HHS Public Access

Author manuscript

J Wildl Dis. Author manuscript; available in PMC 2024 August 01.

Published in final edited form as:

J Wildl Dis. 2022 July 01; 58(3): 646–651. doi:10.7589/JWD-D-21-00148.

Molecular Characterization of a Novel Relapsing Fever *Borrelia* Species from the Desert Cottontail (*Sylvilagus audubonii*) in New Mexico, USA

Irina Goodrich[1,5], **Clifton McKee**[2], **Gabriele Margos**[3], **Michael Kosoy**[4]

[1]Centers for Disease Control and Prevention, 3156 Rampart Road, Fort Collins, Colorado 80521, USA

[2]Department of Epidemiology, Johns Hopkins University Bloomberg School of Public Health, 615 N Wolfe Street, Baltimore, Maryland 21205, USA

[3]Bavarian Health and Food Safety Authority, National Reference Center for Borrelia, Veterinarstrasse 2, 85764 Oberschleissheim, Germany

[4]KB One Health, LLC, 3244 Reedgrass Court, Fort Collins, Colorado 80521, USA

Abstract

The *Borrelia* genus comprises vector-borne, spirochete bacteria infecting vertebrates worldwide. We characterized a novel relapsing fever *Borrelia* species from a desert cottontail (*Syvilagus audubonii*) from New Mexico, US, using an established multilocus sequence analysis approach. Phylogenetic analysis of the flagellin gene (*flaB*) and four other protein-coding loci (*clpX*, *pepX*, *recG*, *rplB*) grouped the novel *Borrelia* species with hard tick relapsing fever borreliae *Borrelia lonestari*, *Borrelia theileri*, and *Borrelia miyamotoi*. The identity of the vectors and other vertebrate hosts, geographic distribution, and zoonotic potential of this novel *Borrelia* species deserve further investigation.

Keywords

Borrelia ; MLSA; multilocus sequence analysis; rabbit; relapsing fever; vector-borne bacteria

Relapsing fever (RF) borreliae comprise several species infecting vertebrates worldwide (Cutler 2015). Most RF borrelioses remain poorly investigated with scant information on their ecology (Talagrand-Reboul et al. 2018). *Borrelia hermsii*, *Borrelia parkeri*, and *Borrelia turicatae* are the main RF borreliae in North America (Talagrand-Reboul et al. 2018). Reservoirs of RF spirochetes in the US include rodents and birds (*B. hermsii*, *B. parkeri*, *B. turicatae*, *Borrelia miyamotoi*), deer (*Borrelia lonestari*), and livestock (*Borrelia theileri*); dogs and humans are probably dead-end hosts (Talagrand-Reboul et al. 2018). Given the

[5]Corresponding author (oaq3@cdc.gov).

SUPPLEMENTARY MATERIAL

Supplementary material for this article is online at http://dx.doi.org/10.7589/JWD-D-21-00148.

diversity of potential vertebrate hosts and tick species in North America, additional RF *Borrelia* species are probably yet to be characterized.

A recent survey of vector-borne bacteria in small mammals of New Mexico, US, detected a RF *Borrelia* species in a desert cottontail (*Sylvilagus audubonii*; Goodrich et al. 2020), prompting our study to characterize the *Borrelia* sp. with multilocus sequence analysis (MLSA). This method more accurately delineates species and reflects deep evolutionary relationships among bacterial taxa than do single-locus analyses (Margos et al. 2009).

Cottontails were captured in the Eldorado subdivision of Santa Fe County, New Mexico, as part of a mark-recapture plague surveillance study between November 2002 and July 2004 (Morway et al. 2008). Animals were captured using Tomahawk traps (10×10×25 cm; Tomahawk Live Trap Company, Hazelhurst, Wisconsin, USA) baited with oats and peanut butter, and then the animals were anesthetized with isoflurane in oxygen. Blood (100–200 μL) was collected from the retro-orbital sinus and kept on dry ice until stored at −80 C in the laboratory. Genomic DNA was extracted from 100 μL of blood using the KingFisher Flex Purification System and MagMAX Pathogen RNA/DNA Kit (both Thermo Fisher Scientific, Waltham, Massachusetts, USA).

