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Unexpected failure of *Ixodes scapularis* nymphs to transmit a North American *Borrelia bissettiae* strain

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ABSTRACT

Globally, the *Borrelia burgdorferi* (*sensu lato*) complex comprises more than 21 species of spirochetes. Although the USA is home to a diverse fauna of Lyme disease group *Borrelia* species, only two are considered responsible for human clinical disease: *Borrelia burgdorferi* (*sensu stricto*) and *Borrelia mayonii*. However, evidence has implicated additional *B. burgdorferi* (*s.l.*) species in human illness elsewhere. While much research has focused on the *B. burgdorferi* (*s.s.*)-tick interface, tick vectors for most of the other North American Lyme disease group *Borrelia* species remain experimentally unconfirmed. In this report we document the ability of *Ixodes scapularis* to acquire but not transmit a single strain of *Borrelia bissettiae*, a potential human pathogen, in a murine infection model. Pathogen-free *I. scapularis* larvae were allowed to feed on mice with disseminated *B. burgdorferi* (*s.s.*) or *B. bissettiae* infections. Molted infected nymphs were then allowed to feed on naïve mice to assess transmission to a susceptible host through spirochete culture and qPCR throughout in ticks collected at various developmental stages (fed larvae and nymphs, molted nymphs, and adults). In this study, similar proportions of *I. scapularis* larvae acquired *B. bissettiae* and *B. burgdorferi* (*s.s.*) but transstadial passage to the nymphal stage was less effective for *B. bissettiae*. Furthermore, *B. bissettiae*-infected nymphs did not transmit *B. bissettiae* infection to naïve susceptible mice as determined by tissue culture and serology. In the tick, *B. bissettiae* spirochete levels slightly increased from fed larvae to molted and then fed nymphs, yet the bacteria were absent in molted adults. Moreover, in contrast to *B. burgdorferi* (*s.s.*), *B. bissettiae* failed to exponentially increase in upon completion of feeding in our transmission experiment. In this specific model, *I. scapularis* was unable to support *B. bissettiae* throughout its life-cycle, and while live spirochetes were detected in *B. bissettiae*-infected ticks fed on naïve mice, there was no evidence of murine infection. These data question the vector competence of *Ixodes scapularis* for *B. bissettiae*. More importantly, this specific *B. bissettiae-I. scapularis* model may provide a tool for researchers to delineate details on mechanisms involved in *Borrelia*-tick compatibility.

1. Introduction

Lyme borreliosis is a tick-borne zoonotic disease caused by spirochetes from the *Borrelia burgdorferi* (*sensu lato*) genospecies complex and transmitted by *Ixodes ricinus* complex ticks (Radolf et al., 2012). The *Borrelia* complex contains at least 21 species, 10 of which are endemic to North America (Parte, 2018; Stanek, 2018; Margos et al., 2020). Despite an estimated 300,000 cases of Lyme borreliosis in the US annually, only two of the North American *Borrelia* species (*Borrelia burgdorferi* (*s.s.*) and *Borrelia mayonii*) are widely considered infectious to humans. Of the remaining species, three (*Borrelia americana*, *Borrelia andersoni* and *Borrelia bissettiae*) have been associated with cases of human illness while the remainder species lack evidence of human pathogenicity (Franke et al., 2013; Clark et al., 2014). In North America, research has focused mainly on the known human pathogenic species, especially *B. burgdorferi* (*s.s.*). As the *Borrelia* species complex continues to expand, the pathogenic potential and vectors for newly discovered and described *Borrelia* species largely remain unknown and untested leaving a critical gap in our understanding on the epidemiology and ecology of Lyme borreliosis.

Borrelia bissettiae is an understudied species in the *B. burdgorferi* (*s.l.*) complex. The species was first isolated and initially described as an atypical *B. burgdorferi* strain in 1987 (Bissett and Hill, 1987). Postic et al.

