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Experimental Demonstration of Reservoir Competence of the White-Footed Mouse, *Peromyscus leucopus* (Rodentia: Cricetidae), for the Lyme Disease Spirochete, *Borrelia mayonii* (Spirochaetales: Spirochaetaceae)

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Abstract

The white-footed mouse, *Peromyscus leucopus* (Rafinesque), is a reservoir for the Lyme disease spirochete *Borrelia burgdorferi* sensu stricto in the eastern half of the United States, where the blacklegged tick, *Ixodes scapularis* Say (Acari: Ixodidae), is the primary vector. In the Midwest, an additional Lyme disease spirochete, *Borrelia mayonii*, was recorded from naturally infected *I. scapularis* and *P. leucopus*. However, an experimental demonstration of reservoir competence was lacking for a natural tick host. We therefore experimentally infected *P. leucopus* with *B. mayonii* via *I. scapularis* nymphal bites and then fed uninfected larvae on the mice to demonstrate spirochete acquisition and passage to resulting nymphs. Of 23 mice fed on by *B. mayonii*-infected nymphs, 21 (91%) developed active infections. The infection prevalence for nymphs fed as larvae on these infected mice 4 wk post-infection ranged from 56 to 98%, and the overall infection prevalence for 842 nymphs across all 21 *P. leucopus* was 75% (95% confidence interval, 72–77%). To assess duration of infectivity, 10 of the *P. leucopus* were reinfested with uninfected larval ticks 12 wk after the mice were infected. The overall infection prevalence for 480 nymphs across all 10 *P. leucopus* at the 12-wk time point was 26% (95% confidence interval, 23–31%), when compared with 76% (95% confidence interval, 71–79%) for 474 nymphs from the same subset of 10 mice at the 4-wk time point. We conclude that *P. leucopus* is susceptible to infection with *B. mayonii* via bite by *I. scapularis* nymphs and an efficient reservoir for this Lyme disease spirochete.

Keywords

Borrelia mayonii; *Ixodes scapularis*; *Peromyscus leucopus*; Lyme disease; reservoir

In the Upper Midwestern United States, the recently discovered Lyme disease spirochete, *Borrelia mayonii*, has been detected in naturally infected blacklegged ticks, *Ixodes scapularis* Say (Acari: Ixodidae), as well as rodents, including the white-footed mouse *Peromyscus leucopus* (Pritt et al. 2016a,b; Johnson et al. 2017, 2018). We previously demonstrated that *I. scapularis* is an experimental vector of *B. mayonii* and that the CD-1

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outbred strain of the house mouse, *Mus musculus*, is an experimental reservoir of this spirochete (Dolan et al. 2016, 2017; Eisen et al. 2017). However, experimental confirmation of reservoir competence for *B. mayonii* has been lacking for natural rodent hosts for *I. scapularis* ticks. We therefore aimed in this study to experimentally assess the reservoir competence for *B. mayonii* of *P. leucopus*, a rodent that occurs throughout most of the eastern United States (Kays and Wilson 2002) and is an important host for *I. scapularis* immatures, as well as a key reservoir for another human-pathogenic Lyme disease spirochete, *Borrelia burgdorferi* sensu stricto (s.s) (Spielman et al. 1985, Donahue et al. 1987, LoGiudice et al. 2003).

Materials and Methods

Origins of *B. mayonii*, *I. scapularis*, and Experimental Rodent Hosts

The *B. mayonii* isolate (MN17-4755) used to start a mouse-tick infection chain in this experiment was originally obtained from a wild-caught *P. leucopus* mouse collected in Pine County, Minnesota (Johnson et al. 2017). A low passage (P1) of this isolate was grown in Barbour–Stoenner–Kelly medium (in-house BSK-R medium with antibiotics: cycloheximide, 20 μg/ml; phosphomycin, 200 μg/ml; rifampicin, 50 μg/ml; and amphotericin B, 2.5 μg/ml), and 100 μl of culture medium containing approximately 1×10^5 spirochetes was inoculated intradermally via needle into 2- to 4-mo-old outbred female CD-1 white mice (Charles River Laboratories, Wilmington, MA). Larval *I. scapularis* ticks were then fed on the *B. mayonii*-infected mice 4 wk post-infection, as described previously (Dolan et al. 2016), and the resulting nymphs were used to start an *I. scapularis*-white mouse infection chain. This infection chain went through two additional cycles before the resulting infected nymphs were used to transmit *B. mayonii* to 2- to 4-mo-old female *P. leucopus* mice (Peromyscus Genetic Stock Center, University of South Carolina, Columbia, SC). Uninfected larvae placed on infected *P. leucopus* mice were obtained from the Medical Entomology Laboratory pathogen-free *I. scapularis* colony at the Centers for Disease Control and Prevention (Atlanta, GA).

