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# WILD TURKEY (*MELEAGRIS GALLOPAVO*) AS A HOST OF IXODID TICKS, LICE, AND LYME DISEASE SPIROCHETES (*BORRELIA BURGDORFERI* SENSU LATO) IN CALIFORNIA STATE PARKS

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**ABSTRACT:** Rio Grande wild turkeys (*Meleagris gallopavo intermedia*) were evaluated as potential hosts of ixodid ticks, lice, and Lyme disease spirochetes (*Borrelia burgdorferi* sensu lato [s.l.]) in three state parks in Sonoma County, California, USA, during 2003 and 2004. In total, 113 birds were collected, 50 (44.2%) of which were found to be infested by 361 ixodid ticks representing three species: the western black-legged tick (*Ixodes pacificus*,  $n=248$ ), the rabbit tick (*Haemaphysalis leporispalustris*,  $n=112$ ), and one American dog tick (*Dermacentor variabilis*). Year-round the prevalence of all ticks combined was unrelated to the age or sex of turkeys, and the prevalence of infestation by *I. pacificus* (35.4%) was significantly higher than it was for either *H. leporispalustris* (14.2%) or *D. variabilis* (0.9%). The proportion of the two prevalent tick species differed significantly by life stage with 86.3% of the *I. pacificus* and 82.1% of the *H. leporispalustris* enumerated being nymphs and larvae, respectively. Three species of lice were collected, including the chicken body louse *Menacanthus stramineus* (12.5% of total), *Chelopistes meleagridis* (37.5% of total), and *Oxylpeurus polytrapezius* (50% of total). The records for all three ticks are the first ever from wild turkeys, and those for the lice are the first from this host in the far-western United States. Wild turkeys potentially were exposed to the feeding activities of *I. pacificus* nymphs infected with *B. burgdorferi* s.l. as 15% of host-seeking nymphs ( $n=200$ ) collected in woodlands used by turkeys as roosting or foraging areas were infected mainly with *B. burgdorferi* sensu stricto (s.s.). However, only one (1%) of 90 turkey blood specimens tested by PCR contained *B. burgdorferi* s.s., and four in vitro, complement-protein assays demonstrated that domestic turkey serum is moderately bacteriolytic for this spirochete. Taken together, these findings indicate that wild turkeys are important avian hosts of *I. pacificus* nymphs, but they appear to be inconsequential hosts of *B. burgdorferi* s.l.

**Key words:** *Borrelia burgdorferi*, complement, ixodid ticks, lice, wild turkey.

## INTRODUCTION

Wild turkeys (*Meleagris gallopavo*) from Mexico apparently were first introduced into California in 1876 (Gardner et al., 2004), and more than 8,000 turkeys were released into the state during the 20th century (Charlton, 2000). Wild turkeys currently are established in an area comprising approximately 75,545 square kilometers or nearly 19% of California's landscape, and the statewide population recently was estimated to be 242,000 birds (Gardner et al., 2004). The wild turkey has become a highly valued game bird in mixed oak and pine woodlands throughout much of California, and in 2003 an all-time high of roughly 25,000 birds were

harvested by about 37,000 hunters during the combined spring and fall seasons.

Despite their contemporary popularity as a game species, a review of the literature published since 1950 revealed that little has been reported about their associated microparasites (e.g., *Mycoplasma* spp., pox virus) and nothing about their macroparasites (e.g., ticks or lice), in the far-western United States (Jessup et al., 1983; Lutz and Crawford, 1987; Charlton, 2000). Most of what is known about the parasites of wild turkeys is based on research conducted in the eastern United States (Davidson and Wentworth, 1992). Furthermore, wild turkeys have not been examined heretofore as either hosts, or reservoir hosts, for the bacterial agent

of Lyme disease, *Borrelia burgdorferi* sensu stricto (s.s.), in North America. Host, as used herein with respect to *B. burgdorferi* s.s., merely denotes a vertebrate species that may become infected naturally with this bacterium. In contrast, a reservoir is a proven natural host of a vector tick, serves as a source of infection for uninfected ticks that feed on it, and amplifies the number of infected ticks in a given area (Kahl et al., 2002).

In 2003, a study was initiated to determine the status, food habits, and other biologic parameters of Rio Grande wild turkeys (*M. gallopavo intermedia*) in three Sonoma County state parks: Annadel State Park (ASP), Jack London State Historic Park (JLSP), and Sugarloaf Ridge State Park (SRSP). Wild turkeys first appeared in these parks around 1992 and became sufficiently abundant by 2002 to arouse concern that this nonnative bird was disrupting park ecosystems by negatively impacting native plants or animals (Barrett, unpubl. data). As one component of the study, we evaluated the capacity of turkeys to serve as a host of ticks, lice, and borreliae belonging to the *B. burgdorferi* sensu lato (s.l.) complex, particularly the human pathogen *B. burgdorferi* s.s.