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(1998) designated the group as a separate *Borrelia* species, and Margos et al. (2016) formally named the species *Borrelia bissettiae* based on type strain DN127. Interestingly, the range of *B. bissettiae* is quite large, and like *B. burgdorferi* (*s.s.*) can be found both in the New and Old World (Margos et al., 2011). *Borrelia bissettiae* spirochetes have been detected in states across the USA (Picken and Picken, 2000; Clark et al., 2002, 2005; Maggi et al., 2010, 2019; Goldstein et al., 2013; Salkeld et al., 2014) and in a variety of host species (Postic et al., 1998; Oliver et al., 2003; Clark et al., 2005). *Borrelia bissettiae*-like spirochetes have also been isolated from a relatively small number of human patients and detected by polymerase chain reaction in human serum, blood, skin and cardiac tissue in both the USA and Europe (Strle, 1999; Rudenko et al., 2008, 2009; Girard et al., 2011). To date, experimental work has described *B. bissettiae* disease in laboratory murine models using artificial (needle) inoculation (Schneider et al., 2008; Leydet and Liang, 2015). At least one study has investigated *B. bissettiae* transmission and reservoir competence in the laboratory mimicking western USA enzootic cycles involving the ticks *Ixodes pacificus* and *I. spinipalpis* and the mammals *Neotoma fuscipes* and *Peromyscus maniculatus* (Eisen et al., 2003). Additionally, Sanders and Oliver (1995) demonstrated the transmission of the *Borrelia* isolate MI-6 which was, at the time, considered a southern *B. burgdorferi* (*s.s.*) strain but in subsequent studies this isolate was determined to be more closely related to *B. bissettiae* strain (Lin et al., 2002).

Ixodes scapularis is arguably the most important human biting tick in the USA, serving as a vector for at least seven tick-borne pathogens. Yet despite evidence associating *B. bissettiae* with human illness (Strle, 1999; Rudenko et al., 2008, 2009, 2016; Hulinska et al., 2009; Girard et al., 2011; Golovchenko et al., 2016), continual detection of the organism in field-collected ticks (Burkot et al., 1997; Postic et al., 1998; Picken and Picken, 2000; Clark et al., 2002, 2005; Lin et al., 2002; Oliver et al., 2003; Maggi et al., 2010; Leydet and Liang, 2013; Salkeld et al., 2014; Lewis and Lloyd, 2019), and the striking similarities between *B. burgdorferi* and *B. bissettiae* infection in animal models (Eisen et al., 2003; Schneider et al., 2008; Leydet and Liang, 2015), there is a lack of understanding on *B. bissettiae* transmission and infection in a well-characterized murine borreliosis model. In an attempt to understand natural tick transmission of *B. bissettiae* in a laboratory murine model, we document the unexpected failure of *B. bissettiae*-infected *I. scapularis* to transmit and cause infection in a susceptible murine host.

2. Materials and methods

For all experiments, pathogen free 5-week-old male C3H/HeJ mice (Jackson Laboratory Bar Harbor, ME, USA) were housed in microisolator cages with HEPA filtered air, food and water provided *ad libitum*. Frozen stocks of low (≤ 3) passage *B. burgdorferi* (*s.s.*) strain B31 and *B. bissettiae* strain CO275 (Maupin et al., 1994; Leydet and Liang, 2015) were cultivated in Barbour Stoenner Kelly (BSK-H) complete media (Sigma-Aldrich, St. Louis, MO, USA) supplemented with antibiotics and fungicide as previously described (Barbour, 1984). Cultures were grown to a concentration of 10^6 spirochetes/ml, centrifuged for 10 min at 10,000× *g*, re-suspended in 100 μl of BSK-H and utilized for inoculation. For initial murine infections, 15 mice were randomly assigned to three groups consisting of five mice each. To ensure infection in all mice, a dose of 10^6 spirochetes/mouse were injected intradermally in the caudoventral region of the abdomen just right of midline with a 27G syringe in 100 μl of BSK inoculum. Mice were inoculated with either *B. burgdorferi* (*s.s.*) spirochetes, *B. bissettiae* spirochetes, or BSK-H (sham) (i.e. the three groups). Infections in mice were allowed to develop for four weeks and monitored by culturing ear punch biopsies (EPB) at 7-day intervals. *Borrelia* isolation from murine tissues (EPB, heart, urinary bladder and joint) was performed as previously described and monitored for growth weekly by darkfield microscopy for eight weeks (Sinsky and Piesman, 1989).