We detected *Borrelia* DNA in rabbit blood via multiplex quantitative real-time PCR targeting the flagellar filament cap gene (*fliD*) and the 16S ribosomal RNA gene (Goodrich et al. 2020). Additional primers were targeted with conventional PCR to further characterize the detected *Borrelia* DNA. We targeted the flagellin gene (*flaB*) and four housekeeping genes used for *Borrelia* MLSA: *clpX*, *pepX*, *recG*, and *rplB* (Margos et al. 2009). Primers are provided in Supplementary Material Table 1; detailed PCR protocols have been published (Schwan et al. 2005; Margos et al. 2015) or are available at PubMLST (Jolley et al. 2018). We separated PCR amplicons in 1.5% agarose gels stained with Biotium GelGreen (Biotium, Hayward, California, USA) and purified them using the QIAquick PCR Purification Kit (QIAGEN, Valencia, California, USA). Products were sequenced in both directions on an Applied Biosystems Model 3130 Genetic Analyzer (Applied Biosystems, Foster City, California, USA) and assembled using Lasergene (DNASTAR, Madison, Wisconsin, USA). Extraction of DNA, PCR, and sequencing were performed in separate laboratories to minimize contamination.

To produce a comprehensive *Borrelia* genus phylogeny, housekeeping gene sequences were compared with named *Borrelia* species available in GenBank (NCBI Resource Coordinators 2016) and PubMLST (Jolley et al. 2018; Supplementary Table 2). We included *Borrelia turcica*, *Candidatus* B. mahuryensis, and *Candidatus* B. tachyglossi, which constitute a third group of *Borrelia* distinct from the relapsing fever and Lyme disease clades (Binetruy et al. 2020). Flagellin gene sequences were compared to closely matching RF *Borrelia* sequences using BLAST (National Center for Biotechnology Information 2018). Sequences were aligned with MAFFT (Katoh and Standley 2013) using the L-INS-i method and trimmed. Phylogenetic model selection and maximum likelihood tree estimation were performed using IQ-Tree for concatenated housekeeping genes and *flaB* sequences separately (Minh et al. 2020).

Nine adult desert cottontails of unknown sex and one juvenile female were captured in April–October 2003; one cottontail, sampled September 2003, was positive for *Borrelia* sp. DNA (Goodrich et al. 2020). None of the 13 rodent species sampled in that study were positive. Sequences obtained from targeted loci were submitted to GenBank with accession numbers MZ408281–MZ408285 and were uploaded to PubMLST as isolate ID 3197. Our BLAST searches for each gene indicated that the novel *Borrelia* species from *S. audubonii* was most closely related to RF spirochetes *B. miyamotoi*, *B. theileri*, *B. lonestari*, and other *Borrelia* species identified in hard ticks or large mammal hosts (Supplementary Table 3). The closest match (>94% sequence identity) for all genes was *Borrelia* sp. tHM16w from *Haemaphysalis megaspinosa* ticks collected from wild boar (*Sus scrofa*) in Japan (Furuno et al. 2017). The closest matching *flaB* sequences (>95% identity) were from Sika deer (*Cervus nippon*) and in *Haemaphysalis* spp. ticks from wild boar and Sika deer in Japan and in *Rhipicephalus sanguineus* ticks collected from vegetation or from sheep in Portugal and Iran (Supplemetary Table 3). Phylogenetic analysis confirmed that the cottontail *Borrelia* belonged to a RF clade including *B. miyamotoi*, *B. theileri*, and *B. lonestari*, species associated with hard ticks (family Ixodidae) rather than the soft ticks (family Argasidae) typically associated with RF *Borrelia* species (Margos et al. 2020; Nakao et al. 2021). Although *clpX*, *pepX*, *recG*, and *rplB* sequences were not available for *B. theileri* and *B. lonestari*, the tree produced from concatenated sequences confirmed that the cottontail *Borrelia* species clustered with *Borrelia* sp. tHM16w and *B. miyamotoi* with 100% bootstrap support (Fig. 1). The *flaB* phylogeny showed that the cottontail *Borrelia* species clustered with the hard tick RF species *B. miyamotoi*, *B. theileri*, and *B. lonestari* with 100% bootstrap support, though it clustered more closely to *Borrelia* sp. tHM16w and other sequences from Eurasian hard ticks and large mammals with 93% bootstrap support (Fig. 2).

Borrelia spp. in the RF clade have not been reported in lagomorphs previously. Previous studies have detected *Borrelia burgdorferi* sensu lato (s.l.) in *Sylvilagus* rabbits and their associated ticks in North America (Burgess and Windberg 1989; Hamer et al. 2012). We did not detect *B. burgdorferi* s.l. in our small sample of *S. audubonii*. Additional surveys of *Syvilagus* spp. in North America will be necessary to determine if the novel cottontail *Borrelia* or other RF *Borrelia* spp. are widespread in this host group, and if *B. burgdorferi* s.l. species are truly absent in the New Mexico population of *S. audubonii* or its ticks.

