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Differences in prevalence of *Borrelia burgdorferi* and *Anaplasma* spp. infection among host-seeking *Dermacentor occidentalis, Ixodes pacificus*, and *Ornithodoros coriaceus* ticks in northwestern California

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Abstract

Previous studies revealed that the Pacific Coast tick (*Dermacentor occidentalis*) is infected occasionally with the agents of Lyme disease (*Borrelia burgdorferi*) or human granulocytic anaplasmosis (*Anaplasma phagocytophilum*) and that it is an inefficient experimental vector of *B. burgdorferi*. The relationship of the pajahuello tick (*Ornithodoros coriaceus*) to each of these bacterial zoonotic agents has not been reported. The primary bridging vector of both bacterial zoonotic agents to humans is the western black-legged tick (*Ixodes pacificus*). Because of the spatial and temporal overlap of *D. occidentalis* and *O. coriaceus* populations with those of *I. pacificus* in natural foci of *B. burgdorferi* and *A. phagocytophilum* in northwestern California, we conducted field and laboratory studies to determine if the Pacific Coast tick or the pajahuello tick potentially may serve as secondary vectors of either bacterium. Our findings reconfirmed that wild-caught *D. occidentalis* ticks are infected infrequently with *B. burgdorferi* or *A. phagocytophilum*, but some adult ticks from dense woodlands or chaparral were found to contain 2 important veterinary pathogens for the first time (*Anaplasma bovis, A. ovis*). The high prevalence of *A. bovis* infection (4.3%, n=185 ticks) within chaparral-derived ticks suggests that *D. occidentalis* could be an efficient vector of this rickettsia. Experimental attempts to transmit borreliae or *Anaplasma* spp. that may have been present in >100 wild-caught *D. occidentalis* adults to naïve rabbits were unsuccessful. *Anaplasma* spp. were not detected in *O. coriaceus*, but one (4.3%) of 23 nymphs was infected with *B. bissettii*. This finding and an antecedent report of a *B. burgdorferi*-like spirochete from the same tick species demonstrate that *O. coriaceus* sometimes acquires and transstadially passes Lyme disease group spirochetes. *I. pacificus* nymphs inhabiting a woodland nidus of *B. burgdorferi* and *A. phagocytophilum* had a 5-fold higher prevalence of borreliae than adult ticks from the same generational cohort. In contrast to the results of preceding studies carried out at the same site, none of the nymphal or adult ticks was

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PCR-positive for *A. phagocytophilum*. This suggests that the distribution of this rickettsia is highly focal or variable from year-to-year within this particular woodland.

Keywords

Borrelia burgdorferi; *Anaplasma* spp.; *Dermacentor occidentalis*; *Ixodes pacificus*; *Ornithodoros coriaceus*

Introduction

In northwestern California, certain subtypes of dense woodlands carpeted with leaf or fir-needle litter have been identified as principal foci for ticks and vertebrates that host the bacteria that cause Lyme disease (*Borrelia burgdorferi*) or human granulocytic anaplasmosis (*Anaplasma phagocytophilum*) (e.g., Clover and Lane, 1995; Eisen et al., 2003, 2004a, 2006, [2010]; Foley et al., 2008, [2009]; Girard et al., 2009; Lane et al., 2001, 2007; Tälleklint-Eisen and Lane, 1999). The primary vectors of *B. burgdorferi* and *A. phagocytophilum* to humans in this region are nymphs or adult females of the western black-legged tick (*Ixodes pacificus*) (Barlough et al., 1997; Burgdorfer et al., 1985; Clover and Lane, 1995; Kramer et al., 1997; Lane et al., 2007; Richter et al., 1995), but it is the nymphal stage that predominates in certain woodlands with relative densities sometimes reaching 32–56 ticks per 100 m^2 (Lane et al., 2004; Tälleklint-Eisen and Lane, 2000).

Two other human-biting ticks that occur in Californian dense woodlands are the Pacific Coast tick (*Dermacentor occidentalis*) and the pajahuello tick (*Ornithodoros coriaceus*). *Dermacentor occidentalis* has been found infected naturally with a diverse suite of microorganisms including several well-known zoonotic pathogens: Colorado tick fever virus, *A. phagocytophilum*, *B. burgdorferi*, *Coxiella burnetii*, *Ehrlichia chaffeensis*, and *Francisella tularensis* (Enright et al., 1971; Holden et al., 2003; Kohls, 1955; Lane and Lavoie, 1988; Parker et al., 1929). Additionally, an unclassified rickettsia of the spotted fever group, designated 364D and associated with *D. occidentalis*, has been implicated as a cause of a Rocky Mountain spotted fever-like illness (Lane et al., 1981a; Philip et al., 1981) and, more recently, of an eschar-associated illness (Shapiro et al., 2010). *O. coriaceus* is a notorious human-biter known for causing systemic allergic reactions in some individuals and, in California during the early 20th Century, its bite reportedly was "…more feared than the rattlesnake by the natives…" (Herms, 1923). This tick is the primary vector of the relapsing-fever group spirochete *Borrelia coriaceae* (Johnson et al., 1987; Lane et al., 1985a), the etiologic agent of Q fever (*C. burnetii*) has been isolated from it (Enright et al., 1971), and it has been incriminated as a vector of a novel alpha-proteobacterium that is believed to be the cause of epizootic bovine abortion (King et al., 2005). Because of the spatial and temporal overlap of *D. occidentalis* and *O. coriaceus* populations with those of *I. pacificus* in woodland foci of Lyme disease and human granulocytic anaplasmosis, we initially sought to determine the relative abundance of host-seeking *D. occidentalis* versus *O. coriaceus* ticks in dense woodlands and their potential involvement as secondary vectors of either bacterium. We subsequently assessed the relative abundance of *D. occidentalis* adults in dense woodlands versus adjacent chaparral habitats, and their prevalence of infection with and capacity to experimentally transmit naturally acquired borreliae or *Anaplasma* spp. For comparative purposes, we also evaluated the relative abundance and prevalence of borrelial and *Anaplasma* infection in *D. occidentalis* nymphs and adults versus those of *I. pacificus* nymphs and adults from a dense woodland known to harbor both *B. burgdorferi* and *A. phagocytophilum*. The results of these multi-year studies are the subject of this report.

