Examining Prevalence and Diversity of Tick-Borne Pathogens in Questing *Ixodes pacificus* Ticks in California

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ABSTRACT   Tick-borne diseases in California include Lyme disease (caused by *Borrelia burgdorferi*), infections with *Borrelia miyamotoi*, and human granulocytic anaplasmosis (caused by *Anaplasma phagocytophilum*). We surveyed multiple sites and habitats (woodland, grassland, and coastal chaparral) in California to describe spatial patterns of tick-borne pathogen prevalence in western black-legged ticks (*Ixodes pacificus*). We found that several species of *Borrelia*—*B. burgdorferi*, *Borrelia americana*, and *Borrelia bissettiae*—were observed in habitats, such as coastal chaparral, that do not harbor obvious reservoir host candidates. Describing tick-borne pathogen prevalence is strongly influenced by the scale of surveillance: aggregating data from individual sites to match jurisdictional boundaries (e.g., county or state) can lower the reported infection prevalence. Considering multiple pathogen species in the same habitat allows a more cohesive interpretation of local pathogen occurrence.

IMPORTANCE Understanding the local host ecology and prevalence of zoonotic diseases is vital for public health. Using tick-borne diseases in California, we show that there is often a bias to our understanding and that studies tend to focus on particular habitats, e.g., Lyme disease in oak woodlands. Other habitats may harbor a surprising diversity of tick-borne pathogens but have been neglected, e.g., coastal chaparral. Explaining pathogen prevalence requires descriptions of data on a local scale; otherwise, aggregating the data can misrepresent the local dynamics of tick-borne diseases.

KEYWORDS *Borrelia miyamotoi*, *Borrelia burgdorferi*, *Anaplasma phagocytophilum*, tick-borne disease surveillance, aggregated data

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In California, the archetypal habitat-host system for natural Lyme disease transmission dynamics is the oak woodland of the northwest—particularly in Mendocino County—where western gray squirrels (*Sciurus griseus*) are the predominant reservoir hosts for the disease agent *Borrelia burgdorferi sensu stricto* (1–6). The western fence lizard (*Sceloporus occidentalis*) is also an important host of the western black-legged tick (*Ixodes pacificus*) vector, though borreliacidal blood factors mean that the lizard host removes *B. burgdorferi* from ticks and does not contribute to further *B. burgdorferi* transmission (5, 7–9). Lyme disease incidence is high in areas of northwestern California and can surpass 50 cases per 100,000 person-years (10).

However, research beyond Mendocino County oak woodlands has illuminated multiple other tick-pathogen disease systems across California's diverse habitats. For example, *I. pacificus* has also been found infected with *Borrelia miyamotoi*—a spirochete that has been strongly implicated as a cause of human disease in California (11). State-wide surveillance for this pathogen shows that it is present in many of the same counties as *B. burgdorferi* (12). In the northeastern United States, *B. miyamotoi* prevalence in ticks is normally lower than that of *B. burgdorferi* from the same locations (13), but in California the relationship is less predictable. State-level observations, and observations in some

TABLE 1 Summary of geographic observations of *Borrelia americana* and *Borrelia bissettiae* in California

Borrelia species	County (site)	Tick species	Mammal species	Reference or source
Borrelia americana	Los Angeles (Malibu Creek)	*I. pacificus*		30
	Marin (Tennessee Valley and Owl Trail)	*I. pacificus*		This study
	Orange (Crystal Cove State Beach)	*I. spinipalpis*		26
	San Mateo (Windy Hill OSP)	*I. pacificus*		26
	Santa Barbara (Coal Oil Point Reserve)	*I. spinipalpis*		27
Borrelia bissettiae	Alameda	*I. pacificus*	*Neotoma fuscipes* (dusky-footed woodrat), *Rattus rattus* (black rat)	14, 23
	Contra Costa	*Ixodes auritulus*		15
	Del Norte	*I. pacificus*		23
	Humboldt		*Neotamias senex* (Allen's chipmunk), *Neotoma fuscipes*	24
	Marin (Fort Baker)	*I. pacificus*		26
	Mendocino	*I. spinipalpis* ex *N. fuscipes*, *I. pacificus*	*Microtus californicus* (California vole), *Neotoma fuscipes, Peromyscus boylii* (brush mouse), *P. maniculatus* (deer mouse), *P. truei* (pinyon mouse)	3, 23
	Monterey (Andrew Molera SP)	*I. pacificus*		This study
	Orange (Crystal Cove State Beach)	*I. spinipalpis*		26
	San Luis Obispo		*Neotoma lepida* (desert woodrat), *P. boylii*	21
	San Mateo (Thornewood OSP)	*I. pacificus*	*P. boylii*	25, 26
	Santa Barbara (Coal Oil Point Reserve, Paradise Reserve)	*Ixodes peromysci, I. spinipalpis*	*R. rattus, Reithrodontomys megalotis* (western harvest mouse), *P. maniculatus*	27
	Santa Clara (Foothills Park)		*P. truei*	25
	Santa Cruz (Wilder Ranch SP)	*I. pacificus*		26

counties (e.g., Alameda County), suggest that prevalence of the two *Borrelia* species is roughly equivalent in adult *I. pacificus* ticks but that *B. burgdorferi sensu lato* is more common in nymphal ticks (12, 14). However, patterns of the relative frequencies of *B. miyamotoi*-*B. burgdorferi* infection appear idiosyncratic, and sometimes *B. miyamotoi* can be the more frequent or the only spirochete in questing tick populations (12, 15–17). Importantly, *B. miyamotoi* can be vertically transmitted from mother to offspring, so questing larvae may also be infected (18, 19).

Other documented species of the *B. burgdorferi sensu lato* complex in California include *Borrelia bissettiae* and *Borrelia americana*. *B. bissettiae* has been observed in human sera in Mendocino County, though its pathological impact is uncertain (20). In California, *B. bissettiae* has been reported from a diverse array of mammals, including wood rats (*Neotoma* spp.), mice (*Peromyscus* spp. and *Reithrodontomys megalotis*), chipmunks (*Neotamias* spp.), and rats (*Rattus rattus*), and in both *I. pacificus* and *Ixodes spinipalpis* (3, 21–27) (Table 1). *B. americana* has been observed in *I. pacificus* and *I. spinipalpis*; human infections have not been reported (26, 27). *Anaplasma phagocytophilum*, which causes human granulocytic anaplasmosis, also occurs in western black-legged tick populations of northern California and has been observed in a variety of habitats (28, 29).

Understanding the host ecology (identifying the species that act as reservoirs and the habitat associations) and the human epidemiology (where, when, and how often people are exposed and whether the bacteria cause illness) of these different tick-borne pathogens is not simple. The landscape is diverse, including chaparral, oak woodland, grasslands, and redwood forest within a county's limits and sometimes on the same hiking trail, with the implication that the reservoir host communities are also heterogeneous (Fig. 1). Consequently, the risk of exposure to tick-borne pathogens is geographically varied (14, 17, 26), and it is not always straightforward to describe local tick-borne pathogen prevalence. Should the infection prevalence in tick populations be described for a single trail, at the county level, or at a regional or state level? And what information is lost if the data are aggregated across these different scales?

FIG 1 Deer in Monte Bello Open Space Preserve, illustrating the habitat heterogeneity of California's landscape: a mosaic of grassland, chaparral, and woodland. (Courtesy of Karl Gohl; reprinted with permission.)

Here, we examined the prevalence patterns of *B. burgdorferi*, *B. americana*, *B. miyamotoi*, *B. bissettiae*, and *A. phagocytophilum* in questing *I. pacificus* ticks at sites in coastal counties of central and northern California. We also explored the impacts of aggregating data from site to regional levels.

RESULTS

***B. burgdorferi sensu lato* prevalence in adult ticks.** Collection sites and infection prevalence for *B. burgdorferi sensu lato* and *Borrelia miyamotoi* are shown in Fig. 2. Aggregated across all sites, real-time PCR prevalence of *B. burgdorferi sensu lato* was 2.9% (95% confidence interval [CI] = 2.3 to 3.7%) in adult ticks (Table 2). A total of 36 *B. burgdorferi sensu lato* samples were successfully sequenced from adult ticks, of which 32 (89%) were *B. burgdorferi sensu stricto* (Table 3). Sequencing also identified the presence of *B. americana* ($n = 3$) and *B. bissettiae* ($n = 1$) (Table 3; Fig. 3); further description of these results is below. Adult tick populations from Marin, Monterey, Napa, Sonoma, and Santa Cruz counties all harbored *B. burgdorferi sensu stricto* (sample sizes were >73 for each county). We did not observe *B. burgdorferi sensu lato* in adult ticks collected in Mendocino or Santa Clara counties, though samples from these two counties were small ($n < 24$ for both counties), and ticks were predominantly collected from coastal grassland or chaparral habitats in Mendocino County.

Several individual sites exhibited *B. burgdorferi sensu lato* prevalence greater than 3.7% in adult ticks, i.e., higher than the confidence intervals generated when all the data were aggregated (2.3 to 3.7%). Some of these sites included different species of *B. burgdorferi sensu lato*, such as *B. burgdorferi sensu stricto*, *B. americana*, and *B. bissettiae* (e.g., Marin Headlands and Tennessee Valley), and some were in habitats not traditionally associated with *B. burgdorferi sensu lato* (e.g., chaparral or redwood forest). We did not observe *B. burgdorferi sensu lato* in adult *I. pacificus* ticks at 11/27 of the sites at which we tested >30 ticks (11 sites representing 768 ticks).

