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Original Article

Novel exposure sites for nymphal *Ixodes pacificus* within picnic areas

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ABSTRACT

Risk of exposure to nymphal *Ixodes pacificus* Cooley and Kohls ticks was investigated at 7 picnic areas in Tilden Regional Park, a heavily used recreation area of over 2000 acres in northwestern California, east of San Francisco Bay. Wooden picnic tables, tree trunks, logs, leaf litter, surrounding vegetation, and rock walls were checked for ticks using standard 1-m^2 flannel tick flags at biweekly intervals from March to August 2008. Results indicate that nymphal *I. pacificus* were commonly found on wooden picnic tables and other wooden materials, such as tree trunks and logs, at an equal proportion to those found in leaf litter. Nymphal *I. pacificus* in picnic areas peaked in April, with a secondary peak in early June. Five of 170 (2.9%) nymphal *I. pacificus* collected at picnic sites were positive for *Borrelia* spirochetes, of which 3 (1.8%) were identified as *B. burgdorferi* sensu stricto using molecular techniques. In addition, a nymphal *I. auritulus* collected from a rock wall in a picnic area tested positive for a mixture of *B. burgdorferi* and *B. bissettii*; this tick species feeds exclusively on birds. This study indicates a moderate risk of acquiring a nymphal tick at Tilden Park picnic areas, but due to the low *B. burgdorferi* infection prevalence, the risk of acquiring Lyme disease appears to be low.

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Introduction

The nymphal stage of the western blacklegged tick, *Ixodes pacificus* Cooley and Kohls is the primary vector of Lyme disease in western North America. Due to its small size, it is often not noticed by humans, but has a relatively high infection prevalence compared to the adult stage (Clover and Lane, 1995). Furthermore, the time of year when *I. pacificus* nymphs are active (spring and early summer) compared to the adult stage (winter) coincides with increased outdoor activity, either peridomestically or in recreational areas. In California, approximately half the reported cases of Lyme disease are acquired during outdoor activities outside the individuals' county of residence (CDPH, unpubl. results). Recreational activities, such as hiking on established trails, appear to present a low risk in terms of acquiring *I. pacificus*, in northern California (Lane, 1996; Li et al., 2000). On the other hand, woodcutting was previously associated with Lyme disease cases in northern California (Lane et al., 1992), and contact with wood by sitting or leaning on logs or tree trunks has been shown to increase the likelihood of acquiring *I. pacificus* nymphs (Lane et al., 1992, 2004, 2007). Previous risk assessment studies on human exposure to *I. pacificus* adult ticks in recreational areas in northwestern California, an area where Lyme disease is endemic, have focused on the behaviors or habitats that increase exposure to adult ticks (Kramer and Beesley, 1993; Lane, 1996; Hui et al., 1998; Li et al., 2000). Although Li et al. (2000) did focus on exposure risk of nymphs within San Francisco Bay Area parks and picnic areas, sampling was restricted to the periphery and a cross-section of picnic areas; wood and wooden materials such as logs, tree trunks, benches, and picnic tables were not sampled.

This study was conducted at Tilden Regional Park (TRP), a large urban park (>2000 acres) located in the hills east of San Francisco Bay in Contra Costa County. Over one million visitors per year visit TRP, which has 20 picnic areas, lakes, hiking trails, a botanical garden, a carousel, and a miniature steam train. Major vegetation is comprised of oaks (*Quercus* spp.), Coastal Redwoods (*Sequoia sempervirens*), Pacific Madrone (*Arbutus menziesii*), Manzanita (*Arctostaphylos* spp.), and native and introduced grasses. Common hosts for *I. pacificus* include Columbian blacktail deer (*Odocoileus hemionus columbianus*), dusky-footed woodrat (*Neotoma fuscipes*), and brush rabbit (*Sylvilagus bachmani*) (Lane, 1996; Peavey et al., 1997). Previous studies have detected *Borrelia burgdorferi* sensu stricto, *B. bissetti*, *B. miyamotoi*, and *Anaplasma phagocytophilum* within *Ixodes* spp. ticks in TRP and at other nearby regional parks (Kramer and Beesley, 1993; Lane, 1996; Peavey et al., 1997; Postic et al., 1998; Hui et al., 1998; Mun et al., 2006; CDPH, unpubl. results). Confirmed human cases of Lyme disease, human granulocytic anaplasmosis, and tularemia have recently been associated with recreational or occupational exposure to ticks at TRP (CDPH, unpubl. data). The prevalence of *B. burgdorferi* sensu lato in questing adult ticks in TRP is approximately 1% (CDPH, unpubl. data). Three

