Journal of Wildlife Diseases, 41(1), 2005, pp. 115–125

COLUMBIAN BLACK-TAILED DEER (*ODOCOILEUS HEMIONUS COLUMBIANUS*) AS HOSTS FOR *BORRELIA* SPP. IN NORTHERN CALIFORNIA

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ABSTRACT: The prevalence of infection of Columbian black-tailed deer (*Odocoileus hemionus columbianus*) with *Borrelia* spp. was evaluated in an area of northwestern California (USA) where Lyme disease is endemic and the relapsing-fever group spirochete *Borrelia coriaceae* is enzootic, and in a far-removed comparison area having a disparate climate and lower density of vector ticks. Blood samples collected from both deer herds in 1987, 1988, and from 2000–02 were assayed for borrelial infection with microscopic and molecular methods. Serum specimens from two (5%) of 39 deer from the Dye Creek Preserve in Tehama County versus 13 (20%) of 64 animals from the Hopland Research and Extension Center (HREC) in Mendocino County, California were polymerase chain reaction (PCR) test positive for *B. burgdorferi* sensu lato. DNA sequencing analyses revealed that eight animals were infected with *B. bissettii*, six with three unclassified genotypes, and one with *B. burgdorferi* sensu stricto. One serum sample (2%) from HREC was positive for a relapsing-fever group spirochete that had a 16S rRNA sequence homology of 99% with the C053 type strain of *B. coriaceae*. Spirochetes undetermined to genospecies were detected in thick-blood drops prepared from three (8%) of 36 deer from the HREC by direct immunofluorescence. Adults of the hippoboscid flies *Lipoptena depressa* ($n=73$) and *Neolipoptena ferrisi* ($n=24$), the Pacific Coast tick (*Dermacentor occidentalis*) ($n=22$), and the western black-legged tick (*Ixodes pacificus*) ($n=1$) that had been removed from deer from both study areas in 2002 were PCR test negative for borreliae. The occurrence of diverse borreliae in deer from northern California confounds and, consequently, reduces the utility of borrelial serosurveys for detecting specific genospecies, unless they are complemented by more specific assays (e.g., immunoblotting, PCR/sequencing analysis).

Key words: *Borrelia bissettii*, *B. burgdorferi*, *B. coriaceae*, Columbian black-tailed deer, deer keds, ixodid ticks.

INTRODUCTION

In California, serologic evidence and an animal-inoculation experiment have implicated the Columbian black-tailed deer (BTD; *Odocoileus hemionus columbianus*) as a probable host of *Borrelia burgdorferi* sensu stricto (s.s.), the spirochete that causes Lyme disease in this region (Lane and Burgdorfer, 1986; Chomel et al., 1994; Lane et al., 1994a). Unlike the relapsing-fever group borreliae that are transmitted almost exclusively by soft ticks in the genus *Ornithodoros* (Sonenshine, 1993), the 11 described genospecies comprising the Lyme disease spirochetal complex, *B. burgdorferi* sensu lato (s.l.), are transmitted mainly by hard ticks in the *Ixodes ricinus* complex (Eisen and Lane, 2002). The primary vector of *B. burgdorferi* in California and Oregon (USA) is the western black-legged tick (*I. pacificus*) (Burgdorfer et al., 1985; Lane and Lavoie, 1988; Clover and Lane, 1995), a tick that feeds abundantly on BTD and other medium to large mammals in its adult stage (Furman and Loomis, 1984; Westrom et al., 1985).

In addition to *B. burgdorferi* s.s., the BTD has been suggested to be a natural host of the relapsing-fever group spirochete *B. coriaceae* (Lane and Burgdorfer, 1986, 1988). The vector of this spirochete is the soft tick *Ornithodoros coriaceus*, which feeds predominantly on deer and occasionally on humans (Furman and Loomis, 1984; Lane et al., 1985; Lane and Manweiler, 1988). Initially, this spirochete was suspected to be a cause of epizootic bovine abortion, an important disease of

rangeland cattle in the far western United States (Lane et al., 1985). Subsequent research aimed at clarifying the possible association of *B. coriaceae* with epizootic bovine abortion, though sometimes suggestive of a causal relation, has been inconclusive (Osebold et al., 1986, 1987; Spezialetti and Osebold, 1989, 1991; Zingg and LeFebvre, 1994), which has led investigators to consider other microorganisms as the potential etiologic agent (Stott et al., 2002).