Lyme disease is endemic in Sonoma County, and adults of the primary regional tick vector, the western black-legged tick (*Ixodes pacificus*), have been found infected naturally there (Burgdorfer et al., 1985). The reported incidence of Lyme disease in Sonoma County was within the range of 1.0 to 5.0 cases per 100,000 person-years between 1990 and 2000 (Fritz and Vugia, 2001). Our specific objectives were to determine the seasonal distribution and abundance of ixodid ticks infesting wild turkeys in these parks, especially ASP, over a 1-yr period; to ascertain what species of lice also parasitize turkeys; to determine if host-seeking *I. pacificus* nymphs inhabiting leaf litter/fir-needle areas at ASP are infected with *B. burgdorferi* s.l.; and to discover by labo-

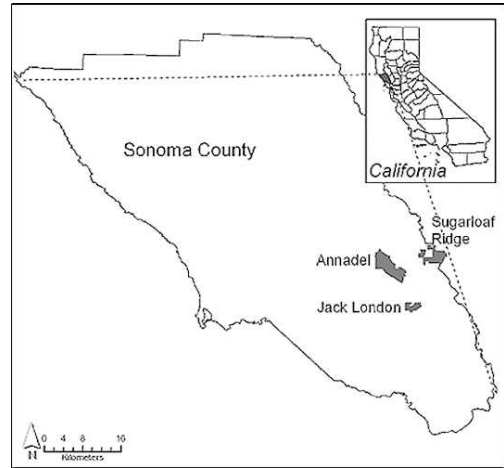


FIGURE 1. Map of Sonoma County, California, USA, showing locations of Annadel State Park, Jack London State Historic Park, and Sugarloaf Ridge State Park.

ratory bioassays if turkeys could serve as natural hosts of *B. burgdorferi* s.s.

## MATERIALS AND METHODS

### Study areas

Turkeys were collected from ASP ( $n=97$ , 85.8% of total), SRSP ( $n=12$ , 10.6%), or JLSP ( $n=4$ , 3.5%) in east-central Sonoma County, California, USA (Fig. 1). Because the borders of all three parks are located within a radius of  $\sim 4.1$  km, their populations of turkeys are treated here as a metapopulation. The principal study area, ASP ( $38^{\circ}25'53''\text{N}$ ,  $122^{\circ}36'49''\text{W}$ ), is a 2,023-ha multipurpose, heavily used recreational area located on the eastern outskirts of the city of Santa Rosa (population,  $\sim 156,000$ ). The topography is characterized by rolling hills with grassland, woodlands, meadows, intermittent streams, and a 10.5-ha lake. At least 14 vegetative types are present with Douglas fir (*Pseudotsuga menziesii*), California black oak (*Quercus kelloggii*), grassland, California bay (*Umbellularia californica*), coast live oak (*Quercus agrifolia*), and common manzanita (*Arctostaphylos manzanita*) comprising most of the ground cover (Barrett et al., 1988). The climate is Mediterranean with cool, moist winters and hot, dry summers. Rainfall occurs largely in winter and early spring, and averages about 762 mm per year.

To the east of ASP, SRSP ( $38^{\circ}26'26''\text{N}$ ,  $122^{\circ}29'43''\text{W}$ ) encompasses 1,093 ha of oak

woodland, chaparral, meadows, and redwood (*Sequoia sempervirens*). To the southeast, JLSP (38°21'25"N, 122°32'31"W) consists of 324 ha of grassy meadows and mixed forests composed of Douglas fir, oaks, Pacific madrone (*Arbutus menziesii*), coast redwood, and manzanita and a 2-ha lake.

### Turkey collections

Turkeys were collected monthly from 30 September 2003 to 9 September 2004 by shooting with a .22-caliber rifle in accordance with collecting permits issued by the California Department of Parks and Recreation, the California Department of Fish and Game, and in compliance with procedures approved by the Animal Care and Use Committee at the University of California, Berkeley. Permission was obtained to collect up to 12 turkeys per month.

Birds were processed in the field after a postmortem interval of approximately 0.25 hr to 3 hr. The age (juvenile or adult), gender, weight, and standard ornithologic measurements were recorded for each bird, and all birds were inspected cursorily for presence of ticks. Any ticks found were stored in 95% ethanol for later specific identification. Blood was withdrawn from the jugular vein, the heart, or an internal blood vessel with a 27 g 1/2 inch needle (Becton Dickinson, Franklin Lakes, New Jersey, USA) and injected into a 4- or 5-ml vacutainer tube containing EDTA (Becton Dickinson). Next, the head of each bird was cut off at the base of the neck and placed into a labeled plastic bag on wet ice. In the laboratory, the blood samples and heads were frozen until they could be tested by PCR (blood) or carefully inspected for ticks and lice (heads and necks).

After thawing, the turkey heads were examined for lice as well as ticks for  $\geq 10$  min with a dissecting microscope at a magnification of 13 $\times$ . The plastic bags in which the heads were frozen also were inspected for presence of ticks or lice. The specific location of attached ticks on the head or neck was noted, and all ectoparasites found were preserved in 95% ethanol for subsequent taxonomic determination. Ticks were identified to species with a dissecting microscope at magnifications up to 90 $\times$  using Furman and Loomis (1984) and an unpublished key to immature ixodid ticks (Kleinjan and Lane, unpubl. data), and voucher specimens were deposited in the collection of R.S.L. Lice were slide mounted and identified with a compound microscope at magnifications ranging from 40 $\times$  to 400 $\times$  using several taxonomic works

(Clay, 1938; Price and Graham, 1997; Price et al., 2003), and by comparison with reference specimens in the collections of the Natural History Museum, London, and the Smithsonian Institution, Washington, D.C. Voucher specimens have been deposited in the Natural History Museum (London).