Phylogenetic analysis indicated that the novel cottontail *Borrelia* sp. is a member of the hard tick RF clade with high bootstrap support (Figs. 1, 2), but the identity of its potential hosts beyond *S. audubonii* is difficult to infer. Close relatives of the novel species have been reported from hard ticks collected from deer, sheep, and wild boar (Supplementary Table 3). Borreliae of the hard tick RF clade have been detected in ungulates, rodents, birds, carnivores, and lemurs (Moore et al. 2003; Kang et al. 2018; Qurollo et al. 2018; Talagrand-Reboul et al. 2018). Detection of a novel *Borrelia* species in the desert cottontail widens the range of potential hosts of hard tick RF borreliae. The vectors of the novel *Borrelia* are unknown. New Mexico has multiple hard tick species, including nine *Ixodes* spp., four *Dermacentor* spp., *Haemaphysalis leporispalustris*, and *R. sanguineus* recorded (Bishopp and Trembley 1945; New Mexico Department of Health 2021). Both *Dermacentor parumapertus* and *H. leporispalustris* are associated with lagomorphs (Bishopp and Trembley 1945). Joint surveys of *Borrelia* spp. in ticks and associated vertebrate hosts

in New Mexico are needed to assess the full host and vector range of this novel cottontail *Borrelia* species.

Placement of the novel *Borrelia* species in the hard tick RF group raises questions about its zoonotic potential, though few *Borrelia* species are known to cause human illness (Cutler et al. 2017). Humans could be exposed to spirochetes in ticks parasitizing lagomorphs in peridomestic areas. At least two hard ticks parasitizing lagomorphs (*H. leporispalustris*, *Haemaphysalis longicornis*) have been reported to bite humans (Brown 1945; Wormser et al. 2020). The potential for this novel *Borrelia* sp. to cause disease in humans is unknown.

We did not attempt to culture spirochetes from rabbit blood in this study due to the age and limited volume of samples, thereby precluding more extensive genetic analysis (e.g., full genome sequencing). Attribution of the novel *Borrelia* sp. to the hard tick RF clade may be strengthened by testing rabbit-associated ticks from the region. Experimental infection of *S. audubonii* and potential tick vectors with isolates of the novel *Borrelia* sp. could then establish host and vector competence (Eisen 2020). We recommend increased surveillance in lagomorphs and their ticks, together with expanded sampling from other geographic locations, to determine the prevalence and distribution of this novel cottontail *Borrelia* species.

We are grateful to Christine Graham, Andrias Hojgaard, and Emma Harris for *Borrelia* spp. DNA. The authors thank the reviewers for careful revision of the manuscript, thoughtful comments, and constructive suggestions. Special thanks go to the staff of the Steven B. Thacker CDC Library for their invaluable assistance in obtaining scientific materials. All animal handling procedures were approved by the Centers for Disease Prevention and Control Institutional Animal Care and Use Committee, protocol number 06–008.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

LITERATURE CITED

Binetruy F, Garnier S, Boulanger N, Talagrand-Reboul É, Loire E, Faivre B, Noël V, Buysse M, Duron O. 2020. A novel *Borrelia* species, intermediate between Lyme disease and relapsing fever groups, in neotropical passerine-associated ticks. Sci Rep 10:10596.

Bishopp FC, Trembley HL. 1945. Distribution and hosts of certain North American ticks. J Parasitol 31:1–54.

Brown JH. 1945. The rabbit tick, *Haemaphysalis leporispalustris Pack.*, as an ectoparasite of man. Can Entomol 77:176.

Burgess EC, Windberg LA. 1989. *Borrelia* sp. infection in coyotes, black-tailed jack rabbits and desert cotton-tails in southern Texas. J Wildl Dis 25:47–51. [PubMed: 2644452]

Cutler SJ. 2015. Relapsing fever borreliae: A global review. Clin Lab Med 35:847–865. [PubMed: 26593261]

Cutler SJ, Ruzic-Sabljic E, Potkonjak A. 2017. Emerging borreliae—Expanding beyond Lyme borreliosis. Mol Cell Probes 31:22–27. [PubMed: 27523487]

Eisen L. 2020. Vector competence studies with hard ticks and *Borrelia burgdorferi* sensu lato spirochetes: A review. Ticks Tick Borne Dis 11:101359.

Furuno K, Lee K, Itoh Y, Suzuki K, Yonemitsu K, Kuwata R, Shimoda H, Watarai M, Maeda K, Takano A. 2017. Epidemiological study of relapsing fever borreliae detected in *Haemaphysalis* ticks and wild animals in the western part of Japan. PLoS One 12:e0174727.