The objectives of this study were twofold; first, to evaluate the prevalence of infection in BTD with *Borrelia* spp. in an area of northwestern California where Lyme disease is endemic and *B. coriaceae* is enzootic, and in a far-removed comparison area of north-central California having a disparate climate and lower density of vector ticks. Second, we sought to determine the presence of borrelial infection in blood-sucking ectoparasites (deer keds, ticks) found on some of the deer from both localities.

MATERIALS AND METHODS

Study areas

In northern California, samples from deer were collected at the University of California, Hopland Research and Extension Center (HREC), Mendocino County, and at the comparison area, the Dye Creek Preserve (DCP) in Tehama County, in 1987, 1988, and 2000–02 (Fig. 1). The HREC is a 2,168-ha agricultural sciences research facility located predominantly in southeastern Mendocino County and in a small part of neighboring west-central Lake County (39°0′35.0″N, 123°4′35.9″W). Situated on the western slopes of the Mayacmas Mountains in the Russian River Valley, the HREC is comprised of rolling hills interspersed with ravines. Seven vegetational types are present, with slightly over half of the ground cover composed of woodland grass and chaparral (Heady, 1961). The climate is Mediterranean with hot, dry summers and cool, moist winters.

The DCP is located at the northeastern end of the Sacramento Valley 21 km southeast of Red Bluff in east-central Tehama County (40°6′11.14″N, 122°2′41.39″W) (Barrett, 1978) at a linear distance of ~151 km northeast of Hopland. A long-time cattle ranch, it now is under the stewardship of the Nature Conservancy. It contains approximately 15,237 ha, and ranges in elevation from 76 m on the Sacramento Valley floor to 762 m in the foothills. Eight vegetational types are present that vary from sparse annual grassland to dense foothill woodland, and the climate is Mediterranean (Barrett, 1978).

Collection and processing of samples

In total, 102 adult BTD, 2 yr of age or older, plus one yearling were taken by shooting. These included 89 bucks collected during either the coastal (HREC) or inland (DCP) hunting seasons in August–September and October–November, respectively, and 13 does and one yearling buck taken at the HREC in late January 1988. The distribution of samples over time by locality is given in Table 1.

Blood samples usually were collected from the vessels or heart chambers that remained intact at a hunter check station (HREC) or from the jugular vein and body cavity by individual hunters (DCP). After clotting at ambient temperature, blood samples were held at approximately 4 C and subsequently centrifuged one to several days later. Sera were frozen at −74 to −80 C before testing. Clots were not tested because they had not been retained from all deer, particularly those (n=33) collected during the 1980s, when our principal objectives were to assay for presence of antibodies against *B. burgdorferi* and to try to cultivate spirochetes from whole-blood specimens (Lane and Burgdorfer, 1986, 1988). We realize that we might have detected more spirochete-infected deer in the present study had we assayed whole blood instead of serum by polymerase chain reaction (PCR). Further, we did not test sera for antiborrelial antibodies because of concerns about the presence of cross-reactive antibodies (Lane and Burgdorfer, 1986, 1988; Rogers et al., 1999). Instead, PCR and DNA sequencing analyses were used to detect and specifically identify any borrelial DNA that might have been present in serum specimens or in whole blood that had been injected into culture medium during isolation attempts.

In 2002 only, 10 deer from the DCP and 13 from the HREC were inspected for up to 5–10 min for the presence of ectoparasites, especially hippoboscid flies (deer keds) and ticks. Keds and ticks were preserved in 70–95% ethanol for later identification and testing. Keds were identified to species using the key to Hippoboscidae provided by Furman and Catts (1982).

