagnostic real-time PCR assay that detects and differentiates *B burgdorferi* sensu lato genospecies by melting temperature analysis. Those PCR assays designed specifically for detection of a single *B burgdorferi* sensu lato genospecies do not have the same ability to identify new or different genospecies. Since many tick-borne human pathogens have a global distribution (eg, *B burgdorferi, Babesia microti, Anaplasma phagocytophilum, Ehrlichia muris*, and *Borrelia miyamotoi*), the emergence of this pathogen highlights the need for widespread surveillance to look for emergence of this organism or related species in other parts of the world.

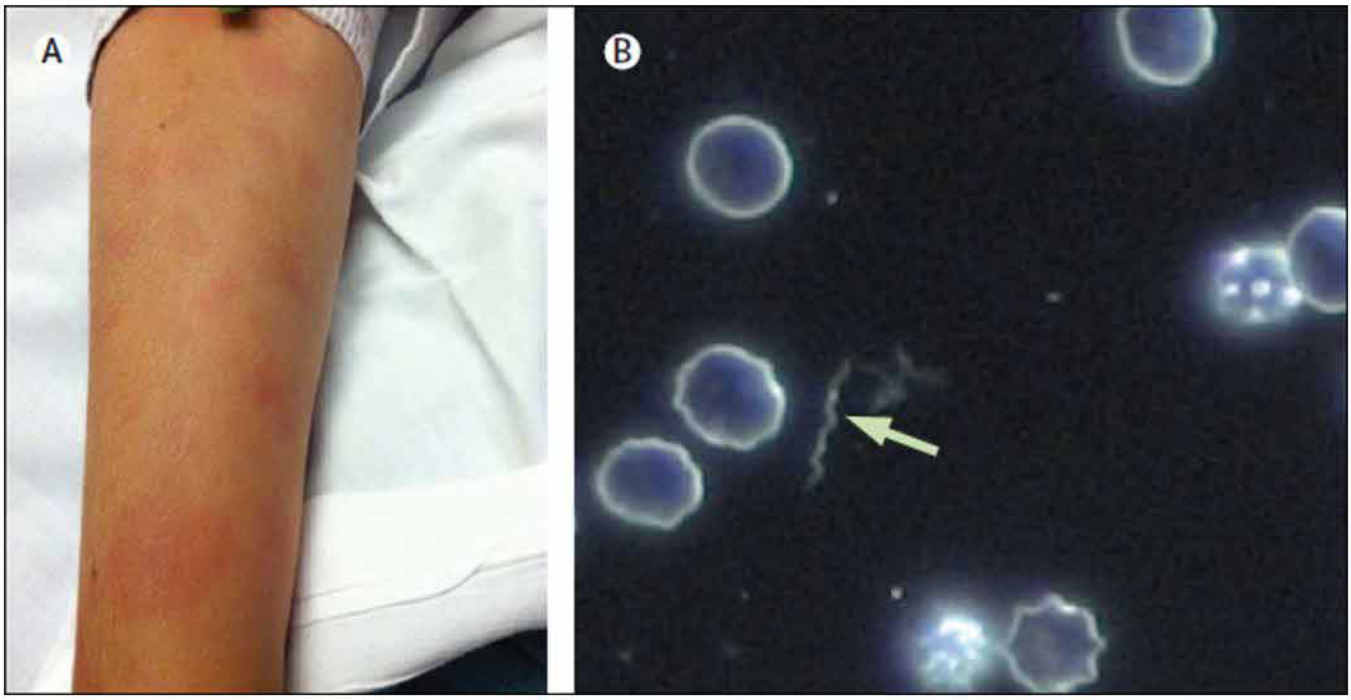

Figure 1. Diffuse macular rash in patient 1 and dark-field microscopic visualisation of a spirochaete in patient 6

(A) Diffuse macular rash seen 4 days after onset of symptoms in patient 1. Rash was reported by patient's caregiver to involve the palms and soles, but this was not documented in the medical record. (B) Dark-field microscopic visualisation (400× magnification) of a single spirochaete in diluted blood from patient 6.