***B. miyamotoi* prevalence in adult ticks.** At the regional level (all counties combined), *B. miyamotoi* in adult ticks occurred at a lower prevalence than *B. burgdorferi sensu lato*: 1.3% (95% CI = 0.8 to 1.8%) (Table 2). When county-level data were compared, *B. miyamotoi* and *B. burgdorferi sensu lato* were observed at comparable prevalences in Monterey (0.7% for both species) and Sonoma (2.8% *B. burgdorferi sensu lato*; 2.0% *B. miyamotoi*) counties.

At individual sites where *B. miyamotoi* was present (and where samples sizes were >30; $n = 9$), the prevalence of *B. miyamotoi* often exceeded the prevalence defined by the confidence intervals generated by the aggregated data (0.8 to 1.8%), ranging from 2.2 to 3.9% at 6 sites (Tables 2 and 3). Like *B. burgdorferi sensu lato*, *B. miyamotoi* was not observed in Mendocino or Santa Clara counties, where samples were small.
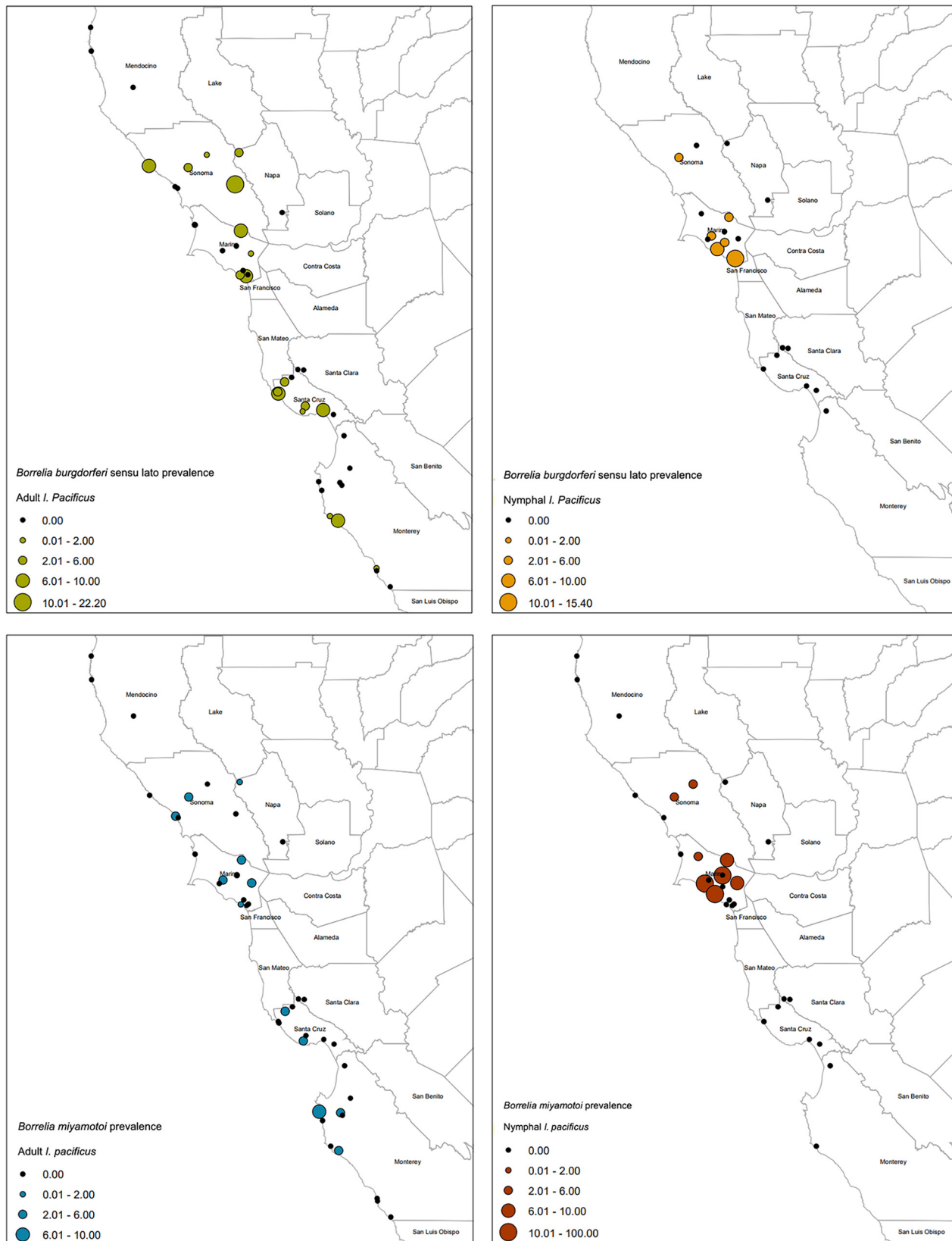
FIG 2 Maps showing collection sites for western black-legged ticks (*Ixodes pacificus*) and the infection prevalence (percentage positive) of *Borrelia burgdorferi sensu lato* (i.e., including *B. burgdorferi sensu stricto*, *B. americana*, and *B. bissettiae*) and *Borrelia miyamotoi*. The maps were created in ArcMap, and the polygon feature class of the California county boundaries was downloaded from ArcGIS (credits: U.S. Bureau of Reclamation, California Department of Conservation, California Department of Fish and Game, California Department of Forestry and Fire Protection, and National Oceanic and Atmospheric Administration).

TABLE 2 County, regional, and state-wide reports of prevalence of *Borrelia burgdorferi sensu lato* and *B. miyamotoi* in questing adult and nymphal *Ixodes pacificus* ticks in California

Level of data aggregation	Prevalence[a]				Reference
	Adult ticks		Nymphal ticks		
	B. burgdorferi sensu lato	*B. miyamotoi*	*B. burgdorferi sensu lato*	*B. miyamotoi*	
Alameda County	29/3,070 (0.9, 0.6–1.4)	13/3,070 (0.4, 0.2–0.7)	189/2,890 (6.5, 5.7–7.5)	11/2,890 (0.4, 0.2–0.7)	14
	3/285 (1.1, 0.2–3.0)	0/73 (0, 0–4.9)	3/342 (0.9, 0.2–2.5)		12, 26
Marin County	42/1,039 (4.0, 2.9–5.4)	17/1,039 (1.6, 1.0–2.6)	17/483 (3.5, 2.1–5.6)	33/483 (6.8, 4.7–9.5)	This study
	14/682 (2.1, 1.1–3.4)	1/406 (0.2, 0.01–1.4)	24/331 (7.3, 4.7–10.6)	2/240 (0.8, 0.1–3.0)	12, 26
Mendocino County	0/23 (0, 0–14.8)	0/23 (0, 0–14.8)			This study
	0/61 (0, 0–5.9)	0/54 (0, 0–6.6)	0/19 (0, 0–17.6)	0/17 (0, 0–19.5)	12, 26
Monterey County	5/693 (0.7, 0.2–1.7)	5/693 (0.7, 0.2–1.7)	0/1 (0, 0–97.5)	0/1 (0, 0–97.5)	This study
	0/140 (0, 0–2.6)	0/49 (0, 0–7.3)	0/35 (0, 0–10.0)	0/2 (0, 0–84.2)	12, 26
Napa County	4/73 (5.5, 1.5–13.4)	1/73 (1.4, 0.03–7.4)	0/20 (0, 0–16.8)	0/20 (0, 0–16.8)	This study
	3/285 (1.1, 0.2–3.0)	0/1 (0, 0–97.5)	3/342 (0.9, 0.2–2.5)	1/101 (1.0, 0.03–5.4)	12, 26
San Mateo County	1/86 (1.2, 0.03–6.3)	1/86 (1.2, 0.03–6.3)	5/203 (2.5, 0.8–5.7)	7/203 (3.4, 1.4–7.0)	17[b]
	15/620 (2.4, 1.4–4.0)	20/316 (6.3, 3.9–9.6)	4/96 (4.2, 1.1–10.3)	1/39 (2.6, 0.06–13.5)	12, 26
Santa Clara County	0/6 (0, 0–45.9)	0/6 (0, 0–45.9)	0/21 (0, 0–16.1)	0/21 (0, 0–16.1)	This study
	2/98 (2.0, 0.2–7.2)	2/98 (2.0, 0.2–7.2)	4/75 (5.3, 1.5–13.1)	5/75 (6.7, 2.2–14.9)	17[b]
	3/182 (1.6, 0.3–4.7)	0/167 (0, 0–2.2)	9/134 (6.7, 3.1–12.4)	1/39 (2.6, 0.06–13.5)	12, 26
Santa Cruz	12/299 (4.0, 2.1–6.9)	4/299 (1.3, 0.4–3.4)	0/33 (0, 0–10.6)	0/33 (0, 0–10.6)	This study
	3/893 (0.3, 0.1–1.0)	7/752 (0.9, 0.4–1.9)	16/476 (3.4, 1.9–5.4)	8/443 (1.8, 0.8–3.5)	12, 26
Sonoma	7/253 (2.8, 1.1–5.6)	5/253 (2.0, 0.6–4.6)	1/80 (1.3, 0.03–6.8	2/80 (2.5, 0.3–8.7)	This study
	1/216 (0.5, 0.01–2.6)	0/53 (0, 0–6.7)	3/337 (0.9, 0.2–2.6)	1/159 (0.6, 0.02–3.5)	12, 26
Region-wide (Marin, Mendocino, Monterey, Napa, Santa Clara, Santa Cruz, Sonoma counties)	70/2,386 (2.9, 2.3–3.7)	30/2,386 (1.3, 0.8–1.8)	18/567 (3.2, 1.9–5.0)	29/567 (5.1, 3.5–7.3)	This study
	24/2,459 (1.0, 0.6–1.4)	8/1,482 (0.5, 0.2–1.1)	55/1,674 (3.3, 2.5–4.3)	13/1,001 (1.3, 0.7–2.2)	12, 26
State-wide	37/6,036 (0.6, 0.5–1.0)	51/6,036 (0.8, 0.6–1.1)	70/2,188 (3.2, 2.5–4.0)	30/2,188 (1.4, 0.9–2.0)	12, 26

[a]Prevalence data are presented as number positive/number tested (percentage positive, 95% confidence interval).
[b]Data obtained from sites where *Borrelia* were identified to the species level, or where zero ticks were infected.

