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A spatially-explicit model of acarological risk of exposure to *Borrelia burgdorferi*-infected *Ixodes pacificus* nymphs in northwestern California based on woodland type, temperature, and water vapor

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Abstract

In the far-western United States, the nymphal stage of the western black-legged tick, *Ixodes pacificus*, has been implicated as the primary vector to humans of *Borrelia burgdorferi* sensu stricto (hereinafter referred to as *B. burgdorferi*), the causative agent of Lyme borreliosis in North America. In the present study, we sought to determine if infection prevalence with *B. burgdorferi* in *I. pacificus* nymphs and the density of infected nymphs differ between dense-woodland types within Mendocino County, California, and to develop and evaluate a spatially-explicit model for density of infected nymphs in dense woodlands within this high-incidence area for Lyme borreliosis. In total, 4.9% (264) of 5431 *I. pacificus* nymphs tested for the presence of *B. burgdorferi* were infected. Among the 78 sampling sites, infection prevalence ranged from 0 to 22% and density of infected nymphs from 0 to 2.04 per 100 m². Infection prevalence was highest in woodlands dominated by hardwoods (6.2%) and lowest for redwood (1.9%) and coastal pine (0%). Density of infected nymphs also was higher in hardwood-dominated woodlands than in conifer-dominated ones that included redwood or pine. Our spatial risk model, which yielded an overall accuracy of 85%, indicated that warmer areas with less variation between maximum and minimum monthly water vapor in the air were more likely to include woodlands with elevated acarological risk of exposure to infected nymphs. We found that 37% of dense woodlands in the county were predicted to pose an elevated

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risk of exposure to infected nymphs, and that 94% of the dense-woodland areas that were predicted to harbor elevated densities of infected nymphs were located on privately-owned land.

Keywords

Borrelia burgdorferi; *Ixodes pacificus*; California; Lyme borreliosis; Spatial risk model

Introduction

In the far-western United States, the nymphal stage of the western black-legged tick, *Ixodes pacificus*, has been implicated as the primary vector to humans of *Borrelia burgdorferi* sensu stricto (hereinafter referred to as *B. burgdorferi*), the causative agent of Lyme borreliosis in North America (Clover and Lane, 1995). Previous studies from north coastal California revealed that human exposure to *I. pacificus* nymphs occurs primarily in woodlands with a ground cover dominated by leaves or fir needles (hereinafter referred to as dense woodland), whereas the risk of humans contacting nymphs is minimal in open grasslands, woodlands carpeted with grass, and chaparral because the nymphs apparently do not ascend vegetation while seeking a host (Clover and Lane, 1995; R.J. Eisen et al., 2004; Lane et al., 1995; Tälleklint-Eisen and Lane, 2000). Furthermore, studies from dense woodlands in Mendocino County, which is located in northwestern California and considered to be a hot-spot for Lyme borreliosis in the Far West (R.J. Eisen et al., 2006a; Fritz and Vugia, 2001), identified environmental factors associated with elevated risk of exposure to vector ticks and *B. burgdorferi*. For example, acarological risk of exposure to *B. burgdorferi*-infected *I. pacificus* nymphs differed across dense woodland types (R.J. Eisen et al., 2003; L. Eisen et al., 2004b), and the timing and magnitude of peak nymphal density also were variable across woodland types with more defined and higher peaks occurring in warmer and drier hardwood-dominated woodlands compared with cooler and more humid redwood-dominated habitats (R.J. Eisen et al., 2003). These and other environmental correlates of risk for exposure to nymphs were later used to produce Geographic Information System (GIS)/Remote Sensing (RS)-based spatial models for predicted acarological risk of encountering *I. pacificus* nymphs in Mendocino County (R.J. Eisen et al., 2006b) and for California (R.J. Eisen et al., 2006a).