Experimental Infection of *P. leucopus* Mice With *B. mayonii* and Subsequent Feeding by Uninfected *I. scapularis* Larvae

In total, 23 *P. leucopus* mice were exposed to *B. mayonii*-infected *I. scapularis* nymphs. Mice were anesthetized, and each mouse was infested with 20 potentially infected nymphs, placed openly on the fur of the mouse. The mice were then held over a water surface for 4 d to collect fed and detached nymphs. Ear biopsies were taken from all 23 mice 3 wk after nymphal ticks were first introduced onto them (Sinsky and Piesman 1989). The biopsies were surface sterilized in 70% ethanol for 5–10 min and then placed into in-house BSK-R culture medium with antibiotics at 34°C. Aliquots of the cultures were examined under dark field microscopy at 400× magnification every 10 d for up to 30 d.

Uninfected *I. scapularis* larvae were introduced onto all 23 mice 4 wk after the start of the feed by the infected nymphs (weeks post-exposure to infected nymphs; w.p.e.). Mice were anesthetized and each mouse was infested with approximately 200–250 larvae placed openly on the fur of the mouse. The mice were then held over a water surface for up to 4 d to collect

fed, detached larvae. Fed larvae were grouped by mouse into 5-ml capacity plastic vials with mesh lids (Corning Falcon Test Tube with Cell Strainer Snap Cap, Thermo Fisher Scientific, Waltham, MA), which then were transferred to desiccators in a growth chamber (90–95% relative humidity; 23–24°C; and a 16:8 [L:D] h cycle) while the larvae molted to nymphs. We tested up to 50 flat nymphs from each of the 21 source mice with an active infection (as determined by ear biopsies producing spirochete-positive cultures) for the presence of *B. mayonii* DNA, as described later. To confirm the presence of viable spirochetes in the molted nymphs, in addition to testing for *B. mayonii* DNA through PCR, we placed into culture groups of five ticks from a subset of 10 mice from which additional nymphs were available (excluding 11 mice from which all molted nymphs were used for PCR-based detection of *B. mayonii* DNA). Nymphs were surface sterilized in 70% ethanol for 5 min, sliced open with a scalpel to facilitate contact with the midgut material, and then placed into in-house BSK II medium with antibiotics. Each culture tube received five nymphs originating from larvae fed on the same mouse. Cultures were examined for spirochetes as described earlier.

To assess if reservoir efficiency of *P. leucopus* for *B. mayonii* decreased over time, we chose a subset of 10 *B. mayonii*-infected mice that yielded robust numbers (≥75) of fed larvae in the first round of larval infestation (4 w.p.e.) and reinfested them with a second set of uninfected larvae 12 w.p.e. Ear biopsies were taken 3 d following completion of the larval feed to confirm that the mice still had active infections; these ear biopsies were processed as described earlier. Fed recovered larvae were allowed to molt to nymphs and up to 50 nymphs from each source mouse were tested for the presence of *B. mayonii* DNA. The presence of viable spirochetes in molted nymphs was examined, using groups of five ticks from five mice, by placing them in culture as described earlier. This included ticks from the five mice for which molted nymphs were still available after the PCR-based detection of *B. mayonii* DNA and where infection rates in the molted nymphs were expected to be >20%. Moreover, there was no result for ticks from one of these mice due to culture contamination. The 4 w.p.e. time point to assess reservoir efficiency represents a scenario with more synchronous feeding by *I. scapularis* nymphs and larvae, such as in the Midwest, whereas the 12 w.p.e. time point aimed to assess longer term reservoir efficiency more representative of a scenario from the Northeast where infected nymphs would feed on *P. leucopus* primarily in late spring (May–June) and larvae most commonly in the summer (July–September; Stafford 2007, Gatewood et al. 2009, Hamer et al. 2012).