***B. burgdorferi sensu lato* prevalence in nymphal ticks.** In total, *B. burgdorferi sensu lato* prevalence in nymphal western black-legged ticks was 3.2% (95% CI = 1.9 to 5.0%) (Table 2). The vast majority of *Borrelia*-positive nymphal ticks were collected in Marin County: 17/18 *B. burgdorferi sensu lato*-positive nymphs and 27/29 *B. miyamotoi*-positive nymphs were observed in Marin County (496 nymphs were collected in Marin County; a total of 155 nymphs were collected from other counties). All the *B. burgdorferi sensu lato* samples sequenced from nymphs were determined to be *B. burgdorferi sensu stricto* (10/10).

At individual sites, nymphal infection prevalence was occasionally higher than the combined prevalence, e.g., 6.7% at Bolinas Lagoon (95% CI = 2.5 to 13.9%; *n* = 90) and 5.6% at Olompali State Park (95% CI = 2.1 to 11.8%; *n* = 107).

***B. miyamotoi* prevalence in nymphal ticks.** In contrast to the adult stage, *B. miyamotoi* was observed in higher prevalence than *B. burgdorferi* in nymphal ticks at the regional level: *B. miyamotoi* prevalence was 5.1% (95% CI = 3.5 to 7.3%) (Table 2), though there was no statistical difference (*B. burgdorferi sensu lato* versus *B. miyamotoi*; Fisher exact test *P* = 0.14; chi-square *P* = 0.10).

Nymphal infection prevalence of *B. miyamotoi* reached 17.8% (95% CI = 10.5 to 27.3; *n* = 90) in Bolinas Lagoon and was also high in China Camp (7.5%; 95% CI = 2.5 to 16.6; *n* = 67) and Olompali state parks (7.5%; 95% CI = 3.3 to 14.2; *n* = 107) (Table 3).

***B. miyamotoi* prevalence in larval ticks.** A pooled sample of two larvae, collected at Olompali State Park, Marin County, tested positive for *B. miyamotoi*. All other larvae (*n* = 85) were negative for *B. miyamotoi*, and these were also collected in Marin County: Olompali State Park (*n* = 22), China Camp State Park (*n* = 16), Cascade Canyon Open Space (*n* = 35), Northern Marin (*n* = 9), Bolinas Lagoon (*n* = 2), and Samuel P. Taylor

TABLE 3 Study sites, habitat types, and prevalence of *Borrelia burgdorferi sensu lato*, *B. miyamotoi*, and *Anaplasma phagocytophilum* in questing adult and nymphal western black-legged ticks (*Ixodes pacificus*)[a]

Site (collection date)	Habitat type	Prevalence[b]					
		Adult ticks			Nymphal ticks		
		B. burgdorferi sensu lato	B. miyamotoi	A. phagocytophilum	B. burgdorferi sensu lato	B. miyamotoi	A. phagocytophilum
Marin Co.							
Bolinas Lagoon (May 2018)	Woodland				6/90 (6.7, 2.5–13.9); 2 Bb ss	16/90 (17.8, 10.5–27.3); 5 Bm	7/90[Bb sl coinf] (7.8, 3.2–15.4)
Cascade Canyon OSP (May 2016)	Woodland				1/34 (2.9, 0.1–15.3); 1 Bb ss	0/34 (0, 0–10.3)	0/34 (0, 0–10.3)
China Camp SP (Jan and May 2016)	Woodland	1/89 (1.1, 0.03–6.1); 1 Bb ss	3/89 (3.4, 0.7–9.5); 2 Bm	2/89 (2.2, 0.3–7.9)	0/67 (0, 0–5.4)	5/67 (7.5, 2.5–16.6); 2 Bm	2/67[Bm coinf] (3.0, 0.4–10.4)
Lucas Valley (Jan and May 2016)	Grassland	0/42 (0, 0–8.4)	0/42 (0, 0–8.4)	0/42 (0, 0–8.4)	0/1 (0, 0–97.5)	1/1 (100, 2.5–100)	0/1 (0, 0–97.5)
Lucas Valley (Jan and May 2016)	Woodland	0/130 (0, 0–2.8)	1/130 (0.8, 0.02–4.2); 1 Bm	0/130 (0, 0–2.8)	0/14 (0, 0–23.2)	0/14 (0, 0–23.2)	0/14 (0, 0–23.2)
Marin Headlands—Owl Trail (Jan 2016)	Coastal chaparral	10/171 (5.8, 2.8–10.5); 5 Bb ss, 2 B am	1/171 (0.6, 0.01–3.2); 1 Bm	2/171 (1.2, 0.1–4.1)			
Northern Marin (May 2016)	Woodland				0/63 (0, 0–5.7)	2/63 (3.2, 0.4–11.0)	0/63 (0, 0–5.7)
Olompali SP (Jan and May 2016, May 2017)	Woodland	26/330 (7.9, 5.2–11.3); 3 Bb ss	10/330 (3.0, 1.5–5.5); 5 Bm	1/325 (0.3, 0.01–1.7)	6/107 (5.6, 2.1–11.8); 6 Bb ss	8/107 (7.5, 3.3–14.2); 5 Bm	1/76 (1.3, 0.03–7.1)
Point Reyes National Seashore—Five Brooks (May 2016)	Woodland				0/4 (0, 0–60.2)	1/4 (25, 0.6–80.6)	0/4 (0, 0–60.2)
Point Reyes National Seashore—McClure Beach (Jan and May 2016)	Coastal chaparral	2/100 (2.0, 0.2–7.0); 1 Bb ss	0/100 (0, 0–3.6)	0/100 (0, 0–3.6)			
Point Reyes National Seashore—Tomales Point (Jan 2016)	Coastal prairie, coastal chaparral	0/35 (0, 0–10.0)	0/35 (0, 0–10.0)	0/35 (0, 0–10.0)			
Samuel P. Taylor SP (Jan and May 2016)	Woodland	0/82 (0, 0–4.4)	2/82 (2.4, 0.3–8.5)	0/82 (0, 0–4.4)	2/90 (2.2, 0.3–7.8); 1 Bb ss	0/90 (0, 0–4.0)	0/90 (0, 0–4.0)
Tamalpais SP (Jan 2016)	Chaparral	0/15 (0, 0–21.8)	0/15 (0, 0–21.8)	0/15 (0, 0–21.8)			
Tennessee Valley (Jan 2016)	Coastal chaparral	3/39 (7.7, 1.6–20.9); 1 Bb ss; 1 B am	0/39 (0, 0–9.0)	0/39 (0, 0–9.0)			
Tennessee Valley (Jan and May 2016)	Woodland	0/6 (0, 0–45.9)	0/6 (0, 0–45.9)	0/6 (0, 0–45.9)	2/13 (15.4, 1.9–45.4)	0/13 (0, 0–24.7)	0/13 (0, 0–24.7)

(Continued on next page)

TABLE 3 (Continued)