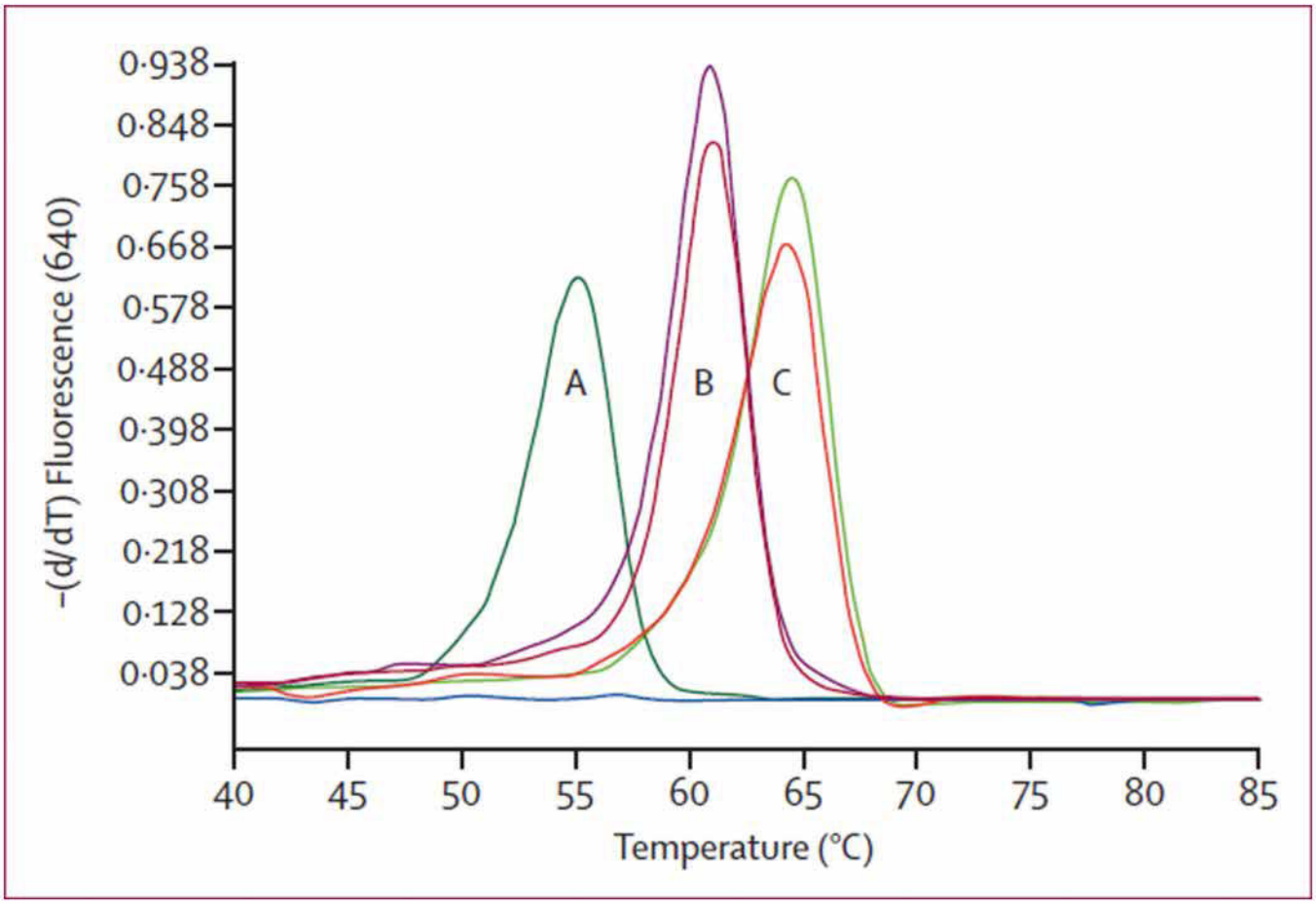

Figure 2. Representative *oppA1* PCR melting temperature peaks of *Borrelia* genospecies

Representative melting temperature peaks in °C for *B afzelii* (A peak; acceptable range 51·7–56·7°C), novel *B burgdorferi* sensu lato genospecies (B peaks; 60·38–61·24°C), and *B burgdorferi* sensu stricto (C peaks; 61·7–66·7°C). Y-axis represents the negative derivative of the ratio of the FRET signal (LC-Red640 flouresence) and background fluorescein fluorescence.

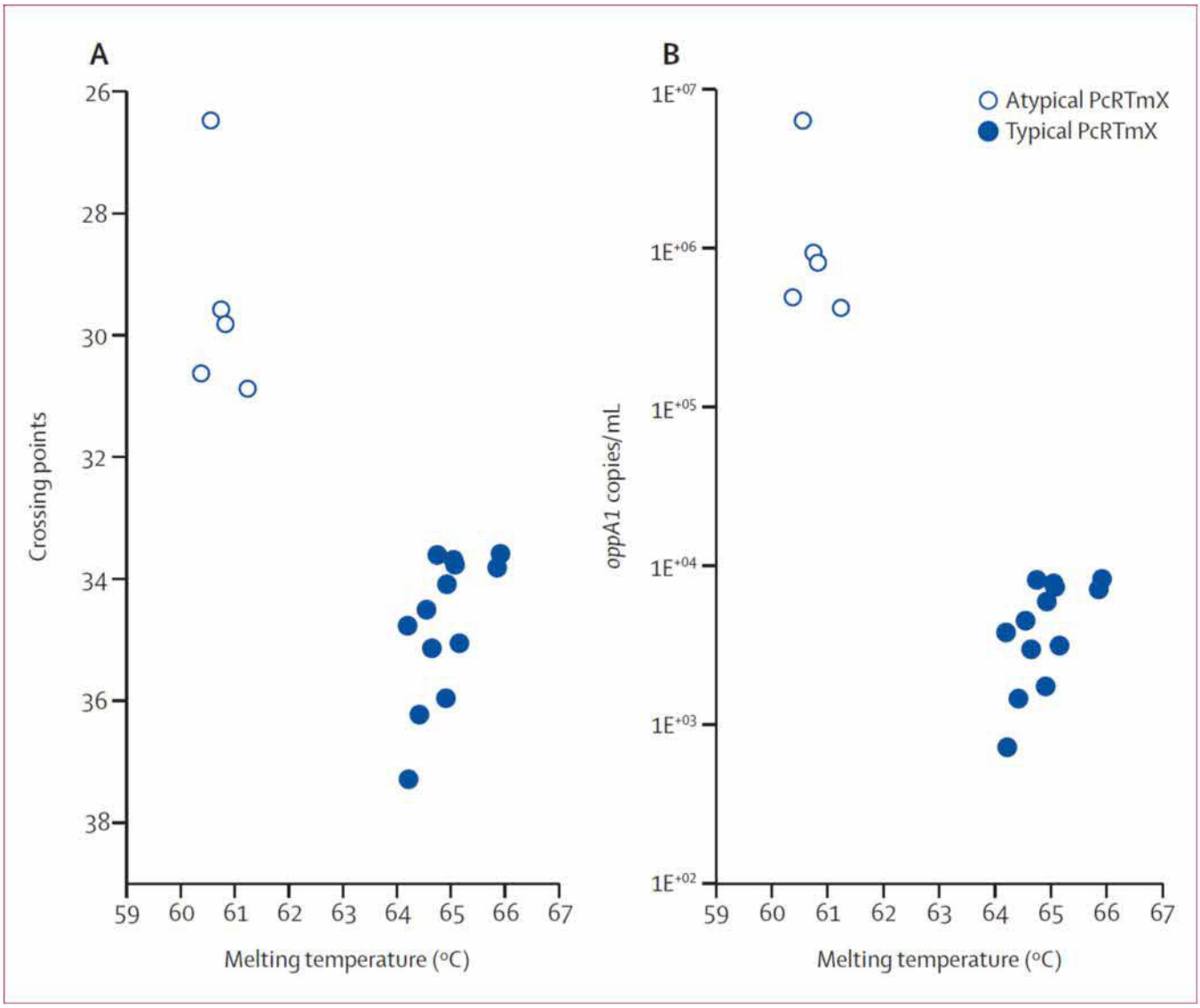

Figure 3. Comparison of *oppA1* PCR melting temperature, crossing points, and estimated *oppA1* copy number in *B burgdorferi*–positive blood specimens

Comparison of melting temperature and crossing point for the five atypical *oppA1* PCR positive blood specimens (open circles) and 13 *B burgdorferi* sensu stricto *oppA1* PCR positive blood specimens (closed circles).

(B) Comparison of melting temperature and estimated *oppA1* copy number (genomes per mL of blood) for five atypical *oppA1* PCR positive blood specimens (open circles) and 13 *B burgdorferi* sensu stricto *oppA1* PCR positive blood specimens (closed circles).