We now are expanding on these studies by developing a spatially-explicit model for acarological risk of exposure to *B. burgdorferi*-infected *I. pacificus* nymphs in dense woodlands in Mendocino County. Risk assessments that combine density of tick vectors and infection prevalence of these vectors with *B. burgdorferi*, typically expressed as density of infected ticks or distance to encounter an infected tick, have been used in California (R.J. Eisen et al., 2003, 2004; L. Eisen et al., 2004b; Lane et al., 2001, 2007) and the eastern United States (Allan et al., 2003; Gatewood et al., 2009; Mather et al., 1996; Paskewitz et al., 2001; Stafford et al., 1998). This represents an improvement over risk assessments based on vector-tick density alone.

In the current study, we sought to determine if, similar to nymphal density, infection prevalence with *B. burgdorferi* in *I. pacificus* nymphs and density of infected nymphs differ between dense-woodland types. In addition, we developed and evaluated a spatially-explicit model for density of *B. burgdorferi*-infected *I. pacificus* nymphs in dense woodlands within Mendocino County. Finally, we determined the distribution of model-defined areas with elevated risk falling within publicly accessible versus privately owned lands.

Materials and methods

Study sites and tick collection

The study was conducted in Mendocino County in northwestern California (Fig. 1). This climatically and ecologically diverse county encompasses ~9090 km² and extends ~130 km along the coast of the Pacific Ocean and up to 80 km inland from the coastline. Cool and moist woodlands with coast redwood (*Sequoia sempervirens*) are common in the western, coastal part of the county, whereas grassland, chaparral, and hardwood or mixed hardwood-Douglas fir (*Pseudotsuga menziesii*) woodlands dominate the warmer and drier eastern, inland part (Mayer and Laudenslayer, 1988). Climatic variability within Mendocino County was described previously (R.J. Eisen et al., 2003; L. Eisen et al., 2004b).

Tick sampling was conducted in 78 dense woodland sites located throughout Mendocino County (Fig. 1). Sites were categorized, based on field determinations of tree species compositions, as redwood (n=10), coastal pine (n=3), inland pine (n=8), hardwood (n=19), hardwood/conifer (n=22), mixed tanoak/madrone or Douglas fir with a redwood influence (n=4), or tanoak (n=12). A more detailed description of each dense woodland category was provided previously (R.J. Eisen et al., 2006b; L. Eisen et al., 2006). In summary, “redwood” areas consisted primarily of coastal redwood (*Sequoia sempervirens*) with Douglas fir (*Pseudotsuga menziesii*), tanoak (*Lithocarpus densiflorus*), or California bay (*Umbellularia californica*). “Coastal pine” areas included mainly Bishop pine (*Pinus muricata*) with an influence of coastal redwood or Douglas fir. “Inland Pine” woodlands were dominated by Ponderosa pine (*Pinus ponderosa*) with an influence of Douglas fir. “Hardwood” sites contained mainly *Quercus* spp. oaks, Pacific madrone (*Arbutus menziesii*), and California Bay. “Hardwood-conifer” sites comprised of mixed *Quercus* spp. oaks, Pacific madrone, California bay, and Douglas fir or Ponderosa pine. “Mixed tanoak/madrone or Douglas fir with a redwood influence” sites contained mixed tanoak, Pacific madrone, Douglas fir with a clear redwood influence in or adjacent to the sampling site.

“Tanoak” woodlands were dominated by tanoak with an influence of coastal redwood, Douglas fir, or Pacific madrone. Site locations were determined with a Trimble Geo XT (Trimble Corp., Sunnyvale, California, U.S.A.) global positioning system receiver and visualized in map format using ArcGIS 9.2 (Environmental Systems Research Institute [ESRI], Redlands, California, U.S.A.). The median distance between sites was 46 km, with a range from <1 to 129 km. In total, 2.5% of sites were separated by 4 km or less from their nearest neighbor.

