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REFRACTORINESS OF THE WESTERN FENCE LIZARD (*SCELOPORUS OCCIDENTALIS*) TO THE LYME DISEASE GROUP SPIROCHETE *BORRELIA BISSETTII*

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ABSTRACT: The western fence lizard, *Sceloporus occidentalis*, is refractory to experimental infection with *Borrelia burgdorferi* sensu stricto, one of several Lyme disease spirochetes pathogenic for humans. Another member of the Lyme disease spirochete complex, *Borrelia bissettii*, is distributed widely throughout North America and a similar, if not identical, spirochete has been implicated as a human pathogen in southern Europe. To determine the susceptibility of *S. occidentalis* to *B. bissettii*, 6 naïve lizards were exposed to the feeding activities of *Ixodes pacificus* nymphs experimentally infected with this spirochete. None of the lizards developed spirochetemias detectable by polymerase chain reaction for up to 8 wk post-tick feeding, infected nymphs apparently lost their *B. bissettii* infections within 1–2 wk after engorgement, and xenodiagnostic *I. pacificus* larvae that co-fed alongside infected nymphs did not acquire and maintain spirochetes. In contrast, 3 of 4 naïve deer mice (*Peromyscus maniculatus*) exposed similarly to feeding by 1 or more *B. bissettii*-infected nymphs developed patent infections within 4 wk. These and previous findings suggest that the complement system of *S. occidentalis* typically destroys *B. burgdorferi* sensu lato spirochetes present in tissues of attached and feeding *I. pacificus* nymphs, thereby potentially reducing the probability of transmission of these bacteria to humans or other animals by the resultant adult ticks.

Borrelia bissettii is 1 of 3 members of the Lyme disease spirochete complex, *B. burgdorferi* sensu lato (s.l.), that have been reported from North America (Postic et al., 1998). The others include the apparently nonpathogenic *B. andersonii* (Marconi et al., 1995) and the human pathogen, *B. burgdorferi* sensu stricto (s.s.) (Burgdorfer et al., 1982; Johnson et al., 1984). The first isolate of *B. bissettii*, designated DN127, was obtained from an adult western blacklegged tick, *Ixodes pacificus*, collected from vegetation in northern California (Bissett and Hill, 1987; Bissett et al., 1988). Since then, *B. bissettii* has been isolated from, or detected in, *Ixodes* spp. ticks; many species of mammals, principally rodents; and a few lizards in North America (Anderson et al., 1990; Postic et al., 1998; Burkot et al., 2000, 2001; Picken and Picken, 2000; Schneider et al., 2000; Lin et al., 2001, 2002, 2003, 2004; DeNatale et al., 2002; Eisen et al., 2003; Oliver et al., 2003; Clark, 2004; Clark et al., 2005; Vredevoe et al., 2004; Lane, Mun et al., 2005; Lane, Mun, Parker, and White, 2005). In Europe, spirochetes closely resembling *B. bissettii* have been isolated from human clinical specimens from Slovenian patients diagnosed with Lyme disease (Picken et al., 1996; Strle et al., 1997; Maraspin et al., 2002), and *B. bissettii* has been detected by polymerase chain reaction (PCR) in a questing *Ixodes ricinus* tick in Slovakia (Hanincová et al., 2003).