Detection of bacteria by microscopic and molecular methods

From 2000 to 2002, one to two drops of whole blood from 48 deer collected at the

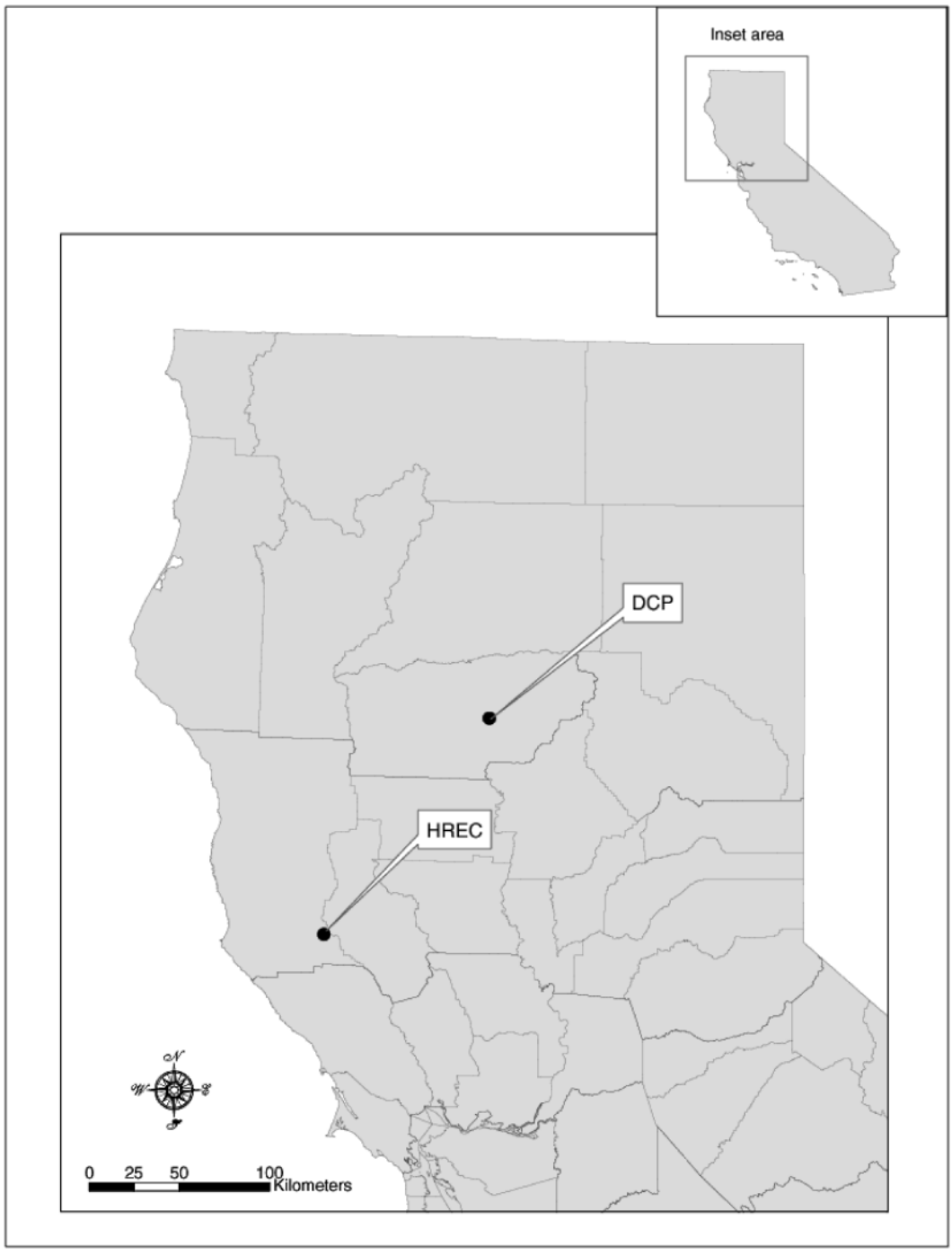

FIGURE 1. Map of northern California (USA) showing locations of the Hopland Research and Extension Center (HREC) and the Dye Creek Preserve (DCP).

