Comparison of Vector Efficiency of *Ixodes scapularis* (Acari: Ixodidae) From the Northeast and Upper Midwest of the United States for the Lyme Disease Spirochete *Borrelia mayonii*

Lars Eisen[1], **Nicole E. Breuner**, **Andrias Hojgaard**, **J. Charles Hoxmeier**, **Mark A. Pilgard**, **Adam J. Replogle**, **Brad J. Biggerstaff**, and **Marc C. Dolan**
Division of Vector-Borne Diseases, National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention, Fort Collins, CO 80521

Abstract

Borrelia mayonii, a recently recognized species within the *Borrelia burgdorferi* sensu lato complex, has been detected in host-seeking *Ixodes scapularis* Say ticks and found to be associated with Lyme disease in the Upper Midwest. This spirochete has, to date, not been documented from the Northeast, but we previously demonstrated that *I. scapularis* ticks originating from Connecticut are capable of serving as a vector of *B. mayonii*. In this follow-up study, we compared the vector efficiency for *B. mayonii* (strain MN14-1420) of *I. scapularis* ticks originating from Minnesota in the Upper Midwest and Connecticut in the Northeast. CD-1 outbred white mice previously infected with *B. mayonii* via tick bite were exposed to simultaneous feeding by Minnesota and Connecticut larvae contained within separate feeding capsules. We found no difference in the ability of Minnesota and Connecticut larvae to acquire *B. mayonii* from infected mice and pass spirochetes to the nymphal stage (overall nymphal infection rates of 11.6 and 13.3%, respectively). Moreover, the efficiency of transmission of *B. mayonii* by single infected nymphs was similar for the Minnesota and Connecticut ticks (33 and 44%, respectively). We conclude that the examined *I. scapularis* ticks from the Upper Midwest and Northeast did not differ in their efficiency as vectors for *B. mayonii*.

Keywords

The blacklegged tick, *Ixodes scapularis* Say, is the primary vector of the Lyme disease spirochete *Borrelia burgdorferi* sensu stricto (hereafter referred to as *B. burgdorferi*) to humans in the United States (Piesman and Gern 2004, Eisen et al. 2016). This tick also most likely serves as the principal vector to humans of another recently described spirochete, *Borrelia mayonii*, associated with Lyme disease in the Upper Midwest (Pritt et al. 2016a,b). Connecticut *I. scapularis* ticks were experimentally demonstrated to be capable of serving as vectors of *B. mayonii* (Dolan et al. 2016), and host-seeking *I. scapularis* nymphs and adults from Wisconsin, including ticks collected from presumed exposure sites for *B. mayonii*-

[1]Corresponding author, evp4@cdc.gov.

infected Lyme disease patients, were found to be infected with *B. mayonii* (Pritt et al. 2016a,b). Natural vertebrate reservoirs for *B. mayonii* have yet to be determined, and potential involvement of additional tick vectors in the enzootic transmission cycle remains to be explored. As *B. mayonii* has been documented from the Upper Midwest but not the Northeast, we sought to evaluate whether *I. scapularis* ticks originating from Minnesota in the Upper Midwest and Connecticut in the Northeast may differ in their ability to acquire and transmit this spirochete.

Materials and Methods

I. scapularis Ticks, *B. mayonii* Source, and Experimental Mouse Host

The *I. scapularis* colony ticks used were of the first or second generations from adults collected in multiple locations in Fairfield County, CT, in the fall of 2014, or in Anoka County (Carlos Avery State Wildlife Management Area) or Washington County (William O'Brien State Park), MN, in the spring of 2015. All larval batches originated from females that, after they had produced their egg batches, tested negative for presence of *B. mayonii* based on detection of the spirochete flagellar filament cap (*fliD*) target, which we previously showed to be present in *B. mayonii* (Dolan et al. 2016) and that also is present in *B. burgdorferi* (Dolan et al. 2011, Hojgaard et al. 2014, Goddard et al. 2015). Detection of the actin gene of *I. scapularis* (Hojgaard et al. 2014) was used as a control for both the DNA purification and the PCR testing. Combined detection of the *I. scapularis* actin and *Borrelia fliD* targets in the females was done using a previously described multiplex TaqMan PCR (Dolan et al. 2016).

The original source of infection to start the mouse–tick transmission chain now maintained in the laboratory was *B. mayonii* strain MN14-1420, which was originally isolated from human blood (Pritt et al. 2016a,b). To infect feeding larvae in this study, we used female CD-1 *Mus musculus* outbred mice (Charles River Laboratories, Wilmington, MA) previously infected via the bite of *B. mayonii*-infected Connecticut *I. scapularis* nymphs (Table 1). Naïve mice used as sentinels to assess transmission by single *B. mayonii*-infected Connecticut or Minnesota nymphs were 1–3-mo-old CD-1 females.