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

Detection of *B. mayonii* DNA in *I. scapularis* Ticks

Nucleic acids were isolated from unfed or fed nymphal ticks as described previously (Lynn et al. 2019). Individual ticks were homogenized in 350 µl of tissue lysis buffer (327.5 µl ATL, 20 µl Proteinase K, 1 µg (1 µl) Carrier RNA, and 1.5 µl DX Reagent; Qiagen, Valencia, CA) using a Mini-Beadbeater-96 (BioSpec Products, Inc., Bartlesville, OK) with 2.0 mm Very High Density Yttria stabilized zirconium oxide beads (GlenMills, Clifton, NJ). DNA

was then extracted from tick lysates (300 µl) using the KingFisher DNA extraction system (Thermo Fisher Scientific) and the MagMAX Pathogen RNA/DNA Kit (Thermo Fisher Scientific) according to manufacturer recommendations and eluted into 90 µl of elution buffer. A blank was included as a negative control to ensure no cross-contamination occurred during the extraction.

The primary multiplex PCR used for detection of *B. mayonii* in ticks included the flagellar filament cap (*fliD*) target for *B. burgdorferi* s.s. (Hojgaard et al. 2014) and a pan-*Borrelia* 16S rDNA target (Kingry et al. 2018). Also included in the multiplex master mix was the *I. scapularis* actin target (Hojgaard et al. 2014), which served as a positive control for DNA quality resulting from the extraction process. The PCR reaction solutions consisted of 5 µl tick DNA, forward and reverse primers each at a concentration of 300 nM, a probe at a concentration of 200 nM, 5.5 µl iQ Multiplex Powermix (Bio-Rad, Hercules, CA), and deionized water to make a total volume of 11 µl. The real-time TaqMan PCR cycling conditions consisted of denature DNA at 95°C for 3 min followed by 40 cycles of 95°C for 10 s, 58°C for 10 s, and 62°C for 30 s on a C1000 Touch thermal cycler with a CFX96 real-time system (Bio-Rad). A second confirmatory PCR for tick samples testing positive in the initial PCR included previously described targets specific to *B. mayonii* and *B. burgdorferi* s.s. (*oppA2*) (Graham et al. 2018), and *B. miyamotoi* (*PurB*) (Graham et al. 2016). This reaction consisted of 5 µl tick DNA; *Borrelia oppA2* target and probe concentrations listed for the M4 assay in Graham et al. (2018); *PurB* forward and reverse primers at a concentration of 200 nM and a probe at a concentration of 130 nM; 5.5 µl iQ Multiplex Powermix; and deionized water to make a total volume of 25 µl. The run cycle followed conditions listed for the M4 assay in Graham et al. (2018): a 3 min 95°C activation step followed by 40 cycles of 95°C for 15 s and 58°C for 1 min.

We analyzed samples using CFX Manager 3.1 software (Bio-Rad) with the quantitation cycle (Cq) determination mode set to regression. Tick samples were considered positive for *B. mayonii* only if the primary PCR reaction resulted in amplification of a *Borrelia* target (16S rDNA or *fliD*) and the secondary PCR reaction resulted in amplification of *B. mayonii oppA2*. Based on Graham et al. (2018), only Cq values < 40 were considered indicative of a target being present in the tested sample.

Statistical Analysis

We calculated the prevalence of infection in tested nymphs, for each mouse and time point, as the number of nymphs infected divided by the total number of nymphs tested. Score confidence intervals (95%) were computed using JMP 13 statistical software (SAS Institute, Inc., Cary, NC). In addition, we compared the proportions of nymphs testing positive for *B. mayonii* at 4 and 12 w.p.e. using a likelihood ratio test. For all analyses, a significance level of $P < 0.05$ was employed.

Results

Infection of *P. leucopus* via Bite by *B. mayonii*-Infected *I. scapularis* Nymphs

As shown in Table 1, we documented infection in 21 *P. leucopus* mice following exposure to 1–10 *B. mayonii*-infected *I. scapularis* nymphs per mouse. However, the recorded numbers of infected nymphs fed on individual mice may be inflated due to cofeeding transmission of *B. mayonii* among nymphs feeding in close proximity to one another on a mouse (as previously observed for *I. scapularis* females feeding on a rabbit; Breuner et al. 2018). Infected fed nymphs were recovered from two additional mice that failed to develop active infections (data not shown) and therefore were not used further in the study. Thus, 91% of the 23 mice fed on by at least one *B. mayonii*-infected nymph were shown to develop active infections.

Acquisition of *B. mayonii* by Uninfected *I. scapularis* Larvae Fed on *P. leucopus* With Active Infections and Passage of Spirochetes to the Nymphal Life Stage

At 4 wk after the mice were exposed to infected nymphs, all 21 *P. leucopus* mice with active infections produced infected larvae which maintained infection to the nymphal life stage (Table 1). The infection prevalence for nymphs resulting from individual mice ranged from 56 to 98%, and the overall infection prevalence for 842 tested nymphs across all 21 *P. leucopus* mice was 75% (95% confidence interval, 72–77%). Moreover, viable spirochetes were documented in nymphs resulting from each of the 10 individual mice for which nymphs were placed in culture (Table 1).