TABLE 1. Prevalence of infection with *Borrelia burgdorferi* s.l. spirochetes in Columbian black-tailed deer from northern California by locality and year as determined by polymerase chain reaction testing of their sera.

Year	Number of sera positive/number tested (% positive)	
	Dye Creek Preserve	Hopland Research and Extension Center
1987	0/6	4/6 (67)
1988	1/7 (14)	3/14 (21)
2000	0/10	3/22 (14)[a]
2001	0/9	3/14 (21)
2002	1/7 (14)	0/8
Totals	2/39 (5)	13/64 (20)

[a] One of the specimens uninfected with *B. burgdorferi* s.l. contained *B. coriaceae*; all other specimens from both localities tested negative for this relapsing-fever group spirochete.

HREC were put into 1.5-ml eppendorf tubes containing complete BSK-H medium (Sigma Corp., St. Louis, Missouri, USA) containing 6% rabbit serum with or without the addition of 25 μg/ml of rifampicin. Cultures were incubated at approximately 34 C, and checked weekly for 4 wk by dark-field microscopy for presence of borreliae at 400×. Additionally, thick-blood drops were prepared from 36 deer, air-dried, fixed in acetone for 10 min, and examined for borreliae by direct immunofluorescence with a polyvalent conjugate (Lane and Burgdorfer, 1986, 1988).

DNA was extracted with a commercial tissue-extraction kit (DNeasy Kits, Qiagen, Valencia, California). Keds and ticks, which were tested individually, were soaked in distilled deionized H_2O for 10 min before they were ground with sterile, disposable plastic pestles in 180 μl of ATL buffer (Qiagen). For the serum, 50 μl were used for extraction following the manufacturer's non-nuclear blood-sample protocol. DNA was eluted in a final volume of 100 μl of AE buffer for all extractions. Polymerase chain reaction protocols using 40 amplification cycles adapted from Lane et al. (2004) were used in attempts to detect both *B. burgdorferi* s.l. and relapsing-fever group spirochetes. For each PCR-positive sample, cycle sequencing was carried out using the Big Dye cycle sequencing kit (Applied Biosystems, Foster City, California) after products had been purified using the QIAquick PCR Purification Kit (Qiagen). Polymerase chain reaction products were sequenced in both directions on an ABI 377 sequencer (Applied Biosystems). The sequences were aligned using the software Sequencher 3.1 (Gene Code, Ann Arbor, Michigan, USA). To determine the relatedness of the novel genotypes of *B. burgdorferi* s.l., each amplicon was characterized by comparing its DNA sequence with those of strains available in GenBank using Paup* 4.0b (Swofford, 2001). The eight derived, unique sequences have been deposited in GenBank (accession numbers AY686674 to AY686681).

Infection with *B. burgdorferi* s.l. spirochetes was detected with two sets of primers in a nested PCR format that specifically targets the 5S–23S rRNA spacer region of the *Borrelia* genospecies group (Postic et al., 1994; Lane et al., 2004). *Borrelia coriaceae* infection was detected with a primer set that targets the 16S rRNA gene of relapsing-fever group spirochetes (Marti Ras et al., 1996). *Borrelia burgdorferi* s.l.- and *B. coriaceae*-infected ticks were used identically as positive controls across all samples (i.e., blood or ectoparasites) in each run. As negative controls, distilled water was included in each run. To minimize the risk of contamination, disposable bench covers were changed between each preparation, and filtered tips were used for all extraction and PCR work.

Prevalence of infection in deer sera with *B. burgdorferi* s.l. by location or within a site was not compared statistically because animals were collected at different times of year and in dissimilar environments at the HREC versus the DCP, several distinct genotypes of borreliae infected deer, and the units sampled would not have been independent of each other at a given site.