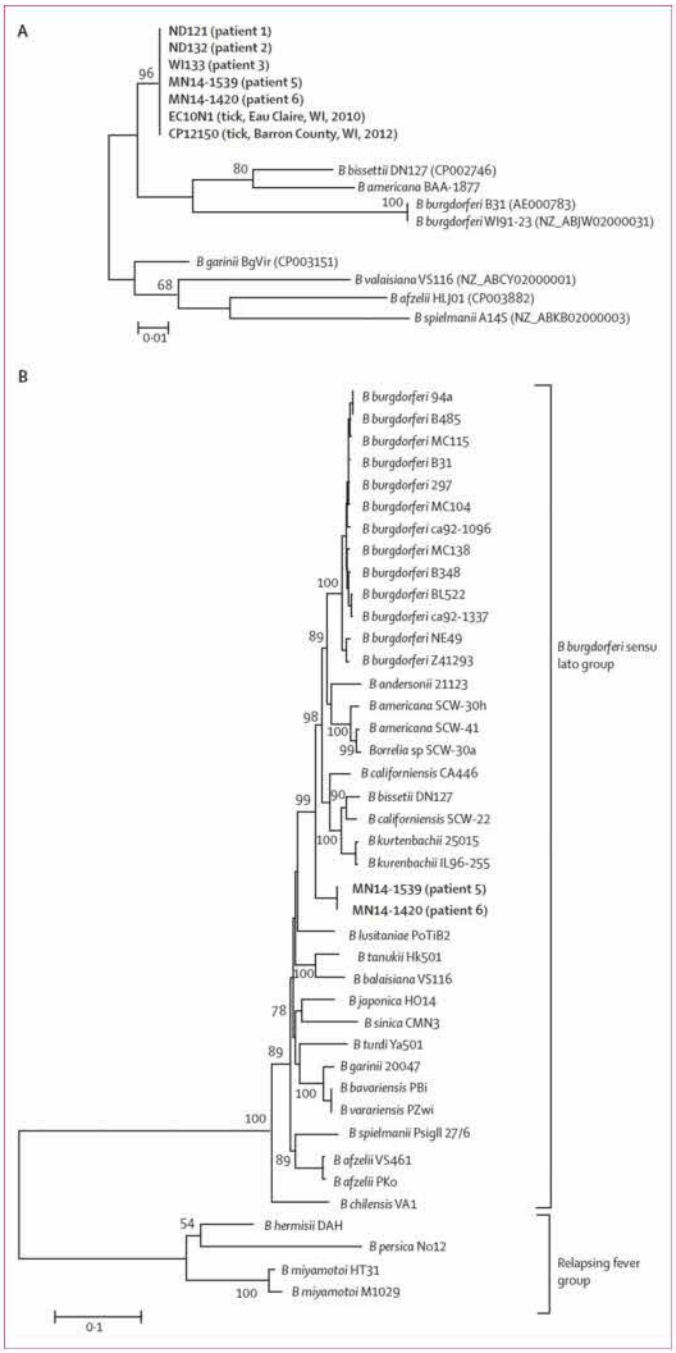

Figure 4. Phylogenetic analyses

(A) Phylogenetic analysis of a 149 base pair fragment of the *oppA1* gene amplified from patient specimens (MN14-1539, MN14-1420, WI133, ND132, and ND121) and tick specimens (CP12150 and EC10N1) compared with seven different species of the *B burgdorferi* sensu lato complex. There is no homologous sequence in relapsing fever borreliae. Bootstrap support values greater than 50% are shown. The scale bar corresponds to 0·01 substitutions per nucleotide position. Accession numbers are indicated for available *Borrelia* species *oppA1* sequences retrieved from GenBank. The *B americana* BAA-1877

oppA1 gene sequence was generated in this study. GenBank does not allow deposition of sequences shorter than 200 bp; *oppA1* sequences generated in this study are available by request. (B) Phylogenetic analysis of eight concatenated housekeeping genes: *uvrA, rplB, recG, pyrG, pepX, clpX, clpA, and nifS*, amplified from patient isolates (MN14-1539, MN14-1420) compared with 18 different *B burgdorferi* sensu lato genospecies and three relapsing fever species. Bootstrap support values greater than 50% are shown. The scale bar corresponds to 0·1 substitutions per nucleotide position. The source of other *Borrelia* species gene sequences is shown in the supplemental methods. Sequence nomenclature (eg, MN14-1539, WI133) represents the state from which the diagnostic specimens were submitted for testing and does not necessarily show the patient's state of residence.

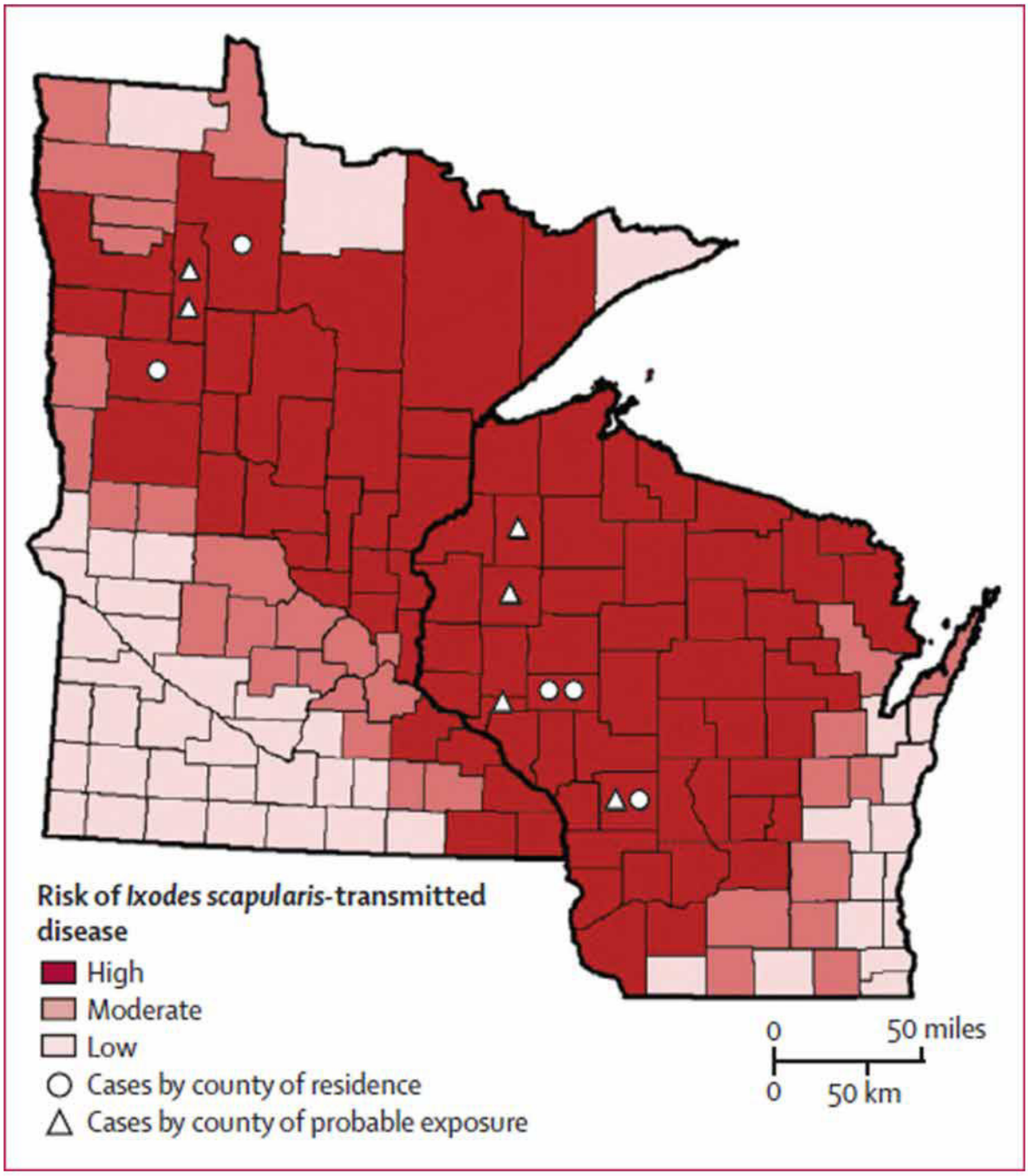

Figure 5. Probable counties of patient exposure to ticks in Minnesota and Wisconsin in relation to risk of diseases transmitted by *I scapularis*

