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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.

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