Ixodes pacificus is an efficient experimental vector of *B. bissettii* (Eisen et al., 2003), and, in California, host-seeking *I. pacificus* nymphs and adults occasionally have been found to be infected naturally with this spirochete (Eisen, Eisen, Chang, et al., 2004; R. N. Brown, M. A. Peot, and R. S. Lane, pers. obs.). The western fence lizard, *Sceloporus occidentalis*, a primary host of *I. pacificus* subadults in many biotopes (Lane and Loye, 1989; Manweiler et al., 1992; Tälleklint-Eisen and Eisen, 1999; Eisen et al., 2001, 2004a, 2004b; Casher et al., 2002), is refractory to infection with *B. burgdorferi* s.s. (Lane, 1990; Lane and Quistad, 1998). The source of this refractoriness, the alternative complement pathway of the innate immune system (Kuo et al., 2000), ultimately destroys spirochetes present in the midgut diverticula of attached nymphal ticks during or after imbibition of the bloodmeal (Lane and Quistad, 1998). This phenomenon, known as zooprophylaxis (Spielman et al., 1985), may reduce the force of transmission of *B. burgdorferi* s.s. to humans by decreasing the prevalence of spirochete infection in the resultant adult ticks (Clover and Lane, 1995; Lane and Keirans, 1999; Eisen, Mun et al., 2004).

Because *I. pacificus* nymphs are apt to attach to *S. occidentalis* in habitats wherever this lizard abounds, we sought to determine, first, whether *B. bissettii*-infected *I. pacificus* nymphs are capable of infecting naïve western fence lizards; second, if such nymphs may lose their infections as a result of having fed on naïve lizards; and third, whether infected nymphs can serve as a source of infection for noninfected *I. pacificus* larvae by means of co-feeding transmission. The latter assay was included to ascertain if co-feeding larvae could become infected in either the presence, or absence of, a demonstrable systemic infection in their lizard hosts.

MATERIALS AND METHODS

Collection and maintenance of preimmune lizards

Six juvenile or adult western fence lizards were collected on 5 dates between 10 February and 11 September 2002 by slip-noosing at Point Isabel Regional Shoreline, a recreational area abutting San Pablo Bay in Richmond, California. Lizards collected there previously were found to be uninfested with *I. pacificus* subadults (Lane and Quistad, 1998). Notwithstanding, each lizard was examined for presence of ticks and other mites, its age ascertained by measuring its snout-to-vent length, i.e., lizards having a snout-to-vent length of <60 mm were considered to be juveniles, and its gender determined. None of the lizards was parasitized by acarines, irrespective of the season of capture. Lizards were maintained separately inside 13- × 48-cm cages having a sandy substrate, a 12:12 light-dark photoperiod, and a temperature regime of ≈27–31 C supplied by overhead lighting (Repti Halogen basking lamps, Zoo Med Laboratories, San Luis Obispo, California) for 4–11 mo. Their diet was made up of crickets and mealworms coated with vitamin dust, and water was provided ad libitum. At the time of experimentation, lizard 1 and lizards 2–5 were adults (male and females, respectively), and lizard 6 was a juvenile (female).

Before potentially infected nymphs were placed on lizards, each an-

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imal was anesthetized with isoflurane (Abbott Laboratories, North Chicago, Illinois) and then at least 10 μl of blood were collected retro-orbitally from adult lizards with a 70-μl-capacity heparinized, microhematocrit capillary tube (Fisher Scientific, Pittsburgh, Pennsylvania) or with a 50-μl-capacity "microcap" tube (Drummond Scientific, Broomall, Pennsylvania) for juvenile lizards. Blood samples were dispensed immediately into 1.0 ml of EDTA tubes (Terumo Medical Corp., Elkton, Maryland) and frozen at −80 C until they were tested by PCR for presence of *B. burgdorferi* s.l. According to the manufacturer (QIAGEN, Chatsworth, Massachusetts), the DNA extraction and purification kit we used (see below) removes potential PCR inhibitors such as heparin or EDTA.