Materials and methods

The study area

This study was conducted in 8 dense woodlands located at the University of California Hopland Research and Extension Center (HREC) in Lake and Mendocino counties (39°0 ´35.0″N, 123°4´35.9″W), northwestern California, from 2003 to 2005 and in 2009 and 2010. The woodlands were composed of oaks (*Quercus* spp.) and Pacific madrone (*Arbutus menziesii*) with an understory of leaf litter and herbaceous vegetation as described earlier (Eisen et al., 2004b, 2009; Lane et al., 2004, 2007). The Beasley, Hunt Club, James II, and Maude sites were bordered primarily by chaparral, whereas the Cell Tower, Parson, Pepperwood, and Tank sites were bounded by grassland (Eisen et al., 2009). The chaparral was comprised of chamise (*Adenostoma fasciculatum*), oaks (*Quercus* spp.), California lilac (*Ceanothus* spp.), and other species. The patch-sizes that were sampled ranged from <0.40 ha (e.g., Hunt Club, Maude) to approximately 6.3 ha (James II). Further details of the James-II site, which was the only woodland site sampled for presence of ticks during all 5 years, have been published previously (Lane et al., 2004, 2005, 2007).

CO_2-baited pitfall trapping, 2003

To determine the relative abundance of *O. coriaceus* nymphs and adults and of *D. occidentalis* adults by pitfall trapping in all 8 dense woodlands, 10 white enamelware pans (18×29×5 cm) were sunk 5 cm into the ground at approximately 10-m intervals on 12 sampling occasions spanning 13 June to 7 August. Each trap within the resultant ≈90-m transect-line was baited with a chunk of dry ice. The dry ice was elevated about 2 cm above the bottom of the pan on 2 wooden strips. The pans were examined for presence of ticks after one and 2 h of operation, and trapping was discontinued thereafter. All ticks found were preserved in 95% ethanol for later identification and testing by polymerase chain reaction (PCR). Two transects were set in 7 of the sites, whereas 5 transects were set in the James-II because it is a well-defined focus of both *B. burgdorferi* and *A. phagocytophilum* (Lane et al., 2004, 2005, 2007).

Flagging woodlands and chaparral, 2004 and 2005

Since few *D. occidentalis* adults were collected in all 8 dense woodlands in 2003 by pitfall trapping, preliminary sampling was carried out in the James-II woodland (one hour) on 26 May and adjoining chaparral (2 hours) on 27 May 2004 to determine if enough adult ticks could be taken by flagging these habitats to enable a more systematic investigation. In 2005, 4 dense woodlands bordered by chaparral (Beasley, Hunt Club, James II, Maude) were flagged on 5 occasions between 12 May and 4 June, as were their adjoining patches of chaparral on 4 occasions between 12 and 26 May. Sampling was conducted either during the morning (≈0800 to 1040 h) or mid- to late afternoon (1540 to 2045 h). Low numbers of *I. pacificus* adults also were collected from both habitat types, but those data are not presented here because our specific aim was to determine if *D. occidentalis* adults may serve as potential secondary vectors of *B. burgdorferi* or *Anaplasma* spp.

Flagging/dragging woodland, 2009 and 2010

The James-II woodland site was sampled on 5 occasions by flagging or dragging so that we could make a direct comparison of the relative abundance of, and the *B. burgdorferi*- versus *Anaplasma* spp. infection prevalences in, host-seeking *D. occidentalis* adults and nymphs with those of the primary vector, *I. pacificus*. In 2009, *D. occidentalis* and *I. pacificus* adults were collected by flagging between ≈0800 and 1030 h on 24 May and 7 June, *I. pacificus* nymphs by dragging between 1150 and 1435 h on 24 May, and *D. occidentalis* and *I. pacificus* nymphs by dragging between 0715 and 0930 h on 19 July. In 2010, *I. pacificus*

and a few *D. occidentalis* adults were flagged between 1255 and 1530 h on 16 and 31 January. The latter sampling events were conducted to coincide more closely with the winter activity peak of *I. pacificus* adults at the HREC (Lane, 1990).

Attempts to culture *B. burgdorferi*

Because the 2004 pilot study produced several *B. burgdorferi*-infected *D. occidentalis* adults from the James-II woodland, we tried to isolate spirochetes from adult ticks flagged at the same site in 2005. Thirty-five host-seeking *D. occidentalis* adults (15 males, 20 females) collected on 12 or 25 May were surface-sterilized by vortexing them for 30 s with ≈5 ml each of hydrogen peroxide, 70% ethanol, and sterile phosphate-buffered saline. Next, ticks were dissected and their unminced tissues placed into 1.5-ml of BSK-H culture medium containing 50 μl of rifampicin in an Eppendorf tube. The cultures were incubated at 34.5°C and examined for presence of spirochetes by dark-field microscopy 6 times for one month at 400×.

Transmission trial

In an attempt to experimentally transmit either *B. burgdorferi* or *Anaplasma* spp. from potentially infected, wild-caught *D. occidentalis* adults to New Zealand white rabbits, 14 or 15 pairs of male and female ticks collected at the James-II site in 2005 were put on each of 4 naïve rabbits inside specially constructed tick-feeding capsules. Each rabbit was bled before tick exposure, and at 2, 4, and 6 weeks post-infestation. The whole-blood samples were frozen at −80°C until they were tested by PCR.