Tick-sampling methodology was described in detail previously (R.J. Eisen et al., 2006a, 2006b). In brief, each sampling site consisted of a 750-m² dense woodland area with a leaf, fir needle, or leaf-fir needle litter ground cover. Ticks were collected by dragging a white flannel blanket, 1 by 1.25 m, along fixed 15-m transect lines (n=50 transects per site). Each site was sampled twice during spring 2004, from 28 April to 14 May and from 17 May to 4 June; this sampling period corresponds to the peak activity period for *I. pacificus* nymphs throughout Mendocino County (R.J. Eisen et al., 2003). Nymphal and adult ticks were stored in 95% ethanol prior to species identification and pathogen detection. In sites where *I. pacificus* nymphs were rare, we conducted additional sampling within or in similar habitat adjacent to the site in order to increase the numbers of nymphs available for detection of *B. burgdorferi*.

Detection of *B. burgdorferi* in *I. pacificus* nymphs

Our goal was to examine 100 nymphs per sampling site for *B. burgdorferi* DNA. This was achieved in 42 of the 78 sites. Numbers of nymphs available for spirochete detection from the remaining 36 sites ranged from 1 to 91, and broke down as follows: 9 sites with 1–9 ticks

examined, 8 sites with 10–19 ticks examined, 5 sites with 20–49 ticks examined, and 14 sites with 50–91 ticks examined.

Total DNA was extracted from individual nymphs using the DNeasy Blood and Tissue Kit (Qiagen, Valencia, California, U.S.A.) according to the manufacturer's protocol for animal tissues and as described earlier (Girard et al., 2009). PCR amplification and sequence analysis of 5S-23S rRNA spacer region of *B. burgdorferi* was performed as previously described (Girard et al., 2009). *B. burgdorferi* genospecies assignment was based on phylogenetic analysis using the neighbor-joining method implemented in PAUP* (version 4.0 beta; Sinauer, Sunderland, MA; uncorrected *p* distances).

Description of predictive variables included in the density of infected nymphs' model

Independent variables that were screened for inclusion in the candidate models included remotely-sensed vegetation indices, topographical features, soil characteristics, climatic variables, and meteorologic variables. The first 4 categories of data were included also in our previous model of nymphal density (R.J. Eisen et al., 2006b). Normalized difference vegetation indices (NDVI) and tasseled cap values for brightness, greenness, and wetness for 4 seasons (May 2002, July 2002, November 2002, and February 2003) were derived from Landsat TM 5 images (30 m spatial resolution). Topographic features, including elevation, slope, aspect, solar insolation, and hours of sun exposure for 4 dates that corresponded with Landsat images, were derived from a U.S. Geological Survey 10 m Digital Elevation Model. Hydrological groupings were based on data from the U.S. Department of Agriculture Natural Resources Conservation Service Soil Survey Geographic database and were described previously (R.J. Eisen et al., 2006b). Dichotomization of the county into coastal or inland areas was based on a previously created GIS layer (R.J. Eisen et al., 2006b), and methods of layer construction can be found within that same reference.

Long-term climatic variables included annual mean precipitation and base 10°C annual mean growing degree days from 1961 to 1990 (Water and Climate Center of the Natural Resources Conservation Service, Portland, Oregon, U.S.A., 2 km resolution). Meteorologic variables were obtained through the PRISM Climate Group (Oregon State University, Portland, Oregon, U.S.A.; 4 km resolution; <http://www.prism.oregonstate.edu/>). These included monthly estimates of precipitation, average dew point (which gauges the variability in available water vapor in the air), and maximum and minimum temperatures for the years 2003 and 2004. These years were selected because they correspond with the period of time when the nymphs collected in this study were seeking hosts (2004) and when the preceding larval stage was questing and potentially acquiring *B. burgdorferi* infection (2003). The data layers for monthly average dew point also were used to calculate the differentials between the months with the highest and lowest average dew point values for 2003 and 2004. To achieve this, grid layers were created for 2003 and 2004 that defined (1) the maximum monthly average dew point and (2) the minimum monthly average dew point. Finally, we created additional grids for each of the 2 years to determine the extent of variation in monthly average dew points; this was calculated as maximum minus minimum monthly average dew point for each grid cell. We also calculated average maximum temperatures for March–August for 2003 and 2004, as spring-summer temperature was found previously to be related to duration of the nymphal host-seeking period and most host-seeking behavior for larvae and nymphs are restricted to these months of the year (R.J. Eisen et al., 2003). All layers were projected to TealAlbers zone 10 NAD 1927.