Goodrich I, McKee C, Kosoy M. 2020. Longitudinal study of bacterial infectious agents in a community of small mammals in New Mexico. Vector Borne Zoonotic Dis 20:496–508. [PubMed: 32159462]

Hamer SA, Hickling GJ, Keith R, Sidge JL, Walker ED, Tsao JI. 2012. Associations of passerine birds, rabbits, and ticks with *Borrelia miyamotoi* and *Borrelia andersonii* in Michigan, U.S.A. Parasit Vectors 5:231. [PubMed: 23057837]

Jolley KA, Bray JE, Maiden MCJ. 2018. Open-access bacterial population genomics: BIGSdb software, the PubMLST.org website and their applications. Well come Open Res 3:124.

Kang J-G, Chae J-B, Cho Y-K, Jo Y-S, Shin N-S, Lee H, Choi K-S, Yu D-H, Park J, et al. 2018. Molecular detection of Anaplasma, *Bartonella*, and *Borrelia theileri* in raccoon dogs (*Nyctereutes procyonoides*) in Korea. Am J Trop Med Hyg 98:1061–1068. [PubMed: 29436346]

Katoh K, Standley DM. 2013. MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. Mol Biol Evol 30:772–780. [PubMed: 23329690]

Margos G, Binder K, Dzaferovic E, Hizo-Teufel C, Sing A, Wildner M, Fingerle V, Jolley KA. 2015. PubMLST.org—The new home for the *Borrelia* MLSA database. Ticks Tick Borne Dis 6:869–871. [PubMed: 26115778]

Margos G, Fingerle V, Cutler S, Gofton A, Stevenson B, Estrada-Peña A. 2020. Controversies in bacterial taxonomy: The example of the genus *Borrelia*. Ticks Tick Borne Dis 11:101335.

Margos G, Vollmer SA, Cornet M, Garnier M, Fingerle V, Wilske B, Bormane A, Vitorino L, Collares-Pereira M, et al. 2009. A new *Borrelia* species defined by multilocus sequence analysis of housekeeping genes. Appl Environ Microbiol 75:5410–5416. [PubMed: 19542332]

Minh BQ, Schmidt HA, Chernomor O, Schrempf D, Woodhams MD, von Haeseler A, Lanfear R. 2020. IQ-TREE 2: New models and efficient methods for phylogenetic inference in the genomic era. Mol Biol Evol 37:1530–1534. [PubMed: 32011700]

Moore VA 4th, Varela AS, Yabsley MJ, Davidson WR, Little SE. 2003. Detection of *Borrelia lonestari*, putative agent of Southern tick-associated rash illness, in white-tailed deer (*Odocoileus virginianus*) from the Southeastern United States. J Clin Microbiol 41:424–427. [PubMed: 12517884]

Morway C, Kosoy M, Eisen R, Montenieri J, Sheff K, Reynolds PJ, Powers N. 2008. A longitudinal study of *Bartonella* infection in populations of woodrats and their fleas. J Vector Ecol 33:353–364. [PubMed: 19263856]

Nakao R, Kasama K, Boldbaatar B, Ogura Y, Kawabata H, Toyoda A, Hayashi T, Takano A, Maeda K. 2021. The evolution of hard tick-borne relapsing fever borreliae is correlated with vector species rather than geographical distance. BMC Ecol Evol 21:105. [PubMed: 34058972]

National Center for Biotechnology Information. 2018. Basic local alignment search tool. http://blast.ncbi.nlm.nih.gov/Blast.cgi. Accessed October 2021.

NCBI Resource Coordinators. 2016. Database resources of the National Center for Biotechnology Information. Nucleic Acids Res 44(D1):D7–19. [PubMed: 26615191]

New Mexico Department of Health. 2021. New Mexico vector control manual: Ticks and tick-borne disease. https://www.nmhealth.org/publication/view/guide/1000/. Accessed October 2021.

Qurollo BA, Larsen PA, Rakotondrainibe HH, Mahefarisoa K, Rajaonarivelo T, Razafindramanana J, Breitschwerdt EB, Junge RE, Williams CV. 2018. Molecular surveillance of novel tick-borne organisms in Madagascar's lemurs. Ticks Tick Borne Dis 9:672–677. [PubMed: 29477959]

Schwan TG, Raffel SJ, Schrumpf ME, Policastro PF, Rawlings JA, Lane RS, Breitschwerdt EB, Porcella SF. 2005. Phylogenetic analysis of the spirochetes *Borrelia parkeri* and *Borrelia turicatae* and the potential for tick-borne relapsing fever in Florida. J Clin Microbiol 43:3851–3859. [PubMed: 16081922]