DNA extraction, and pathogen detection in ticks, cultures and rabbit blood

All biological specimens were tested individually for presence of *B. burgdorferi* sensu lato (s.l.) and *Anaplasma* spp. DNA was extracted from ticks or whole blood following previously published protocols (Lane et al., 2004, 2005). In 2005, not all *D. occidentalis* adults were tested by PCR to keep the sample sizes manageable, i.e., usually to minima of ≈30–50 individuals per site when large samples of ticks were obtained by flagging. Also, we used 140 ticks for the borrelial isolation attempts and the *B. burgdorferi* and *Anaplasma* spp. transmission trial. Details of the PCR assays and sequencing analysis followed those of Lane et al. (2004, 2005). Ear-punch biopsies from wild-caught western gray squirrels that had been infected with either *B. burgdorferi* or *A. phagocytophilum*, or *B. burgdorferi* isolate CA4 and *A. phagocytophilum* strain MRK, were employed as positive controls from 2003 to 2005 and 2009–2010, respectively. PCR water was employed as a negative control with each run. *B. burgdorferi* s.l. infection was assessed with a nested PCR format that specifically targets the 5S-23S rRNA spacer region (Lane et al., 2004). *Anaplasma* spp. infection was evaluated by targeting the 16S rRNA gene (Massung et al., 1998). Products from positive samples were purified using either the QIAquick PCR Purification Kit or QIAquick Gel Extraction Kit (Qiagen, Valencia, CA). Positive amplicons were sequenced on an ABI 3700 Sequencer (Applied Biosystems, Foster City, CA) or at the University of California, Berkeley, DNA Sequencing Facility using internal PCR primers. The contigs were compared with sequences available in GenBank using the software Sequencher 4 or 4.6 (Gene Code, Ann Arbor, MI).

Statistical analyses

In numerous pair-wise comparisons, McNemar's test with continuity correction was used to determine the significant values for the prevalence of *B. burgdorferi* versus *Anaplasma* spp. infection in *D. occidentalis* adults within sites, and *B. burgdorferi* or *Anaplasma* spp. infection in adult ticks between sites, in either chaparral or woodland in 2004 and 2005. Likewise, the same test was used to compare the prevalence of the same bacteria in *D.*

occidentalis or *I. pacificus* by life stage during each sampling occasion in 2009 or 2010; the prevalence of *B. burgdorferi* infection in *I. pacificus* ticks from the same generational cohort (spring 2009 nymphs versus winter 2009–2010 adults); the prevalence of *B. burgdorferi* infection in *D. occidentalis* versus *I. pacificus* nymphs in summer 2009; and the prevalence of *Anaplasma* spp. in *D. occidentalis* adults versus *I. pacificus* adults in spring 2009. No attempt was made to adjust for multiple statistical tests. The 5% level of probability was set for rejecting the null hypothesis, and only statistically significant probability values are presented below. Differences in the bacterial prevalence data for the *D. occidentalis* adults used during the transmission trial (Table 5) were not analyzed statistically because each group of those ticks had fed upon the same individual animals.

Results

CO_2-baited pitfall trapping, 2003

In total, 190 traps yielded 34 *O. coriaceus* nymphs or adults and 12 *D. occidentalis* adults (Table 1); 79% of the *O. coriaceus* and 75% of the *D. occidentalis* ticks were collected at the James-II woodland. The remaining *O. coriaceus* were captured at one of the other woodlands bordered by chaparral (Beasley), whereas the *D. occidentalis* adults were caught at 2 sites bordered either by chaparral (Hunt Club) or grassland (Tank). The percentage of traps that produced ≥1 tick was similar for both tick species (7.4%, *O. coriaceus*; 4.7%, *D. occidentalis*), but the mean number of ticks collected per trap was 3 times higher for *O. coriaceus* than it was for *D. occidentalis* (Table 1).

All individuals of both tick species were PCR-negative for *Anaplasma* spp., but one of 23 *O. coriaceus* nymphs from the James II produced a *B. burgdorferi* s.l.-positive PCR product that sequenced as *B. bissettii*. Although the 12 *D. occidentalis* adults also were tested for *B. burgdorferi* s.l., the results are not included here because of suspected contamination.

Flagging woodlands and chaparral, 2004–2005

Preliminary sampling of the adult *D. occidentalis* populations in the James-II woodland (one hour) and its adjoining chaparral (two hours) in 2004 produced 30 and 29 adults, respectively. Of these, 13.3% (4/30) from the woodland were infected with *B. burgdorferi*, but not with *Anaplasma* spp., whereas 3.4% (1/29) of ticks from chaparral were infected with *A. phagocytophilum*, but not with *Borrelia* spp.

These findings spurred a more in-depth examination of *D. occidentalis* as a potential vector of *Borrelia* and *Anaplasma* spp. the following year. In total, 587 adult ticks were flagged from 4 woodlands and their adjacent patches of chaparral and, of these, one-half were tested for presence of these bacteria (Tables 2 and 3). The relative abundance of ticks was 5 times greater in chaparral than in woodland (56.4 versus 11.2 ticks per h, Tables 2 and 3).

In dense woodlands, *B. burgdorferi* was detected in zero, and *Anaplasma* spp. in 3 (2.3%) of 132 ticks. Sequencing revealed that 2 of the positive ticks were infected with *A. bovis* and one with *A. ovis* (Table 2). In chaparral, *B. burgdorferi* was detected in one (0.6%), and *Anaplasma* spp. in 9 (5.8%), of 156 ticks (p=0.026). Among the 4 individual sites, only Maude yielded a statistically significant pair-wise difference in *B. burgdorferi* versus *Anaplasma* spp. prevalence (p=0.041). Sequencing disclosed that 8 of the positive amplicons were *A. bovis* and one was *A. ovis* (Table 3).