Pathogen-free larval *I. scapularis* were purchased from Oklahoma State University's Tick Rearing Facility (Sillwater, OK, USA) and maintained in an environmental chamber (Percival, Perry IA) with a 12:12 L:D schedule at $\geq$ 96% RH. Larval ticks were free-fed until repletion on individual mice (4 weeks post-inoculation) caged on a wire floor over water. Immediately following detachment, a subset of engorged larvae from each mouse were either subjected to culture, stored in 100% ethanol at −20 °C until DNA extraction and qPCR could be performed, or allowed to molt. Two-three weeks post-larval molt, nymphal ticks were either subjected to culture, stored in 100% ethanol at −20 °C for DNA extraction, or utilized in transmission experiments.

Borrelia isolation from ticks was performed after surface sterilization (washing in 10% bleach, phosphate-buffered saline (PBS), 70% ethanol, and rinsed in molecular grade water). Individual ticks were minced in BSK-H with a sterile scalpel and monitored for spirochetal growth weekly for eight weeks by dark field microscopy. Nymphs derived from larvae fed on infected mice were utilized to assess their ability to transmit *Borrelia* spirochetes to naïve mice. After larval molt, nymphs were allowed to rest for a minimum of two weeks to facilitate post-molt development. Fifteen mice were randomly allocated into three groups of five mice. Each mouse was infested with 20 nymphal *I. scapularis* that had previously fed on either *B. burgdorferi* (*s.s.*), *B. bissettiae* or sham inoculated mice. Upon detachment, engorged ticks were collected and either subjected to culture, stored in 100% ethanol at −20 °C for DNA extraction, or allowed to molt to the adult stage. Two-three weeks post-molt, the remaining adults were subjected to culture or DNA extraction and qPCR. EPBs were collected weekly for 6 weeks to monitor for murine infection After 6 weeks, mice were euthanized and EPB, heart, joint and bladder tissues were cultured as described above. gDNA was extracted from individual ticks following GeneElute gDNA Miniprep kit (Sigma-Aldrich, St. Louis, MO, USA) protocols. qPCR was performed in triplicate on a Roche Light Cycler 480i (Roche, Basel, Switzerland).

Spirochetes/tick were estimated by absolute quantification with the LightCycler 480i software based on the Ct-values of a single copy *Borrelia* gene (decorin binding protein B) using standard curve serial dilutions of plasmid constructs assembled following methods previously described (Zanettii et al., 2008; Leydet and Liang, 2015). Both western blot and ELISA were utilized to detect exposure to *Borrelia* spirochetes in tick-exposed mice and were performed as previously described except primary antibody (infected murine serum) was diluted 1:50 (Liang et al., 1999). Membranes were visualized on a LI-COR Odyssey CLx (LI-COR, Lincoln, NE, USA) imaging system.

Mann-Whitney U-tests were performed to compare mean spirochete burdens between tick infections, and a Fisher's exact test was performed to compare infection proportions. Statistical tests and graphs were compiled in Graphpad Prism v5 (La Jolla, California, USA).

3. Results

Larval acquisition of spirochetes from needle infected mice were similar between infections (Fisher's exact test: $P = 0.35$, OR = 2.25, 95% CI = 0.623–8.06). As shown in Table 1, 20 of the 25 tested larvae fed on *B. burgdorferi* (*s.s.*)-infected mice were culture-positive for spirochetes, an acquisition rate of 80%. In comparison, 16 of the 25 tested larvae fed on

Table 1
Spirochete prevalence and burden in replete *Ixodes scapularis* larvae fed on needle inoculated mice.