		Prevalence[b]					
		Adult ticks				Nymphal ticks	
Site (collection date)	Habitat type	B. burgdorferi sensu lato	B. miyamotoi	A. phagocytophilum		B. burgdorferi sensu lato	B. miyamotoi	A. phagocytophilum
Mendocino Co.							
Glass Beach (Jan 2018)	Coastal prairie, coastal chaparral	0/13 (0, 0–24.7)	0/13 (0, 0–24.7)	0/13 (0, 0–24.7)			
Hendy Woods SP (Dec 2017)	Woodland	0/7 (0, 0–41.0)	0/7 (0, 0–41.0)	0/7 (0, 0–41.0)			
Mendocino Headland (Jan 2018)	Coastal prairie, coastal chaparral	0/3 (0, 0–70.8)	0/3 (0, 0–70.8)	0/3 (0, 0–70.8)			
Monterey Co.							
Andrew Molera SP (Jan 2018)	Woodland	1/149 (0.7, 0.02–3.7); 1 Bbis	0/149 (0, 0–2.4)	2/149 (1.3, 0.2–4.8)			
Elkhorn Slough National Estuarine Research Reserve (Dec 2015 and May 2016)	Coastal chaparral, coastal prairie, woodland	0/72 (0, 0–5.0)	0/72 (0, 0–5.0)	0/72 (0, 0–5.0)		0/1 (0, 0–97.5)	0/1 (0, 0–97.5)
Garrapata SP (Dec 2015)	Coastal chaparral	0/155 (0, 0–2.4)	0/155 (0, 0–2.4)	0/155 (0, 0–2.4)			
Garland Ranch Regional Park (Dec 2015 and May 2016)	Woodland	0/70 (0, 0–5.1)	2/70 (2.8, 0.3–10.0); 1 Bm	0/70 (0, 0–5.1)			
Garland Ranch Regional Park— Garzas Creek (Dec 2015)	Woodland	0/53 (0, 0–6.7)	0/53 (0, 0–6.7)	0/53 (0, 0–6.7)			
Pfeiffer Big Sur SP (May 2016)	Woodland	3/46 (6.5, 1.4–17.9); 2 Bb ss	1/46 (2.2, 0.1–11.5); 1 Bm	0/46 (0, 0–7.7)			
Point Lobos SP (Jan 2016)	Coastal prairie	0/28 (0, 0–12.3)	2/28 (6.7, 0.8–22.1); 2 Bm	0/28 (0, 0–12.3)			
Prewitt Loop Trail (Dec 2015)	Coastal chaparral	1/55 (1.8, 0.05–9.7)	0/55 (0, 0–6.5)	0/55 (0, 0–6.5)			
Salmon Creek Falls (Dec 2015, Jan and May 2016)	Woodland	0/57 (0, 0–6.3)	0/57 (0, 0–6.3)	1/57 (1.8, 0.04–9.4)			
Sand Dollar Beach (Dec 2015)	Coastal chaparral	0/3 (0, 0–70.8)	0/3 (0, 0–70.8)	0/3 (0, 0–70.8)			
Toro Park (Dec 2015)	Woodland	0/5 (0, 0–52.2)	0/5 (0, 0–52.2)	0/5 (0, 0–52.2)			

TABLE 3 (Continued)

Site (collection date)	Habitat type	Prevalence[b] Adult ticks B. burgdorferi sensu lato	B. miyamotoi	A. phagocytophilum	Nymphal ticks B. burgdorferi sensu lato	B. miyamotoi	A. phagocytophilum
Napa Co.							
Robert Louis Stevenson SP (May and Dec 2017)	Woodland	3/71 (4.2, 0.9–11.9); 3 Bb ss	1/71 (1.4, 0.4–7.6); 1 Bm	0/57 (0, 0–6.3)	0/11 (0, 0–28.5)	0/11 (0, 0–28.5)	
Skyline Wilderness Park (May 2017)	Woodland	0/2 (0, 0–84.2)	0/2 (0, 0–84.2)		0/9 (0, 0–33.6)	0/9 (0, 0–33.6)	
Santa Clara Co.							
Sanborn Creek (May 2017)	Woodland	0/3 (0, 0–70.8)	0/3 (0, 0–70.8)		0/3 (0, 0–70.8)	0/3 (0, 0–70.8)	
Skyline Boulevard (May 2017)	Woodland	0/3 (0, 0–70.8)	0/3 (0, 0–70.8)		0/18 (0, 0–18.5)	0/18 (0, 0–18.5)	
Santa Cruz Co.							
Big Basin Highway (May 2017)	Woodland	0/4 (0, 0–60.2)	0/4 (0, 0–60.2)		0/3 (0, 0–70.8)	0/3 (0, 0–70.8)	
Big Basin SP (Dec 2016)	Redwood forest/meadow	1/28 (3.6, 0.1–18.3); 1 Bb ss	1/28 (3.6, 0.1–18.3); 1 Bm	0/28 (0, 0–12.3)			
Big Basin SP—Rancho del Oso (Dec 2016 and May 2017)	Woodland	4/73 (5.5, 1.5–13.4); 4 Bb ss	0/73 (0, 0–4.9)		0/15 (0, 0–21.8)	0/15 (0, 0–21.8)	
Forest of Nisene Marks SP (Dec 2016 and May 2017)	Redwood forest, woodland	2/20 (10.0, 1.2–31.7); 1 Bb ss	0/20 (0, 0–16.8)	0/6 (0, 0–45.9)	0/4 (0, 0–60.2)	0/4 (0, 0–60.2)	
Henry Cowell SP (Dec 2016)	Redwood forest	1/38 (2.6, 0.1–13.8); 1 Bb ss	0/38 (0, 0–9.3)				
Larkin Valley (Jan and May 2017)	Woodland	0/20 (0, 0–16.8)	0/20 (0, 0–16.8)	0/20 (0, 0–16.8)	0/11 (0, 0–28.5)	0/11 (0, 0–28.5)	
Waddell Creek (Dec 2016)	Woodland	3/42 (7.1, 1.5–19.5); 2 Bb ss	0/42 (0, 0–8.4)	0/42 (0, 0–8.4)			
Wilder Ranch SP (Dec 2016)	Woodland/coastal chaparral	1/74 (1.4, 0.03–7.3)	3/74 (4.1, 0.8–11.4); 2 Bm				
Sonoma Co.							
Austin Creek (May and Dec 2017)	Woodland	2/76 (2.6, 0.3–9.2); 2 Bb ss	3/76 (3.9, 0.8–11.1); 3 Bm	0/68 (0, 0–5.3)	1/36 (2.8, 0.1–14.5)	1/36 (2.8, 0.1–14.5); 1 Bm	
Goat Rock (Dec 2017)	Coastal chaparral	0/38 (0, 0–9.3)	1/38 (2.6, 0.1–13.8); 1 Bm	0/38 (0, 0–9.3)			
	Woodland	1/64 (1.6, 0.04–8.4); 1 Bb ss	0/64 (0, 0–5.6)	0/60 (0, 0–6.0)	0/39 (0, 0–9.0)	1/39 (2.6, 0.1–13.5)	

TABLE 3 (Continued)

Site (collection date)	Habitat type	Prevalence[b]					
		Adult ticks			Nymphal ticks		
		B. burgdorferi sensu lato	B. miyamotoi	A. phagocytophilum	B. burgdorferi sensu lato	B. miyamotoi	A. phagocytophilum
Healdsburg Ridge OSP (May and Dec 2017)							
Pomo Canyon (Jan 2017)	Grassland/ coastal chaparral	0/34 (0, 0–10.3)	0/34 (0, 0–10.3)	0/34 (0, 0–10.3)			
Salt Point SP (Jan 2018)	Coastal prairie	2/32 (6.25, 0.7–20.8); 2 Bb ss	0/32 (0, 0–10.9)	1/32[Bb ss coinf] (3.1, 0.1–16.2)			
Trione-Annadel SP (Dec 2017)	Woodland	2/9 (22.2, 6.0–60.0); 2 Bb ss	0/9 (0, 0–33.6)	0/9 (0, 0–33.6)			

[a]Abbreviations: Bb sl, B. burgdorferi sensu lato; Bb ss, B. burgdorferi sensu stricto; Bm, B. miyamotoi; Bbis, B. bissettiae; Bam, B. americana; OSP, Open Space Preserve; SP, State Park.

[b]Prevalence data are presented as number positive/number tested (percentage positive, 95% confidence interval). Superscripts indicate coinfections and represent one coinfected tick in the total sample. Sequenced samples are described following the reported prevalence, e.g., 6/90 ticks positive for Borrelia burgdorferi sensu lato, 2 of which were sequenced as B. burgdorferi sensu stricto. Only a subset of positive samples were sequenced.

FIG 3 Maximum-likelihood tree and Tamura-Nei model demonstrating the relationship between previously and newly characterized sequences of the *Borrelia* 16S-23S intergenic spacer (IGS; *rrs-rrlA*). Newly characterized sequences were isolated from *Ixodes pacificus*. Blue sequences are previously characterized sequences. The tree is drawn to scale, with branch lengths measured in number of substitutions per site.

State Park ($n = 1$). *B. miyamotoi* prevalence in *I. pacificus* larvae was therefore minimally 4.2% (1/24; 95% CI = 0.1 to 21.1%) in Olompali State Park, assuming just a single infected larva in the tested pool, and 1.1% (1/87; 95% CI = 0.03 to 6.2%) overall in Marin County.