I scapularis-transmitted diseases in the figure were Lyme borreliosis, babesiosis, and anaplasmosis. The county of residence for each patient (indicated with a circle) is deemed a county of potential exposure except for the patient from North Dakota, whose county of residence is not shown. Some patients had probable exposures in one or more county in addition to their county of residence (indicated with a triangle). The risk of disease transmitted by *I scapularis* is based on county-specific mean annual reported incidence of confirmed Lyme borreliosis and confirmed and probable human anaplasmosis and babesiosis

in Minnesota and Wisconsin in 2007–13. Counties with 10·0 or fewer cases per 100 000 people were classified as low risk, counties with 10·1–24·9 cases per 100 000 people were classified as moderate risk, and counties with 25·0 or more cases per 100 000 people were classified as high risk.

Table 1

Demographic, clinical, and laboratory features in patients infected with suspected novel *B burgdorferi* sensu lato genospecies

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6
Demographic features						
Age (years)	10	65	11	21	67	51
Sex	Male	Male	Male	Female	Female	Male
Tick exposure	Probable	Probable	Known bite	Probable	Probable	Known bite
Symptoms						
Fever	Yes	Yes	Yes	No	Yes	Yes
Headache	Yes	Yes	Yes	No	Yes	Yes
Neck pain	Yes	Yes	Yes	No	No	No
Fatigue	Yes	Yes	Yes	No	Yes	No
Myalgia	Yes	Yes	No	No	Yes	Yes
Nausea or vomiting	Yes	No	Yes	No	Yes	Yes
Arthralgia (site)	No	No	No	Yes (left knee)	No	No
Other	Profound somnolence	..	Confused speech	..	Chills, abdominal and lumbar back pain, flashing lights	..
Physical findings						
Measuredtemperature (°C)	40	NA	39·7	Afebrile	38·2	NA
Rash	Many erythematous macules on face, trunk, arms (figure 1A)*	NA	Initial macule, enlarged to erythema migrans; diffuse macular rash after single dose of doxycycline; many erythema migrans 28 days later	NA	Many erythematous macules on trunk and upper extremities	2 × 2 cm macule on leg at site of possible tick bite
Other	..	..	..	Swelling left knee	..	..
Laboratory results [normal range for adults and children aged 10–11 years combined]						
Days of illness before specimen collection for PCR	1	4	2	34	3	1
oppA1 PCR melting temperature (°C)	61·24	60·75	60·83	61·19	60·56	60·38
Crossing point	30·88	29·58	29·82	34·20	26·48	30·63
Estimated number of oppA1 copies per mL	$4{\cdot}2\times10^5$	$9{\cdot}4\times10^5$	$8{\cdot}1\times10^5$	Not determined	$6{\cdot}4\times10^6$	$4{\cdot}9\times10^5$

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	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6
White blood cell count ($\times 10^{-9}$/L) [3·4–10·5]	7·4	3·4	4·6	NA	12·4	5·3
Lymphocyte count ($\times 10^{-9}$/L) [0·90–6·50]	0·74	0·31	0·44	NA	0·93	0·30
Platelet count ($\times 10^{-9}$/L) [150–450]	184	113	122	NA	215	150
Haemoglobin (g/dL) [12·0–17·5]	14	NA	14·7	NA	9·6	15·5
Aspartate aminotransferase (U/L) [8–60]	46	NA	NA	NA	118	23
Alanine aminotransferase (U/L) [7–55]	33	NA	NA	NA	69	27
Treatment and outcome						
Antimicrobial therapy	Ceftriaxone (1 day), amoxicillin (21 days; dosage NA)	Doxycycline (dosage and duration NA)	Initial treatment: doxycycline (discontinued after 1 × 50 mg dose)[†]	Initial treatment: doxycycline (100 mg twice per day for 28 days)[‡]	Doxycycline (100 mg twice per day for 21 days)	Doxycycline (100 mg twice per day for 14 days)
Hospital admission	4 Days	No	No	No	1 day	No
Outcome	Recovered	Recovered	Recovered	Persistent joint pain	Improved, lingering fatigue (pre-existing anaemia)	Recovered

Clinical findings and symptoms were recorded by medical staff at time of initial patient presentation. NA=not available.

[*] Rash was reported by patient's caregiver to involve the palms and soles, but this was not documented in the medical record.

[†] Subsequent treatment for patient 3 was initiated 3 weeks after illness onset, and consisted of cefuroxime, 500 mg twice per day for 21 days.

[‡] For patient 4, subsequent treatment consisted of amoxicillin, 500 mg three times per day for 21 days.

Table 2

Serological test results from patients infected with the novel *B burgdorferi* sensu lato genospecies

	Days from onset of illness to collection of specimen	*B burgdorferi* EIA—whole cell	*B burgdorferi* EIA-C6	*B burgdorferi* IgM immunoblot (number of bands detected/number of possible bands); specific antigens detected	*B burgdorferi* IgG immunoblot (number of bands detected/number of possible bands); specific antigens detected
Patient 1	6	Not available	Positive	Positive (2/3); 23, 41	Negative (1/10); 41
Patient 3	2	Not available	Equivocal	Negative (0/3)	Negative (0/10)
Patient 3	29	Not available	Positive	Positive (3/3); 23, 39, 41	Negative (2/10); 23, 41
Patient 3	104	Positive	Positive	Negative (0/3)	Negative (4/10); 18, 23, 39, 41
Patient 4	266	Positive	Positive	Negative (1/3); 23	Positive (5/10); 23, 39, 41, 45, 58
Patient 5 (plasma)	3	Negative	Positive	Negative (0/3)	Negative (0/10)
Patient 5	32	Positive	Positive	Positive (2/3); 23, 39	Negative (2/10); 23, 41
Patient 6 (plasma)	1	Negative	Negative	Negative (0/3)	Negative (1/10); 41

Specimens from patient 2 were not available for testing. IgM immunoblot was deemed second tier positive for *B burgdorferi* if two or more of a possible three bands (21–25 kDA[OspC], 39 kDA[BmpA], and 41 kDA[Fla]) are detected within 30 days of onset.[17] IgG immunoblot was deemed second tier positive for *B burgdorferi* if five or more of a possible ten bands (18 kDa, 21 kDa[OspC], 28 kDa, 30 kDa, 39 kDa[BmpA], 41 kDa[Fla], 45 kDa, 58 kDa[not GroEL], 66 kDa, and 93 kDa) were detected.