RESULTS

Overall, the prevalence of *B. burgdorferi* s.l. infection was four times higher in deer from the HREC (20%) than in animals from the DCP (5%, Table 1). *Borrelia coriaceae* was detected in just one (2%, deer HREC00–14) of 64 animals from the HREC and in zero from the DCP; the single positive amplicon had a 16S rRNA sequence homology of 99% with the C053 type strain of *B. coriaceae*.

Deer blood samples obtained at the HREC from 2000–02 that were not always paired were evaluated for presence of *Borrelia* spp. with two microscopic methods. Efforts to cultivate borreliae from the blood of 48 deer in BSK-H medium yielded two isolates that were lost during passage. Serum from each of these deer tested positive for borreliae by PCR, i.e., the

TABLE 2. Distribution of *Borrelia* spp. in sera of polymerase chain reaction-test positive Columbian black-tailed deer by locality as determined by DNA sequence analysis.

Locality (n)[a]	*Borrelia burgdorferi* s.l.					*Borrelia coriaceae*
	B. bissetti	*Bb*[b] s.s.	*Bb* s.l.(1)	*Bb* s.l.(2)	*Bb* s.l.(3)	
Dye Creek Preserve (2/2)		1		1		
Hopland Research & Extension Center (13/13)	8		3		2	1

[a] n, number of amplicons sequenced successfully divided by the total number of positive amplicons evaluated. In total, sera from 39 and 64 deer were tested from the Dye Creek Preserve and the Hopland Research & Extension Center, respectively.
[b] *Bb*, *Borrelia burgdorferi*; *Bb* s.l.(1), (2), and (3) represent uncharacterized genotypes.

aforementioned HREC00–14 for *B. coriaceae* and HREC00–24 for an uncharacterized member of the *B. burgdorferi* s.l. complex. Also, spirochetes were observed in three (8%) of 36 duplicate, thick-blood drops examined by direct immunofluorescence. Spirochetes observed in the paired drops from each of these deer resembled *B. coriaceae* morphologically, averaged 7 μm in length (range, 5–8 μm; n=30), and ranged in number from 10–100 per drop. Sera were available from two of these deer, but neither was PCR positive for borreliae.

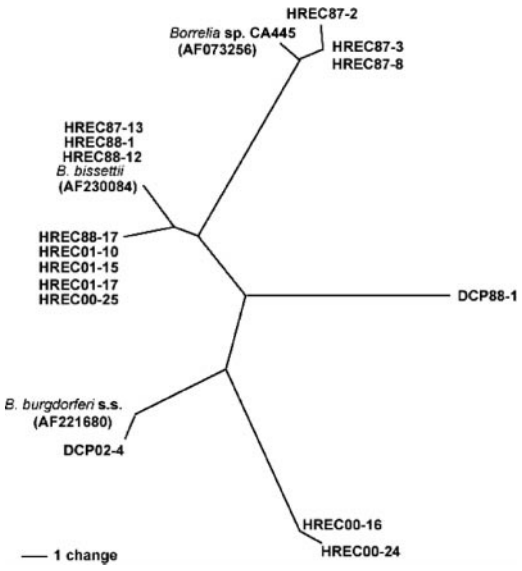

FIGURE 2. Unrooted most parsimonious phylogenetic tree based on 187 base-pair region of 5S–23S rRNA spacer region of Lyme disease spirochetes from sera of Columbian black-tailed deer. Obtained from a heurisitic TBR branch-swapping analysis using stepwise addition in Paup* 4.0b (Swofford, 2001).

In 2002, 47 keds of two species (32 *Lipoptena depressa,* prevalence 100%; 15 *Neolipoptena ferrisi,* prevalence 20%) and 23 ixodid ticks belonging to three species (21 *Dermacentor occidentalis* [19 males, two females], prevalence 50%; one *D. albipictus* nymph, prevalence 10%; one *I. pacificus* female, prevalence 10%) were collected from 10 deer at the DCP. Sixty-four keds of two species (42 *L. depressa*, prevalence 85%; 22 *N. ferrisi*, prevalence 46%) and one *D. occidentalis* male (prevalence 8%) were removed from 13 deer at the HREC. Of these, 97 keds of two species (*L. depressa*, 73; *N. ferrisi*, 24) removed from deer from both localities, and 22 adult Pacific Coast ticks (*D. occidentalis*) and one *I. pacificus* tick obtained from deer at the DCP were PCR negative for *Borrelia* spp.