Flagging/dragging woodland, 2009–2010

In spring 2009, more than twice as many *D. occidentalis* adults as compared with *I. pacificus* adults, and 21 times as many *I. pacificus* nymphs versus adult ticks were collected

per hour (Table 4). Additionally, 1.6 times more *D. occidentalis* nymphs as compared with *I. pacificus* nymphs were caught in summer. In winter 2009–2010, the relative abundance of *D. occidentalis* and *I. pacificus* adults were 1/15th and 2.6 times greater than what they were in spring 2009, respectively.

None of the *D. occidentalis* or *I. pacificus* adults was PCR-positive for borreliae, whereas 2.2% of the *D. occidentalis* adults contained *A. bovis* in spring 2009. The *Anaplasma* spp. prevalence in *D. occidentalis* did not differ significantly from the zero prevalence for the 20 *I. pacificus* adults collected simultaneously. Among the nymphs, 2.6% of the *D. occidentalis* and 8.3–10.0% of the *I. pacificus* were infected with *B. burgdorferi* in spring or summer 2009. The prevalence of spirochete infection in the *D. occidentalis* nymphs (2.6%) versus that in the *I. pacificus* nymphs (8.3%) collected in summer did not differ significantly, nor did the prevalence in *I. pacificus* nymphs (10.0%, spring 2009) and adult ticks (2.0%, winter 2009–2010) from the same generational cohort (Table 4).

Attempts to culture *B. burgdorferi*

Borreliae were not cultured from the tissues of 35 *D. occidentalis* adults that had been placed in BSK-H medium.

Transmission trial

Four rabbits were exposed to the feeding activities of either 28 or 30 *D. occidentalis*, half of which were females (Table 5). Blood samples taken from each rabbit before and at 2, 4, or 6 weeks post-tick exposure were PCR-negative for both *B. burgdorferi* or *Anaplasma* spp. The number of fed or partially-fed ticks recovered from each rabbit ranged from 22 to 29. All 105 ticks were PCR-negative for *B. burgdorferi*, but one male tick (3.7%) from rabbit 840 was positive for *A. bovis* and 2 male ticks (6.9%) from rabbit 841 were infected with *A. ovis*.

Discussion

CO_2 trapping studies, 2003

The use of carbon dioxide as a host-seeking attractant is the only method known to be suitable for efficiently collecting the soft tick *O. coriaceus* (Garcia, 1962; Hokama and Howarth, 1977). In the present study, nearly 200 h of $C0_2$-baited, pitfall-trapping in 8 dense woodlands yielded few *O. coriaceus* or *D. occidentalis* ticks, which suggests that oak-dominated dense woodlands are not a preferred habitat for either tick species. In preceding investigations aimed at determining the vector specificity of ticks for *B. burgdorferi* at the HREC, basically the same CO_2-baited pitfall-trapping procedure was used to sample populations of *O. coriaceus* and, coincidentally, *D. occidentalis* (Lane and Manweiler, 1988; R.S. Lane, unpubl. data). In 1984, for example, 40 trapping sessions were conducted in each habitat type on 8 dates spanning 29 June to 6 September. The mean numbers of *O. coriaceus* nymphs/adults collected per trapping period in chaparral (8.05) and woodland-grass (2.28) were 45 and 13 times greater than the mean number (0.18) caught in dense woodlands in 2003 (R.S. Lane, unpubl. data; present study). Although the chaparral/woodland-grass collections were made 19 years earlier, we nevertheless posit that woodland-grass and particularly chaparral are more favored habitats than dense woodlands for the pajahuello tick in the Hopland area.

Moreover, the unpublished 1984 data for *D. occidentalis* from chaparral (mean, 0.13 ticks per trapping session) and woodland-grass (mean, 0.00) mirror the 2003 dense-woodland data (mean, 0.06) from the current study. Similarly, Garcia (1962) noted that CO_2 trapping yielded much lower frequencies of *D. occidentalis* adults than those of *O. coriaceus* in chaparral-covered foothills in the San Francisco Bay region. Our findings and those of

Garcia (1962) demonstrate that CO_2 trapping is ineffective for determining the relative abundance of the Pacific Coast tick in these habitat types.

Flagging woodlands and chaparral, 2004–2005

Not surprisingly, flagging produced many more *D. occidentalis* adults per timed-sampling effort than pitfall trapping in dense woodlands. The actual area covered by flagging on an hourly basis was orders of magnitude greater than pitfall trapping, the effective radius of CO_2 trapping for collecting *D. occidentalis* adults was just 4 m in chaparral (Lane et al., 1985b), and some of the *D. occidentalis* adults attracted to pitfall traps escaped (Hokama and Howarth, 1977; R.S. Lane, pers. observations). In 2005, a mean of 11.2 *D. occidentalis* adults was flagged hourly from the 4 dense woodlands sampled, which was higher than the mean numbers of *D. occidentalis* ticks typically flagged per hour (≤10.4) in northern California, except from chaparral (Lane, 1992; Lane and Lavoie, 1988; present study).

Flagging woodland, 2009–2010

In spring 2009, the relative abundance of *D. occidentalis* adults in the James II was one-half what it was in spring 2005 but 15 times greater than it was in January 2010. At the HREC, *D. occidentalis* adults are active in chaparral during winter and spring with a peak in March (Lane, 1990). If the seasonal peak activity period of *D. occidentalis* adults is similar in dense woodlands and chaparral, we likely missed the acme of host-seeking in both habitat types and in all years by having sampled for adult Pacific Coast ticks in January, May, and June. The collection of 9.8 *D. occidentalis* nymphs per hour by dragging the forest floor in mid-July is the highest relative abundance of host-seeking *D. occidentalis* nymphs ever recorded from any habitat. Previously, Castro et al. (1997) collected 23 *Dermacentor* spp. nymphs from 3 (chaparral, Douglas fir, oak woodland) of 4 habitats sampled semi-monthly by flagging over a one-year period in neighboring Sonoma County, California. Twenty (87%) of the nymphs were flagged from chaparral. Subsequently, Castro et al. (2001; M.B. Castro, pers. comm.) collected an average of 0.4 *D. occidentalis* nymphs per hour by flagging leaf litter in 2 oak-woodland habitats (designated sites B and C) in the same county.