Murine infection	No. of positive ticks/No. of ticks examined	*P*-value	Mean spirochete no. per tick	*P*-value
B. bissettiae	16/25	0.35	2305	<0.0001
B. burgdorferi	20/25		18,467	

Notes: Larvae free-fed on mice infected with either *B. bissettiae* or *B. burgdorferi*. Engorged ticks were examined for spirochete acquisition rates by culture and spirochete loads by qPCR. A Fisher's exact test and Mann-Whitney *U*-test were performed to compare acquisition rates and spirochete burdens, respectively.

B. bissettiae-infected mice demonstrated spirochete growth in culture, an acquisition rate of 64%. qPCR data from fed *I. scapularis* larvae also showed similar (Fisher's exact test: $P = 0.46$, OR = 1.56, 95% CI = 0.527–4.189) infection prevalence (32/40 ticks, 80% for *B. burgdorferi* (*s.s.*) and 36/50, 72% for *B. bissettiae*). However, mean *B. burgdorferi* (*s.s.*) spirochete load was eight times higher than *B. bissettiae* (Mann-Whitney test: $U = 92.5$, $Z = -13.02$, $n_1 = n_2 = 32$, $P < 0.0001$) infected ticks (Table 1).

Based on combined culture and qPCR of replete nymphs collected from naïve mice (five mice per group), each mouse was exposed to at least six infected nymphs. All five mice exposed to the *B. burgdorferi* (*s.s.*)-infected ticks had multiple spirochete-positive tissue cultures (Table 2). In contrast, none of the five mice exposed to the *B. bissettiae*-infected ticks had evidence of infection by tissue culture (Table 2), indicating that either *I. scapularis* was unable to transmit this *B. bissettiae* strain to mice or that the mice had cleared the infection prior to tissue collection. To address these possibilities, we sought additional evidence of infection. Our previous work indicated that *B. bissettiae* infection induces a strong anti-C6 response in C3H mice (Leydet and Liang, 2015). None of the five mice that were exposed to the *B. bissettiae*-infected ticks in the present study had evidence of an anti-C6 response (Supplementary Figure S1), suggesting again that infection had not developed. Serum samples from the five mice exposed to *B. bissettiae*-infected nymphs were also analyzed by immunoblotting against *B. bissettiae* whole cell lysates in which only one mouse had a single band detected at ~33 kDa (Supplementary Figure S2), again suggesting that mice had not developed *B. bissettiae* infection.

qPCR allowed us to investigate the temporal dynamics of spirochetal burden throughout the tick's life-cycle. As depicted in Fig. 1, mean spirochete loads of *B. bissettiae* were eight times lower (Mann-Whitney test: $U = 92.5$, $Z = -13.02$, $n_1 = n_2 = 32$, $P < 0.0001$) than that of *B. burgdorferi* (*s.s.*) in engorged larvae. In molted unfed nymphs, *B. bissettiae* mean burden increased 2-fold from the larvae stage (Mann-Whitney test: $U = 124$, $Z = -5.77$, $n_1 = 32$, $n_2 = 16$, $P = 0.0032$), while *B. burgdorferi* (*s.s.*) spirochete burdens did not change (Mann-Whitney test: $U = 294.5$, $Z = -0.998$, $n_1 = 32$, $n_2 = 20$, $P = 0.6378$). Additionally, unfed *B. burgdorferi* (*s.s.*)-infected nymphs had mean spirochete burdens twice as high a *B. bissettiae*-infected nymphs (Mann-Whitney test: $U = 56.5$, $Z = -7.71$, $n_1 = 16$, $n_2 = 20$, $P = 0.0006$). A much more dramatic difference was observed when spirochete levels from engorged nymphs from each infection cohort were analyzed immediately after detachment from naïve mice. While mean *B. bissettiae* spirochete burdens showed a significant, albeit modest, 13% increase from unfed to engorged nymph (Mann-Whitney test: $U = 70.5$, $Z = -5.65$, $n_1 = 16$, $n_2 = 39$, $P < 0.0001$), the mean number of *B. burgdorferi* spirochetes increased 45-fold (Mann-Whitney test: $U = 0$, $Z = 13.85$, $n_1 = 20$, $n_2 = 39$, $P < 0.0001$) after nymphal blood-meal. This resulted in engorged nymphs harboring 100 times more *B. burgdorferi* (*s.s.*) spirochetes, on average, than *B. bissettiae* (Mann-Whitney test: $U = 6.5$, $Z = -19.21$, $n_1 = 39$, $n_2 = 39$, $P < 0.0001$) (Fig. 1 and Supplementary Table S1). After nymphs molted to adults, *B. bissettiae* was undetectable by qPCR and culture (Supplementary Table S1), suggesting *B. bissettiae* was unable to survive molt from nymph to adults. In contrast, *B. burgdorferi* (*s.s.*) load in adult ticks remained unchanged (Mann-Whitney test: $U = 296$, $Z = -2.05$, $n_1 = 39$, $n_2 = 18$, $P = 0.3528$).