### Collection of questing *Ixodes pacificus* nymphs

On 8 May 2004, host-seeking *I. pacificus* nymphs were collected from fir-needle/leaf-litter areas beneath two turkey-roosting sites and two foraging areas at ASP. Two of us used standardized flannel tick-drags, 1 m by 1.25 m, to sample each site for 1 hr apiece. Sampling at sites 1–4 was begun at 11:30 AM, 2:00 PM, 3:12 PM, and 4:51 PM, respectively. The predominant tree species at all sites were Douglas fir and California bay with lesser elements of coast live oak and madrone. Besides leaf or fir-needle litter, the ground at each site contained a variable amount of branches and tree limbs. All ticks found were preserved in 95% ethanol and identified as described above. Fifty nymphs obtained from each site were assayed for borrelial infection by PCR.

### DNA extraction and PCR

Blood specimens from turkeys were stored in 4- or 5-ml EDTA tubes at  $-20^{\circ}\text{C}$ , and ticks were stored in 95% ethanol, prior to DNA extraction. DNA was extracted from 10  $\mu\text{l}$  of turkey blood and the entire bodies of ticks using the DNeasy Tissue Kit (QiaGen, Chatsworth, Massachusetts, USA) according to the manufacturer's instructions. Our total DNA extraction protocol requires 5–10  $\mu\text{l}$  of bird blood to yield  $\sim 20$   $\mu\text{g}$  to 100  $\mu\text{g}$  of DNA. DNA from blood samples and ticks was eluted in final volumes of 200  $\mu\text{l}$  and 100  $\mu\text{l}$  of AE buffer, respectively.

Presence of borreliae was determined by PCR using primer sets targeting the 5S–23S rRNA intergenic spacer region (Lane et al., 2004). PCR assays used 3  $\mu\text{l}$  of each DNA extract as a template in a total reaction volume of 25  $\mu\text{l}$ . All PCR mixtures contained 2.5  $\mu\text{l}$  of 10 $\times$  PCR buffer (Applied Biosystems, Foster City, California, USA), 2.5  $\mu\text{l}$  of 8 mM dNTP, 1.5  $\mu\text{l}$  of 25 mM  $\text{MgCl}_2$ , 1  $\mu\text{l}$  of 10  $\mu\text{M}$  primers, and 0.2  $\mu\text{l}$  of 5 unit/ $\mu\text{l}$  *Taq* polymerase (Applied Biosystems). Cycling conditions involved an initial 4 min denaturation at 94  $^{\circ}\text{C}$  followed by amplification cycles, each consisting of a 40-sec denaturation at 94  $^{\circ}\text{C}$ , a 40-sec annealing at 52–58  $^{\circ}\text{C}$ , and a 1-min extension at 72  $^{\circ}\text{C}$ . These cycles were followed by a 10-min extension at 72  $^{\circ}\text{C}$ . Positive

controls (CA4 strain) and negative controls (autoclaved distilled water) were included in each run.

### Sequence alignment and phylogenetic analysis

Positive amplicons were characterized by sequence analysis of the 5S–23S rRNA intergenic spacer region. The amplicons were purified with the Qiaquick PCR Purification Kit (QiaGen, Valencia, California, USA). Each 10- $\mu$ l cycle sequencing reaction contained 6.5  $\mu$ l of PCR-grade water, 0.5  $\mu$ l of Big Dye Terminator Ready Reaction Mix (Applied Biosystems), 0.5  $\mu$ l of 3.2  $\mu$ mol of the primer that was used to produce the PCR product, 1.5  $\mu$ l of 5 $\times$  Sequencing Dilution Buffer (Applied Biosystems), and 1  $\mu$ l of purified PCR product. All cycle-sequencing products were purified with Sephadex Centri-Sep columns (Edge Biosystems, Gaithersburg, Maryland, USA) and run on an ABI 3100 (Applied Biosystems).

### Complement sensitivity

To determine if turkeys could serve as reservoir hosts of Lyme disease spirochetes, the sensitivity of *B. burgdorferi* s.s. isolate CA4 to complement proteins present in domestic turkey serum was determined. To that end, four in vitro serum assays were performed in 96-well microtiter plates (Dynatech Laboratories & Co., Chantilly, Virginia, USA) as described previously (Kuo et al., 2000), that is, untreated (preimmune serum), heat treated (56 C for 30 min), EDTA treated (10 mM), and EGTA treated (10 mM) with MgCl<sub>2</sub> (4 mM). Each well contained a final volume of 100  $\mu$ l, 50% of which was either treated or untreated serum and the remainder BSK-II culture medium containing viable spirochetes. The survivability of spirochetes was determined with a Petroff-Hausser counting chamber (Hausser Scientific Co., Horsham, Pennsylvania, USA) at 1 and 2 hr postinoculation by examining the viability of 100 individual spirochetes in each of 10- $\mu$ l aliquots. Spirochetes were considered dead if they were nonmotile or lysed. Spirochetes were inoculated into BSK-II culture medium as a positive control, and into preimmune serum from a western fence lizard (*Sceloporus occidentalis*), which previously had been demonstrated to be highly bacteriolytic for *B. burgdorferi* s.s. (Lane and Quistad, 1998; Kuo et al., 2000).

Blood specimens were drawn from four Nicholas large white domestic turkeys at the Nicholas Turkey Breeding Farms in Sonoma, California, USA. All birds were males 17 wk of age. Although their feed had been medicated

with an antiprotozoal drug (amprolium hydrochloride) during the first 6 wk to 8 wk of life, none of them had been treated with antibiotics or exposed to vector ticks (Dr. Yan Ghazikhanian, pers. commun.) The blood samples were transported to the Berkeley campus on wet ice, refrigerated at 4 C for several hours, centrifuged and then the sera were removed and stored at –80 C prior to testing.