Experimental infection of nymphs with *B. bissettii*

To experimentally infect cohorts of *I. pacificus* nymphs, noninfected larvae were fed xenodiagnostically on 5 laboratory-reared adult male deer mice (*Peromyscus maniculatus*) that had been infected with *B. bissettii* by *I. spinipalpis* nymphs. The latter ticks had fed as larvae on 2 naturally infected dusky-footed wood rats (*Neotoma fuscipes*) collected at the University of California Hopland Research and Extension Center in Mendocino County (Eisen et al., 2003). Spirochetal isolates from these wood rats were determined to be *B. bissettii* by restriction digestion of the *rrf* (5S)-*rrl* (23S) intergenic spacer region, amplification by PCR, and sequencing of the *rrf-rrl* spacer region by using previously described methods (Postic et al., 1998; Lane et al., 2004). In the present study, the *I. pacificus* larvae were derived from 2 egg masses determined to be free of *B. burgdorferi* s.l. by testing 50 larvae per mass in 5 pools of 10 larvae each by PCR.

After detaching from the 5 deer mice, fed larvae were held inside plastic vials at a relative humidity of 98% until they molted to nymphs. Next, an aliquot of 10 nymphs from each experimentally infected cohort was assayed for *B. bissettii* infection by PCR to determine the approximate prevalence of infection. The infection prevalence for each of the different nymphal cohorts put on lizards usually was 40 or 50%, except for lizard 2, which was exposed to a mixture of 31 nymphs selected in a convenience sample from 3 cohorts having an infection prevalence of 10% (n = 24), 40% (n = 3), or 50% (n = 4). It was impossible to use a single cohort of infected nymphs for these trials or those involving mice (see below) because too few nymphs were available per cohort.

Transmission experiments

Six naïve lizards were each exposed to an average of 18 (range, 15–31) *I. pacificus* nymphs potentially infected with *B. bissettii* inside separate muslin bags for up to 24 hr. Next, lizards were held individually inside cages modified from standard mouse cages (18 × 28 cm) with a tray of water below until attached ticks (n = 49) had fed fully and dropped off. Because of technical error, only 37% of these nymphs were preserved in 95% ethanol within 1–2 wk and assayed later for *B. bissettii* infection by PCR.

To determine whether lizards exposed to feeding by spirochete-infected nymphs can serve as a source of spirochete infection for noninfected larvae by co-feeding transmission, and whether fed xenodiagnostic larvae can maintain the infection transstadially, ≈50 to 85 spirochete-free larvae were placed on each lizard 4 days before they were exposed to the nymphs. For lizard 2, however, none of the larvae or nymphs attached successfully. Therefore, a second batch of nymphs (n = 31) was put on this lizard 1 wk before it was exposed to more larvae (≥80). About 30% of the fed larvae (n = 394) that detached from all lizards were preserved in 95% ethanol within 1–2 wk and assayed subsequently for borrelial infection by PCR. Of the remainder, 73 larvae that survived the transstadial molt were preserved similarly within 4 to 6 wk postmolting and tested eventually by PCR.

Lizards were bled retro-orbitally 2, 4, and 8 wk after all potentially infected nymphal ticks had detached from them. Blood specimens were processed and stored like those taken pretick exposure until they could be tested by PCR.

Controls

We used the deer mouse, a competent experimental host of *B. bissettii* (Eisen et al., 2003), as a positive control to validate that some of the *I. pacificus* nymphs from the same *B. bissettii*-infected nymphal cohorts placed on lizards indeed contained infectious spirochetes. Naïve deer mice were each exposed to either 15 or 16 potentially infected nymphs inside 2 cork feeding capsules (9.5 mm in height × 8.0 mm in width) affixed to the shaven, dorsal surface of the head with Krazy Glue® (Elmer's Products, Columbus, Ohio). For mice 1 and 3, the infection prevalence of the experimentally infected nymphal cohorts put on them was 40%, whereas those for mice 2 and 4 ranged from 10 to 50%. One and 15 of the nymphs put on mouse 2 were from cohorts that had overall respective infection prevalences of 40 and 50%, whereas 7 and 8 of the nymphs placed on mouse 4 were from cohorts that had infection prevalences of 10 and 40%, respectively. Ear-punch biopsies (2 mm, n = 2 per animal) obtained from mice pre- and 4 wk postnymphal infestation, respectively, were assayed for infection by PCR. Because mouse #1 tested negative at 4 wk, additional biopsies and a blood specimen taken at 8 wk post-tick exposure also were tested by PCR.