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Original article

Vector competence of the blacklegged tick, *Ixodes scapularis*, for the recently recognized Lyme borreliosis spirochete Candidatus *Borrelia mayonii*

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ABSTRACT

A novel species within the *Borrelia burgdorferi* sensu lato complex, provisionally named *Borrelia mayonii*, was recently found to be associated with Lyme borreliosis in the Upper Midwest of the United States. Moreover, *B. mayonii* was detected from host-seeking *Ixodes scapularis*, the primary vector of *B. burgdorferi* sensu stricto in the eastern United States. We therefore conducted a study to confirm the experimental vector competence of *I. scapularis* for *B. mayonii* (strain MN14-1420), using colony ticks originating from adults collected in Connecticut and CD-1 white mice. Larvae fed on mice 10 weeks after needle-inoculation with *B. mayonii* acquired spirochetes and maintained infection through the nymphal stage at an average rate of 12.9%. In a transmission experiment, 40% of naïve mice exposed to a single infected nymph developed viable infections, as compared with 87% of mice fed upon by 2–3 infected nymphs. Transmission of *B. mayonii* by one or more feeding infected nymphs was uncommon up to 48 h after attachment (one of six mice developed viable infection) but occurred frequently when nymphs were allowed to remain attached for 72–96 h or feed to completion (11 of 16 mice developed viable infection). Mice infected via tick bite maintained viable infection with *B. mayonii*, as determined by ear biopsy culture, for at least 28 weeks. Our results demonstrate that *I. scapularis* is capable of serving as a vector of *B. mayonii*. This finding, together with data showing that field-collected *I. scapularis* are infected with *B. mayonii*, indicate that *I. scapularis* likely is a primary vector to humans of this recently recognized Lyme borreliosis spirochete.

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1. Introduction

The blacklegged tick, *Ixodes scapularis*, is the primary vector of the Lyme borreliosis spirochete, *Borrelia burgdorferi* sensu stricto (hereafter referred to as *B. burgdorferi*) to humans in the United States (Piesman and Gern, 2004). This tick species also has been confirmed as an experimental vector – including demonstrations of spirochete acquisition in larvae or nymphs that had been fed on infected hosts, transstadial spirochete passage, and spirochete transmission to susceptible hosts by the resulting nymphs or females – of three other spirochetes within the *B. burgdorferi* sensu lato (s.l.) species complex, *Borrelia afzelii*, *Borrelia bissettii*, and *Borrelia garinii*, as well as the relapsing fever spirochete *Borrelia miyamotoi* (Dolan et al., 1998; Scoles et al., 2001; Eisen and Lane, 2002; Rollend et al., 2013). Pritt et al. (2016) recently reported on the discovery of a novel species within the *B. burgdorferi* s.l. complex, provisionally named *Borrelia mayonii*, associated with Lyme borreliosis in six patients from the Upper Midwest of the United States. Moreover, *B. mayonii* was detected in host-seeking *I. scapularis* collected from presumed tick exposure sites for two patients in Wisconsin, with demonstrable infection rates of 4% in nymphs and 5% in adults collected in 2014 (Pritt et al., 2016). Corresponding infection rates for *B. burgdorferi* in the same examined ticks were more than six-fold higher, 27% for nymphs and 33% for adults. Two individual adult *I. scapularis* were co-infected with both species. The primary objective of this study was to confirm the vector competence of *I. scapularis* for the recently recognized Lyme borreliosis spirochete *B. mayonii*.

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2. Materials and methods

2.1. B. mayonii isolate, I. scapularis ticks, and experimental mouse host

We used strain MN14-1420 of *B. mayonii*, which was originally isolated from human blood (Pritt et al., 2016). The *I. scapularis* colony ticks used were of the first or second generations from adults collected in Connecticut in the northeastern United States. Experimental ticks originated from females that tested negative, after they had produced their egg batch, for infection with *B. burgdorferi*, *B. mayonii*, and *B. miyamotoi* by polymerase chain reaction (PCR) as described below. Mice used in these experiments were 1–3 month old females of the CD-1 strain of *Mus musculus* (Charles River Laboratories, Wilmington, MA, USA).

2.2. PCR-based confirmation that female I. scapularis used to generate non-infected colony larvae for transmission studies were free of Borrelia infection

After blood fed adult females produced their egg batch, spent females were tested for *Borrelia* infection. Single females were added to tubes with 400 μL of Roche Tissue Lysis Buffer (Roche Diagnostics, Indianapolis, IN, USA). Samples were homogenized by repeated bead-beating (agitation with beads) using 1.4 mm ceramic beads, interspersed with cooling to prevent sample overheating, using a Roche MagNA Lyser (Roche Diagnostics) or a BioSpec MixerMill (BioSpec Products, Inc., Bartlesville, OK, USA). The samples were then centrifuged and 300 μL aliquots of tick homogenate were removed and placed in new tubes with 200 μL tissue lysis buffer. A Roche MagNA Pure Compact Nucleic Acid Isolation Kit I – Large Volume (Roche Diagnostics) using the Blood v. 3.2 program with an elution volume of 200 μL was used for DNA extraction in a Roche MagNA Pure Compact (Roche Diagnostics). Two different PCR targets were used to screen the tick samples for presence of *Borrelia* DNA: *B. miyamotoi glpQ* (modified from Ullmann et al., 2005) and *B. burgdorferi OspA* (Ivacic et al., 2007). The *B. burgdorferi OspA* PCR target was confirmed by us to be present also in *B. mayonii*. The PCR was performed using an Applied Biosystems® 7500 Fast DX (Thermo Fisher Scientific, Waltham, MA, USA), with primers in a final concentration of 500 nM, and probes in a final concentration of 100 nM. The PCR cycling conditions were 95 °C for 3 min to denature the DNA followed by 50 cycles of 95 °C for 10 s and 57 °C for 30 s.