Feeding of *I. scapularis* Larvae on Infected Mice to Assess the Rate of Spirochete Acquisition and Passage to the Nymphal Stage

To avoid bias due to changes over time in spirochetemia of individual mice, we fed Connecticut and Minnesota larvae simultaneously on eight infected mice (Table 1). This was achieved by affixing two feeding capsules to the shaved flanks of individual mice as described previously (Mbow et al. 1994, Soares et al. 2006). The target per mouse for larval feeding was to obtain roughly 50 fed larvae from each of the capsules receiving Connecticut larvae and Minnesota larvae. Fed larvae harvested from within the capsules were grouped by mouse and tick geographic origin 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 photoperiod of 16:8 (L:D) h. Depending on the available numbers of molted nymphs for a given mouse and tick geographic origin, all nymphs or subsets of the

nymphs were examined, 3–4 wk after the molt, for presence of the *I. scapularis* actin and *Borrelia fliD* targets as described previously (Dolan et al. 2016).

Nymphal Transmission of *B. mayonii* to Naïve Mice

As the purpose was to assess the likelihood of transmission by single *B. mayonii*-infected nymphs, we used nymphs originating from larvae that fed on the mouse (2084) yielding the highest infection rate (~50%) in the unfed nymphs (Table 1). Based on the expectation that half of the nymphs would be infected, we exposed each of 20naïve mice to two nymphs; 10 of the mice were exposed to Connecticut nymphs and 10 to Minnesota nymphs. Fully fed detached nymphs were collected and examined for presence of the *I. scapularis* actin and *Borrelia fliD* targets. Of the 20 mice, 10 were found to have been fed upon by a single infected nymph (Table 2) whereas the remaining mice either were fed upon by no infected nymph or two infected nymphs. An additional five mice (2064, 2066, 2071, 2072, 2075) were previously exposed to feeding by a single *B. mayonii* (MN14-1420)-infected Connecticut nymph in the study by Dolan et al. (2016), and these mice were included in the data presented here (Table 2).

For the mice that were exposed to the feeding of a single infected nymph, ear biopsies were taken 3–4 wk after the nymphal feed (Sinsky and Piesman 1989). Ear biopsies were cultured in modified Barbour-Stoenner-Kelly (BSK) medium with antibiotics to detect live spirochetes as described previously (Dolan et al. 2016). Cultures were examined by dark-field microscopy, at 400× magnification, weekly for up to 3 wk.

Serum samples from mice that were exposed to an infected nymph but still yielded spirochete-negative ear biopsies were taken 8–10 wk after the nymphal feed and examined for serological reactivity to *B. mayonii* using the MarDx *B. burgdorferi* (IgG) Marblot Strip Test System (MarDX Diagnostic Inc., Carlsbad, CA). The Marblot strip test system was developed to detect antibodies to different *B. burgdorferi* proteins, but we found it to also be reactive to antibodies generated against *B. mayonii* (Table 2). As the Marblot strip test system was developed for detection of human antibodies, testing of mouse serum was accomplished with a modification to the manufacturer's instructions by using alkaline-phosphatase labeled goat anti-mouse IgG + IgM (H + L) (Kirkegaard and Perry Laboratories, Gaithersburg, MD) as the detection antibody at 1:2,000 dilution. Serum samples taken 8–10 wk after the nymphal feed from mice that yielded spirochete-positive ear biopsies were used as a positive control (Table 2). Marblot strip banding patterns were analyzed and scored as positive, according to the manufacturer's recommendations, when 5 distinct bands were evident.

Statistical Evaluation

The proportions of nymphs that were infected with *B. mayonii* after the larvae had fed on infected mice were compared between Connecticut and Minnesota ticks using mixed effects binomial regression with a log link and a random effect for mouse to account for anticipated within-mouse correlation. Using the log link provides a direct estimate of the ratio of the tick geographic origin infection probabilities. Model parameters were estimated using maximum likelihood; models were compared using the likelihood ratio test, and 95%

confidence intervals (CI) were computed using profile likelihood. Standard diagnostics were used to evaluate model adequacy (Pinheiro and Bates 2009). Intraclass correlation coefficients (and CIs) were used to evaluate the magnitude of within-mouse correlation (Zou and Donner 2004).

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.

Results

Borrelia mayonii Acquisition From Infected Mice by Larval *I. scapularis* and Transstadial Passage to the Nymphal Stage

There was substantial variability in infection rates for nymphs having fed as larvae on individual infected mice, ranging from 1.3–47.5% for Connecticut and Minnesota nymphs combined (Table 1). However, infection rates for nymphs having fed as larvae on the same individual infected mouse were similar for Connecticut and Minnesota nymphs. For the seven mice with more than 15 resulting nymphs examined for each tick geographic origin, three mice yielded nymphs with infection rates <4% for both Connecticut and Minnesota ticks, one mouse yielded nymphs with infection rates <8% for both Connecticut and Minnesota ticks, and three mice yielded nymphs with infection rates 20% for both Connecticut and Minnesota ticks (Table 1). Moreover, there was no clear trend among these mice for infection rates being higher for either Connecticut or Minnesota nymphs; infection rates were numerically higher for the Connecticut ticks for three of the mice and for the Minnesota ticks for four of the mice.