At Olompali State Park, where we observed *B. miyamotoi* in all the tick life stages, prevalence was 4.2% in larvae (1/24), 7.5% in nymphs (8/107), and 3.0% in adults (10/330). *B. miyamotoi* prevalence was not statistically different across life stages (Fisher's exact test, $P = 0.12$). Comparing *B. miyamotoi* prevalence in larvae from May 2016 (1/24) with nymphs in May 2017 (0/31), i.e., the same tick cohort, there was also no statistical difference in prevalence (Fisher's exact test, $P = 0.44$). Aggregating data from across Marin County, *B. miyamotoi* prevalence was 1.1% in larvae (1/87; 95% CI = 0.03 to 6.2%), 6.8% in nymphs (33/483; 95% CI = 4.7 to 9.5%), and 1.6% in adults (17/1,039; 95% CI = 1.0 to
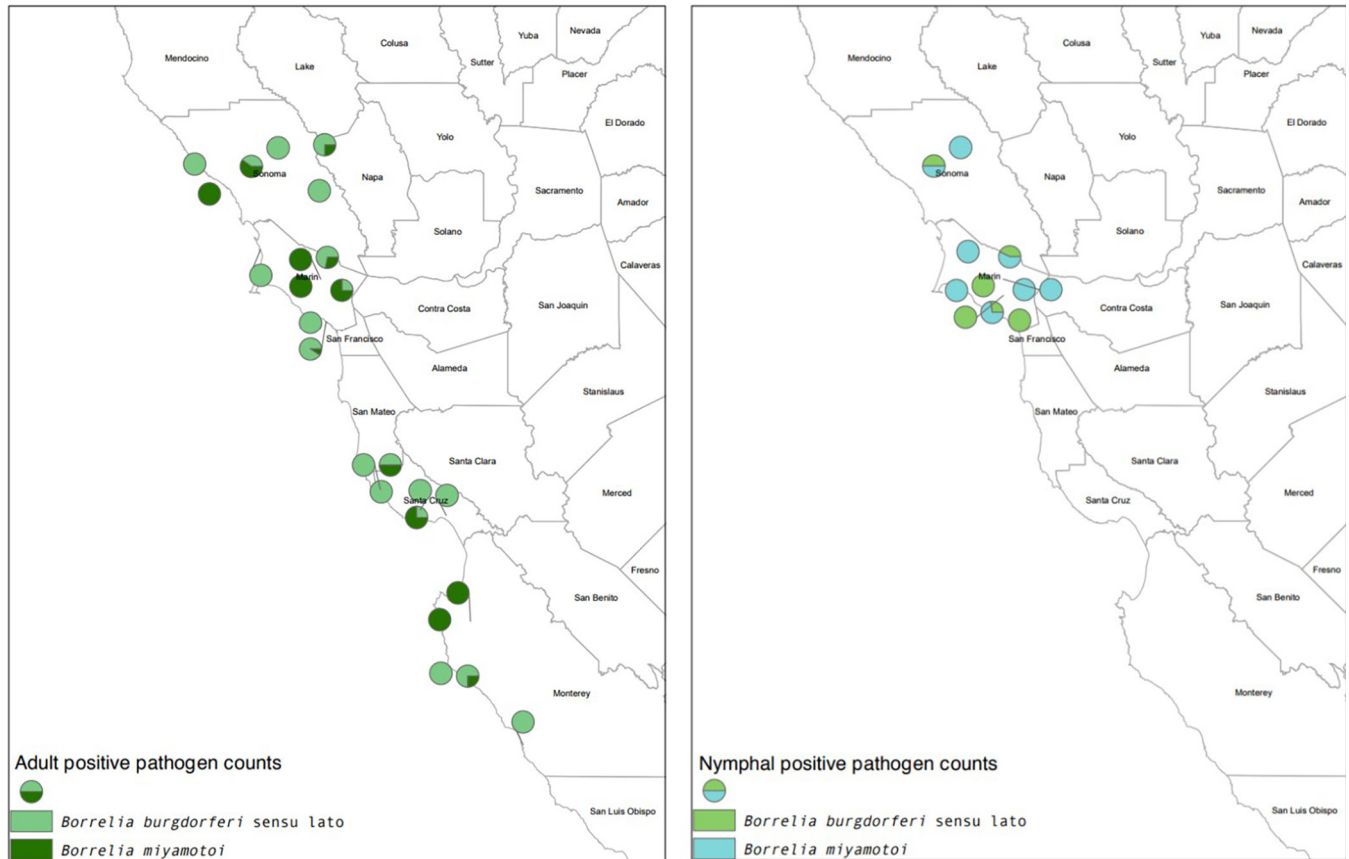
FIG 4 Maps showing proportional counts of positive samples from study sites for *Borrelia burgdorferi sensu lato* and *B. miyamotoi* in nymphal (left) and adult (right) western black-legged ticks (*Ixodes pacificus*). The maps were created in ArcMap, and the polygon feature class of the California county boundaries was downloaded from ArcGIS (credits: U.S. Bureau of Reclamation, California Department of Conservation, California Department of Fish and Game, California Department of Forestry and Fire Protection, and National Oceanic and Atmospheric Administration).

2.6%), revealing a significant difference in prevalence between life stages (Fisher's exact test, $P < 0.001$).

B. americana and B. bissettiae. *B. americana* was observed in three *I. pacificus* ticks, all collected in coastal chaparral habitat in Marin County. *B. americana* constituted 2/7 (28.6%) of the sequenced *B. burgdorferi sensu lato* samples from adult ticks on the Owl Trail, Marin Headlands, and 1/2 (50%) of the sequenced *B. burgdorferi sensu lato* samples from adult ticks in nearby Tennessee Valley. At both sites, both *B. americana* and *B. burgdorferi sensu stricto* were observed in the adult tick populations, and *B. miyamotoi* was also observed at Owl Trail.

B. bissettiae was observed just once, in an adult tick from Andrew Molera State Park in Monterey County, where it accounted for the only *Borrelia*-positive result.

***Anaplasma phagocytophilum* prevalence and coinfections.** *Anaplasma phagocytophilum* was observed in Marin County at prevalence up to 7.8% (95% CI = 3.2 to 15.4%; $n = 90$; Bolinas Lagoon), though its presence was sporadic (4/14 sites), as well as in Monterey County (2/11 sites) and in Sonoma County (1/6 sites).

We observed three coinfected ticks: one adult tick from Salt Point State Park with *B. burgdorferi sensu stricto* (sequenced) and *A. phagocytophilum*, one nymph with a similar microbial combination (*B. burgdorferi sensu lato*—not sequenced) from Bolinas Lagoon, and one nymph with *B. miyamotoi* (sequenced) and *A. phagocytophilum* from China Camp State Park.

Coexisting tick-borne pathogens. There was no obvious pattern to coexistence of *B. burgdorferi sensu lato* and *B. miyamotoi* (Fig. 4). At sites where *Borrelia* species were observed, 13 sites had only *B. burgdorferi sensu lato*, six sites had only *B. miyamotoi*, and the two species coexisted at 10 sites.
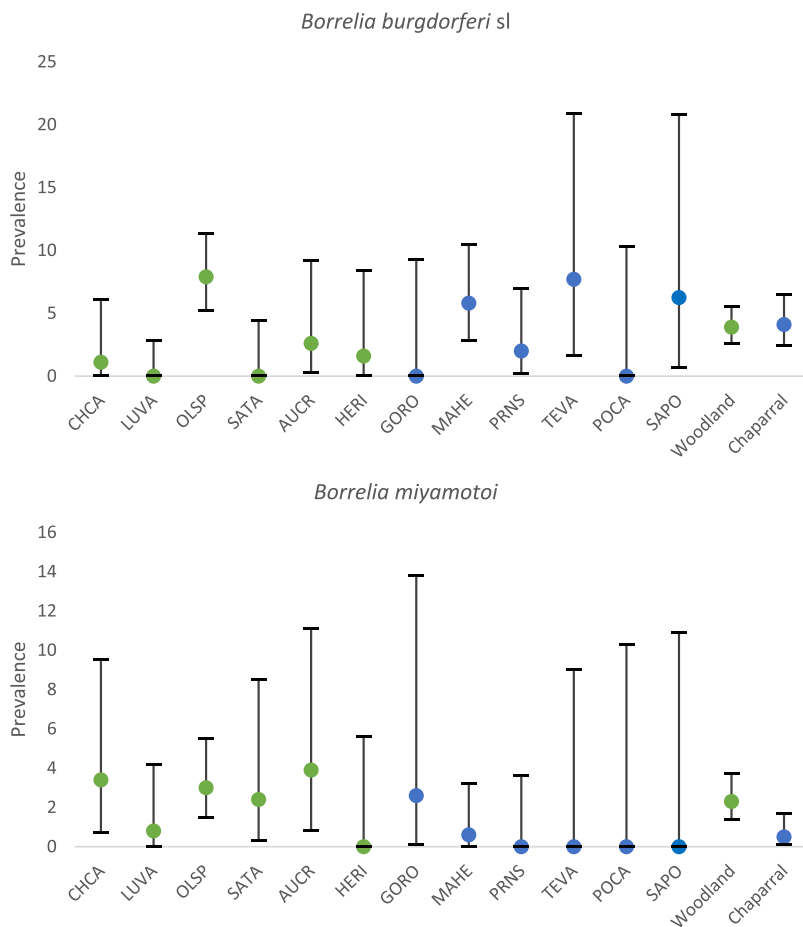
FIG 5 Comparisons of the prevalence of *Borrelia burgdorferi sensu lato* (sl) and *Borrelia miyamotoi* in adult *Ixodes pacificus* in woodland (green) and chaparral (blue) habitats of Marin and Sonoma counties, California. Bars show 95% confidence intervals for several sites; $n > 30$ for all sites. Sites were in Marin County (CHCA, China Camp State Park; LUVA, Lucas Valley woodland; OLSP, Olompali State Park; SATA, Samuel P. Taylor State Park; MAHE, Marin Headlands; PRNS, Point Reyes National Seashore [McClure Beach]; TEVA, Tennessee Valley [chaparral]) and Sonoma County (AUCR, Austin Creek; HERI, Healdsburg Ridge; GORO, Goat Rock; POCA, Pomo Canyon; SAPO, Salt Point).