DNA extraction and PCR

Fed and unfed ticks, and ear-punch biopsies, were stored in 95% ethanol before DNA extraction. All ticks and tissue biopsies were tested individually. For the tick xenodiagnosis, however, 50 *I. pacificus* larvae originating from a single egg mass were tested initially in 10 pools of 5 each to verify that they were spirochete-free before other larvae from the same mass were placed on lizards. Blood specimens from lizards and mice were stored in 500-μl EDTA tubes (Terumo Medical Corp.) at −80 C before DNA extraction.

DNA was extracted using the DNeasy tissue kit (QIAGEN) according to the manufacturer's instructions. For preparing DNA extracts from blood samples, either 10 μl (lizards) or 50 μl (mice) of blood was used. Samples stored in 95% ethanol were soaked in double-distilled H_2O for 10 min before they were ground in 180 μl of buffer ATL. The DNA was eluted in a final volume of 100 μl of AE buffer. Presence of borreliae in ticks, tissue biopsies, or blood was determined by PCR by using primer sets targeting the 5S–23S rRNA intergenic spacer region (Lane et al., 2004). PCR reaction assays used 3 μl of each tick DNA extract as a template in a total reaction volume of 25 μl. All PCR reaction mixtures contained 2.5 μl of 10× PCR buffer (Applied Biosystems, Foster City, California), 2.5 μl of 8 mM dNTPs, 1.5 μl of 25 mM $MgCl_2$, 1 μl of 10 μM primers, and 0.2 μl of 5 units/μl Taq polymerase (Applied Biosystems). Cycling conditions involved an initial 4-min denaturation at 94 C followed by 40 amplification cycles, each consisting of a 40-sec denaturation at 94 C, a 40-sec annealing at 52 C for the first and 58 C for the second amplification, and a 1-min extension at 72 C. These cycles were followed by a 10-min extension at 72 C. Positive controls (known infected tick DNA extract) and negative controls (autoclaved distilled water) were included with each PCR run.

RESULTS

Susceptibility of *S. occidentalis* to *B. bissettii*

Six naïve *S. occidentalis* lizards were fed upon by a mean ± SD of 8.2 ± 3.7 (range, 4–14) nymphs potentially infected with *B. bissettii* (Table I). Forty-nine (46%) of 107 nymphs placed on all 6 lizards fed fully and detached. Of these, 18 replete nymphs (range, 2–7 per lizard) preserved 1–2 wk postdetachment tested negative for *B. bissettii* by PCR. The probability that all 18 of the replete nymphs actually tested, and found to be spirochete free, would have been uninfected before feeding was evaluated with the bionomial probability distribution. Assuming a prefeeding infection prevalence of 30%, which was more modest than what was detected by PCR (Table I), the probability that none of the 18 fed nymphs tested contained spirochetes before attaching to lizards was only 0.0016. Thus, it is highly improbable that all 18 nymphs were uninfected with *B. bissettii*.

Whole-blood specimens obtained retro-orbitally from each lizard 2, 4, and 8 wk post-tick feeding likewise tested negative for *B. bissettii* by PCR. Moreover, 113 xenodiagnostic *I. pacificus* larvae that had fed alongside nymphs on all 6 lizards test-

TABLE I. Results of attempts to experimentally infect naïve western fence lizards by exposing them to feeding by *Borrelia bissettii*-infected *Ixodes pacificus* nymphs.

