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Original article

# Vector biodiversity did not associate with tick-borne pathogen prevalence in small mammal communities in northern and central California

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ABSTRACT

Vector and host abundance affect infection transmission rates, prevalence, and persistence in communities. Biological diversity in hosts and vectors may provide "rescue" hosts which buffer against pathogen extinction and "dilution" hosts which reduce the force of infection in communities. *Anaplasma phagocytophilum* is a tick-transmitted zoonotic pathogen that circulates in small mammal and tick communities characterized by varying levels of biological diversity. We examined the prevalence of *A. phagocytophilum* in *Ixodes* spp. ticks in 11 communities in northern and central California. A total of 1020 ticks of 8 species was evaluated. Five percent of ticks (5 species) were PCR-positive, with the highest prevalence (6–7%) in *I. pacificus* and *I. ochotonae*. In most species, adults had a higher prevalence than nymphs or larvae. PCR prevalence varied between 0% and 40% across sites; the infection probability in ticks increased with infestation load and prevalence in small mammals, but not tick species richness, diversity, evenness, or small mammal species richness. No particular tick species was likely to "rescue" infection in the community; rather the risk of *A. phagocytophilum* infection is related to exposure to particular tick species and life stages, and overall tick abundance.



## Introduction

The fate of a pathogen upon introduction into a community can be extinction, enzootic persistence, or emergence into an epizootic depending on biotic and abiotic factors that influence host, vector, and pathogen survival and how many individuals in each host species are susceptible, infected, or immune. Biological diversity could reduce the probability of particular hosts acquiring infection, increase this risk, or "rescue" infection in a community (Keesing et al., 2006). A highly biodiverse disease system provides opportunity to evaluate whether infection prevalence varies due to dilution or amplification effects. In this paper, we consider the prevalence of an obligately tick-transmitted bacterial pathogen, *Anaplasma phagocytophilum*, in communities of small mammal hosts and ixodid ticks in California. The distribution of *A. phagocytophilum* is Holarctic and the *Ixodes* spp. ticks which transmit this bacterium feed only once per stage as larvae, nymphs, and adults, transmitting the infection transstadially, but not transovarially (Munderloh and Kurtti, 1995; Foley et al., 2004). Most studies of *A. phagocytophilum* ecology have focused on bridge vectors, i.e. *Ixodes* spp. ticks such as the western black-legged tick (*I. pacificus*) in the western U.S. which has diverse feeding habits as adults, typically questing on vegetation seasonally for a variety of large mammals (Foley et al., 2004). These bridge vectors do contribute to maintenance cycles with small mammal hosts, but often host-specialist or nidicolous ticks also may be responsible for pathogen maintenance (Foley et al., 2011). California has 20 species of ticks in the *Ixodes* genus including the known vector-competent *I. pacificus* and *I. spinipalpis* and other relatively common small mammal-feeding species such as *I. woodi* and *I. angustus*. Small mammal diversity is high, and the bacteria themselves are actually a diverse set of closely related strains or genospecies (Foley et al., 2009; Rejmanek et al., 2011). Biological diversity in many areas is very high and overall highly variable.

In the present study, we collected small mammals and ticks from 11 sites where there was evidence of *A. phagocytophilum* and varying levels of tick biological diversity. We assessed ticks for the presence of *A. phagocytophilum* DNA and determined whether individual-level (tick species, stage, or capture method) or site-level factors (tick species richness, evenness, and diversity, number of ticks per host, small mammal species richness, or prevalence of *A. phagocytophilum* in small mammals) could account for the patterns of infection we obtained.

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## Materials and methods

### *Study sites and trapping*

Small mammal trapping and tick collection were performed at 11 sites in northern and central California from February 2005 to January 2012 (Table 1). Sampling was performed at each site at least 6 times in that interval. At each site, transects were established along deer trails and poorly used human trails and roads. Flagging for ticks was performed over herbaceous and shrubby vegetation as well as duff and litter using a 1-$m^2$ white cotton flag. In order to obtain small mammals and their attached ticks, extra-large (10 cm × 10.4 cm × 38 cm) Sherman (HB Sherman, Tallahassee, FL) and Tomahawk (Tomahawk Live Trap, Tomahawk, WI) live traps were set overnight at locations of observed active rodent usage and baited with peanut butter and oats. Rodents were anesthetized with approximately 20 mg/kg ketamine and 3 mg/kg xylazine delivered SC, examined for ectoparasites, and given a permanent individually numbered metal ear tag. Blood samples were collected from the retroorbital sinus into EDTA. Ticks were removed with forceps and preserved in 70% ethanol. *Ixodes* spp. were identified to species using keys (Furman and Loomis, 1984; Webb et al., 1990). Larvae were examined under both a dissecting and a compound microscope in a depression slide. All work with small mammals was performed under the oversight of the UC Davis Attending Veterinarian and the Institutional Animal Care and Use Committee.