All 15 *B. burgdorferi* s.l.-positive amplicons from deer were sequenced successfully (Table 2, Fig. 2). One (DCP02–4) of two positive amplicons originating from deer taken at the DCP was found to represent *B. burgdorferi* s.s., the agent of Lyme disease in the far-western US, whereas the other (DCP88–1) represented a novel genotype. Among the 13 positive amplicons from the HREC, eight were identified as *B. bissettii* and five represented two uncharacterized genotypes. An uncorrected pairwise-distance matrix (Paup 4.0) revealed 89–99% homology among the genotypes detected in deer versus three previously described genospecies (Fig. 2).

DISCUSSION

Deer as hosts of *Borrelia* spp.

Black-tailed deer are exposed frequently to *B. burgdorferi* s.l. at the HREC, as 20% were PCR positive for one of three genospecies. Moreover, there was no evidence to suggest that long-term storage of serum specimens affected the PCR results from either locality. In contrast, 1/13 (8%) and 7/20 (35%) of the combined 1987 and 1988 samples from the DCP and HREC, respectively, were positive, versus only 1/26 (4%) and 6/44 (14%) of those collected from 2000–02 (Table 1). The substantial amount of annual variation in the prevalence of infection (0% to 67%) at the HREC doubtless reflects the low sample sizes, except for the year 2000 ($n=22$), plus numerous ecologic factors (e.g., the specific habitat types where deer were collected in different years, interannual variation in tick abundance, and infection prevalences).

Notably, borreliae detected in eight (e.g., HREC88–1 and HREC00–25) of the 13 infected deer from Hopland represented *B. bissettii* (Fig. 2), a spirochete previously associated with *I. spinipalpis,* but seldom with *I. pacificus* ticks in California (Postic et al., 1998; Eisen et al., 2004; Peot, Brown, and Lane, unpubl. data). Although *I. pacificus* adults feed plentifully on BTD in fall and winter at the HREC, and the nymphs feed on them occasionally in winter or spring (Westrom et al., 1985), there are no records of any life stage of *I. spinipalpis* attaching to deer in California (Furman and Loomis, 1984). Three additional species of ticks are known to feed on deer with some frequency at the HREC, that is, the ixodids *D. albipictus* and *D. occidentalis* and the argasid *O. coriaceus* (Westrom, 1975), but *B. bissettii* has not been detected in or isolated from any of them to date. Thus, the source of the *B. bissettii* infections in the Hopland deer is enigmatic given the current status of our knowledge.

This is the first time *B. bissettii* has been associated with deer in North America. Previously it had been isolated from numerous species of rodents from the western, southeastern, and upper midwestern US (reviewed by Eisen et al., 2003; Oliver et al., 2003; Vredevoe et al., 2004). In Europe, spirochetes closely related to, if not identical with, *B. bissettii* reportedly infect humans and can cause clinical illness (Picken et al., 1996; Strle et al., 1997). At the HREC, *B. bissettii* is maintained primarily in an intensely focused transmission cycle involving *I. spinipalpis,* the dusky-footed wood rat (*Neotoma fuscipes*), and chaparral (Brown and Lane, 1992; Postic et al., 1998; Peot, Brown, and Lane, unpubl. data). The immature stages of *I. pacificus,* particularly the larvae, also feed on the dusky-footed wood rat in chaparral (e.g., Leprince and Lane, 1996; Lane et al., 1998), and the resultant adult ticks infest BTD abundantly in the same vegetational type (Westrom et al., 1985). Since *I. pacificus* is as efficient an experimental vector of *B. bissettii* as is *I. spinipalpis* (Eisen et al., 2003), perhaps *I. pacificus* is infected naturally with *B. bissettii* in certain habitats (e.g., chaparral) more often than has been recognized. If true, this might account for the presence of *B. bissettii* in deer at HREC.