In addition to the GIS/RS-derived layers, we also included a single field-derived variable in the candidate models. Although this layer could not be extrapolated within a GIS, it served the purpose of comparing how a variable that is easily identifiable on the ground compares with GIS- or RS-derived variables. This variable, referred to as “hardwood” in Table 1, compares

the combined “hardwood” and “hardwood-/conifer” categories with all other woodland categories. These categories were described above.

Model construction

Our model seeks to classify Mendocino County dense woodlands into elevated or low risk of encountering *B. burgdorferi*-infected *I. pacificus* nymphs. For each of the collection sites, the observed peak density of infected nymphs was calculated by multiplying the higher of the 2 sampled values of density of host-seeking nymphs per 100 m² by site-specific infection prevalence in the nymphs (number infected per number tested). Previous work in some of the sites included in this study demonstrated significant inter-annual variation in prevalence of infection with *B. burgdorferi* in *I. pacificus* nymphs (L. Eisen et al., 2004b). Based on the shortcoming in this study of only having infection prevalence data available for a single year, we chose to dichotomize the response variable into elevated versus low risk, where low risk represents sites below the median density of infected nymphs (0–0.0627 infected nymphs per 100 m²) and elevated risk sites represent those above the median value (0.0628–2.0400 infected nymphs per 100 m²).

Following methods described previously (R.J. Eisen et al., 2007), logistic regression models were constructed to evaluate the association between the probability of a site being classified as elevated risk and its environmental, climatic, or meteorologic attributes (described below). Forward stepwise logistic regression models were constructed to screen for variables with greatest association with elevated risk. Spearman correlation tests were used to eliminate variables that were significantly associated with risk ($P < 0.05$) but highly correlated with other covariates ($\rho \geq 0.80$). Six candidate models were constructed (Table 1). Each is described by the following equation:

$$\text{Logit}(P) = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \dots + \beta_k x_k \quad [\text{expression 1}]$$

where P is the probability that a grid cell will be classified as elevated risk, and β_0 is the intercept. The values $\beta_1 \dots \beta_k$ represent coefficients assigned to each independent variable, $x_1, \dots, x_k$ included in the regression model. The probability of each cell being classified as elevated or low risk was derived from expression 1 using the following equation:

$$P = \exp^{\text{Logit}(P)} / (1 + \exp^{\text{Logit}(P)}) \quad [\text{expression 2}]$$

For each candidate model, a goodness of fit test was used to determine whether the covariates included in the model adequately described the distribution in the data. The goodness of fit test compares the pure error negative log-likelihood with the fitted model log-likelihood; sufficient explanatory variables are considered to be included in the model when the chi-square test is not significant.

The overall discrimination ability of each model was assessed using area under the curve (AUC) estimates derived from receiver operating characteristic curves (ROCs). The AUC provides a threshold-independent measure of the overall accuracy of the model; values range from 0.5 to 1, where a value of 1 indicates that all points were correctly classified. ROCs were also used to determine the optimal probability cut-off value for characterizing each grid cell as elevated or low risk. Based on the selected probability cut-off value that simultaneously maximized sensitivity and specificity (Fielding and Bell, 1997), we compared sensitivity (percentage of elevated risk sites that were accurately classified by the model as elevated risk), specificity (percentage of low risk sites that were accurately classified by the model as low risk), positive

and negative predictive values (percentage of sites that the model predicted to be elevated or low risk, and the actual risk value was either elevated or low, respectively) for each of the candidate models (Table 1).