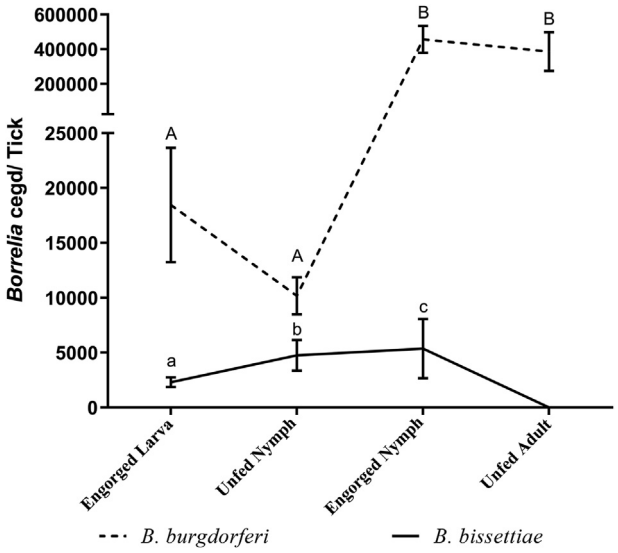

Fig. 1. Spirochete burdens in infected tick stages as determined by qPCR. Whole ticks at each developmental stage were analyzed by qPCR to determine spirochete burdens (exact numbers tested can be found in Supplementary Table S1). Data are presented as *Borrelia* copies of extracted gDNA (cegd) per tick. A Mann-Whitney *U*-test was performed to compare between spirochete burdens at time points. Burdens at each tick life stage between species were significantly different ($P < 0.01$). Letters denote statistical differences between tick life stages. Error bars represent standard error of the mean (SEM).

Table 2
Spirochete growth in tissue cultures from mice infested with *Borrelia*-infected nymphal ticks.

Tick infection	No. of positive specimens/No. of samples examined					
	Ear biopsy	Skin	Heart	Bladder	Joint	No. of mice infected/No. of mice inoculated
B. bissettiae	0/5	0/5	0/5	0/5	0/5	0/5
B. burgdorferi	5/5	5/5	5/5	2/5	5/5	5/5

Notes: Groups of 5 mice each were challenged with 20 nymphs that were previously collected from mice infected with either *B. bissettiae* or *B. burgdorferi*. Infection was monitored by weekly ear biopsy. Four weeks after tick feeding, mice were sacrificed, and tissues were cultured for spirochetes.

4. Discussion

Despite biological and ecological similarities between *B. bissettiae* and *B. burgdorferi* (*s.s.*) only two previous studies have investigated *B. bissettiae* transmission in controlled tick-mammalian host models. Eisen et al. (2003) described the ability of both *I. pacificus* and *I. spinipalpis* (both distributed in the Western USA) to acquire *B. bissettiae* (two tick isolates from CA and two woodrat isolates from CO) from and transmit to naïve dusky-footed woodrats and deer mice. Interestingly, the authors describe very low acquisition rates (as determined by examination of molted nymphs) of both spirochete species in both tick species from needled inoculated mice (*Mus musculus* CD-1 and ICR and *P. maniculatus*). In contrast, larval ticks fed on rodents infected through tick-bite were more likely to result in infected nymphal ticks. Furthermore, Sanders and Oliver (1995) described the ability of *I. scapularis* to acquire (from hamsters) and transmit a southern *B. bissettiae* isolate (MI-6) to laboratory mice and cotton rats, albeit at significantly lower rates when compared to a northern *B. burgdorferi* (*s.s.*) isolate.