2.3. PCR-based detection of B. mayonii in tick immatures or ear biopsy cultures in the transmission experiments

Combined detection of the *I. scapularis* actin and the spirochete flagellar filament cap (*fliD*) target in tick samples was done using a multiplex TaqMan PCR modified from previously described methodology (Hojgaard et al., 2014; Goddard et al., 2015). The *fliD* PCR target has been used extensively for detection of *B. burgdorferi* in ticks (Dolan et al., 2011; Hojgaard et al., 2014; Goddard et al., 2015) and we found that it is present also in *B. mayonii* (unpublished results). The *fliD* PCR target is not present in *B. miyamotoi* (Hojgaard et al., 2014). Nucleic acids were isolated from ticks using a modified version of a previously described protocol (Hojgaard et al., 2014). One tick was added to a well in a 1.1 ml Axygen 96 well plate (BioSpec Products, Inc.) with 352 μL ATL buffer, 20 μL proteinase K, 1 μg carrier RNA, 1.88 μL DX reagent (Qiagen, Valencia, CA, USA), 410 mg 2.3 mm Chrome Steel beads and 260 mg 1.3 mm Chrome Steel beads (BioSpec Products, Inc.). Samples were homogenized by bead-beating for 2 cycles of 1 min each (with cooling between and after cycles) using a Mini-Beadbeater-96 (BioSpec Products, Inc.), and then incubated for 10 min at 56 °C. Following incubation, the sample was centrifuged at 1000 × *g* for 30 s and 150 μL of the sample was mixed with 150 μL AL buffer and incubated at 70 °C for 10 min. After incubation, the sample was centrifuged for 15 s at 1500 × *g* and processed with a QIAcube HT robot using the *cador* Pathogen Kit (Qiagen). A modified version of the *cador* Pathogen 96 QIAcube HT V3 software program was used where no VXL lysis buffer or TopElute was added and 100 μL of AVE buffer was used to elute the sample. A set of primers and probe against the actin gene of *I. scapularis* (Hojgaard et al., 2014) was used as a control for both the DNA purification and the PCR. A set of primers and probe for *fliD* (Dolan et al., 2011) was used to detect infection with *B. mayonii* in flat nymphs having fed as larvae on mice infected with this spirochete species via needle or tick bite. The multiplex PCR was performed using iQ™ Multiplex Powermix (Bio-Rad, Hercules, CA, USA), with primers in a final concentration of 300 nM and probes in a final concentration of 200 nM. The PCR cycling conditions were 95 °C for 3 min to denature DNA followed by 40 cycles of 95 °C for 10 s and 60 °C for 1 min on a C1000 Touch thermal cycler with a CFX96™ real time system (Bio-Rad).

We also tested culture media containing ear biopsies from mice infected with *B. mayonii* via needle or tick bite with the *fliD* target. Nucleic acid from 50 μl of ear biopsy culture medium was isolated as described above but without using chrome steel beads. Detection of *fliD* was performed as described above.

2.4. Needle inoculation of CD-1 mice with B. mayonii and confirmation of infection by culture of ear biopsy

Low culture passage (P3) *B. mayonii* spirochetes grown in modified Barbour-Stoenner-Kelly (BSK IIR) medium without antibiotics were used in these experiments. A total of six mice (designated 884–889; Table 1) were inoculated intradermally with 100 μl of culture medium containing approximately 5×10^4 spirochetes. Ear biopsies were taken from individual mice 3 weeks after inoculation (Sinsky and Piesman, 1989), surface sterilized in 70% ethanol for 5–10 min, and placed in BSK IIR medium with antibiotics. Spirochetes were cultured at 34 °C in 5 ml tubes containing 4.5 ml medium and maintained in a microaerophilic environment. Cultures were examined by dark-field microscopy, at 400× magnification, weekly for 4 weeks. A second set of ear biopsies was performed from these same mice 9 weeks post-inoculation and processed as described above. Aliquots from positive cultures were confirmed to contain *B. mayonii* by real-time PCR using a protocol described previously (Babady et al., 2008; Pritt et al., 2016).

2.5. B. mayonii acquisition from needle-inoculated mice by larval I. scapularis and transstadial passage to the nymphal stage

At 4 weeks after needle-inoculation, ear biopsy culture-positive mice were infested with approximately 200 uninfected *I. scapularis* larvae, allowed to feed ad libitum, and held over a water surface for up to 4 days to collect fed, detached larvae. All collected fed larvae died due to a desiccator failure. A second larval feeding conducted 10 weeks after *B. mayonii* was inoculated into the mice produced adequate numbers of fed larvae and resultant nymphs from all six mice. Fed larvae were grouped by mouse into small glass vials (equipped with plaster of Paris and activated charcoal and fitted with a lid and mesh to allow for air exchange), which then were transferred to desiccators (90–95% relative humidity) in a growth chamber maintained at 21–22 °C with a 16:8 h light:dark cycle. The resulting nymphs were examined for presence of *B. mayonii*, 3–4 weeks after the molt, by PCR based on combined detection of tick actin and spirochete *fliD* as described above.

Table 1
Needle inoculation of mice with *B. mayonii* and *I. scapularis* larval acquisition of *B. mayonii* during feeding on needle-infected mice and transstadial passage to the nymphal stage.

Mouse	Detection of *B. mayonii* in mouse ear biopsy taken 3 or 9 weeks post-inoculation[a]		*B. mayonii* infection in flat *I. scapularis* nymphs fed as larvae on infected mice 10 weeks post-inoculation[b]			*B. mayonii* infection in *I. scapularis* nymphs after they fed to completion on other naïve mice[c]		
	3 wk	9 wk	No. examined nymphs	No. infected nymphs	% infected nymphs	No. examined nymphs	No. infected nymphs	% infected nymphs
884	+	+	40	5	12.5	40	6	15.0
885	+	+	40	5	12.5	197	48	24.4
886	+	+	40	10	25.0	34	5	14.7
887	+	+	41	3	7.3			
888	+	+	40	7	17.5	39	6	15.4
889	+	+	40	1	2.5			

[a] Determined by culture of ear biopsy to detect live spirochetes (+ indicates infection).
[b] Determined by PCR assay of nymphs 3–4 weeks after they molted.
[c] Determined by PCR assay of freshly fed nymphs.

Table 2
Transmission of *B. mayonii* by infected *I. scapularis* nymphs to naïve mice, and larval acquisition of *B. mayonii* during feeding on tick bite-infected mice and transstadial passage to the nymphal stage.