	Nymphs put on lizards			Infection status of lizards postnymphal tick exposure or of xenodiagnostic ticks as determined by PCR		
Lizard no.	Total no.	Infection prevalence (%)	No. fed fully (%)*	Lizard blood	No. replete xenodiagnostic larvae (pos./no. tested)	No. xenodiagnostic larvae after molting to nymphs (pos./no. tested)
1	16	50	14 (88)	Negative	0/20	0/10
2	31	10–50	9 (29)	Negative	0/20	0/10
3	15	40	4 (27)	Negative	0/15	0/3
4	15	50	7 (47)	Negative	0/19	0/10
5	15	50	10 (67)	Negative	0/21	0/10
6	15	50	5 (33)	Negative	0/18	0/30

* Eighteen of these replete nymphs (range, 2–7 per lizard) were assayed for *B. bissettii* with negative results.

ed negative for *B. bissettii* infection, as did 73 recently molted nymphs that had fed as larvae on the same lizards (Table I).

Peromyscus controls

In contrast to lizards, deer mice were highly susceptible to experimental infection with *B. bissettii* (Table II). Three of 4 mice fed upon by at least 1 to 5 *B. bissettii*-infected *I. pacificus* nymphs developed patent infections within 4 wk. The 1 mouse (1) that did not become infected had been fed upon by a single infected nymph; ear-punch biopsies excised from this animal at 4 and 8 wk and blood drawn from it 8 wk post-tick exposure were PCR negative. Spirochetes were detected in 10 (67%) of 15 blood-fed nymphs that had fed on mice.

DISCUSSION

The interrelations of *B. bissettii*, *S. occidentalis*, and *I. pacificus* mirror those of *B. burgdorferi* s.s. with the same species of lizard and vector tick. Previously, the western fence lizard was found to be refractory to experimental infection with *B. burgdorferi* s.s., and *I. pacificus* nymphs containing this bacterium lost their infections after having fed on naïve lizards and molting to adult ticks (Lane, 1990; Lane and Quistad, 1998). In the present study, 6 naïve lizards exposed to the feeding activities of up to ≈8 *B. bissettii*-infected *I. pacificus* nymphs, i.e., estimated from the number of nymphs that had fed on them times the infection prevalence of the nymphal cohorts from which they had been derived, apparently did not become infected. Eighteen engorged nymphs that had fed on all 6 lizards were spirochete free 1–2 wk after detaching, and noninfected *I. pacificus* larvae that had fed alongside infected nymphs on lizards did not acquire and maintain *B. bissettii*.

TABLE II. Results of attempts to experimentally infect naïve deer mice by exposing them to feeding by *Borrelia bissettii*-infected *Ixodes pacificus* nymphs.

	Nymphs put on mice				
Mouse no.	Total no.	Infection prevalence (%)	No. fed fully (%)	No. fed that were pos./no. tested	Infection status of mice post-tick exposure
1	15	40	1	1/1	Negative*
2	16	40–50	5	5/5	Positive
3	15	40	3	3/3	Positive
4	15	10–40	8	1/6	Positive

* As determined by PCR testing of ear-punch biopsies taken 4 and 8 wk post-tick exposure and of a blood specimen taken 8 wk post-tick exposure.

Although only 37% of the 49 potentially infected nymphs that had fed on lizards were tested, all of them were PCR negative, which is consistent with previous findings demonstrating that nonimmune serum from this reptile is highly lytic for *B. bissettii* and *B. burgdorferi* s.s. (Lane and Quistad, 1998; Kuo et al., 2000; Ullmann et al., 2003). Accordingly, 100% and 98% of 2 *B. bissettii* strains (CO-501 and DN127) were immobilized or lysed within 2 hr after their inoculation into 50% lizard test-serum (Ullmann et al., 2003), and ≈98 to 100% of 2 *B. burgdorferi* s.s. strains (CA4 and B31) injected into lizard serum at concentrations of 50 or 80% usually died within 1–2 hr (Lane and Quistad, 1998; Kuo et al., 2000; Ullmann et al., 2003). Comparable findings have been reported for the southern alligator lizard, *Elgaria multicarinata*, another lizard that is infested abundantly with *I. pacificus* subadults in some Lyme disease endemic foci in California (Wright et al., 1998; Kuo et al., 2000).