These observations were reinforced by the statistical analysis. Accounting for anticipated within-mouse correlation, the proportions of infected nymphs did not differ statistically by tick geographic origin in the binomial regression (RR = 1.12, 95% CI 0.75–1.65; likelihood ratio test *P*-value = 0.57). The variance of the random effect for mouse was estimated to be 1.86 (95% CI 0.66–6.83), characterizing the noticeable variability seen among the proportions of infected nymphs among the mice. Model diagnostics indicated no serious departures from model assumptions. Based on this result, we estimated the intraclass (intramouse) correlation coefficient (ICC) as 0.25 (95% CI 0.01–0.77), combining results for both tick geographic origins within mice. Note that the CI for the ICC is wide in this context, reflecting relatively few mice in this experiment ($n = 8$) and relatively large variability in the results among mice.

Nymphal Transmission of *B. mayonii* to Naïve Mice

Single *B. mayonii*-infected nymphs that were allowed to feed to completion on naïve mice transmitted spirochetes to 4/9 mice (44%) for the Connecticut ticks and 2/6 mice (33%) for the Minnesota ticks (Table 2). Both Connecticut and Minnesota ticks thus were capable of transmitting *B. mayonii*. For both Connecticut and Minnesota ticks combined, the overall

transmission efficiency for single infected nymphs was 40% (transmission occurring in 6/15 mice).

Discussion

We present experimental evidence indicative of similar vector efficiency of *I. scapularis* ticks from the Upper Midwest (Minnesota) and Northeast (Connecticut) for the recently recognized Lyme disease spirochete *B. mayonii* (strain MN14-1420). This is perhaps not surprising because 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, Jacobs et al. 2003, Goddard et al. 2015). If the current geographic range of *B. mayonii* should prove to be restricted to the Upper Midwest, as suggested by Pritt et al. (2016a,b), this is most likely due to factors other than variation in the vector efficiency of *I. scapularis* across its range. An ongoing convergence of the previously distinct geographic foci for *I. scapularis* in the Upper Midwest and Northeast (Dennis et al. 1998, Eisen et al. 2016) could facilitate spread of *B. mayonii* from the Upper Midwest into the Northeast via infected ticks or vertebrates. Important study caveats include the use of an experimental mouse host, a single source isolate for *B. mayonii*, and tick colonies from only two states. As we learn more about the natural maintenance of *B. mayonii*, follow-up studies should be able to address some of these study weaknesses. Urgent research needs include determination of the natural vertebrate reservoirs for *B. mayonii* and clarification of its current geographic range by surveillance of vector ticks and putative vertebrate reservoirs.

Acknowledgments

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Table 1

Acquisition of *B. mayonii* by larval *I. scapularis* ticks originating from Connecticut and Minnesota during feeding on infected outbred white mice and transstadial passage to the nymphal stage

Mouse	Duration of infection in mouse prior to larval feeding (d)	B. mayonii infection in *I. scapularis* nymphs fed as larvae on infected mice[a]								
		Connecticut ticks			Minnesota ticks			Connecticut and Minnesota ticks combined		
		No. nymphs examined	No. nymphs infected	% nymphs infected	No. nymphs examined	No. nymphs infected	% nymphs infected	No. nymphs examined	No. nymphs infected	% nymphs infected
2111	70	87	3	3.4	88	2	2.3	175	5	2.9
2112	70	40	14	35.0	17	10	58.8	57	24	42.1
2114	70	20	10	50.0	20	4	20.0	40	14	35.0
2117	70	40	0	0	40	1	2.5	80	1	1.3
2071	224	14	2	14.3	3	1	33.3	17	3	17.6
2073	224	40	3	7.5	40	1	2.5	80	4	5.0
2074	224	40	0	0	40	1	2.5	80	1	1.3
2084	224	20	8	40.0	20	11	55.0	40	19	47.5
All mice		301	40	13.3	268	31	11.6	569	71	12.5

[a] Determined by PCR assay of nymphs 3–4 wk after they molted.

Table 2

Transmission of *B. mayonii* by single infected nymphal *I. scapularis* ticks originating from Connecticut and Minnesota to naïve outbred white mice

Mouse	Tick geographic origin	No. infected nymphs fed upon the mouse (total no. nymphs fed upon the mouse)	Culture of ear biopsy (taken 4 wk after the nymphal feed) to detect live spirochetes[a]	Serological reactivity to *B. mayonii* (8–10 wk after the nymphal feed)[a]
2064[b]	Connecticut	1 (10)	−	−
2066[b]	Connecticut	1 (7)	−	−
2071[b]	Connecticut	1 (11)	+	Not tested
2072[b]	Connecticut	1 (8)	+	Not tested
2075[b]	Connecticut	1 (10)	−	−
B19	Connecticut	1 (2)	−	−
B21	Connecticut	1 (2)	+	+
B22	Connecticut	1 (2)	−	−
B27	Connecticut	1 (2)	+	+
B09	Minnesota	1 (1)	−	−
B12	Minnesota	1 (1)	−	−
B13	Minnesota	1 (2)	−	−
B14	Minnesota	1 (2)	+	+
B15	Minnesota	1 (2)	−	−
B16	Minnesota	1 (2)	+	+

[a]+indicates infection in culture or reactive serology.

[b]From Dolan et al. (2016).