Borreliae detected in the five remaining PCR-positive deer from Hopland belonged to two uncharacterized genotypes in the *B. burgdorferi* s.l. complex. All five animals were bucks taken in summer. Three of these deer (HREC87–2, HREC87–3, HREC87–7) were infected with borreliae most closely related to *Borrelia* sp. isolate CA445, which originated from a California kangaroo rat (*Dipodomys californicus*) inhabiting a grassland biotope at the HREC (Lane et al., 1999). Uncharacterized borreliae detected in the other two deer (HREC00–16, HREC00–24) were more closely related to *B. burgdorfersi* s.s. than they were to *Borrelia* sp. isolate CA445.

The prevalence of *B. burgdorferi* s.l. infection in deer from the DCP was low

(5%) and only one-quarter that in deer from the HREC. One of the animals (DCP02–4) contained *B. burgdorferi* s.s. and the other (DCP88–1) harbored an uncharacterized *Borrelia* sp. whose nucleotide sequence was distinct from all previously characterized genospecies. Deer at the DCP invariably were collected in late October or early November when *I. pacificus* adults were just beginning their autumnal host-seeking activities, and consequently few animals were infested with them. In 2002, for instance, a single *I. pacificus* adult was found on the 10 animals examined. The low prevalence of borrelial infection in deer at the DCP also may reflect low population densities of *I. pacificus* year-round, not just in fall. *Ixodes pacificus* thrives in humid, temperate climates like those present in central and north coastal California, whereas the DCP is located in an inhospitable climatic zone having protracted periods of hot, dry weather in summer with temperature maxima exceeding 32 C for nearly 100 days annually in the nearby city of Red Bluff (Barrett, 1978). Not surprisingly, few collection records exist for *I. pacificus* in this part of north-central California (Furman and Loomis, 1984).

Earlier investigations conducted at the HREC, the type locality of *B. coriaceae* (Johnson et al., 1987), demonstrated that this spirochete occurs in all three trophic stages of *O. coriaceus*, including up to 35–39% of the female ticks from certain habitats (Lane et al., 1985; Lane and Manweiler, 1988). This soft tick commonly inhabits soil, litter, and the bedding areas of deer and cattle (Furman and Loomis, 1984). There is a summer peak in the prevalence of borrelial antibodies in BTD that coincides with the feeding activities of *O. coriaceus* (Lane and Burgdorfer, 1986). Moreover, spirochetes closely resembling *B. coriaceae* morphologically and immunologically are present in the blood of some deer during the summer (Lane and Burgdorfer, 1988; present study), and a blood specimen from which spirochetes were cultivated was PCR positive for *B. coriaceae* (present study). These findings provide presumptive evidence that deer can serve as a natural host of *B. coriaceae*, and possibly as a source of infection for noninfected *O. coriaceus* ticks that feed on them.

Experiments using low-passage borrelial isolates, their primary tick vectors, and preimmune deer must be conducted before the precise role of BTD in the enzootic transmission cycles of *B. burgdorferi* s.l. or *B. coriaceae* spirochetes can be understood. At present, we cannot reconcile the presence of such a diverse and prevalent borrelial flora in deer from Hopland (Table 2) with the findings of two recent studies in which preimmune sera from two cervid species exhibited complement-mediated killing of *B. burgdorferi* s.l. (Nelson et al., 2000; Ullmann et al., 2003). Equally puzzling is the fact that serum specimens from two of the Hopland deer tested negative by PCR even though thick-blood drops prepared from them contained up to 100 *B. coriaceae*-like spirochetes per drop. Perhaps BTD serum is not a particularly useful clinical sample for detecting *B. coriaceae* by PCR as compared with other specimens (e.g., skin) as has been proposed for the PCR-based identification of *Ehrlichia chaffeensis* and *Borrelia lonestari* in white-tailed deer (Little et al., 1998; Moore et al., 2003). In that regard, *B. burgdorferi* s.i. have been detected in skin biopsies taken from wild deer (*Cervus* spp., *Capreolus capreolus*) in Japan and France, which demonstrates that some Lyme disease group spirochetes have a tropism for cervid skin (Kimura et al. 1995; Pichon et al., 2000).