In contrast to previous studies, we were unsuccessful in demonstrating natural transmission of a North American strain of *B. bissettiae* from infected ticks to naïve hosts. Although the proportion of larval ticks that acquired spirochetes from an infected host did not differ between *Borrelia* species, the number of spirochetes in the tick post-larval blood-meal was significantly lower in *B. bissettiae*-exposed ticks. These findings do support results in at least one surveillance study that detected no *B. bissettiae* in *I. scapularis* or *I. pacificus* from multiple regions of the USA while identifying *B. bissettiae* in 31% of tested *Ixodes affinis* ticks (Maggi et al., 2019).

To date, specific tick/*Borrelia* species and strain compatibility is a highly understudied topic, and most research involving the exact mechanisms behind tick acquisition and transmission of *Borrelia* spirochetes has focused solely on interactions between *B. burgdorferi* (*s.s.*) and

I. scapularis. While evidence supports the "generalist" nature of *I. scapularis* in its ability to transmit a variety of geographically and genetically diverse *Borrelia* species and strains (Sanders and Oliver, 1995; Dolan et al., 1997, 1998, 2017; Piesman and Happ, 1997; Jacobs et al., 2003; Derdáková et al., 2004), experimental studies with *B. burgdorferi* (*s.s.*) in non-vector ticks demonstrate absent or severely inefficient transstadial transmission from larvae to nymph (Mather and Mather, 1990; Ryder et al., 1992; Lane et al., 1994; Dolan et al., 1997; Piesman and Happ, 1997; Soares et al., 2006). In this study, transstadial survival from larvae to nymphs was similar between the two *Borrelia* species yet was not seen for *B. bissettiae* during molt from nymph to adult. To our knowledge, life-stage-specific defects in transstadial transmission have not been described in ticks or other vector systems. Mutagenesis studies have identified *B. burgdorferi* (*s.s.*) plasmids and genes that are essential to tick midgut colonization as well as transmission during nymphal feeding (reviewed in Kung et al., 2013 and Caimano et al., 2016). Although we employed a low culture passage, original isolate of *B. bissetiae* CO275, it did have to be passed through BALB/c scid mice to recover an infectious phenotype, so there remains the question of whether this isolate is deficient in genes and/or plasmids required for efficient colonization, replication and transmission in *I. scapularis*. Unfortunately, detailed plasmid profiling of the isolate was not conducted as part of this study; however, we did confirm the presence of major plasmids and orthologs associated with *B. burgdorferi* (*s.s.*) infectivity in this isolate in a previous study (Leydet and Liang, 2015). While unlikely, this isolate may lack important genetic elements required for successful transmission in the tick. Conducting a 'complete' plasmid profile of this isolate was outside of the scope of this study but certainly would be warranted. Unfortunately, plasmid presence and gene organization can significantly vary both between and within species from the *B. burgdorferi* (*s.l.*) complex (Casjens et al., 2012, 2018). To date only a single *B. bissettiae* genome has been sequenced and annotated, the type-strain DN127 (Schutzer et al., 2012). Moreover, DN127 at time of sequencing was described as only 'low passage' and has yet to be tested for its infectivity.