At the site level, *B. burgdorferi sensu lato* was sometimes present in adult tick samples but absent from nymphs (e.g., China Camp State Park). The contrasting pattern (infected nymphs and uninfected adults) also appeared (e.g., Samuel P. Taylor State Park). Similarly, *B. miyamotoi* was observed in adult ticks at Samuel P. Taylor State Park but was not found in the collected nymphal ticks.

Habitat associations. *Borrelia*-positive ticks were observed in coastal chaparral and prairie habitats in Sonoma, Marin, Santa Cruz, and Monterey counties. Species identified by sequencing in these habitats represented the full gamut of *Borrelia* species identified in this study: *B. burgdorferi sensu stricto*, *B. miyamotoi*, *B. americana*, and *B. bissettiae* (Table 3; Fig. 3).

We compared tick-borne-pathogen prevalence in woodland and coastal chaparral habitat for our sample sites in Marin and Sonoma counties, using adult tick populations where n was >30. We restricted analyses to these two counties because they are coastal and the two habitats were well represented ($n=6$ for woodland and chaparral sites), and we used just adult ticks because nymphs are difficult to collect in chaparral. There was no significant difference in the prevalence of *B. burgdorferi sensu lato* in the two habitats after aggregation of the data ($\chi^2 = 0.03$; $P = 0.86$), and site-level prevalence was similar (Fig. 5). In contrast, *B. miyamotoi* prevalence was higher in woodland habitats ($\chi^2 = 5.57$; $P = 0.018$) (Fig. 5). Prevalence of *A. phagocytophilum* did not differ

according to habitat, though prevalence was low across sites (Fisher's exact test, $P = 0.67$).

Comparisons to previous reports. Our regional estimates of *B. miyamotoi* prevalence in *I. pacificus* ticks were 1.3% in adult ticks (95% CI, 0.8 to 1.8%), and 5.1% (95% CI, 3.5 to 7.3%) in nymphal ticks; compared to prior reports of 0.5% (95% CI, 0.2 to 1.1%) and 1.3% (95% CI, 0.7 to 2.2%) in adult and nymphal ticks, respectively, from these same counties, and statewide estimates of 0.8% (95% CI, 0.6 to 1.1%) and 1.4% (95% CI, 0.9 to 2.0%) (12) (Table 2). Prevalence of *B. miyamotoi* in adult ticks was not quite statistically significantly different from the regional aggregation and statewide levels reported by Padgett et al. (12) (Fisher's exact test, $P = 0.058$). However, our nymphal *B. miyamotoi* infection prevalence was significantly higher (Fisher's exact test, $P < 0.001$).

For *B. burgdorferi sensu lato*, our regional estimates of prevalence in *I. pacificus* ticks were 2.9% (95% CI, 2.3 to 3.7%) in adult ticks and 3.2% in nymphal ticks (95% CI, 1.9 to 5.0%), compared to prior reports of 1.0% (95% CI, 0.6 to 1.4%) in adults ticks and 3.3% (95% CI, 2.5 to 4.3%) in nymphal ticks from these same counties and statewide estimates of 0.6% (95% CI, 0.5 to 1.0%) and 3.2% (95% CI, 2.5 to 4.0%) in adult and nymphal ticks, respectively (12, 26). Our study observed a higher *B. burgdorferi sensu lato* prevalence in adult ticks than the prior studies (Fisher's exact test, $P < 0.001$), but there was no difference in nymphal *B. burgdorferi sensu lato* infection prevalence (Fisher's exact test, $P = 0.99$).

DISCUSSION

Describing infection prevalence. The tick-borne pathogens *Borrelia burgdorferi sensu lato*, *B. miyamotoi*, and *Anaplasma phagocytophilum* were observed sporadically in questing tick populations across northern California. Aggregating across the region, we found higher infection prevalence of *B. miyamotoi* in nymphal ticks and higher *B. burgdorferi sensu lato* infection prevalence in adult ticks than reported in recent studies (12, 26). Because the sampled ticks were collected at different times, these differences in prevalence may reflect trends in *Borrelia* infection patterns, interannual fluctuations in prevalence, or simply variation due to chance. Nevertheless, multiple measures of tick-borne infection prevalence are useful to gain a broader picture of local and regional pathogen prevalence, rather than relying on a single data source.

A key component and rationale of surveillance of ticks and tick-borne pathogens in the wild is to be able to represent the risk of exposure to humans. Often the data are summarized across the largest spatial extent. So, for example, despite sampling at multiple sites, data are reported as "In total, *x*% of ticks were infected with *B. burgdorferi*...," a behavior we have been guilty of (e.g., see references 17 and 29). However, aggregation of data into a single statistic can underrepresent the risk of exposure to tick-borne pathogens at sites where prevalence is higher, in part because the portrayed prevalence is deflated by including data from sites where tick abundance and/or pathogen prevalence is low. As an illustration, *B. burgdorferi sensu stricto* is known to be extremely rare in southern California—documented in only a single *I. pacificus* tick, from 5,571 ticks screened during three different studies (0.02%; 95% CI = 0.0005 to 0.1) (26, 27, 30). Consequently, describing *B. burgdorferi* prevalence in ticks for the state of California could dramatically underrepresent Lyme disease risk for northern Californians if all data are aggregated. This phenomenon can occur at a smaller scale: for example, *B. miyamotoi* prevalence in nymphal *I. pacificus* in Bolinas Lagoon, Marin County, was measured as 17.8%, with a 95% CI of 10.5 to 27.3% and a decent sample size of 90 nymphs. However, when aggregated across the county, nymphal infection prevalence of *B. miyamotoi* falls to 6.6% (95% CI = 4.4 to 9.4%), and in the Bay Area region, it slips to 5.1% (95% CI = 3.5 to 7.3) (Fig. 6; Table 3). Counties are often used as the spatial unit for reporting vector-borne and other disease metrics, but doing so can obfuscate smaller-scale patterns of disease risk (31) or result in erroneous interpretations of disease drivers (32). One solution to portray disease prevalence is to portray the 95% confidence intervals, which inherently demonstrate the range of interpretable prevalence. However, this method
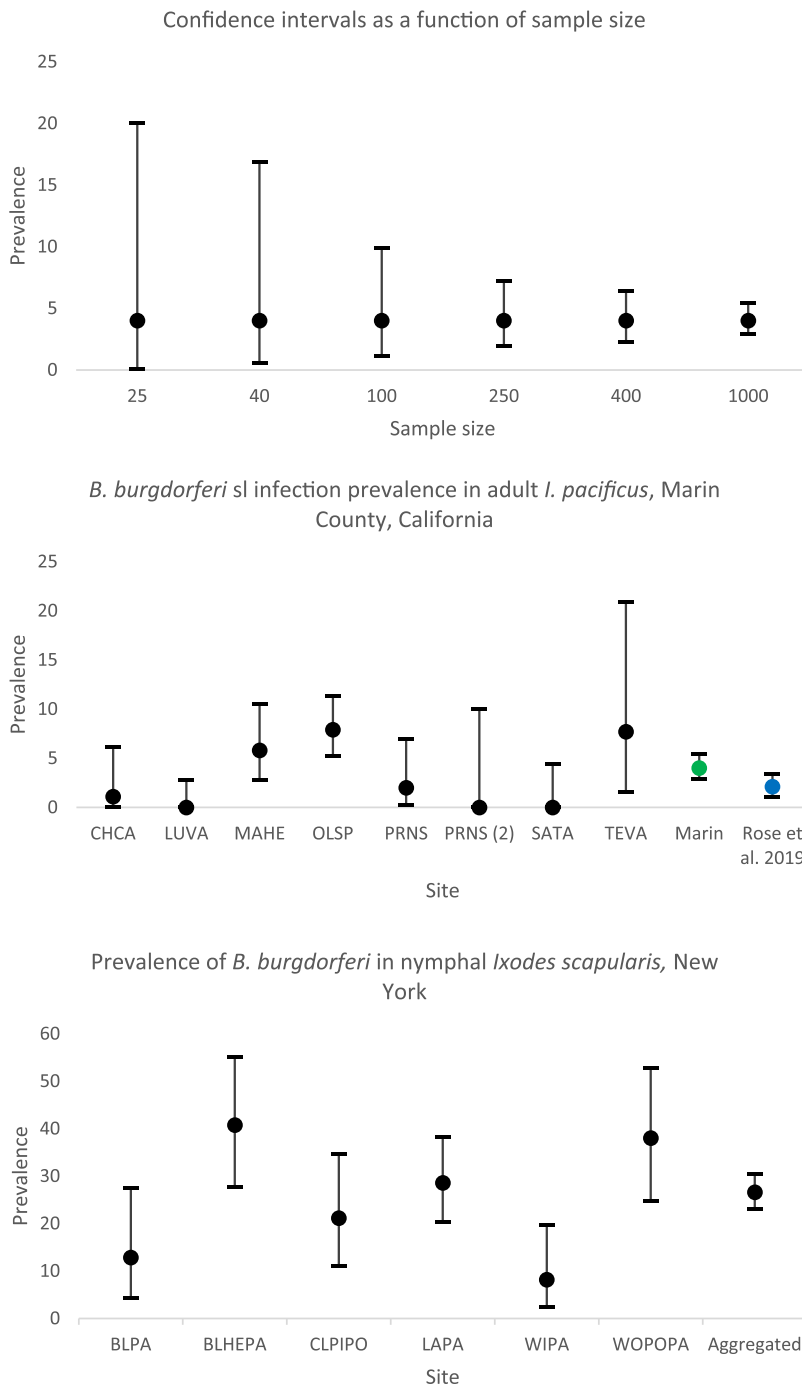
FIG 6 (Top) Patterns of 95% confidence intervals as a function of growing sample size (range, 25 to 1,000) for a set prevalence of 4%. (Middle) Prevalence (95% CI) of *B. burgdorferi sensu lato* in adult western black-legged ticks (*I. pacificus*) from sites in Marin County (sample sizes > 30), as well as aggregated prevalence for the entire county (green, this study; blue, reference 26) (CHCA, China Camp State Park; LUVA, Lucas Valley woodland; MAHE, Marin Headlands; OLSP, Olompali State Park; PRNS, Point Reyes National Seashore [McClure Beach]; PRNS (2), Point Reyes National Seashore [Tomales Point]; SATA, Samuel P. Taylor State Park; TEVA, Tennessee Valley [chaparral]). (Bottom) Prevalence (95% CI) of *B. burgdorferi* in black-legged ticks (*I. scapularis*) from a subset of sites in New York as well as aggregated prevalence across all sites (53) (BLPA, Bloomingdale Park; BLHEPA, Blue Heron Park; CLPIPO, Clay Pit Ponds; WIPA, Willowbrook Park; WOPOPA, Wolfe's Pond Park).