The most parsimonious model with the highest predictive power was selected using Akaike's Information Criterion (AIC) (Akaike, 1974). An AIC value was calculated for each candidate model. The model with the lowest AIC value was deemed the best, however, models within 2 AIC units were considered to be competing models with substantial support (Burnham and Anderson, 2002). To select the best model among competing models, we selected the model with highest sensitivity that had the best balance in sensitivity, specificity, and positive and negative predictive values. A leave-one-out evaluation method (Fielding and Bell, 1997) was used to determine how sensitive the model was to any particular sampling site. In summary, the best model was repeatedly fitted by removing a single site, recording the AUC value, replacing the site and removing the next site in the data set. The average and range in AUC values are reported. Finally, the best model was extrapolated to dense woodlands in Mendocino County (30 m resolution) by applying expression 2 to a previously developed analysis mask that excluded all habitats not falling into the category of dense woodland (R.J. Eisen et al., 2006b). This was accomplished using the raster calculator function of ArcGIS version 9.2. Uniform inclusion of non-dense woodland habitats into the category of low acarological risk of exposure to infected nymphs is justified because the host-seeking behavior of the nymphs (reluctance to ascend vegetation) leads to human risk of nymphal exposure being very low in non-dense woodland habitats. Finally, within Mendocino County, the majority of Lyme borreliosis cases are believed to be acquired in peridomestic settings. A land stewardship layer (Davis et al., 1998) was used to determine the percentages of woodland areas classified as elevated risk on public versus privately-owned land. These categories serve as proxies for recreational or peridomestic exposure, respectively.

Data analysis

Contingency table analyses were conducted to determine if the proportion of nymphs infected with *B. burgdorferi* differed among the woodland types described above. Fisher's exact tests were used to compare proportions of ticks infected between particular pairs of woodland types. Statistical analyses were carried out using the JMP® statistical package (SAS, Cary, North Carolina, U.S.A.), and results were considered significant when $P < 0.05$. Specific tests used are indicated in the text.

Results

Infection prevalence with *B. burgdorferi* in *I. pacificus* nymphs, and density of infected nymphs by woodland type

In total, 4.9% (264) of 5431 *I. pacificus* nymphs tested for the presence of *B. burgdorferi* were infected. Among the 78 sampling sites, infection prevalence ranged from 0 to 22%. Overall infection prevalence by dense-woodland type is shown in Fig. 2. Statistical tests revealed that prevalence of infection was significantly lower for nymphs collected in woodlands dominated by redwoods (1.9%) compared with hardwood (6.2%), tanoak (5.0%), or mixed class (5.4%) (Fisher's exact test; $\chi^2 \geq 4.2$, d.f.=1; $P \leq 0.04$ in all cases). Infection prevalence was highest in woodlands dominated by hardwoods (6.2%), but statistically significant differences between this and other dense-woodland types occurred only for inland pine (3.9%), hardwood-conifer (4.2%), and redwood (1.9%) ($\chi^2 \geq 4.3$, d.f.=1; $P \leq 0.04$ in all cases).

Among the 78 sites, density of *B. burgdorferi*-infected *I. pacificus* nymphs ranged from 0 to 2.04 per 100 m². The median density of infected nymphs was 0.06 per 100 m². The percentages of sites classified as elevated risk (above the median value) or low risk (below the median

value) by woodland type is shown in Fig. 3. The percentage of sites classified as having an elevated density of infected nymphs was similar among hardwood (84%), mixed class (75%), and hardwood-conifer (64%) woodland types. However, a significantly higher percentage of hardwood sites were classified as elevated risk compared with redwood (20%), inland pine (25%), coastal pine (0%), or tanoak (17%) sites ($\chi^2 \geq 8.80$, d.f.=1; $P \leq 0.003$ in all cases).

Spatially-explicit model for density of infected nymphs in dense woodlands

The model selected as the best among the candidate models (Table 1, model 3) indicated that risk of exposure to *B. burgdorferi*-infected *I. pacificus* nymphs was associated with the extent of variation between maximum and minimum monthly average dew point (water vapor in the air) during the year when the collected nymphs were seeking hosts (2004 in our case), and temperature during the preceding year when the larval stage of these nymphs were seeking hosts (2003 in our case). Specifically, the odds of a site being classified as elevated risk increased with temperature and showed a negative association with the extent of variation between maximum and minimum monthly average dew point values (Table 2). It is important to note that we found, for both predictor variables, strong correlations between 2003 and 2004 (Spearman correlations; $\rho > 0.88$, $P < 0.0001$ in both cases); the year selected for inclusion in the models for each predictor variable was the one that provided the best statistical fit.