### Statistical analyses

All prevalence data were evaluated in either  $2 \times 2$ , or  $2 \times 4$ , chi-square contingency tables. A probability value of 5% was set for rejection of the null hypothesis.

## RESULTS

### Tick infestations

In total, 113 turkeys were collected including 69 males and 44 females; of these, 25 were juveniles and 88 adults. Significantly fewer females ( $n=2$ ) than males ( $n=21$ ) ( $P=0.009$ ), and more adults ( $n=22$ ) than juveniles ( $n=1$ ) ( $P=0.075$ ), were collected in fall. Most adults were males (61/88), whereas the majority of juveniles were females (17/25,  $P=0.0007$ ).

Fifty (44.2%) turkeys were infested by 361 ixodid ticks representing three species; these included 248 western black-legged ticks (*I. pacificus*), 112 rabbit ticks (*Haemaphysalis leporispalustris*), and one American dog tick (*Dermacentor variabilis*) (Table 1). Ignoring season, the prevalence of all ticks combined was unrelated to the age ( $P=0.16$ ) or sex of turkeys ( $P=0.86$ ). Most ticks (290/361, 80.3%) were found on the head or neck during the  $\geq 10$ -min laboratory inspections; the remainder were collected during the cursory field inspections. Attached ticks ( $n=101$ , 28.0% of total) were distributed almost equally on the head (53) and neck (48) regions. Among those affixed to the head, 83% were found on the crown ( $n=23$ , 43.4%), around the eye ( $n=11$ , 20.8%), and on the auricular ( $n=10$ , 18.9%). Low percentages were removed from the dewlap (5.7%), snood (5.7%), around the mandibles (3.8%), or in the suborbital region (1.9%).



TABLE 1. Prevalence and abundance of ixodid ticks on 113 wild turkeys collected from September 2003 to September 2004, Sonoma County, California, USA.

Tick species by stage	No. ticks collected (no. birds infested)	Prevalence of infestation (%)	Mean per bird $\pm$ SD	Mean intensity (range)
<i>Ixodes pacificus</i>				
Larva	33 (15)	13.3	$0.29 \pm 1.12$	2.2 (1–10)
Nymph	214 (38)	33.6	$1.89 \pm 12.24$	5.6 (1–130)
Adult	1 (1)	0.9	$0.01 \pm 0.09$	1.0
Total	248 (40)	35.4	$2.19 \pm 12.45$	6.2 (1–131)
<i>Haemaphysalis leporispalustris</i>				
Larva	92 (10)	8.8	$0.81 \pm 5.50$	9.2 (1–55)
Nymph	20 (8)	7.1	$0.18 \pm 0.86$	2.5 (1–7)
Total	112 (16)	14.2	$0.99 \pm 5.61$	7.0 (1–55)
<i>Dermacentor variabilis</i>				
Adult	1 (1)	0.9	$0.01 \pm 0.09$	1.0
Totals	361 (50)	44.2	$3.19 \pm 14.13$	7.2 (1–138)

The prevalence of infestation by *I. pacificus* (35.4%) was significantly higher than it was for *H. leporispalustris* (14.2%) ( $P=0.0004$ ) or *D. variabilis* (0.9%) ( $P\leq 0.0001$ ). Seven (14.0%) of the infested birds were co-infested with *I. pacificus* and *H. leporispalustris*. The proportion of the two most prevalent tick species differed significantly by life stage ( $P\leq 0.0001$ ). Specifically, 86.3% of the *I. pacificus* were nymphs, whereas 82.1% of the *H. leporispalustris* were larvae (Table 1). Two turkeys contributed about one half of all the *I. pacificus* or *H. leporispalustris* obtained from all birds. A juvenile female collected at ASP on 19 May 2004 was parasitized by 131 *I. pacificus* (1 larva, 130 nymphs) and seven *H. leporispalustris* nymphs. This bird was the lightest (2,950 g) of nine juvenile females (mean  $\pm$  SD =  $3,722 \pm 446$ ; range, 2,950–4,200 g) taken at ASP in spring 2004. An adult female collected at ASP on 17 August 2004 was parasitized by 55 *H. leporispalustris* larvae. The weight of this bird (3,750 g) was slightly higher than the average weight (mean  $\pm$  SD =  $3,514 \pm 273$ ; range, 3,250–4,000 g) of seven adult females collected at ASP in summer 2004.

Larvae and nymphs of *I. pacificus* were present on turkeys in winter and spring but were most prevalent in spring

(Fig. 2A). The nymphs also occurred on birds in summer, but neither life stage was found on birds in fall. One *I. pacificus* female was removed from a turkey in spring. Larvae of *H. leporispalustris* infested turkeys in summer and fall, especially the former, whereas nymphs were found on low percentages of birds taken in spring, summer, and fall (Fig. 2B). The prevalence of *I. pacificus* and *H. leporispalustris* larvae or nymphs on turkeys (Table 2) did not differ significantly by the age or sex of the bird ( $P$  values range from 0.69 to 0.90). A single *D. variabilis* male was collected from a turkey in fall.