also has shortfalls if samples are aggregated, as the confidence intervals shrink with increasing sample size (Fig. 6), suggesting improved confidence but ignoring the fact that the source data are combined from multiple sites.

We advocate for transparently sharing data from all sites so that scientists, concerned citizens, physicians, public health agencies, and vector control districts can make appropriate judgments regarding the relevant risk of tick-borne disease. For example, it is important to understand that outdoor recreation in southern California has a lower risk for tick-borne disease exposure than outdoor recreation in northern California. These nuances can be important for treatment, control, and educational opportunities. In addition, zoonotic disease systems often exhibit fine-scale spatial patterns, and sharing these data at the site level may help future studies examining disease ecology and environmental drivers (33). Similarly, prevalence patterns of *Borrelia* likely will vary across time even at the same sites (34, 35).

Typically, infection prevalence is reported for a single pathogen, e.g., prevalence of *B. miyamotoi* in a tick population or sample. This method of data presentation fails to recognize the fact that a population of ticks can often harbor multiple pathogens (35) and that reporting on a single pathogen species underestimates local risk of tick-borne disease. To provide an example, for the same Bolinas Lagoon tick population, pathogen prevalence is 6.7% for *B. burgdorferi sensu lato*, 17.8% for *B. miyamotoi*, and 7.8% for *A. phagocytophilum*. The overall prevalence of ticks with human pathogens in this population is 31.1% (28/90, as one tick was coinfected; 95% CI = 21.8 to 41.7%). The difference when multiple pathogens are considered is not always so pronounced; e.g., cumulative tick-borne-pathogen prevalence in China Camp State Park is 9.0% (6/61; 95% CI = 3.4 to 18.5%), compared to 7.5% for *B. miyamotoi* and 3.0% for *A. phagocytophilum* (one tick was coinfected). However, it is important to consider multiple pathogens when assessing local disease risk.

Vertical transmission of *B. miyamotoi*. *B. miyamotoi* is known to be vertically transmitted in *Ixodes scapularis* (18, 19), has been observed in *I. pacificus* larvae (36), and is able to infect small mammals that ticks feed on (18, 25, 36, 37). It is unclear whether infection dynamics in natural populations require amplification by horizontal transmission from the vertebrate hosts.

Recently, data from the Bay Area were used to argue that horizontal transmission is required for *B. miyamotoi* transmission in California, based on an increase in infection prevalence across developing tick life stages (36). However, this pattern was generated from data that included a single infected larva and aggregation of infection prevalence in tick life stages from eight different sites spanning five counties. At the site where the *B. miyamotoi*-infected larva was observed (Heinz Open Space, Santa Clara County), infection prevalence was 0.5% (1/201) in larvae, 0% in nymphs (0/19), and 0% (0/1) in adults (Fisher's exact test, $P = 1.0$). At sites with higher *B. miyamotoi* prevalence, e.g., Windy Hill, San Mateo County, there were significant differences between the life stages (Fisher's exact test, $P = 0.01$), though this statistical difference is driven by the lack of observed infected larvae (0/58 larvae, 5/57 nymphs, 16/137 adults; Fisher's exact test for only nymph and adult stages, $P = 0.62$).

In our study, at Olompali State Park, *B. miyamotoi* was observed in all *I. pacificus* life stages, though in only a single larva, and there was no significant change in infection prevalence. Because we also identified only a single *B. miyamotoi*-infected larva, interpretations of both studies on *B. miyamotoi* transmission across tick life stages should be viewed with caution. Though we suspect that small mammals do indeed play a role in *B. miyamotoi* infection dynamics, there are not yet enough field data from the California system to support this hypothesis. Increased surveillance for *B. miyamotoi* in larval *I. pacificus* and experimental tests of reservoir competence for *B. miyamotoi* in vertebrate hosts are required to demonstrate that horizontal transmission is important in the California system (25).

Borrelia ecology and habitat type. We observed a diversity of *Borrelia* species in coastal habitats. Coastal prairie and coastal chaparral have received relatively little attention compared to woodland habitats in northern California (e.g., see references 3,
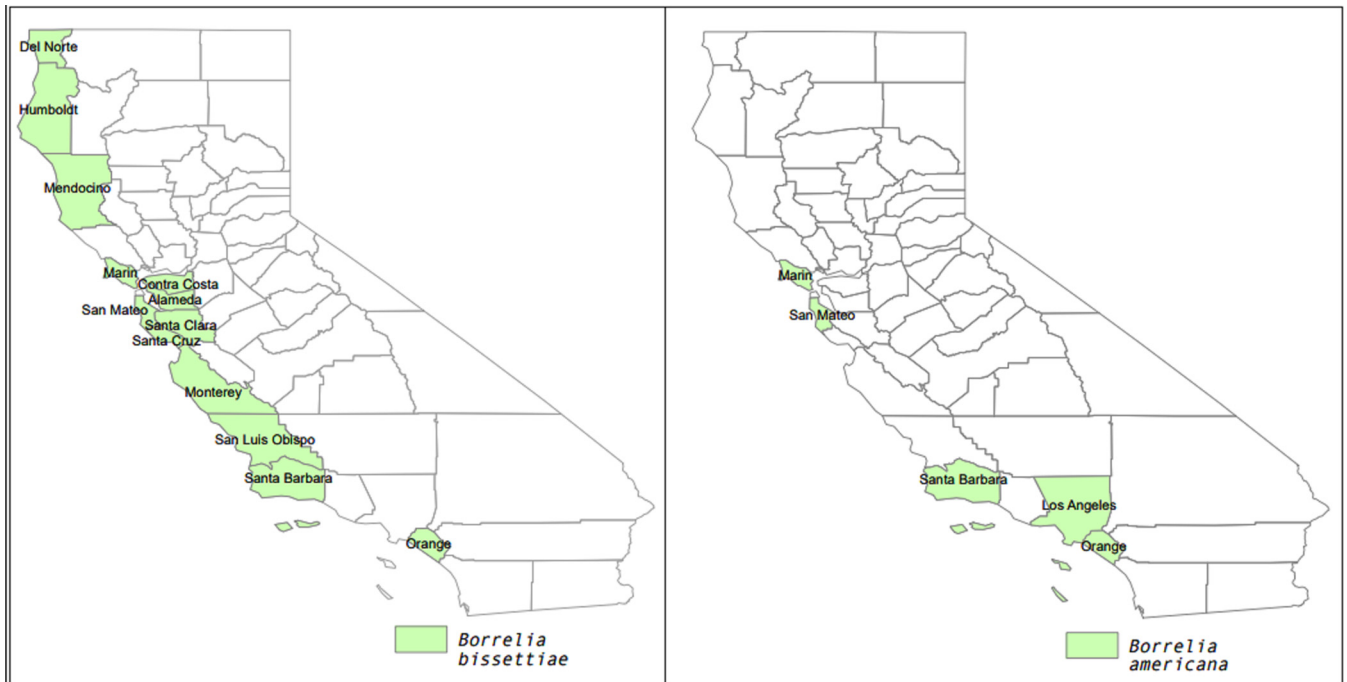
FIG 7 Maps showing reported presence of *Borrelia bissettiae* (left) and *B. americana* (right) in California counties, based on data from this study combined with previous published reports (see Table 1 for details and references). The maps were created in ArcMap, and the polygon feature class of the California county boundaries was downloaded from ArcGIS (credits: U.S. Bureau of Reclamation, California Department of Conservation, California Department of Fish and Game, California Department of Forestry and Fire Protection, and National Oceanic and Atmospheric Administration).

17, and 38), and at first glance these habitats would appear to be low risk for *Borrelia* exposure due to the lack of recognized mammalian reservoir hosts; e.g., western gray squirrels are not common in these habitats. However, the prevalence of *B. burgdorferi sensu lato* in adult ticks in coastal chaparral in Marin and Sonoma counties was equivalent to that in woodlands, suggesting that this habitat may pose a risk for Lyme borreliosis exposure when adult tick populations are abundant in the winter.