The actual mechanism underlying the apparent destruction of *B. bissettii* in the tissues of infected *I. pacificus* nymphs as a result of feeding on western fence lizards is not known, but most likely it is due to the borreliacidal activity of proteins making up the alternative complement pathway of the reptilian innate immune system. Previously, complement-mediated killing of *B. burgdorferi* s.s. was suggested when this spirochete was killed soon after it had been injected into nonimmune serum from the western fence lizard and the southern alligator lizard; this lytic activity was abolished by preheating the serum first (Lane and Quistad, 1998; Kuo et al., 2000; Ullmann et al., 2003). Two other in vitro assays, in which lizard sera were treated with either EDTA or EGTA/$MgCl_2$ before spirochetes were introduced, established that complement proteins generally, and alternative-complement proteins specifically, were the source of the observed lytic activity (Kuo et al., 2000). The complement system, which is involved in many biological, in-

flammatory, and noninflammatory processes, plays an important role in the innate immunity against invading pathogens by opsonizing them and thereby facilitating their phagocytosis (Inal, 2004). Furthermore, the putative reservoir competence of different species of wildlife and domesticated animals has been found to be dependent upon the sensitivities of different genospecies of *B. burgdorferi* s.l. to complement proteins (Kurtenbach et al., 1998; Kuo et al., 2000; Nelson et al., 2000).

In contrast, the deer mouse proved to be an efficient host of *B. bissettii*, thereby corroborating that some of the *I. pacificus* nymphs put on both lizards and mice contained infectious spirochetes. Deer mice exposed to the feeding activities of as few as 1 *B. bissettii*-infected *I. pacificus* nymph develop patent infections (Eisen et al., 2003; present study), and wild deer mice occasionally are infected with *B. bissettii* in California (R. N. Brown, M. A. Peot, and R. S. Lane, pers. obs.). Not surprisingly, nonimmune serum from 2 species of deer mice (*Peromyscus* spp.) was not lytic for *B. bissettii*, *B. burgdorferi*, or both (Lane and Quistad, 1998; Kuo et al., 2000; Ullmann et al., 2003).

To determine whether co-feeding transmission could occur, we exposed lizards to xenodiagnostic larvae either 4 days before (lizards 1, 3–6), or 7 days after (lizard 2), placement of nymphs. Virtually all *I. pacificus* subadults that feed on this lizard do so in proximity within the lateral nuchal pockets (Lane and Loye, 1989), which are small pouchlike structures composed of fine scales located anterior to each foreleg. Because it took larvae, on average, 12.0 days (range, 7–17 days; n = 374) and nymphs 11.8 days (range, 9–15 days; n = 49) to feed to repletion, detach, and drop off the host (data not shown), most larvae and nymphs fed concurrently for ≈5 to 8 days. Prolonged feeding by *I. pacificus* larvae on laboratory-maintained western fence lizards has been noted previously (Lane, 1994) and doubtless is a consequence of the lizards' poikilothermic physiology. Co-feeding transmission and transstadial passage of spirochetes did not occur because zero of 186 fed xenodiagnostic larvae or nymphs that had molted from them contained *B. bissettii* (Table I).

Efficient co-feeding transmission of closely related *B. burgdorferi* s.l. spirochetes (reported as *B. burgdorferi* s.s.) occurs between infected and noninfected *Ixodes persulcatus* or *Ixodes ricinus* ticks while feeding on various small-to-large mammals, such as sika deer (Kimura et al., 1995), laboratory mice (Gern and Rais, 1996), and domestic sheep (Ogden et al., 1997). Alternatively, the efficacy of co-feeding among *Ixodes scapularis* larvae and nymphs for maintaining *B. burgdorferi* s.s. while feeding on 1 of its primary reservoir hosts, the white-footed mouse (*Peromyscus leucopus*), was nil when experiments were conducted using densities of ticks similar to those encountered in nature (8 nymphs, 30–40 larvae per mouse) (Piesman and Happ, 2001). A low percentage of the *I. scapularis* larvae (5%) did become infected, but only when abnormally high densities of ticks (40 nymphs, >200 larvae) were put on mice. To maximize the possibility of localized or horizontal transmission, we also placed large numbers of uninfected larvae alongside several potentially infected nymphs on lizards to no avail.