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common human-biting tick species are present in TRP (*I. pacificus*, *Dermacentor occidentalis*, and *D. variabilis*) and are capable of transmitting a range of zoonotic disease agents to visitors. Other tick species present in this park that are primarily animal parasites include *I. spinipalpis*, *I. auritulus*, *I. brunneus*, and *Haemaphysalis leporispalustris*.

The overall objective of this study was to identify activities and habitats that put people at risk of adult and nymphal tick exposure at picnic areas in a large northern California recreational park where *B. burgdorferi* has been documented. Specific goals were to determine: (1) abundance of adult and nymphal ticks collected at picnic sites, (2) abundance of nymphal *I. pacificus* in various habitats associated with wood (logs, tree trunks, benches, picnic tables), leaf litter, vegetation, or rock walls; and (3) prevalence of *Borrelia* spp. infection in *I. pacificus* adults and nymphs collected at picnic sites.

Materials and methods

Beginning in November 2006, VBDS staff collected adult and nymphal ticks from various sites in TRP to provide samples for diagnostic evaluation. Abundance and prevalence data provided background tick abundance data necessary for study site selection (CDPH, unpubl. results).

Seven heavily used picnic areas ranging in elevation from 176 m to 501 m above sea level were selected within TRP (Big Springs, Brook, Carousel, Fern, Indian Camp, Island, and Steam Trains). All picnic areas were visited on a single day, every 2 weeks from March 28 to August 18, 2008, coincident with the time of year when nymphal *I. pacificus* are active. Upon arrival at each picnic area, a 50 cm × 60 cm woven cotton blanket was placed at random within the picnic area. A biologist sat in the middle of the blanket and took notes while the other biologist flagged the picnic area for ticks with a 1-m^2 white cotton flannel flag. For 1/2 h, each picnic area was flagged around the perimeter and along 2 bisecting linear transects. Habitat flagged included vegetation (shrubs, grasses, small plants) and leaf litter. Habitat type where ticks were collected was recorded. All trees within the picnic site were flagged by placing the flannel flag against the tree trunk at a height of 1.5 m several times, covering the entire circumference of trunk. Similarly, logs were flagged as well as rock walls and wooden benches. All wooden picnic tables and benches were sampled on the top of all surfaces as well as the ground underneath. The sites where ticks were collected were recorded; ticks were collected and retained separately according to habitat type. Lastly, before leaving each picnic area, the blanket was carefully lifted, placed on a picnic table, and checked for ticks. While picnic areas were sampled for uniform time per visit (1/2 h), the amount of sampling per habitat was not uniform, precluding statistical analysis of ticks per unit area per habitat type.

The seven picnic areas had perimeters ranging from 25.91 m (Big Springs) to 209.21 m (Fern) (mean = 115.65 m; *SD* = 60.39). Each picnic area had 2–7 picnic tables (mean = 4.4; *SD* = 2.3; *n* = 29). Wooden picnic table tops and their benches ranged from 299 to 355 cm long. Table tops were 88–91 cm wide and 66–95 cm tall. The attached benches were 28–30 cm wide and 26–61 cm tall. Carousel and Brook were the only 2 sites with rock walls. These walls measured 588 cm long × 30 cm wide × 38 cm tall and 896 cm long × 30 cm wide × 38 cm tall, respectively. Three picnic areas (Carousel, Fern, and Indian Camp) had wooden benches not associated with tables; these benches ranged from 242 to 197 cm in length, 28–38 cm in width, and 46–54 cm in height. At each picnic area, 0–14 tree trunks (average = 4.6; *SD* = 4.5; *n* = 32) and 0–5 logs were flagged (average = 2.1; *SD* = 2.2; *n* = 15) on each sample day.