Ten of the *P. leucopus* mice were reinfested with uninfected larval ticks on a second occasion 12 wk after the mice were exposed to infected nymphs. All 10 mice still produced infected larvae, which maintained infection to the nymphal life stage (Table 1). However, for 9 of the 10 mice the infection prevalence in the nymphs (range, 10–56%) had decreased significantly ($P < 0.05$) compared with the time point at 4 wk after the same mouse was infected (Table 1). The last mouse had few nymphs ($n = 24$) tested 4 w.p.e., which yielded a wide confidence interval and resulted in only a nonsignificant trend toward a decrease from 4 w.p.e. (66%) to 12 w.p.e (56%). The overall infection prevalence for 480 tested nymphs across all 10 *P. leucopus* mice at 12 w.p.e. was 26% (95% confidence interval, 23–31%), when compared with 76% (95% confidence interval, 71–79%) for 474 tested nymphs from the same subset of 10 mice at 4 w.p.e. (Table 1).

Discussion

We showed that *P. leucopus* is an efficient experimental reservoir for *B. mayonii*: this rodent species was found to be highly susceptible to infection via *I. scapularis* nymphal bites and a large proportion of larvae fed on infected mice acquired *B. mayonii* and passed spirochetes to the resulting nymphal stage. Previous similar experimental studies with *B. mayonii* (Dolan et al. 2016, 2017; Eisen et al. 2017) were restricted to a laboratory mouse model (CD-1 white mice), but here we demonstrated reservoir competence of a host naturally infested by immature *I. scapularis* ticks using an isolate originating from a naturally infected *P. leucopus* mouse (Johnson et al. 2017). Long-term persistence of *B. mayonii* infectivity in

P. leucopus, as observed in this study over a 3 mo-period, allows this important tick host to contribute to the natural maintenance of *B. mayonii* in the Upper Midwest, where this Lyme disease spirochete presently is known to occur (Pritt et al. 2016a,b; Johnson et al. 2017, 2018). Moreover, as both *P. leucopus* and *I. scapularis* occur commonly in the Northeast there appears to be no barrier to spirochete establishment should *B. mayonii* be introduced to that region (or discovered to already occur).

Consistent with the results of previous similar studies on the duration of infectivity for *P. leucopus* experimentally infected with *B. burgdorferi* sensu lato (s.l.) or *B. burgdorferi* s.s. (Donahue et al. 1987, States et al. 2017), spirochete acquisition by *I. scapularis* larvae and transstadial spirochete passage (hereafter simply referred to as host infectivity) was highly efficient for *B. mayonii*-infected *P. leucopus* mice at a time point 4 wk after the mice were exposed to infected ticks. In all three studies (Table 1; Donahue et al. 1987, States et al. 2017), the infectivity of *P. leucopus* exceeded 70% by 2–4 wk after mice were first infected. The infectivity then consistently decreased over time, falling to approximately 25% at the 6-wk time point for *B. burgdorferi* s.s. strain B348 (States et al. 2017), 50% at the 9-wk time point for *B. burgdorferi* s.l. (Donahue et al. 1987), 25% at the 12-wk time point for *B. mayonii* in our study (Table 1), and 40% at the 14-wk time point for *B. burgdorferi* s.s. strain BBC13 (States et al. 2017). Moreover, Lindsay et al. (1997) similarly reported decreasing infectivity over a 7-wk period for *P. leucopus* infected with *B. burgdorferi* s.l. via needle inoculation or tick bite. Low-level infectivity persisted for at least 7 mo for the *P. leucopus* infected with *B. burgdorferi* s.l. (Donahue et al. 1987) and was similarly reported to persist for up to 7 mo for hamsters infected with the JD1 strain of *B. burgdorferi* s.s. (Piesman 1988) and up to 12 mo for white mice infected with the MN14-1420 strain of *B. mayonii* (Dolan et al. 2017).