The role of lizards in the ecology of *B. burgdorferi* s.l. seems to be markedly different in the southeastern United States, where *B. burgdorferi* s.l. DNA was detected recently in 54% of 160 lizards belonging to 9 species and 6 genera (Clark et al., 2005). *Borrelia andersonii*, *B. bissettii*, and *B. burgdorferi* s.s. were identified in lizard blood by PCR amplification and sequence analysis of partial flagellin, outer surface protein A, or 66-kDa protein gene fragments. Notably, 93% of the lizards were tick free at the time of capture; several species of the lizards evaluated seldom, if ever, host the regional tick vector *I. scapularis*; and attempts to isolate spirochetes from 12 PCR-positive broad-headed skinks (*Eumeces laticeps*) were unsuccessful. However, ≥21% of 28 *I. scapularis* nymphs that had fed xenodiagnostically as larvae on PCR-positive lizards reportedly acquired and transstadially passed the infection. These findings lend support to those of an earlier experimental study in which the green anole (*Anolis carolinensis*) and the southeastern five-lined skink (*Eumeces inexpectatus*) were found to be susceptible to *B. burgdorferi* s.s. infection and to be capable of serving as sources of spirochete infection for xenodiagnostic *I. scapularis* larvae for at least 5 wk (Levin et al., 1996). We conclude that the conflicting results with respect to the status of lizards in the ecology of *B. burgdorferi* s.l. in different regions of the United States underscore the necessity to assess each species of vertebrate individually, under both field and laboratory conditions, before any meaningful conclusions can be drawn.

We chose the *rrf-rrl* intergenic spacer region as a genetic marker because this target has been adopted widely for detecting and typing *B. burgdorferi* s.l. in both cultured and uncultured material during the past decade (Schwartz et al., 1992; Postic et al., 1994, 1998; Wang et al., 1999; Aguero-Rosenfeld et al., 2005). We have had considerable success to date by using this spacer region for detecting borrelial DNA in host-seeking or blood-fed ticks (Eisen, Eisen, Chang et al., 2004; Eisen, Mun et al., 2004; Lane et al., 2004; Lane, Mun, Eisen, and Eisen, 2005; Lane, Mun, Parker, and White, 2005; present study), in mammalian blood and tissues (Eisen et al., 2003; Lane, Mun, Eisen, and Eisen, 2005; Lane, Mun, Parker, and White, 2005), and, more recently, in bird blood (data not shown). In our hands, its sensitivity has proven to be similar to that of a direct detection assay, i.e., direct immunofluorescence, for visualizing the presence of *B. burgdorferi* s.l. within tick tissues (Eisen, Eisen, Chang et al., 2004).

In conclusion, the western fence lizard fulfills the criteria for a zooprophylactic host of *B. bissettii* as it does for *B. burgdorferi* s.s. (Lane and Quistad, 1998; Kuo et al., 2000). This lizard is refractory to experimental infection with *B. bissettii*, and its innate immune system probably destroys spirochetes present in infected *I. pacificus* nymphs that engorge on it, presumably as a by-product of complement proteins delivered with the bloodmeal. To the extent that nymphal ticks feed preferentially on lizards (Casher et al., 2002; Eisen et al., 2004a, 2004b) rather than on reservoir-competent hosts such as the western gray squirrel (Lane, Mun, Eisen, and Eisen, 2005) in a particular biotope, humans or other animals bitten subsequently by *I. pacificus* adults from the same tick population are less likely to be exposed to *B. bissettii*.

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