The overall hourly means recorded for *I. pacificus* adults from the James-II site on paired dates in spring 2009 (i.e., 2.5) and winter 2009–2010 (6.4) are on the low side compared with previous studies from other habitats in which up to an average of 106.5 adult ticks were collected per hour (Lane, 1992; Lane and Lavoie, 1988). This probably represents a true difference in the size of adult-tick populations present in dense woodlands versus the other habitat types, though it may reflect the months or time-of-day when samples were taken or a dearth of suitable vegetative perches for host seeking in the James-II woodland. At the HREC, *I. pacificus* adults are active in chaparral from January through May, with a pronounced peak in early March and a slight peak in November (Lane, 1990). Since we sampled specifically for *I. pacificus* adults in January, we may have inadvertently bypassed the annual peak-activity period for this tick, too. As anticipated (Lane et al., 2004), the James-II population of *I. pacificus* nymphs was high (106 ticks per h) in late May, low in mid-July when its seasonal activity period was winding down, and inactive in January.

Prevalence of *B. burgdorferi* s.l. in ticks

In northwestern California, host-seeking *D. occidentalis* or *O. coriaceus* have been evaluated before as possible secondary vectors of *B. burgdorferi*, but to our knowledge not in dense woodlands carpeted with leaf litter (e.g., Barlough et al., 1997; Kramer et al., 1999; Lane, 1992, 1996; Lane and Lavoie, 1988; Lane and Manweiler, 1988; Lane et al., 1985a, 2004, 2005; Nicholson et al., 1999). Among hundreds of host-seeking *D. occidentalis* adults collected from chaparral, grassland, or woodland-grass in several studies (reviewed by Lane, 1996), only 0.8% (2/253) *D. occidentalis* adults flagged from chaparral at the HREC tested

positive for *B. burgdorferi* with monoclonal antibodies by indirect immunofluorescence (Lane and Lavoie, 1988).

Overall, 0.9% (5/536) of the *D. occidentalis* adults and 2.6% (1/38) of the nymphs we collected from woodlands or chaparral in all years except 2003 were found to be infected with *B. burgdorferi* by 3 methods (culture, PCR alone, or PCR post-feeding on rabbits). Similarly, 0.8% (3/353) of *D. occidentalis* adults collected from recreational areas in central coastal California were PCR positive for *B. burgdorferi* (Holden et al., 2003). By comparison, 9.5% (7/74) of the *I. pacificus* nymphs and 1.4% (1/71) of the adults collected during spring, summer, or winter in 2009/2010 were PCR positive for *B. burgdorferi*.

We conclude that *D. occidentalis* larvae or nymphs occasionally acquire and transstadially pass *B. burgdorferi* after having fed on reservoir hosts in dense woodlands (Lane et al., 2005; Salkeld et al., 2008). For example, one of 5 *D. occidentalis* larvae removed from western gray squirrels at the HREC was infected with *B. burgdorferi* (Lane et al., 2005). The viability, infectivity, and transmissibility of *B. burgdorferi* in or by *D. occidentalis* adults remain unknown because all ticks used in isolation attempts (n=35) or recovered from rabbits (n=105) during the transmission trial were not infected with borreliae. However, several vector competence studies, and a seroepidemiologic investigation using anti-arthropod saliva antibodies as a biologic marker for tick exposure in a rural community at high risk for Lyme disease, demonstrated that this tick is an inefficient experimental or natural vector of *B. burgdorferi* (Brown and Lane, 1992; Lane et al., 1994, 1999; Li and Lane, 1996).

Although the pajahuello tick (*O. coriaceus*) is the primary vector of the relapsing-fever group spirochete *B. coriaceae*, we report here for the first time the detection of a Lyme disease group spirochete (*B. bissettii*) in this human-biter or, for that matter, in any *Ornithodoros* species, by PCR. The infected nymph was collected in the James-II woodland, a highly enzootic site for the human pathogen *B. burgdorferi* (Lane et al., 2007). This woodland, and 3 others we investigated (Beasley, Hunt Club, Maude), are bordered largely by chaparral wherein exists a *B. bissettii* transmission cycle driven primarily by *I. spinipalpis* ticks, dusky-footed wood rats (*Neotoma fuscipes*), and *Peromyscus* spp. mice (Eisen et al., 2009). Likewise, host-seeking *I. pacificus* nymphs inhabiting such woodlands at the HREC and elsewhere in Mendocino County occasionally are infected with *B. bissettii*, as was an *I. pacificus* larva removed from a western gray squirrel in the James-II woodland (Eisen et al., 2004a, 2009; Lane et al., 2005).

In an earlier study at the HREC, tissue smears prepared from 5.6% (1/18) spirochete-infected *O. coriaceus* ticks collected from either chaparral or woodland–grass habitats reacted with a monoclonal antibody (H5332) believed to be specific for *B. burgdorferi* (Lane and Manweiler, 1988). Thus, *O. coriaceus* occasionally acquires and maintains *B. burgdorferi* s.l., and therefore this tick cannot be ruled out as a potential secondary vector of Lyme disease group spirochetes pending the results of experimental transmission studies.

To our knowledge, the only other soft ticks that have been implicated in the ecology or epidemiology of *B. burgdorferi* s.l. are either bat- or bird-feeding parasites in the genus *Argas* (*A. reflexus, A. vespertilionis*) (Genchi et al., 1989; Hubbard et al., 1998; Stanek and Simeoni, 1989). In Europe, circumstantial evidence suggests that the pigeon tick (*A. reflexus*) occasionally may transmit *B. burgdorferi* s.l. to people inside buildings where pigeons roost (Genchi et al., 1989; Stanek and Simeoni, 1989).