The presence in the Upper Midwest of both an efficient tick vector (*I. scapularis*) and an efficient reservoir (*P. leucopus*) for *B. mayonii*, thus sharing an enzootic transmission cycle with *B. burgdorferi* s.s., raises the question of why *B. mayonii* appears to be much less prevalent in host-seeking *I. scapularis* ticks compared with *B. burgdorferi* s.s. (Pritt et al. 2016a,b; Johnson et al. 2018). The reason for this disparity remains unknown, but it would be interesting in future studies to determine whether *B. burgdorferi* s.s. may have a fitness advantage over *B. mayonii* in either coinfected reservoirs or coinfected ticks.

In our previous transmission experiments with *B. mayonii*, we used an isolate (MN14-1420) obtained from human blood (Pritt et al. 2016a). The use in the present study of another, rodent-derived isolate (MN17-4755) resulted in greater *B. mayonii* infectivity in a rodent model. In several previous studies with the human-derived MN14-1420 isolate, we never recorded an infection prevalence of >60% for *I. scapularis* nymphs having fed as larvae on an infected white mouse (Dolan et al. 2016, 2017: Eisen et al. 2017), whereas in this study, the infectivity of the *P. leucopus*-derived MN17-4755 isolate exceeded 60% for 17 of 21 examined *P. leucopus* mice and was >90% for 4 of the mice (Table 1). Albeit based on small sample sizes, we also note that infectivity was consistently high for the *P. leucopus*-derived MN17-4755 isolate when maintained routinely in a white mouse-tick transmission chain in preparation for the present study (data not shown). Variable infectivity for isolates of a given *B. burgdorferi* s.l. species in rodent models has been reported in several previous studies

(Piesman and Happ 1997, Derdakova et al. 2004, Hanincova et al. 2008, Tonetti et al. 2015, States et al. 2017). Such variability could be attributed to genetic differences among isolates, which may affect spirochete fitness in a particular species of reservoir host.

Our study had some notable limitations. The assessment of duration of infectivity of *P. leucopus* was limited to two time points (4 and 12 w.p.e.) and it would be interesting to generate more granular data including time points before 4 w.p.e. as well as between 4 and 12 w.p.e. and extending out beyond 12 w.p.e. Moreover, the *P. leucopus* mice were exposed to infected ticks on a single occasion whereas in nature they may be repeatedly infested by *B. mayonii*-infected nymphs, potentially counteracting the decrease in infectivity over time observed in our study. A more natural scenario with continuous infestations of infected mice by uninfected larval ticks also could positively affect spirochete acquisition at later time points after infection, as shown previously for *B. burgdorferi* s.l.-infected *Apodemus* spp. mice and *Ixodes ricinus* (L.) ticks (Gern et al. 1994). Finally, because our study was limited to a single *B. mayonii* isolate, we cannot be certain how representative the results are for the enzootic transmission cycle.

The recent recognition of the human-pathogenic *B. mayonii* in the Upper Midwest has implications both for surveillance of tickborne pathogens and control of infected ticks. Surveillance for Lyme disease spirochetes in *I. scapularis* ticks and wild animals should employ assays capable of differentiating *B. burgdorferi* s.s. from *B. mayonii* (CDC 2018, Graham et al. 2018). The implication of *P. leucopus* as a reservoir for *B. mayonii* suggests that methods developed to treat this rodent species with topical acaricides to interrupt enzootic transmission of *B. burgdorferi* s.s. among *P. leucopus* and *I. scapularis* (reviewed by Eisen and Dolan 2016) should be effective against *B. mayonii*. However, as other small mammals also probably contribute to the sylvatic maintenance of *B. mayonii* (Johnson et al. 2017), additional field research is needed to determine how effectively existing rodent-targeted tick control methods will suppress this Lyme disease spirochete.

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Table 1.

Prevalence of infection with *Borrelia mayonii* in nymphal *Ixodes scapularis* ticks fed as larvae on *Peromyscus leucopus* mice with active infections