After successfully generating cohorts of *B. bissettiae* and *B. burgdorferi* (*s.s.*)-infected nymphs, we attempted a natural transmission experiment to naïve mice. *Borrelia burgdorferi* (*s.s.*)-infected nymphs successfully infected all naïve mice, whereas mice exposed to *B. bissettiae*-infected nymphs developed no apparent infection, as indicated by serology and tissue culture. This lack of transmission was surprising considering live spirochetes were recovered by culture from *B. bissettiae*-infected nymphs fed on all five naïve mice. True refractory ticks rarely have detectable bacteria even during infectious blood-meals (Soares et al., 2006). Spirochete burdens (as estimated by qPCR) showed that *B. bissettiae*-infected ticks had a modest increase in spirochetes throughout the tick's development cycle until the adult stage where none were detected. In contrast, *B. burgdorferi* (*s.s.*) spirochete levels in infected ticks were not significantly different from larval acquisition through molt to the nymphal stage. The most striking difference was the exponential increase in spirochete burdens in fed *B. burgdorferi* (*s.s.*)-infected nymphs after nymphal blood-meal, which was not detected in *B. bissettiae*-infected nymphs. Overall, at each developmental stage *B. bissettiae* spirochete burdens were significantly lower than *B. burgdorferi* (*s.s.*) levels in ticks, with the most dramatic differences occurring in replete nymphs (~100-fold difference) and molted adults. Admittedly the *B. burgdorferi* (*s.s.*) strain used in this report (B31) is known to colonize at high levels in both ticks and mammals; however, the exponential increase in spirochetes during feeding has been demonstrated in other *B. burgdorferi* (*s.s.*) strains (i.e. JD1) (Dunham-Ems et al., 2009).

In this study, unexpectedly, mice exposed to *B. bissettiae*-infected ticks did not develop infection. One mouse showed evidence of antibodies to *B. bissettiae via* western blot, which may indicate exposure to live spirochetes; however, this single band at low serum dilution may be a result of cross-reaction, a phenomenon seen in human sera collected from non-endemic countries and regions of the USA (Burkot et al., 1997; Lantos et al., 2015). Host immune system evasion is a critical step for microbes to establish in a host. A potential explanation for the lack of transmission in our study is that the murine innate immune system may have cleared initial injection of *B. bissettiae* spirochetes before the adaptive system was activated. Utilizing immunodeficient mice in future experiments could help confirm this as true exposure. Future work could also assess bacterial presence at the tick-bite site through multiple time points during tick feeding.