The overall accuracy of the model based on the AUC was 85%. Based on a probability cut-off value of 0.52, 35 of 39 sites that were identified through field sampling as having elevated acarological risk of exposure to infected nymphs were correctly predicted by the model to pose an elevated risk (sensitivity = 90%). Of the 39 sites classified as low risk, 29 were predicted by the model to pose low risk (specificity = 75%). Among the 45 sites predicted by the model to pose an elevated risk, 35 were characterized in the field as elevated risk (positive predictive value = 78%). Finally, the model predicted 33 sites to pose low risk, and 29 of these were deemed low risk based on field sampling (negative predictive value = 88%). The leave-one-out evaluation revealed that the model was not particularly sensitive to the characteristics of any single site (mean AUC = 85.3%, range: 85–88%). The semivariograms of the model residuals and the Moran's I statistic showed no evidence of spatial autocorrelation (Moran's I = 0.12; $P = 0.42$). Although dense woodlands occur throughout Mendocino County and in fact are most abundant in coastal areas (R.J. Eisen et al., 2006a), extrapolation of the predictive model to the county revealed that most of the elevated risk areas are located in the central portion of the county (Fig. 1). In total, 37% of dense woodlands were predicted to pose an elevated risk of exposure to infected nymphs. Moreover, 94% of dense woodlands predicted to harbor elevated densities of infected nymphs were located on privately owned land.

We also evaluated the relationship between the percentage of sites classified by the model as elevated risk and the actual risk category for each woodland type (Fig. 3). The greatest difference between actual and observed values shown in Fig. 3 includes a 50% discrepancy within the redwood class. That is, 20% (n=2 sites) of sites were observed to have a high density of infected nymphs, while only 10% (n=1 site) were expected to harbor a high density of infected nymphs. Thus, it is important to note that the small sample size and the low density of infected nymphs is based on the classification of a single site and the differences between observed and expected within this class may not be as large as they appear in Fig. 3. Overall, 86% of woodland-type specific variation in the percentage of sites classified as high risk was explained by the model (Linear regression; actual percentage of sites by woodland type with high risk = $0.82 * \text{predicted high risk} + 7.99$; $r^2 = 0.86$, $F_{1,6} = 31.94$, $P = 0.002$).

Comparison of meteorologic variables between dense woodland types

We compared site-specific estimates of the average maximum temperature from March to August in 2003 and the extent of variation between maximum and minimum monthly average

dew points in 2004 between hardwood/hardwood-conifer sites and sites from all other dense woodland types combined (redwood, coastal pine, inland pine, tanoak, mixed class). This showed that temperature was significantly higher in hardwood/hardwood-conifer sites ($n=41$, median= 24.2°C , range= $21.7\text{--}26.5^{\circ}\text{C}$) compared with sites from the other woodland types ($n=37$, median= 21.9°C , range= $16.8\text{--}25.2^{\circ}\text{C}$; Wilcoxon rank sum test with chi-square approximation: $\chi^2=31.64$, d.f.=1, $P<0.001$). The extent of variation between maximum and minimum monthly average dew point was similar between hardwood/hardwood-conifer and all other woodland types, but there was a trend towards higher variability in the former category.

Discussion

Our main findings were that: (1) consistent with a previous, less extensive study from northwestern California (R.J. Eisen et al., 2003), density of *B. burgdorferi*-infected *I. pacificus* nymphs is typically higher in hardwood-dominated woodlands than in coniferous woodlands including redwood and pine; (2) site-specific meteorological variables, such as average maximum spring-summer temperature and the degree of variability in water vapor, are better predictors of acarological risk of exposure to infected nymphs within dense woodlands than woodland type classifications; and (3) the vast majority of elevated risk areas in Mendocino County falls on privately owned lands which likely promotes peridomestic exposure to *B. burgdorferi*.