I. pacificus nymphs inhabiting the James-II woodland had a 5-fold higher prevalence of borreliae (10%, n=50, spring 2009) than did adult ticks (2%, n=51, winter 2009–2010) from the same generational cohort. Similarly, the combined infection prevalence with *B.*

burgdorferi s.l. was 5-fold higher in nymphs (7.4%) than in adult ticks (1.6%) from the same generational cohort in 2 woodlands at the HREC (Eisen et al., 2004c). The adult ticks were collected in ecotones bordering the leaf-litter areas. Reservoir-incompetent and spirochete-cleansing western fence lizards are primary hosts of *I. pacificus* immatures in certain woodlands including the James II, which presumably accounts for much of the reduction in *B. burgdorferi* infection prevalence from the nymphal to adult-tick stage (Eisen et al., 2004b; Lane et al., 2007).

Prevalence of *A. phagocytophilum* in ticks

A. phagocytophilum was not detected in the 34 *O. coriaceus* or 12 *D. occidentalis* collected from dense woodlands in 2003, but the following year one (3.4%) of 29 *D. occidentalis* adults from chaparral and none of 30 adults from woodland were infected with this rickettsia during the pilot study. In 2005, none of 288 *D. occidentalis* adults from chaparral or dense woodland was infected, nor were 105 adult ticks from the James II that had been put on rabbits. In 2009–2010, 49 *D. occidentalis* adults likewise were PCR-negative for *A. phagocytophilum*. Hence, just one (0.2%) of 513 *D. occidentalis* adults tested from both habitats in all years contained *A. phagocytophilum*. In central coastal California, *A. phagocytophilum* was detected in a slightly higher percentage (1.1%, 4/353) of host-seeking *D. occidentalis* adults (Holden et al., 2003). In other Californian studies involving lower sample sizes, host-seeking *D. occidentalis* adults invariably were PCR-negative for *A. phagocytophilum* [e.g., Barlough et al., 1997 (n=57 ticks); Kramer et al., 1999 (n=162); Nicholson et al., 1999 (n=9)].

Taken together, these findings, like those presented above for *B. burgdorferi*, suggest that *D. occidentalis* is not a primary vector of *A. phagocytophilum*, and its involvement in the transmission cycles of both bacteria is tangential to that of *I. pacificus* (Lane et al., 2007; present study). Although all 145 *I. pacificus* nymphs or adults tested from the James II during 2009 or 2010 were PCR-negative for *A. phagocytophilum*, an earlier study carried out there revealed that up to 15.6% of the *I. pacificus* nymphs from various biotopes (e.g., logs) are infected with it in certain years (Lane et al., 2007). This finding suggests that year-to-year transmission cycles of *A. phagocytophilum* are either highly focal or variable within this particular woodland.

Prevalence of *A. bovis* and *A. ovis* in ticks

Our detection of *A. bovis* and *A. ovis* in *D. occidentalis* ticks are the first such associations for these veterinary pathogens. Prior to 2003, *A. bovis* had been recognized primarily as a parasite of cattle and buffalo in Africa, the Middle East, and South America, and *A. ovis* as a parasite of domestic sheep and goats worldwide and bighorn sheep in North America (Davidson and Goff, 2001; Goethert and Telford, 2003; Goff et al., 1993; Uilenberg, 1997). Since then, the known geographic and vertebrate host/vector tick distributions of *A. bovis* or closely related rickettsiae have expanded to include cottontail rabbits (*Sylvilagus floridanus*), rabbit ticks (*Haemaphysalis leporispalustris*), and *Ixodes* spp. in North America (Goethert and Telford, 2003), *Haemaphysalis* ticks from Thailand (Parola et al., 2003), Korea (Kim et al., 2003), and Russia (Shpynov et al., 2006), and cattle, sika deer (*Cervus nippon nippon, C. nippon yesoensis*), and *Haemaphysalis* ticks from Japan (Kawahara et al., 2006; Jilintai et al., 2009).

Similarly, *A. ovis* has been detected recently in an increasing number of wild ruminants around the globe (e.g., de la Fuente et al., 2007, 2008; Haigh et al., 2008; Yabsley et al., 2005). Neither rickettsia has been considered to be a zoonotic pathogen, but Chochlakis et al. (2010) presented intriguing molecular evidence that a patient from Cyprus who experienced a febrile illness accompanied by hepatosplenomegaly and lymphadenopathy

was infected with *A. ovis*. Here, we incriminate *D. occidentalis* as a potentially important vector of *A. bovis*. The high prevalence of *A. bovis* infection (4.3%, n=185 ticks) within chaparral-derived ticks in 2004 and 2005 suggests that *D. occidentalis* could be an efficient vector of this rickettsia. The occasional presence of this rickettsia in Pacific Coast ticks in adjacent woodlands may represent spill-over from reservoir hosts and vector ticks that predominate in chaparral. In the northeastern and southern United States, lagomorphs (cottontails, *Sylvilagus* spp.; black-tailed jack rabbits, *Lepus californicus*) and the ubiquitous rabbit tick *H. leporispalustris* have been implicated in the enzootiology of *A. bovis* (Goethert and Telford, 2003; Yabsley et al., 2006). Although the enzootic cycle of *A. bovis* awaits clarification in the Far West, brush rabbits (*Sylvilagus bachmani*) and black-tailed jack rabbits are resident at the HREC, they overlap spatially in chaparral, and they are infested by both *H. leporispalustris* and *D. occidentalis* (Furman and Loomis, 1984; Lane and Burgdorfer, 1988; Lane et al., 1981b).