Mouse	No. fed nymphs infected with *B. mayonii*[a]	Feeding history of infected nymphs	Source animal for infected nymphs	Detection of *B. mayonii* in mouse ear biopsy taken 4 weeks after exposure to infected nymphs[b]	Follow-up tests for samples taken 9 weeks after exposure to infected nymphs		*B. mayonii* infection in flat nymphs fed as larvae on mice infected by tick bite[d]		
					Detection of *B. mayonii* in ear biopsy[b]	Serological reactivity to *B. mayonii*[c]	No. examined nymphs	No. infected nymphs	% infected nymphs
2063	2	Complete feed	884	+			37	2	5.4
2064	1	Complete feed	884	–	–	–			
2065	2	Complete feed	884	+			39	4	10.3
2066	1	Complete feed	884	–	–	–			
2067	2	Complete feed	886	+			40	7	17.5
2070	2	Complete feed	886	+					
2071	1	Complete feed	886	+			40	3	7.5
2072	1	Complete feed	888	+					
2073	2	Complete feed	888	+					
2074	2	Complete feed	888	+					
2075	1	Complete feed	888	–	–	–			
2076	6	Removed after 24 h	885	+					
2077	8	Removed after 24 h	885	–	–	–			
2078	2	Removed after 24 h	885	–	–	–			
2079	2	Removed after 48 h	885	–	–	–			
2080	4	Removed after 48 h	885	–	–	–			
2081	9	Removed after 48 h	885	–	–	–			
2082	3	Removed after 72 h	885	–	–	–			
2083	3	Removed after 72 h	885	+					
2084	6	Removed after 72 h	885	+					
2085	3	Complete feed (96 h)	885	+					
2086	2	Complete feed (96 h)	885	–	–	–			
2062	0			–	–	–			
2087	0			–	–	–			

[a] Determined by PCR assay of freshly fed nymphs.
[b] Determined by culture of ear biopsy to detect live spirochetes (+ indicates infection). Results were the same for microscopic examination of cultures for live spirochetes and PCR detection of *B. mayonii* DNA in culture aliquots.
[c] Determined by in-house serological assay for reactivity of mouse plasma to *B. mayonii*.
[d] Determined by PCR assay of nymphs 3–4 weeks after they molted.

2.6. Nymphal transmission of *B. mayonii* to naïve mice

Holding of mice and collection of fed nymphs were as described previously for half of the mice ($n = 12$, designated 2062–2067 and 2070–2075: Table 2), for which nymphs were allowed to feed to completion. Based on the infection rate in the nymphs prior to feeding (15–25%), these mice each received 10 nymphs in order to achieve feeding by low numbers (1–3) of infected nymphs. The other half of the mice ($n = 12$, designated 2076–2087; Table 2) were used in a preliminary assessment of the time required for *B. mayonii* transmission to occur after infected nymphs attach. These mice were exposed to larger numbers (~20) of potentially infected nymphs. All attached nymphs were removed using forceps (with mice under isoflurane-induced anesthesia) after 24 h ($n = 3$ mice), 48 h ($n = 3$ mice), 72 h ($n = 3$ mice), or 96 h ($n = 3$ mice). All mice were monitored daily for 4 days to ensure that no feeding nymphs had been overlooked at the allotted removal times. All nymphs were examined for presence of *B. mayonii* by PCR based on combined detection of tick actin and spirochete *fliD* as described above. Two mice (2062 and 2087) were found to not have been exposed to infected nymphs and served as negative controls in additional experiments (Table 2).

Ear biopsies were performed on all 24 mice 4 weeks after nymphal exposure. These ear biopsy samples were processed and

cultured as described above for the needle-inoculated mice, with the exception that they were bisected and that one half of each ear biopsy was cultured in BSK IIR medium with antibiotics and the other half in standard BSK II medium with antibiotics. Outcomes were identical with regards to positive versus negative results for the modified BSK IIR medium and the standard BSK II medium. Cultures were examined weekly by dark field microscopy for 4 weeks. In addition, aliquots from all cultures were examined for presence of *B. mayonii* by PCR based on detection of *fliD* as described above. Dark-field microscopy-positive cultures were confirmed to contain *B. mayonii* using the real-time PCR protocol described previously (Babady et al., 2008; Pritt et al., 2016). Mice with spirochete-positive ear biopsy cultures were retained and subsequent ear biopsies were taken 28 weeks after nymphal exposure. Mice with ear biopsies that failed to produce evidence of spirochetes were subjected to additional ear biopsy and blood draw 9 weeks after nymphal exposure. Ear biopsies were cultured in BSK IIR medium and examined for spirochetes as described above, with the exception that culture aliquots were examined for presence of *B. mayonii* by PCR only at the 2 week culture time-point. Plasma was examined for serological reactivity to *B. mayonii*. Whole *B. mayonii* cells were cultivated in BSK IIR medium, sonicated, run on 12% SDS agarose resolving gel, transferred to a 15 × 15 cm nitrocellulose membrane blocked with powdered milk, and cut into 3 × 110 mm strips; each strip containing approximately 5 μg of protein. Strips were hydrated in TBS-t wash buffer and incubated in wash buffer with test serum at a concentration of 1:200 for 30 min. This was followed by a series of washes, a 15 min incubation with wash buffer and phosphatase-labeled goat anti mouse IgG (H + L) conjugate at a concentration of 1:5000, followed by a second wash series. As a final step, strips were developed for 3 min using BCIP/NBT phosphatase substrate. Previously verified *B. mayonii*-infected mouse serum served as a positive control and banding patterns were analyzed.

2.7. *B. mayonii acquisition from tick bite-infected mice by larval I. scapularis and transstadial passage to the nymphal stage*

Four mice infected with *B. mayonii* by the bite of infected nymphs (2063, 2065, 2067, and 2071; Table 2) were exposed to larvae 7 weeks after they were fed on by infected nymphs. Recovered larvae were allowed to molt to nymphs and examined for *B. mayonii* infection 3–4 weeks after the molt, as described above.

2.8. *Regulatory compliance*

Animal use and experimental procedures were in accordance with an approved protocol on file with the Centers for Disease Control and Prevention Division of Vector-Borne Diseases Animal Care and Use Committee.

3. Results

3.1. *Needle inoculation of CD-1 mice with B. mayonii*

All six mice that were needle-inoculated with *B. mayonii* developed viable infection as determined by culture of ear biopsies taken 3 and 9 weeks after inoculation (Table 1). Observed spirochete density was higher in cultures with ear biopsies taken 9 weeks after inoculation, as compared with 3 weeks after inoculation (data not shown).

3.2. *B. mayonii acquisition from needle-inoculated mice by larval I. scapularis and transstadial passage to the nymphal stage*

I. scapularis larvae acquired *B. mayonii* from needle-inoculated mice and infection was maintained transstadially in the resultant nymphs (Table 1). All six mice exposed to larvae 10 weeks post-inoculation produced infected nymphs, with infection rates ranging from 2.5 to 25.0% (Table 1).

3.3. *Nymphal transmission of B. mayonii to naïve mice*

Nine out of 13 (69%) mice on which 1–3 *B. mayonii*-infected nymphs were allowed to feed to completion developed viable infection as determined by ear biopsy taken 4 weeks after nymphal exposure (Table 2). Seven of eight (87%) mice fed upon by 2–3 *B. mayonii*-infected nymphs developed viable infection, as compared with two out of five mice (40%) fed upon by a single *B. mayonii*-infected nymph. Additional ear biopsies performed on the culture negative mice 9 weeks after exposure to infected nymphs remained negative (Table 2). Moreover, none of the culture-negative mice were serologically reactive to *B. mayonii*, indicating they had not been exposed to spirochetes via tick bite (Table 2).