#### Louse infestations

Three species of lice (Insecta: Phthiraptera) were removed from the heads or necks of 20 (17.7%) of the turkeys (Table 3). Fourteen birds were infested by one species of louse, five birds by two species, and one bird by all three species. These included the cosmopolitan chicken body louse *Menacanthus stramineus*, which accounted for 12.5% of the 64 lice enumerated, the large turkey louse *Chelopistes meleagridis* (37.5% of total), and the slender turkey louse *Oxylipaeurus polytrapezius* (50% of total). Forty-seven (73.4%) of the lice were collected in summer versus 4.7, 7.8, and 14.1% in fall,

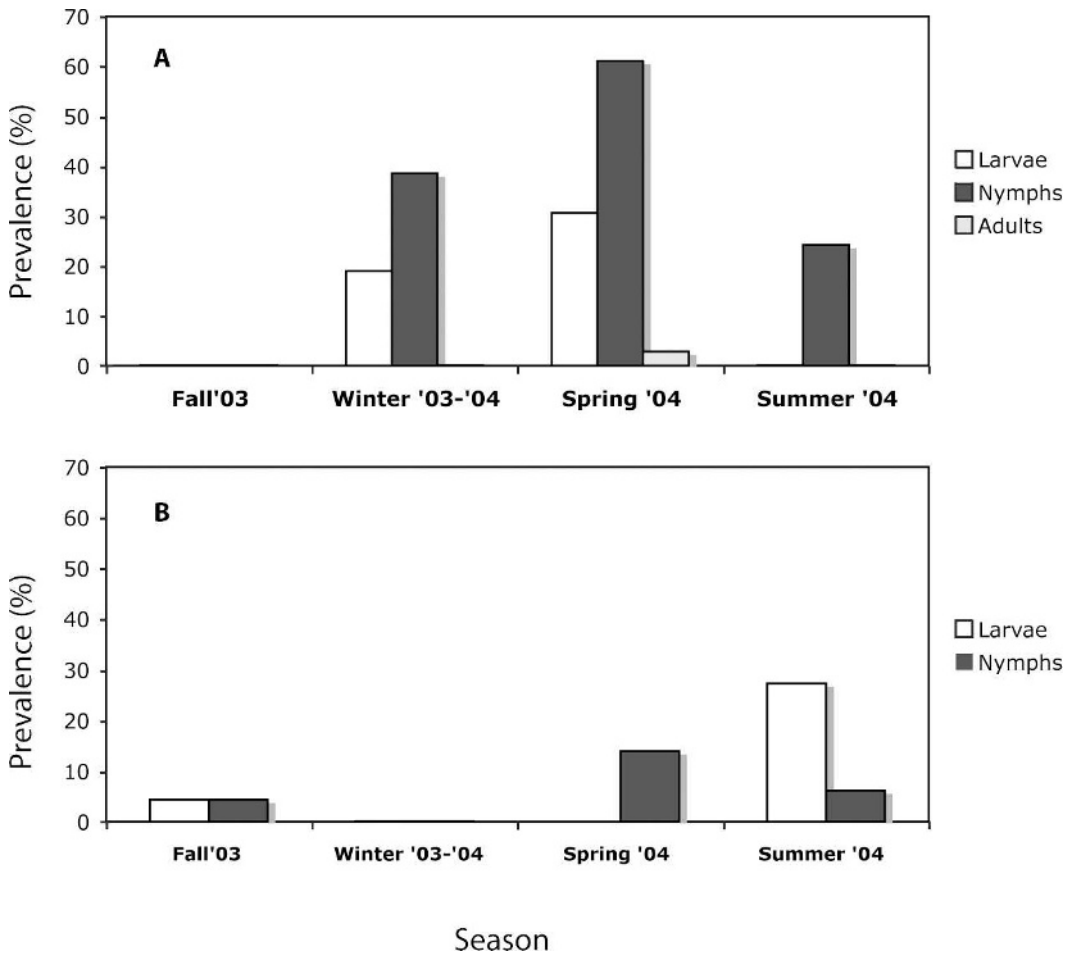


FIGURE 2. (A) Seasonal prevalence of *Ixodes pacificus* by life stage on wild turkeys ( $n=113$ ), Sonoma County, California, USA, 2003–2004. (B) Seasonal prevalence of *Haemaphysalis leporispalustris* by life stage on wild turkeys ( $n=113$ ), Sonoma County, California, USA, 2003–2004.

TABLE 2. Prevalence of ixodid ticks on 113 wild turkeys by age and sex of bird, Sonoma County, California, USA, 2003–2004.

Tick species by stage	Comparison of infestation levels (%)			
	Males ( $n=69$ ) <sup>a</sup>	Females ( $n=44$ ) <sup>b</sup>	Juveniles ( $n=25$ )	Adults ( $n=88$ )
<i>Ixodes pacificus</i>				
Larva	14.5	11.4	16.0	12.5
Nymph	31.9	36.4	28.0	35.2
Adult	1.4	0.0	4.0	0.0
<i>Haemaphysalis leporispalustris</i>				
Larva	8.7	9.1	4.0	10.2
Nymph	7.2	6.8	12.0	5.7

<sup>a</sup> 61 adults, 8 juveniles.

<sup>b</sup> 27 adults, 17 juveniles.

TABLE 3. Lice collected from 113 wild turkeys in Sonoma County, California, USA, 2003–2004.

Louse species (suborder)	Number collected				Prevalence (%) <sup>a</sup>
	Nymph	Male	Female	Total	
<i>Menacanthus stramineus</i> (Amblycera)	3	0	5	8	4.4
<i>Chelopistes meleagridis</i> (Ischnocera)	10	1	13	24	11.5
<i>Oxylipeurus polytrapezius</i> (Ischnocera)	25	0	7	32	8.0

<sup>a</sup> Since the entire body of each bird was examined cursorily in the field, these data should be viewed as reflecting the prevalence of lice on the head and neck regions only.

winter, and spring, respectively. Further, 14 of the louse-infested birds were parasitized by one ( $n=11$  birds) or two ( $n=3$  birds) species of ticks.