D. occidentalis also is the most likely vector of *A. ovis* in northwestern California given earlier experimental data and our molecular evidence demonstrating that this tick can transmit *A. ovis* intrastadially (D. Stiller, unpublished, cited by Davidson and Goff, 2001) and transstadially (present study), respectively. Previously, *D. hunteri*, a parasite mainly of bighorn sheep (*Ovis canadensis*) and occasionally of mule deer (*Odocoileus hemionus*), was the suspected vector of *A. ovis* in the western United States (Stiller et al., 1999; Yabsley et al., 2005). *A. ovis* has been detected in mule deer in the western United States (de la Fuente et al., 2007; Yabsley et al., 2005), but collection records of *D. hunteri* ticks from California are restricted to the arid southeastern region inhabited by desert bighorn sheep (Furman and Loomis, 1984). At the HREC, the only ungulates present are domestic sheep and a subspecies of mule deer (Columbian black-tailed deer, *O. hemionus columbianus*). *D. occidentalis* seldom parasitizes sheep, but the adult ticks abundantly infest Columbian black-tailed deer (Furman and Loomis, 1984; Westrom, 1975). This cervid reportedly is susceptible to infection with *A. marginale*, deer exposed to this rickettsia develop long-term rickettsemias without manifesting clinical illness, and naturally infected *D. occidentalis* adults can transmit *A. marginale* to cattle (Davidson and Goff, 2001; Howarth and Roby, 1972; Howarth et al., 1969).

A caveat concerning *Anaplasma* studies conducted before the 1990s was that calf inoculation, Giemsa-stained blood smears, and serologic methods were used to diagnose such infections in wild ungulates (Howarth et al., 1969; Kuttler, 1984) because DNA probes and monoclonal antibodies had not been developed yet. Accordingly, some of the *Anaplasma* infections attributed to *A. marginale* in deer in California (e.g., Howarth and Roby, 1972; Howarth et al., 1969) may have represented other *Anaplasma* spp. Indeed, *A. bovis* and *A. ovis* infections were not detected in wild deer until recently (de la Fuente et al., 2007, 2008; Haigh et al., 2008; Kawahara et al., 2006; Yabsley et al., 2005). In California, the diagnostic dilemma is obfuscated further by the presence of 2 additional *Anaplasma* spp. in deer, i.e., *A. phagocytophilum* (reported as *Ehrlichia equi*) and *Anaplasma* sp. (reported as *Ehrlichia*-like sp. of white-tailed deer) (Foley et al., 1998).

Transmission trial

Our initial attempts to experimentally transmit *Anaplasma* spp. and *B. burgdorferi* from potentially infected, wild-caught *D. occidentalis* adults to 4 naïve rabbits were unsuccessful. Three adult male ticks recovered from 2 of the rabbits were PCR-positive for either *A. bovis* or *A. ovis*, whereas none of the ticks was discovered to contain *B. burgdorferi*. Our findings suggest that the New Zealand white rabbit may not be susceptible to infection with either *A. bovis* or *A. ovis*, or if rabbits develop a rickettsemia, it may be transient, of low titer, or occur more than 6 weeks after the attachment of an infectious tick. *D. occidentalis* is a competent experimental vector of the bovine and deer rickettsia *A. marginale* (referenced by

Davidson and Goff, 2001), and males of the Rocky Mountain wood tick (*Dermacentor andersoni*) are primary vectors of *A. marginale* to cattle. Moreover, *D. andersoni* males become persistently infected, feed intermittently, freely transfer among cattle, and therefore can transmit the infection to more than one animal (Kocan et al., 1992a, 1992b). A similar infection cycle has been described for *A. ovis* in *D. andersoni* males (Kocan and Stiller, 1992). We chose rabbits instead of mice as our laboratory animal model because *D. occidentalis* adults rarely attach to wild rodents, but they readily feed upon rabbits both in the field and laboratory (Lane and Burgdorfer, 1988; Casher et al., 2002; present study).

Conclusions

We and other researchers have demonstrated that in dense woodlands in northwestern California, *D. occidentalis* occasionally acquires and maintains *B. burgdorferi* and *A. phagocytophilum*, and that *O. coriaceus* sometimes acquires and transstadially passes *B. burgdorfer*i s.l. Experimental proof that these ticks are efficient vectors of either agent is lacking, however. On the other hand, *D. occidentalis* adults, particularly in chaparral, may be primary vectors of the veterinary pathogens *A. bovis* and *A. ovis*. The 5-fold greater prevalence of *B. burgdorferi* infection in *I. pacificus* nymphs as compared with the adults from the same generational cohort in 2009–2010 reconfirms antecedent observations, but the absence of *A. phagocytophilum* in the nymphs contravenes recent findings from the same woodland suggesting that nidi of this bacterium are either highly focal or variable from year to year.

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Table 1

Number of *O. coriaceus* and *D. occidentalis* ticks collected in carbon dioxide-baited pitfall traps in 8 dense woodlands bordered by chaparral or grassland, Hopland area, California, 13 June to 7 August 2003.

Woodland site[a]	Number of traps set	Number of ticks collected by species[b] (stage[c])		Percentage of traps yielding ≥1 tick		Mean number of ticks/trap ± SD	
		O-cor	D-occ	O-cor	D-occ	O-cor	D-occ
Beasley	20	7 (n)	0	5.0	–	0.35±1.56	–
Hunt Club	20	0	1 (f)	–	5.0	–	0.05±0.22
James II	50	27 (23n, 2m, 2f)	9 (3m, 6f)	26.0	12.0	0.54±1.51	0.18±0.63
Maude	20	0	0	–	–	–	–
Cell Tower, Parson, Pepperwood, Tank	80	0	2 (m, f)	–	2.5	–	0.03±0.17
Total	190	34 (30n, 2m, 2f)	12 (4m, 8f)	7.4	4.7	0.18±0.95	0.06±0.35

[a] Beasley, Hunt Club, James II and Maude were bordered by chaparral, the remaining four sites by grassland. Data for the grass-bordered sites were pooled because they yielded only two ticks.

[b] O-cor, *Ornithodoros coriaceus*, D-occ, *Dermacentor occidentalis.*

[c] n, nymph; m, male; f, female.