The time-course experiment showed viable infection detectable by culture of ear biopsies taken 4 weeks after exposure to infected nymphs for only one of six (17%) mice fed upon by infected *I. scapularis* nymphs for 24–48 h, as compared with 11 of 16 mice (69%) fed upon by infected nymphs for 72–96 h (Table 2). Despite higher numbers of infected nymphs feeding on mice exposed for 24–48 h (range = 2–9, median = 5) as compared with ≥72 h (range = 1–6, median = 2), the mice fed upon for ≥72 h were significantly more likely to have viable infection detectable by culture of ear biopsy than those fed upon for 24–48 h (Fisher's exact test, one-tailed, $P = 0.043$).

All 12 mice that had a viable *B. mayonii* infection 4 weeks after exposure to infected nymphs (as determined by ear biopsy culture; Table 2) maintained viable infection 24 weeks later (28 weeks after exposure to infected nymphs).

3.4. *B. mayonii acquisition from tick bite-infected mice by larval I. scapularis and transstadial passage to the nymphal stage*

In these trials, the acquisition and subsequent transstadial passage of *B. mayonii* did not differ between larvae fed on mice infected by needle inoculation versus tick bite. The prevalence of *B. mayonii* infection for nymphs fed as larvae upon mice infected by tick bite ranged from 5.4 to 17.5% (Table 2). The overall infection rate for flat nymphs fed as larvae on mice infected by tick bite (10.3%; 16/156; Table 2) was similar to that for flat nymphs fed as larvae on needle-inoculated mice (12.9%; 31/241; Table 1).

4. Discussion

We present experimental evidence for the vector competence of *I. scapularis* for the recently recognized Lyme borreliosis spirochete *B. mayonii*, including acquisition of *B. mayonii* from infected mice by feeding larvae, transstadial passage to resulting nymphs, and transmission of *B. mayonii* to naïve mice by single or multiple infected nymphs. Our experimental demonstration of vector competence complements the previous report of infection with *B. mayonii* in field-collected, host-seeking *I. scapularis* from the Upper Midwest of the United States (Pritt et al., 2016). These laboratory and field results collectively suggest that, similar to *B. burgdorferi*, *I. scapularis* nymphs likely serve as primary vectors of *B. mayonii*.

Evidence for the occurrence of *B. mayonii* in the northeastern United States is still lacking, but we show that a tick strain

originating from Connecticut can effectively acquire and transmit *B. mayonii*. Should the geographic range of *B. mayonii* ultimately be found to be restricted to the Upper Midwest, our demonstration of transmission of *B. mayonii* by a Connecticut strain of *I. scapularis* indicates that regional differences in vector efficiency of *I. scapularis* for this spirochete is not the main reason for its absence in the northeastern United States. Previous experimental studies reported comparable vector efficiency of *I. scapularis* from different parts of its extensive range in the eastern United States for *B. burgdorferi* (Piesman and Sinsky, 1988; Sanders and Oliver, 1995; Goddard et al., 2015). Natural vertebrate reservoirs for *B. mayonii* are still unknown but the robust performance of *M. musculus* as an experimental host suggests that the same suite of rodent species involved in enzootic transmission of *B. burgdorferi* (Piesman and Gern, 2004) should be considered prime suspects in the enzootic transmission of *B. mayonii*.

We found that, albeit based on a very limited sample size, viable infection with *B. mayonii* occurred more commonly for mice on which *I. scapularis* nymphs were allowed to feed for ≥72 h (69%) as compared with mice fed upon by infected nymphs for 24–48 h (17%) (Table 2). These findings agree with previous studies on transmission of *B. burgdorferi* by *I. scapularis* nymphs, in which the likelihood of transmission occurring from single infected nymphs to naïve mice increased from 24 h of nymphal feeding (no transmission recorded) to 48 h (range of 0–26% nymphs transmitting) and 66–72 h (56–89% nymphs transmitting) (Piesman et al., 1987; des Vignes et al., 2001; Piesman and Dolan, 2002; Hojgaard et al., 2008). In our study, the single occurrence of transmission within 48 h was for a mouse that, to the best of our knowledge, was fed upon by six infected nymphs but for no more than 24 h. Transmission within the first day of nymphal feeding needs to be confirmed in subsequent studies to ensure that no nymphs escaped detection in our mass-feeding scenario and accidentally were able to feed for more than 24 h. There are no records of transmission within the first 24 h of feeding for single *B. burgdorferi*-infected *I. scapularis* nymphs (Piesman et al., 1987; des Vignes et al., 2001; Piesman and Dolan, 2002; Hojgaard et al., 2008) but transmission was recorded at the 24 h time point on a single occasion for feeding by multiple *B. burgdorferi*-infected *I. scapularis* nymphs on a naïve hamster (Piesman et al., 1987). Transmission to a naïve host within 24 h of feeding by multiple-infected ticks also was recorded for *B. afzelii* and *B. burgdorferi* s.l. by *I. ricinus* nymphs (Kahl et al., 1998; Crippa et al., 2002). More extensive studies using single infected nymphs are needed to clarify the duration of nymphal attachment required for transmission of *B. mayonii*.

In contrast to a previous study with *B. burgdorferi* (Piesman, 1993), mice infected with *B. mayonii* via tick bite were not more infectious to feeding ticks as compared with mice infected by needle. We aim to maintain *B. mayonii* in a tick-mouse cycle in preparation for downstream vector and reservoir efficiency studies with this spirochete and *B. burgdorferi* in a standardized transmission model, including comparison of vector efficiency of *I. scapularis* tick strains originating from the Northeast versus Upper Midwest, the potential for rapid spirochete transmission within 24–48 h of nymphal attachment, the occurrence of transovarial transmission from an infected female to her offspring, and examination of the reservoir efficiency of key rodent hosts for transmission to immature *I. scapularis* ticks.

Disclaimer

The findings and conclusions of this study are by the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.

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Your CDC FOIA Request #23-01704-FOIA

christine: massey <cmssyc@gmail.com> Sat, Oct 14, 2023 at 9:58 AM
To: uls9@cdc.gov

Hi,

The studies provided are not responsive to my request. They are not controlled experiments showing that purified bacterial cultures cause Lyme disease.

Also, my request was not limited to studies by scientists inside the CDC. I specifically asked for

> "*All studies - **authored by anyone, anywhere, ever,** in the possession, custody or control of ..."*

So the claim that

> "*The principle science has been driven by scientists outside of CDC and therefore no responsive documents were found"*

is nonsensical and irrelevant. I also also asked that

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