#### Prevalence of borrelial DNA in turkey blood

Blood was drawn successfully from 90 (79.6%) of the turkeys and tested by PCR. One specimen (1%) was positive for *B. burgdorferi* s.l., and sequencing analysis revealed that the genospecies was *B. burgdorferi* s.s. This bird, an adult male, was collected at ASP on 21 October 2003. It was parasitized by a *D. variabilis* male, which proved to be PCR negative for borrelial DNA.

#### Sensitivity of *B. burgdorferi* s.s. to turkey-serum complement

Over 50% of spirochetes inoculated into domestic turkey sera were alive after 1 hr of incubation at room temperature, as compared with zero of those injected into lizard serum and ~86% introduced into BSK-II culture medium (Table 4). Similar results were observed after 2 hr of incubation (data not shown). Heat treatment (56 C, 30 min) abolished the moderate

bactericidal activity of turkey serum and the complete bactericidal activity of lizard serum (Table 4). Adding EDTA to sera from turkeys or the western fence lizard similarly reduced spirochetal mortality. The addition of EGTA plus  $MgCl_2$  to turkey or lizard sera resulted in bactericidal activity like that observed for untreated sera.

#### Abundance of questing *I. pacificus* nymphs at ASP

Drag-sampling leaf litter/fir-needle areas in four turkey-roosting or foraging areas for 8 hr at ASP yielded 430 *I. pacificus* nymphs for a mean of 53.8 ticks/hr (SD=23.1, range=23–91/hr). Additionally, two larvae and four males of *I. pacificus*, one *Ixodes spinipalpis* nymph, nine *H. leporispalustris* nymphs, and 13 nymphs, three males and one female of *Dermacentor occidentalis* were collected.

#### Prevalence of *B. burgdorferi* s.l. in questing *I. pacificus* nymphs

*Borrelia burgdorferi* s.l. DNA was detected in 15.0% (range, 8–28% per site) of the 200 nymphs tested (Table 5). Twenty-eight of the 30 positive amplicons

TABLE 4. Complement sensitivity of *B. burgdorferi* s.s. (isolate CA4) after 1 hour of incubation in sera of domestic turkeys and the western fence lizard subjected to different treatments.

Source of serum (number specimens)	Survivability (%) $\pm$ SD (range) by serum treatment <sup>a</sup>			
	Untreated	Heat	EDTA	EGTA/ $MgCl_2$
Domestic turkey (4)	55.5 $\pm$ 16.9 (41–79)	82.0 $\pm$ 11.7 (69–95)	84.3 $\pm$ 10.7 (72–98)	63.3 $\pm$ 20.5 (36–81)
Lizard (1) <sup>b</sup>	0	93	93	0

<sup>a</sup> Spirochetal survivability (%) among the BSK-II controls averaged 85.6  $\pm$  6.7 (range, 76–90) for the domestic-turkey serum runs, and it was 93% during the single lizard-serum run.

<sup>b</sup> Serum from the western fence lizard was assayed for comparative purposes because of its proven bacteriolytic activity.



TABLE 5. Prevalence of *Borrelia burgdorferi* s.l. infection in host-seeking *Ixodes pacificus* nymphs collected by dragging fir-needle/leaf-litter areas in dense woodlands, Annadel State Park, California, USA, 8 May 2004.

Site	Number ticks tested	Number ticks positive (%)	Genospecies <sup>a</sup>
1	50	14 (28)	14 Bb ss
2	50	7 (14)	5 Bb ss, 2 ud
3	50	4 (8)	4 Bb ss
4	50	5 (10)	4 Bb ss, 1 Bb sl
Totals	200	30 (15)	27 Bb ss, 1 Bb sl, 2 ud

<sup>a</sup> Bb ss = *Borrelia burgdorferi* s.s.; Bb sl = uncharacterized *B. burgdorferi* s.l.; ud = undetermined.

were sequenced and, of these, 27 were determined to be the human pathogen *B. burgdorferi* s.s. and one was identified as an unclassified *B. burgdorferi* s.l.

# DISCUSSION

## Turkeys as hosts of ectoparasites

The wild turkey is indigenous to the North American continent (Dickson, 1992). Following numerous translocations during the 20th century, this bird now is widespread throughout much of the continental United States (Stangel et al., 1992) including the three major geographic foci where humans contract Lyme disease, the Northeast, the Upper Midwest, and the Far West. Nonetheless, wild turkeys have not been evaluated heretofore as potential hosts of Lyme disease spirochetes, and there have been no previously published records of wild turkeys being naturally infested with either of the two primary *Ixodes* spp. tick vectors of *B. burgdorferi* s.s. in the United States, *I. pacificus* in the Far West and the black-legged tick *Ixodes scapularis* in the East. Here we report new records for three species of ixodid ticks parasitizing wild turkeys including *I. pacificus*, the detection of *B. burgdorferi* s.s. DNA in a low percentage of birds (1%,  $n=90$ ), and the moderate sensitivity of *B. burgdorferi* s.s. to complement proteins present in sera from domestic turkeys. Moreover, three species of lice common on wild turkeys in the eastern United States are recorded for the first time from this host in California.