Nymphal *I. pacificus* ticks were not collected in the coastal grass- or shrublands. We suspect that they are present but that tick flagging is not an effective way to collect this life stage in chaparral or grassland. Future investigations should attempt to survey tick hosts, e.g., western fence lizards, to examine the ecology of nymphal *I. pacificus* in these habitats (30).

Multiple *Borrelia* species (*B. bissettiae*, *B. americana*, *Borrelia californiensis*, and *B. burgdorferi sensu stricto*) have also been observed in coastal habitats in southern California (27) (Fig. 7; Table 1). Wood rats (*Neotoma* spp.) may play a role in *Borrelia* transmission in these environments, as they have been found to be infected with *B. bissettiae*, *B. miyamotoi*, and *B. burgdorferi* (21, 22, 25). *Peromyscus* mice may also be important in these habitats (25, 27). Verification of host reservoir roles in coastal habitats requires further investigation, but the existing data suggest that *Borrelia* transmission dynamics are very different from the archetypal black oak woodland study systems, where wood rats and mice are believed to play largely peripheral roles in Lyme disease ecology (3, 5).

B. americana was observed in three *I. pacificus* ticks, all collected in coastal chaparral habitat in Marin County. Prior observations of *B. americana* in *I. pacificus* were also linked to chaparral/grassland habitat in San Mateo and Los Angeles counties (26, 30). In southern California, *B. americana* has also been observed in *I. spinipalpis* (26, 27). Though data are still admittedly sparse, *B. americana* has been consistently observed in grassland/chaparral habitat, presumably because its reservoir host is associated with this habitat. Human infections with *B. americana* have not been reported (39).

A single *B. bissettiae*-infected tick was recovered from Monterey County. *B. bissettiae* has been associated with wood rats (*Neotoma* spp.) and other small mammals (Table 1)

and is potentially also a zoonotic pathogen, as it has been found infecting humans in northern California (20). It appears to be widely distributed in California's coastal region (Fig. 7).

We did not observe a pattern of dominance by either *B. burgdorferi* or *B. miyamotoi*, echoing previous reports from both California and the northeastern United States (13, 40). Furthermore, based on the phylogeny, we found no evidence of geographic clustering of *B. burgdorferi* by latitude or sampling location (Fig. 3 and 4).

Surveillance of adult versus nymphal ticks. Despite our best efforts, we struggled to find nymphs in Monterey County with tick flagging. However, adult *I. pacificus* ticks are abundant in Monterey County, and a variety of tick-borne pathogens are present (*B. burgdorferi sensu stricto*, *B. miyamotoi*, *B. bissettiae*, and *A. phagocytophilum*). Tick flagging is regarded as a sampling method that is representative of human exposure to ticks, and if this is the case, then human exposure to nymphs is rare in Monterey County Indeed, patterns of nymphal tick submissions from citizen scientists were rare in Monterey County (and counties further south) and were seen only in May (which is when we carried out surveillance for this study) (41). In contrast, citizen scientists reported adult ticks from Monterey County for several months (and from a broader swath of California) (41).

Although nymphs are regarded as the life stage that is most responsible for Lyme disease transmission (42), adult ticks are often easier to collect in abundance due to their habit of questing on higher vegetation and because they are more noticeable on tick flags. As such, adult ticks are good sentinels to demonstrate the local presence and diversity of *Borrelia* species. We observed *B. bissettiae* and *B. americana* only in adult ticks, though this may have been due to the larger samples as well as the habitat associations that appear to be important for *B. americana* ecology; i.e., it is difficult to collect nymphs in grassland/chaparral. Given the opportunity, we recommend that both adult and nymphal stages be included in tick-borne disease surveillance in California.

MATERIALS AND METHODS

Field sites and tick collection. Sampling sites were predominantly recreational areas or hiking trails, e.g., California state parks (SP) and midpeninsula open space preserves (OSP), in Marin, Mendocino, Monterey, Napa, Santa Clara, Santa Cruz, and Sonoma counties in northwest California (Table 3; Fig. 2). Some privately owned sites were also surveyed. Data are presented as belonging to a particular site which represents a single trail.

The study was conducted between December 2015 and May 2018 (Table 3; see also Data Set S1 in the supplemental material). Adult western black-legged ticks were predominantly collected each winter (December and January), when they are questing in greatest abundance (43). Collections in spring (May) were focused on nymphal ticks, though adults and larvae were also present and collected opportunistically. We attempted to visit each site during both winter and spring, but heavy rains and/or damage from wildfires precluded repeat visits in many locales. Olompali State Park was visited on three occasions.

Ticks were collected by dragging a 1-m^2 white flannel blanket along vegetation abutting trails for 20 m; ticks that attached themselves to the flannel were removed. We also collected ticks that were observed on vegetation, as well as any ticks found crawling on clothes or skin. We recorded the GPS coordinates and habitat type for observed ticks—either at the point that the ticks were observed or when a 20-m drag was successful in finding a tick. To prevent pseudoreplication of geographic data, we discarded GPS coordinates within 1.415 km of each other (44), unless the observed tick was a different life stage recorded in a different sampling period. Habitat classifications were coarse and included (i) coastal scrub/chaparral, where dominant species are coyote brush (*Baccharis pilularis*), California sagebrush (*Artemisia californica*), coastal buckwheat (*Eriogonum parvifolium*), sawtooth goldenbush (*Hazardia squarrosa*), and poison oak (*Toxicodendron diversilobum*); (ii) coastal grassland/prairie, which is dominated by annual grasses and forbs, with various amounts of native perennials; (iii) redwood forest, where dominant species are coastal redwood (*Sequoia sempervirens*) with associated Douglas fir (*Pseudotsuga menziesii*) and tanoak (*Lithocarpus densiflorus*); and (iv) oak-bay forest, where dominant species are coast live oak (*Quercus agrifolia*) or other *Quercus* species, California bay (*Umbellularia californica*), madrone (*Arbutus menziesii*), California blackberry (*Rubus ursinus*), and poison oak. At some sites, the trailside habitat was mixed, normally combining patches of coastal chaparral and grassland that could not be separated.

Ticks were stored in 70% ethanol. All ticks were identified to species and stage levels via morphology, and here we describe only observations of ticks identified as *I. pacificus*. DNA was extracted from

ticks following manufacturer's protocols (DNeasy blood and tissue kit; Qiagen, Valencia, CA) and stored at −20°C until molecular analysis.

Pathogen detection and identification. To detect *Borrelia* pathogens, we used real-time PCR protocols described previously (17). In brief, we amplified a segment of the 16S rRNA gene of *Borrelia* sp. DNA (13), which enabled detection and classification of *B. burgdorferi sensu lato* (Lyme disease group) and *B. miyamotoi* (tick-borne relapsing fever group) through the detection of separate hybridization probes. Samples were considered positive if they had a cycle threshold (C_T) value of <40 and logarithmic distributions on the amplification plots.

To identify *Borrelia* species and strain genotypes, we amplified and sequenced the 16S-23S intergenic spacer (IGS; *rrs-rrlA*) of a subset of the real-time PCR-positive tick samples using a nested-PCR protocol with a 25-μl reaction volume (45). The subset of *Borrelia*-positive ticks was chosen to represent as many different sites as possible across the geographical range of sampling. Prior to amplification of the inner target region, we used a 1× magnetic bead cleanup to purify and concentrate the target DNA. During this magnetic bead cleanup, targets were annealed to the beads, washed twice with 70% ethanol (EtOH) and diluted into 12.5 μl of molecular-grade H_2O before being added to the inner PCR mixture. Amplified samples were sequenced using capillary Sanger sequencing on an ABI 3730 sequencer with both forward and reverse primers (EnGGen, Northern Arizona University). Successfully sequenced forward and reverse *Borrelia* sp. samples were trimmed, and forward/reverse reads were assembled using Geneious prime (version 2019.1.1). For phylogenic reconstruction, sequences from this study were chosen from each location and were aligned (muscle alignment using default settings) with sequences obtained from GenBank NCBI (HQ012505.1 [46], KC416410.1 [47], EU886969.1 [48], EU377803.1, and EU377801.1 [49]) using MEGAX (version 10.1.8). A phylogenic tree was constructed with MEGAX using the maximum-likelihood method and the Tamura-Nei model (50, 51).

Anaplasma phagocytophilum was detected using a previously described real-time PCR assay (52). We did not screen all ticks for *A. phagocytophilum*, so sample sizes differ from those for *Borrelia* sp. screening.

Analyses. Prevalence is reported as the percentage of ticks testing positive for the disease agent (i.e., number of positives/number tested × 100). Some analyses were restricted to sites where sample sizes were >30, as this removes the impact of considering sites with an inflated pathogen prevalence because a single positive was observed in a small sample, and this seems to be an informal threshold at which we are normally able to detect *Borrelia* if it is present (16).

Binomial proportion 95% confidence intervals were calculated using binom.test in R. We used Fisher's exact test or the chi-square test to evaluate differences among proportions (i.e., infection prevalence).

Data availability. Sanger sequencing data have been uploaded in the NCBI database under accession numbers MW862414 to MW862434.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.
SUPPLEMENTAL FILE 1, XLSX file, 0.3 MB.

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