Deer ectoparasites as hosts of *Borrelia* spp.

Borreliae were not detected in *D. occidentalis* adults removed from deer at the DCP, which included three ticks removed from the only animal (DCP02–4) that was PCR positive for *B. burgdorferi* s.s. during this study. In fact, borreliae rarely have been detected in this tick (e.g., reviewed

in Lane, 1996; Holden et al., 2003), and it is an inefficient experimental vector of *B. burgdorferi* s.s. (Brown and Lane, 1992; Lane et al., 1994b). The paucity of *I. pacificus* adults on BTD from both localities in 2002 also was anticipated because animals were collected when the adult ticks normally are either inactive (summer) or minimally active (mid-fall). The absence of *O. coriaceus* nymphs or adults on deer likewise was expected because most trophic stages of this soft tick (except for the prolonged feeding larvae and the nonfeeding first nymphal instar) feed to repletion, detach, and drop off the host within less than an hour (Furman and Loomis, 1984).

Borreliae were not detected in 97 keds representing two species, but only 8% of the keds (seven *L. depressa,* one *N. ferrisi*) were collected from two spirochete-infected BTD. The serum from one of the infected deer (DCP02–4) was PCR positive for *B. burgdorferi* s.s., whereas a thick-blood drop prepared from the other animal (HREC02–15) contained ~50 spirochetes undetermined to genospecies. A larger series of keds must be assayed before either hippoboscid species can be eliminated from consideration as a potential enzootic vector of *Borrelia* spp. In the Czech Republic, one (5%) of 22 specimens of the ked *L. cervi* that apparently had been removed from roe deer (*Capreolus caprelous*) was infected with the Eurasian Lyme disease spirochete *B. garinii* (Hulinska et al., 2002). The public health significance, if any, of this novel finding is unknown. Nonetheless, humans are not normal hosts of any of the approximately 150 described species of Hippoboscidae (Lloyd, 2002). Any species of obligatory blood-sucking arthropod feeding on a spirochetemic host potentially may acquire, but not necessarily maintain and transmit, such spirochetes to a naïve host.

Conclusions

We did not assay deer sera for presence of anti-borrelial antibodies to *B. burgdorferi* s.l. or *B. coriaceae* antigens because of well-founded concerns about cross-reactivity between them (e.g., Lane and Burgdorfer, 1986; Rogers et al., 1999), and *B. burgdorferi* s.s. antibodies are short-lived in BTD, at least after needle inoculation (Lane et al., 1994a). Our findings underscore why BTD in the far-western US cannot be used as sentinel animals to determine the occurrence of Lyme disease spirochetes or of *B. coriaceae* on the basis of serologic test results alone. The presence of diverse borreliae in deer from northern California confounds, and consequently reduces the utility of, borrelial serosurveys for detecting specific genospecies. By using PCR and sequencing analysis in tandem to assay serum specimens, we identified the relapsing-fever group spirochete *B. coriaceae* and eight distinct genotypes of *B. burgdorferi* s.l., including three hitherto uncharacterized ones in deer from two widely separated herds. We conclude that carefully controlled experiments involving naïve deer are needed to determine the viability of these spirochetes in deer blood or tissues and their infectivity for vector ticks.

ACKNOWLEDGMENTS

We thank C. Brooks, R. J. Eisen, I. Jones, R. J. Keiffer, J. E. Kleinjan, T. R. McWilliams, E. Omi-Olsen, S. P. Poor, R. L. Rittenhouse, C. L. Simerson, A. Swei, and T. Wilder for their much appreciated technical assistance, and D. R. McCullough and numerous public hunters at the HREC and members of the Ballard Unit at the DCP for contributing blood specimens or ectoparasites from deer. This research was funded in part by a grant from the National Institutes of Health (AI22501) and by a generous gift from A. Henry.

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Received for publication 17 February 2004.