Adult and nymphal *Ixodes* spp. ticks collected at TRP during this study were screened for *Borrelia* spirochetes using a direct fluorescent antibody assay (DFA) (Persing et al., 1990). Ticks were identified to species, life stage, and sex and washed with phosphate buffered saline (pH 7.4) solution (PBS). Ticks were placed individually in one of two etched wells on fluorescent antibody microslides (Thermo Fisher, Waltham, MA) with 20 μl of sterile PBS and dissected with forceps and a scalpel. The tick exoskeleton and a small drop of PBS was removed from the microslide and placed in a separate 2 mL microcentrifuge tube (Eppendorf, Hamburg, Germany) containing 20 μl sterile PBS and preserved at −80 °C. Forceps and scalpels were wiped on 70% ethanol-saturated cotton balls and flamed between ticks to prevent cross-contamination with *Borrelia*. Microslides were allowed to dry and placed in acetone for a minimum of 10 min to fix. Slides were either prepared for DFA or frozen at −80 °C until ready to be read.

To check for *Borrelia* on acetone-fixed slides, slides were placed on moistened paper towels within a sealed incubation chamber. Twenty microliters of 1:20 anti-*Borrelia* antibody conjugate was dispensed on each well (fluorescein isothiocyanate-conjugated anti-*Borrelia* genus antibody KPL, Inc., Gaithersburg, MD) and incubated for up to 2 h (Persing et al., 1990). Slides were rinsed with PBS, and a drop of mounting media was placed on each specimen and covered with a glass coverslip. Slides were screened for spirochetes under a fluorescent microscope (minimum of 100 random fields of view at 400× magnification). Positive control slides were made using *B. burgdorferi* cellular antigen (KPL, Inc.). Ticks positive for *Borrelia* spirochetes by DFA were also tested by a nested polymerase chain reaction (PCR) that targeted the 16S–23S intergenic spacer region (IGS) (Travinsky et al., 2010). In addition, all DFA-positive ticks were tested by multilocus PCR followed by electrospray ionization mass spectrometry (PCR/ESI-MS) by Ibis Biosciences (Carlsbad, CA). This technique has been shown to resolve *Borrelia* genotypes as well as to detect mixtures of *Borrelia* species and genotypes (Crowder et al., 2010).

The risk of exposure to *B. burgdorferi* was calculated by using the entomological risk index (the number of nymphs collected per person-hour multiplied by prevalence of ticks infected with *B. burgdorferi*) (Mather et al., 1996).

Results

A total of 1758 adult *I. pacificus* was collected at TRP during 2006–2008. Of those, 11 of 814 adult *I. pacificus* tested (1.4%) were positive for *Borrelia* spirochetes by DFA. All were tested by molecular methods, and 5 were confirmed by PCR. Four of these were identified as *B. miyamotoi* and one as *B. burgdorferi* sensu stricto. In the 7 picnic areas, 18 adult *I. pacificus*, 32 *D. occidentalis*, and 14 *D. variabilis* were collected from March to August. The mean number of adult ticks per picnic site per day was: 0.4 *I. pacificus* (range: 0–0.7; *SD* = 0.26), 0.4 *D. occidentalis* (range: 0–0.7; *SD* = 0.34), and 0.2 *D. variabilis* (range: 0–0.4; *SD* = 0.12). Adult ticks were generally collected on leaf litter and vegetation around the periphery. Of note, one adult *I. pacificus* was collected from a tree trunk, 2 adult *D. variabilis* were collected off wooden picnic tables, and 3 adult *D. variabilis* were collected from a rock wall. No ticks were flagged in the 2 bisecting linear transects covering the main section of the picnic areas.

The number of nymphal *I. pacificus* collected in picnic sites peaked in late April, with a secondary peak in early June (Fig. 1). *I. pacificus* nymphs (*n* = 170) were collected from each of 7 habitat types sampled: leaf litter (37.9%), vegetation (20.1%), logs (19.0%), picnic tables (12.1%), tree trunks (8.6%), rock wall (1.7%), and picnic blanket (0.6%). (Fig. 2A) The highest number of *I. pacificus* nymphs was collected from leaf litter (*n* = 66), but when wooden materials were combined (logs, tree trunks, and picnic tables), the abundance of nymphs on wood products was similar to that found in leaf