Talagrand-Reboul E, Boyer PH, Bergström S, Vial L, Boulanger N. 2018. Relapsing fevers: Neglected tick-borne diseases. Front Cell Infect Microbiol 8:98. [PubMed: 29670860]

Wormser GP, McKenna D, Piedmonte N, Vinci V, Egizi AM, Backenson B, Falco RC. 2020. First recognized human bite in the United States by the Asian longhorned tick, *Haemaphysalis longicornis*. Clin Infect Dis 70:314–316. [PubMed: 31150055]

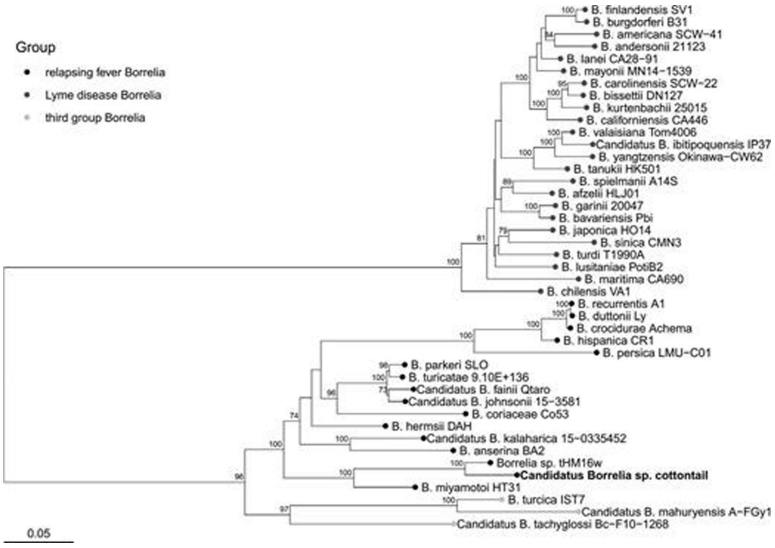

FIGURE 1.

Maximum likelihood phylogenetic tree of concatenated *Borrelia* sequences produced from a 2468 base pair (bp) alignment of housekeeping genes (623 bp *clpX*, 570 bp *pepX*, 651 bp *recG*, 624 bp *rplB*). The best model of sequence evolution was GTR+F+I+G4 based on the Bayesian information criterion. The tree was rooted at the midpoint and bootstrap branch support values greater than 70% are shown next to branches. Strain names are labeled next to each *Borrelia* species name. GenBank accession numbers for genes from each strain are provided in Supplementary Material Table 2.

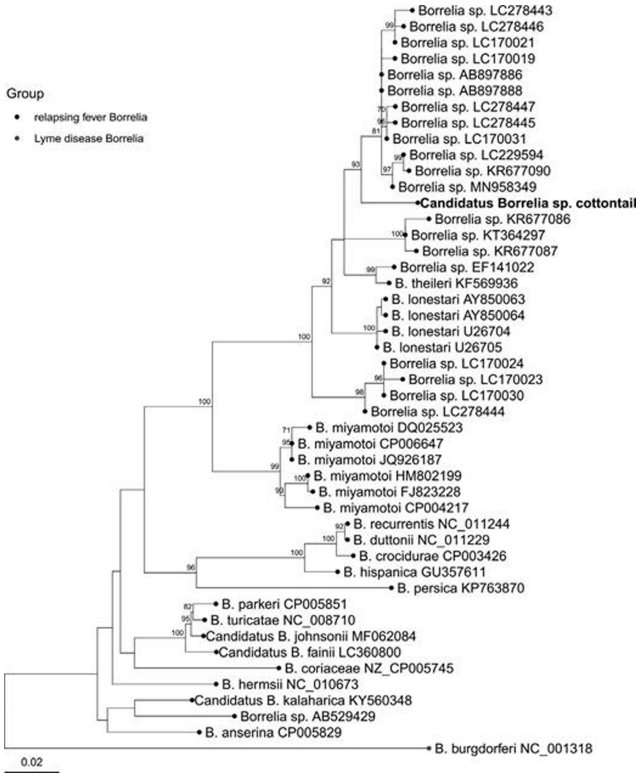

FIGURE 2.

Maximum likelihood phylogenetic tree of hard tick relapsing fever *Borrelia flaB* sequences (1017 base pair, gaps included). The best model of sequence evolution was TIM3+F+G4 on the Bayesian information criterion. The tree was rooted at the midpoint and bootstrap branch support values greater than 70% are shown next to branches. GenBank accession numbers are given next to each *Borrelia* species name.