### *Polymerase chain reaction for A. phagocytophilum infection*

Ticks and small mammal blood samples were assessed for *A. phagocytophilum* infection by polymerase chain reaction (PCR). DNA was extracted from mammalian blood using a kit (Qiagen Blood and Tissue Kit, Valencia, CA, USA) following manufacturer's instructions. DNA was extracted from ticks using a protocol modified from Humair et al. (2007). Ticks were surface-cleaned with 70% ethanol, the ethanol was allowed to evaporate, ticks were frozen in liquid nitrogen for 3 min, and then crushed with a pestle. The ticks were then boiled for 15 min in 100 μl of 0.7 M $NH_4OH$, cooled quickly for 30 s on ice, and then boiled again for 15 min in open vials to evaporate ammonia. We previously showed that ammonium hydroxide boiling did not affect DNA yield from questing ticks compared with Qiagen extraction, when we compared the cycle threshold (CT) from TaqMan PCR of the 18S rDNA gene using a purchased primer and probe set (Applied Biosystems; Cleopatra del Prado and Foley, unpubl. data). For this study, we randomly selected fed adult *I. pacificus* and subjected half to Qiagen extraction which might better remove PCR inhibitors from blood and the other half to the ammonium hydroxide method. The mean CT from Qiagen (17.4) was slightly, but significantly ($p = 0.003$) lower than from ammonium hydroxide (20.3).

Real-time quantitative PCR was performed targeting the multiple-copy *msp2* gene of *A. phagocytophilum* as previously described (Drazenovich et al., 2006). Each 12-μl reaction contained 5 μl DNA, 1X TaqMan Universal Master Mix (Applied Biosystems), 2 nmol of each primer, and 400 pmol of probe. The amplification cycle consisted of 50 °C for 2 min, 95 °C for 10 min, and 40 cycles at 95 °C for 15 s, followed by 60 °C for 1 min. Samples were considered positive if they had a CT value <40 and characteristic amplification plots. For all reactions, 3 water negative controls and a DNA sequence-confirmed positive DNA control were included during each run.

### *Data analysis*

Data were maintained in Excel (Microsoft, Redmond, WA) and analyzed with the statistical package "R" (R-Development Core Team, http://www.r-project.org). The cutoff for statistical significance was $p = 0.05$.

Summary statistics were calculated for each site and overall. PCR prevalence was calculated in ticks by species, stage, and site; in hosts by genus; and in reservoir hosts (i.e. woodrats, chipmunks, and squirrels). The stage distribution of flagged ticks was compared to that for ticks removed from small mammals with a chi-square contingency test. With the non-simultaneous sampling across sites, we could not analyze for temporal trends, but acknowledge that there may have been seasonal or other dynamic influences we cannot account for.

We used a mixed logistic regression model, function glmer in the R package lme4 (Bates et al., 2011), with site as a random effect, to evaluate the dependence of PCR results in ticks on individual and site-level predictors. Prior to analysis, we examined potential predictor variables for collinearity and suitability for analysis. Individual-level predictors were tick stage, species, and capture method (i.e., flagging vs. rodent trapping). Due to low sample sizes, the rare species *I. auritulus* ($n = 1$), *I. sculptus* ($n = 1$), and *I. soricis* ($n = 2$) were dropped from the analysis. While DNA extraction methods yielded slightly different quality and quantity DNA from flagged vs. host-fed ticks, by retaining capture method and stage in the model, we were able to detect whether there was an effect

**Table 1**
Characteristics of 11 study sites evaluated for *Anaplasma phagocytophilum* in ticks and small mammals from 2005 to 2012. Abbreviations for study sites are given in this table and used for subsequent tables.

| Study site | Dominant vegetation | Region | County | Centroid latitude and longitude | Elevation (m) |
|---|---|---|---|---|---|
| Big Basin State Park (BB) | Redwood, chaparral live oak, tanoak, madrone, | Central coast range | Santa Cruz | 37.1668; 122.2243 | 360 |
| Boggs Mountain Demonstration Forest (BM) | Live oak, chaparral, Jeffrey pine | Northern interior coast range | Lake | 38.8155; 123.6828 | 1136 |
| Cold Canyon Preserve (CC) | Live oak, gray pine | Northern interior coast range | Yolo | 38.5127; 122.0972 | 61 |
| Green Diamond (GD) | Ponderosa pine, Douglas fir, tanoak, true oak | Far northwest | Del Norte, Humboldt | 41.1261; 123.8134 | 10–690 |
| Henry Cowell/Fall Creek State Park (HC) | Redwood, tanoak, Douglas fir | Central coast range | Santa Cruz | 37.0442; 122.0725 | 83 |
| Humboldt Redwoods State Park (HR) | Redwood, Douglas fir, tanoak, madrone, live oak | Northern coast range | Humboldt | 41.0998; 123.9083 | 230 |
| Hendy Woods State Park (HW) | Redwood, Douglas fir, live oak | Northern coast range | Mendocino | 39.0691; 123.4637 | 168 |
| Montgomery Woods State Park (MW) | Douglas fir, redwood | Northern coast range | Mendocino | 35.3658; 123.8879 | 249 |
| Quail Ridge Reserve (QR) | Chaparral, gray pine, live oak | Interior northern coast range | Napa | 38.4812; 122.1035 | 600 |
| Soquel Demonstration Forest (SD) | Redwood, live oak, tanoak, madrone | Central coast range | Santa Cruz | 37.0478; 121.9343 | 450–600 |
| Samuel P. Taylor State Park (SPT) | Redwood, live oak, tanoak, madrone | Northern coast range | Marin | 38.0247; 122.7238 | 134 |