Table 2

Relative abundance of *D. occidentalis* adults collected by flagging low vegetation in 4 dense woodlands, Hopland area, California, 12 May to 4 June 2005, and prevalence of infection with *B. burgdorferi* or *Anaplasma* spp. in 43% of those ticks.

Site (n[a])	Number of ticks collected by sex[b]	Mean number ticks per hour (range)	Number of ticks PCR-positive/number tested (percentage positive)		Species of *Anaplasma*[c]
			B. burgdorferi	***Anaplasma* spp.**	
Beasley (2)	49 (21m, 28f)	24.5 (12–37)	0/46	0/46	–
Hunt Club (3.75)	43 (21m, 22f)	11.5 (8–17)[d]	0/43	1/43 (2.3)	Ao
James II (17)	203 (99m, 104f)	11.9 (3–21)	0/33	1/33 (3.0)	Ab
Maude (4.5)	10 (6m, 4f)	2.2 (0–4)	0/10	1/10 (10.0)	Ab
Total (27.25)	305 (147m, 158f)	11.2 (0–37)	0/132	3/132 (2.3)	2 Ab, 1 Ao

[a] Number of hours spent flagging at each site.

[b] m, male; f, female.

[c] Determined by sequencing analysis; Ab, *A. bovis*; Ao, *A. ovis*.

[d] Number of ticks for one sample extrapolated from 0.75 hour.

Table 3

Relative abundance of *D. occidentalis* adults collected by flagging chaparral abutting 4 dense woodlands, Hopland area, California, 12 to 26 May 2005, and prevalence of infection with *B. burgdorferi* or *Anaplasma* spp. in >50% of those ticks.

Site (n[a])	Number of ticks collected by sex[b]	Number of ticks positive/number tested (percentage positive)		Species of *Anaplasma*[c]
		B. burgdorferi	***Anaplasma* spp.**	
Beasley (2)	86 (47m, 39f)	0/41	2/41 (4.9)	2 Ab
Hunt Club (1)	77 (38m, 39f)	1/39 (2.6)	1/39 (2.6)	Ao
James II (1)	38 (20m, 18f)	0/38	0/38	–
Maude (1)	81 (40m, 41f)	0/38	6/38 (15.8)	6 Ab
Total (5)	282 (145m, 137f)	1/156 (0.6)	9/156 (5.8)	8 Ab, 1 Ao

[a] Number of hours spent flagging each site.

[b] m, male; f, female.

[b] Determined by sequencing analysis; Ab, *A. bovis*; Ao, *A. ovis*.

Table 4

Relative abundance of, and prevalence of *B. burgdorferi* or *Anaplasma* spp. infection in, *D. occidentalis* versus *I. pacificus* nymphs and adults collected by flagging low vegetation (adults) or dragging the forest floor[a] (nymphs) in the James-II woodland, Hopland area, California, 2009–2010.

Tick species by stage (n[b])	Season and year[c]	Number of ticks collected[d]	Mean number ticks per hour ± S.D. (range)	Number of ticks PCR-positive/number tested (percentage positive)	
				B. burgdorferi	***Anaplasma* spp.**
D. occidentalis					
Adults (8)	spring 2009	46 (19m, 27f)	5.6 ± 5.8 (1–19)	0/46	1/46 (2.2)[e]
Adults (8)	winter 2009–10	3 (1m, 2f)	0.4 ± 0.5 (0–1)	0/3	0/3
Nymphs (4)	summer 2009	39	9.8 ± 11.3 (0–20)	1/38 (2.6)	0/38
I. pacificus					
Adults (8)	spring 2009	20 (13m, 7f)	2.5 ± 2.6 (0–8)	0/20	0/20
Adults (8)	winter 2009–10	51 (25m, 26f)	6.4 ± 4.4 (2–14)	1/51 (2.0)	0/51
Nymphs (4)	spring 2009	423	105.8 ± 54.8 (48–156)	5/50 (10.0)	0/50
Nymphs (4)	summer 2009	24	6.0 ± 6.7 (2–16)	2/24 (8.3)	0/24

[a] Primarily leaf litter, though wood (e.g., logs) occasionally was sampled.

[b] Number of hours spent dragging or flagging.

[c] Sampling for *D. occidentalis* and *I. pacificus* adults was performed on 24 May and 7 June 2009 and on 16 and 31 January 2010; for *D. occidentalis* nymphs on 19 July 2009; and for *I. pacificus* nymphs on 24 May and 19 July 2009. The collection and test results for the *D. occidentalis* adults in spring 2009 and for the *I. pacificus* adults in winter 2009–2010 were combined because the 2 sampling occasions during each of those seasons were separated by only 2 weeks.

[d] m, male; f, female.

[e] Sequenced as *A. bovis*.

Table 5

Results of experimental attempts to infect New Zealand white rabbits with *B. burgdorferi* or *Anaplasma* spp. by feeding potentially infected, wild-caught *D. occidentalis* adults on them.

Rabbit number	Number of ticks put on rabbit[a]	Number of replete ticks tested/ number PCR-positive (%) for:		PCR test results of rabbit blood[b] for:	
		B. burgdorferi	*Anaplasma* spp.	*B. burgdorferi*	*Anaplasma* spp.
838	14m, 14f	0/22	0/22	negative	negative
839	14m, 14f	0/27	0/27	negative	negative
840	15m, 15f	0/27	1/27 (3.7)[c]	negative	negative
841	15m, 15f	0/29	2/29 (6.9)[d]	negative	negative
Total	58m, 58f	0/105	3/105 (2.9)		

[a] m, male; f, female.

[b] PCR was performed on blood specimens taken before and at 2, 4, and 6 weeks after rabbits had been infested with ticks.

[c] Sequenced as *A. bovis*.

[d] Both positive amplicons sequenced as *A. ovis*.