During preceding investigations of the role of birds in the ecology of *B. burgdorferi* s.l. in northern California, *I. pacificus* immatures (i.e., larvae, nymphs or both) were removed from three (13%) of 24 avian species in an oak-woodland (Manweiler et al., 1990), eight (24%) of 34 species in an oak/pine woodland (Wright et al., 2000), 13 (38%) of 34 species from either chaparral or woodland-grass habitats (Slowik and Lane, 2001), and 23 (51%) of 45 species in an isolated canyon containing mixed hardwoods (Wright et al., 2006). In two of those studies, when the avian sample size was  $\geq 10$  for birds infested by *I. pacificus*, the prevalence and the mean intensities (mean number of ticks per infested bird species) were relatively low and ranged from 7.1–10.0% and 1.0–3.0 ticks (Manweiler et al., 1990) and 4.2–30.8% and 1.0–2.0 ticks (Slowik and Lane, 2001), respectively. In another study, the prevalence (2.2–100%) and intensity data (1.0–7.8) were considerably higher (Wright et al., 2006). In the fourth study, these data were not provided, but the average number of ticks removed from infested birds representing five species ranged from 0.08 to 1.14 when the avian sample size exceeded 10 (Wright et al., 2000). The foregoing studies are not strictly comparable because sampling was not uniform with respect to the seasons when birds were sampled.

Among potential avian hosts in California, the prevalence (35.4%), and especially the mean intensity (6.2 ticks) of *I. pacificus* immatures on wild turkeys examined year-round during the present

study were moderately high. In spring alone, 66.7% of the turkeys collected were infested by *I. pacificus*, and the mean number of ticks per bird was 9.0 (data not shown). To determine the actual significance of any vertebrate species as a host of a particular tick, the area-wide abundance of the host must be determined relative to that of other potential hosts, as well as the tick burdens present on each host species. Our abundance data for *I. pacificus* larvae and nymphs must be considered underestimates because the entire body of each bird was examined superficially in the field, whereas only the head/neck regions were inspected thoroughly in the laboratory with the aid of a dissecting microscope and proper lighting conditions. In future studies involving wild turkeys, the entire body of each bird should be inspected exhaustively to obtain a more reliable estimate of tick burdens.

Overall, birds seem to contribute less to the maintenance of *I. pacificus* larvae or nymphs in oak/Pacific-madrone woodlands, and adjacent grasslands or chaparral than do lizards and western gray squirrels (*Sciurus griseus*) (Eisen et al., 2004). Infestation of lizards (western fence lizards; alligator lizards [*Elgaria* spp.]) and western gray squirrels by *I. pacificus* immatures (means of 9–35 larvae and 5–6 nymphs per animal) was several times higher than it was for dusky-footed wood rats (*Neotoma fuscipes*), deer mice (*Peromyscus* spp.), and birds (means of 0.9–3.5 larvae and 0–0.3 nymphs). In dense woodlands, lizards alone hosted 84% of *I. pacificus* larvae and 91% of nymphs removed from all animals (lizards, birds, rodents) collected simultaneously (Eisen et al., 2004).

Elsewhere in the continental United States, wild turkeys have been recorded as hosts of four ixodid (*Amblyomma americanum*, *Amblyomma cajennense*, *Haemaphysalis chordeilis*, *Rhipicephalus sanguineus*) and two argasid ticks (*Argas miniatus*, *Argas persicus*) (Cooley and Kohls, 1944; Bishopp and Trembley,

1945; Kellogg et al., 1969; Jacobson and Hurst, 1979; Davidson and Wentworth, 1992; Mock et al., 2001). Two of these tick species (*A. americanum*, *H. chordeilis*) reportedly cause losses among wild turkeys (Bishopp and Trembley, 1945). Losses or incapacity directly attributable to high tick burdens were not observed during the present study. The most heavily tick-infested turkey (138 ticks) was the lightest juvenile female collected in spring, and it weighed 21% less than the average bird in its age/sex cohort taken at that time of year. However, the weight of the only other abundantly parasitized bird (55 ticks) was close to the average for its cohort/sex.

Although the other primary vector of *B. burgdorferi* s.s. in the United States, *I. scapularis*, has never been reported from wild turkeys, an experimental study in which hundreds of *I. scapularis* immatures were placed directly on five captive wild birds demonstrated that no larvae and few nymphs actually engorged on turkeys (Ostfeld and Lewis, 1999). The authors concluded that wild turkeys are unlikely to serve as natural hosts of *I. scapularis* larvae or nymphs, which is consistent with the absence of published collection records indicative of such a tick/host association.

Lice typically are the most commonly reported ectoparasites of wild turkeys. Eight species of chewing lice have been recorded from wild turkeys in the United States (Davidson and Wentworth, 1992). The three species we found (*M. stramineus*, *C. meleagridis*, *O. polytrapezius*) were known previously to infest wild turkeys in seven to 10 states from other geographic regions (Davidson and Wentworth, 1992). Our data indicate that lice were most prevalent on birds in summer, but both the prevalence and abundance data should be regarded circumspectly, if not as gross underestimates. We did not attempt to collect lice from turkeys in the field, and our search was confined solely to the head and neck regions in the laboratory. Moreover, *M. stramineus* feeds

in part on feathers, *C. meleagridis* is a niche specialist restricted in distribution to feathers on the neck and breast of its host, and *O. polytrapezius* exploits turkey wing feathers (Johnson and Clayton, 2003).