Results from this report indicate that naïve mice were likely never infected with *B. bissettiae* spirochetes after exposure to infected *I. scapularis*. The lack of exponential increase in spirochete numbers in *B. bissettiae*-infected nymphs during feeding is an interesting finding that deserves follow up. The exponential increase we observed in *B. burgdorferi* (*s.s.*)-infected ticks is hypothesized to be an essential step in the spirochete penetration of the tick's midgut, a major barrier to *Borrelia* transmission (De Silva and Fikrig, 1995; Dunham-Ems et al., 2009). Lack of this in *B. bissettiae*-infected ticks may explain their inability to transmit. *Borrelia* replication defects in the tick have been described in several experimental studies (all employing *B. burgdorferi* (*s.s.*) strains) and were related to the presence and ability to utilize various nutrient resources (i.e. transition metals, glycerol, purines, etc.) (Jewett et al., 2009; Ouyang et al., 2009; Pappas et al., 2011). Because *Borrelia* spirochetes are almost completely dependent on their environment for nutrients, any defect in genes or pathways involved in transport or scavenging of these essential nutrients may result in severe phenotypic changes. However, overall lower spirochete burdens in *B. bissettiae*-infected ticks at all stages suggest multiple factors may also contribute. For example, in other vector competence studies needle inoculation of animals results in decreased spirochete acquisition and transstadial transmission in the tick (Gern et al., 1993; Shih and Liu, 1996). Additionally, *Borrelia* species strain to strain variation in infectivity to mammals and ticks has also been described (Lane et al., 1994; Dolan et al, 1997, 1998; Wang et al., 2002; Eisen et al., 2003; Derdáková et al., 2004; Baum et al., 2012). Moreover, acquisition and colonization of *B. bissettiae* spirochetes in this study may have been influenced by the presence of anti-OspA antibodies, which could develop as a result of parenteral delivery of 10^6 spirochetes (Fikrig et al., 1992; Pal et al., 2004). Lastly, multiple groups have highlighted the importance of using ticks, pathogens, and hosts from a common geographical source to avoid potential problems with compatibility (Lane et al., 1994; Eisen et al., 2003, 2019). The *B. bissettiae* isolate used in our study was isolated from an *I. spinipalpis* collected off a woodrat in Colorado (Maupin et al., 1994). We chose this isolate because it is low passage, closely related to the *B. bissettiae* type-strain (DN127) and known infectious to laboratory mice. Despite the ability of *I. scapularis* to transmit various geographical isolates of *B. burgdorferi* (*s.s.*), geographical differences resulting from historical evolutionary/adaptive processes could explain compatibility issues described in this report (Becker et al., 2016). The relationship between microbe and host is complex and evolves over time. The peculiar *B. bissettiae* dynamics in *I. scapularis* we describe in this study may represent a unique time in the evolution of the bacteria and/or its vector tick. Margos et al. (2019) reviewed the suspected evolutionary history of *Borrelia bavariensis* in Europe. *Borrelia bavariensis* is very closely related to *Borrelia garinii* from which it appears to have diverged due to a switch in vector and host. *Borrelia bissettiae* is one of the closest related species to *B. burgdorferi* (*s.s.*) and likely utilizes different vectors in areas of sympatry. Field studies suggest *B. bissettiae* enzootic cycles in the Eastern and Southern USA rely on a different tick-vector such as *Ixodes affinis* (Oliver et al., 1987, 2003; Clark et al., 1998, 2001; Lin et al., 2002; Harrison et al., 2010; Nadolny et al., 2011). Studies have detected *B. bissettiae* in these ticks (Maggi et al., 2010, 2019) yet experimental transmission has not been assessed. Unlike *B. bavariensis*, *B. bissettiae* spirochetes can be found across the USA and in many regions of Europe. Because *B. bissettiae* will ultimately utilize different hosts and vectors across this range, it is likely that there exist evolutionary driven compatibilities between bacteria, tick, and host across the spirochetes range.

5. Conclusions

As we continue to uncover the genetic complexity of the *B. burgdorferi* (*s.l.*) complex we will also begin to appreciate and hopefully understand variations in vector/reservoir competence and susceptibility between ticks, hosts, and *Borrelia* species/strains. It is important to point out that studies like ours involving a single isolate are likely not generalizable to the infectivity of all the numerous strains within the *B. bissettiae* and *B. burgdorferi* (*s.s.*) groups. This notion is evidently clear in a recent scoping review of tick and *Borrelia* competence studies (Eisen, 2019). Combining our results with others is the first step in understanding the complexity of *Borrelia* genotype-tick and -host compatibility. Additionally, we describe an intriguing temporal phenomenon between an infectious and well-characterized *B. bissettiae* strain and *I. scapularis* ticks. Assessing the vector competence of newly described *Borrelia* species should be an area of continued study. While it appears that multiple factors may contribute to our observation that *B. bissettiae*-infected ticks were unable to transmit infection to susceptible hosts, the exact mechanism(s) remains unknown. With the advent of technologies like next-generation sequencing and advanced microscopy techniques, this "transmissionless" tick infection model may provide an important glimpse into questions of *Borrelia* vector competence and compatibility.

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Ethical approval

All animal research was approved by Louisiana State University's Institutional Animal Care and Use Committee (#14-028).

CRediT author statement

Brian Leydet: Conceptualization, Methodology, Validation, Formal Analysis, Investigation, Data Curation, Writing-Original Draft, Writing-Review & Editing, Funding acquisition, Visualization. Fang-Ting Liang: Formal Analysis, Investigation, Resources, Writing-Original Draft, Supervision, Project administration, Funding acquisition.

Declaration of competing interests

Brian F. Leydet declares no competing interests. Fang-Ting Liang has a financial interest in the C6 peptide.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crpvbd.2021.100039.

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