Mouse number	No. fed B. *mayonii*-infected nymphs recovered from the mouse[a]	Outcome for molted nymphs resulting from uninfected larvae fed at different time intervals after mice were exposed to infected nymphs									
		4 wk after infection					12 wk after infection				
		Detection of *B. mayonii* spirochetes from mouse ear biopsy[b]	No. fed larvae recovered	Detection of *B. mayonii* DNA in molted nymphs		Detection of *B. mayonii* spirochetes from molted nymphs[b]	Detection of *B. mayonii* spirochetes from mouse ear biopsy[b]	No. fed larvae recovered	Detection of *B. mayonii* DNA in molted nymphs		Detection of *B. mayonii* spirochetes from molted nymphs[b]
				No. positive nymphs/no. tested nymphs	Infection prevalence (95% CI)[c]				No. positive nymphs/no. tested nymphs[e]	Infection prevalence (95% CI)[c]	
42	10	+	175	32/50	0.64 (0.50–0.76)	+	+	129	12/50***	0.24 (0.14–0.37)	+
45	7	+	97	28/50	0.56 (0.42–0.69)	+	+	144	11/49***	0.22 (0.13–0.36)	+
48	3	+	146	44/50	0.88 (0.76–0.94)	+	+	77	6/50***	0.12 (0.06–0.24)	NT
49	4	+	136	36/50	0.72 (0.58–0.83)	+	+	45	17/41**	0.41 (0.28–0.57)	NT
51	1	+	81	36/50	0.72 (0.58–0.83)	+	+	52	1/49***	0.02 (0.00–0.11)	NT
52	4	+	125	47/50	0.94 (0.84–0.98)	+	+	115	7/50***	0.14 (0.07–0.26)	NT
56	9	+	200	49/50	0.98 (0.90–1.00)	+	+	162	26/50***	0.52 (0.39–0.65)	NR[f]
59	8	+	129	31/50	0.62 (0.48–0.74)	+	+	79	15/50**	0.30 (0.19–0.44)	+
60	8	+	75	16/24	0.66 (0.47–0.82)	+	+	75	28/50NS	0.56 (0.42–0.69)	+
84	7	+	87	39/50	0.78 (0.65–0.87)	+	+	51	4/41***	0.10 (0.04–0.23)	NT
41	4	+	32	17/29	0.59 (0.41–0.74)	NT[d]	NT	NT	NT	NT	NT
65	6	+	49	38/47	0.81 (0.67–0.90)	NT	NT	NT	NT	NT	NT
78	4	+	27	18/23	0.78 (0.58–0.90)	NT	NT	NT	NT	NT	NT
79	3	+	38	28/34	0.82 (0.66–0.92)	NT	NT	NT	NT	NT	NT

Mouse number	No. fed B. *mayonii*-infected nymphs recovered from the mouse[a]	Outcome for molted nymphs resulting from uninfected larvae fed at different time intervals after mice were exposed to infected nymphs									
		4 wk after infection					12 wk after infection				
		Detection of *B. mayonii* spirochetes from mouse ear biopsy[b]	No. fed larvae recovered	Detection of *B. mayonii* DNA in molted nymphs		Detection of *B. mayonii* spirochetes from molted nymphs[b]	Detection of *B. mayonii* spirochetes from mouse ear biopsy[b]	No. fed larvae recovered	Detection of *B. mayonii* DNA in molted nymphs		Detection of *B. mayonii* spirochetes from molted nymphs[b]
				No. positive nymphs/no. tested nymphs	Infection prevalence (95% CI)[c]				No. positive nymphs/no. tested nymphs[e]	Infection prevalence (95% CI)[c]	
81	3	+	16	12/16	0.75 (0.51–0.90)	NT	NT	NT	NT	NT	NT
83	6	+	52	30/50	0.60 (0.46–0.72)	NT	NT	NT	NT	NT	NT
86	8	+	33	30/32	0.94 (0.80–0.98)	NT	NT	NT	NT	NT	NT
87	3	+	42	18/37	0.49 (0.33–0.64)	NT	NT	NT	NT	NT	NT
94	7	+	54	39/50	0.78 (0.65–0.87)	NT	NT	NT	NT	NT	NT
95	6	+	31	27/28	0.96 (0.82–0.99)	NT	NT	NT	NT	NT	NT
98	10	+	24	13/22	0.59 (0.39–0.77)	NT	NT	NT	NT	NT	NT
Subset of 10 mice examined at both time points				358/474	0.76 (0.71–0.79)				127/480 ***	0.26 (0.23–0.31)	
All examined mice				628/842	0.75 (0.72–0.77)						

[a] Twenty potentially infected nymphs were placed on each mouse. Fed infected nymphs recovered from the mice may have included nymphs infected via cofeeding during the bloodmeal.

[b] +, live *B. mayonii* spirochetes detected by culture.

[c] Prevalence of *B. mayonii* infection in tested nymphs; 95% confidence intervals computed using score confidence intervals.

[d] NT, not tested.

[e] Comparison between proportions of *B. mayonii*-infected nymphs resulting from larvae fed at 4 versus 12 wk after mice were infected; likelihood ratio test:

NS $P \geq 0.05$,

* $P < 0.05$,

** $P < 0.01$,

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***$P < 0.001$.

[f]NR, no result due to contaminated culture.