*Menacanthus stramineus* parasitizes many species of domestic poultry worldwide, but the wild turkey generally is considered to be its native host because this louse has never been recorded from any other wild avian species (Price and Graham, 1997). It is the most prevalent and destructive species of louse infesting modern poultry in the United States (Axtell, 1999). *Menacanthus stramineus* also is considered to be a possible vector of various avian viral and bacterial disease agents (Derylo, 1970) because, unlike most chewing lice, its diet includes host blood as well as feathers, hair, and skin debris (Derylo and Gogacz, 1974).

Two species of *Chelopistes* occur in North America North of Mexico, and *C. meleagridis* is by far the most common (Price et al., 2003). This louse is a cosmopolitan parasite of domestic turkeys and is a common parasite of wild turkeys in the United States. It also has been recorded from the ocellated turkey (*Meleagris ocellata*) of southern Mexico and Central America (Clay, 1941). *Oxylipeurus polytrapezius* was the most prevalent louse among the three species collected. Although it is a common parasite of both domestic and wild turkeys, its life history and habits are poorly known. Its slender morphology enables it to exploit the wing feathers, where it can slot between barbules to escape the preening activities of its host (Johnson and Clayton, 2003).

#### **Turkeys as hosts of *B. burgdorferi* s.s.**

The fact that *B. burgdorferi* s.s. DNA was detected in blood from only 1% of wild turkeys collected year-round suggests that this bird is not an important host of this particular Lyme disease spirochete. The wild turkey is just the second avian species from the far western United States found to contain *B. burgdorferi* s.s. DNA

in its blood. A specimen from a hermit thrush (*Catharus guttatus*) collected from a wooded canyon in the Sutter Buttes of northern California was PCR positive for this spirochete (Wright et al., 2006). The low prevalence of spirochetal infection detected in turkeys during the present study is noteworthy because most turkeys were collected at ASP where 15% of the *I. pacificus* nymphs assayed were infected with *B. burgdorferi* s.l. in spring, and 86% of all the *I. pacificus* removed from birds were nymphs. Thus, at least some of the turkeys had been, or were being, fed upon by spirochete-infected nymphs at the time of collection. We did not test the relatively few *I. pacificus* larvae ( $n=33$ ) obtained from turkeys because no larval ticks were found attached to the one infected bird. Curiously, that bird was collected in fall when *I. pacificus* nymphs usually are inactive, and the only tick found on it, a *D. variabilis* male, was PCR negative for *B. burgdorferi* s.s.

The complement system plays an important role in defense against infection (Sim and Dodds, 1997). Among other functions, it promotes and regulates the lysis or phagocytosis of foreign cells including gram-negative bacteria (e.g., borreliae), and it interacts with the adaptive immune system. The level of complement activity in serum has been found to be an important intrinsic factor determining the reservoir competence of lizards, birds, and mammals for several genospecies of *B. burgdorferi* s.l. (Isogai et al., 1994; Kurtenbach et al., 1998; Kuo et al., 2000; Nelson et al., 2000). Our complement-protein assays demonstrated that untreated (preimmune) serum from domestic turkeys was moderately bacteriolytic for *B. burgdorferi* s.s. because 45% of spirochetes introduced into it were dead within 1 hr. In contrast, 100% of spirochetes injected into untreated lizard serum died within 1 hr, as expected (Lane and Quistad, 1998; Kuo et al., 2000). Taken together, the assays implicate components of the alternative com-

plement pathway as a significant source of much of the observed bacteriolytic activity: heat or EDTA treatment of domestic-turkey serum largely arrested bacteriolysis, whereas a lack of treatment or the addition of EGTA/MgCl<sub>2</sub> allowed turkey serum to retain its moderate borreliacidal effects.

In related studies, serum from northern bobwhite quail (*Colinus virginianus*) was nonlytic for the B-31 type strain of *B. burgdorferi* s.s. and only partially lytic for two strains of the related spirochete *Borrelia bissettii* (Ullmann et al., 2003). Like turkeys, serum from pheasant was moderately lytic for *B. burgdorferi* s.s., whereas it was either nonlytic or highly lytic for four other genospecies of *B. burgdorferi* s.l. (Kurtenbach et al., 1998). In vitro complement studies such as these, and reservoir-competence trials using xenodiagnostic larval ticks and wild-caught birds (e.g., Ginsberg et al., 2005), demonstrate that avian species differ characteristically in their capacity to host and disseminate various genospecies of *B. burgdorferi* s.l. group spirochetes.

We used sera from domestic turkeys rather than sera from wild turkeys in our complement assays because these birds represent a single species (*M. gallopavo*), and we did not have a source of pre-immune serum from captive wild birds that had been held in a tick-free environment. Nevertheless, the complement-test results, when considered alongside the low spirochetal infection prevalence detected in blood specimens of field-derived turkeys and the low infestation prevalence by *I. pacificus* larvae, suggest that the Rio Grande wild turkey is an inefficient host of *B. burgdorferi* s.s. in northern California woodlands. A competent reservoir of a tick-borne disease agent must not only be susceptible to the agent and capable of serving as a source of infection for uninfected ticks that feed on it, but it also must be fed upon by at least two life stages of a vector tick in order to amplify the infection in nature.

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## ADDENDUM

While this paper was *in press*, we decided to test the *I. pacificus* larvae that had infested 15 of the wild turkeys (Table 1). All 33 larvae were PCR negative for Lyme disease-group spirochetes, which reconfirms our conclusion that the Rio grande wild turkey is an inefficient host of *B. burgdorferi* s.s.

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