Predictive variables in the spatially-explicit risk model

Warmer areas with less variation between maximum and minimum monthly dew point (water vapor in the air) were more likely to be classified as having elevated risk of exposure to infected nymphs. This result is consistent with previous studies of *I. pacificus* nymphs in Mendocino County which showed that peak densities were typically higher in warmer and drier areas such as oak woodlands, compared with cooler and moister conifer-dominated sites (L. Eisen et al., 2002; R.J. Eisen et al., 2003). Although our study focused on peak densities of infected nymphs, which is presumed to be a relevant risk measure as human contact rates should increase with increasing nymphal density, it is important to recognize that the total duration of host-seeking activity is also important in risk assessment. Cooler areas with consistent moisture in the air may experience longer periods of host-seeking activity than hotter and drier areas where the peak questing activity is compressed with a higher proportion of the population questing during a short window of time (R.J. Eisen et al., 2003). Based on data from the entire season of host-seeking activity, the differences in risk of exposure to infected nymphs between warmer and cooler areas may therefore be less pronounced than indicated in this study. Furthermore, care should be taken to extrapolate the results to areas with hotter and drier climates than Mendocino County, such as southwestern California, where extreme arid conditions indeed commonly may lead to desiccation-induced mortality.

In addition to directly impacting tick survival and host-seeking behavior (Kahl and Knülle, 1988; Lees and Milne, 1951; Randolph and Storey, 1999; Stafford, 1994), these same climatic variables (temperature and water vapor in the air) also may be indicative of areas with higher abundances of tick hosts and/or *B. burgdorferi* reservoirs which influences tick population build-up and the likelihood of larval ticks acquiring infection while feeding. For example, warmer hardwood areas may support higher abundances of Columbian black-tailed deer (*Odocoileus hemionus columbianus*), which is a key host for *I. pacificus* adults (Lane and Burgdorfer, 1986; Westrom et al., 1985). Indeed, in a previous study from Mendocino County that included both hardwood and redwood habitats, we found that both the peak and cumulative density of infected nymphs were higher in areas where deer signs were present which occurred more commonly in hardwood forests (R.J. Eisen et al., 2003).

The other key factor is abundance of *B. burgdorferi* reservoirs, which also may be higher in warmer hardwood habitats than in cooler coastal conifer-dominated ones. In contrast to the northeastern United States where the white-footed mouse, *Peromyscus leucopus*, often serves as the primary reservoir of Lyme borreliosis spirochetes (LoGiudice et al., 2003; Mather et al., 1989), *Peromyscus* spp. mice and other small rodents only rarely are infected with *B. burgdorferi* in dense woodlands of northwestern California (L. Eisen et al., 2004a, 2009). Instead, because of their high prevalence of *B. burgdorferi* infection and heavy larval and nymphal *I. pacificus* loads, the western gray squirrel (*Sciurus griseus*) has been implicated as the primary reservoir of Lyme borreliosis spirochetes in northwestern California (L. Eisen et al., 2009; Lane et al., 2005; Salkeld et al., 2008). In Mendocino County, the frequency with which western gray squirrels were observed in woodland types that were identified as elevated risk by our model (e.g., oak woodlands or oak-Douglas fir woodlands) was variable. However, none was observed in the cooler and moister redwood habitats that were classified as low risk by our model. Although GIS-based maps of projected distributions of vertebrates are available through the California Gap Analysis Project (http://www.biogeog.ucsb.edu/projects/gap/gap_home.html), they were not deemed to be of a quality adequate for inclusion in our fine-scale models because they are restricted to species presence/absence rather than abundance and also are based on a series of assumptions that introduce significant uncertainty regarding the quality of the output distribution maps.

Model outcomes and field risk indicators

Our model highlighted the spatial heterogeneity in human risk of encountering infected nymphs within Mendocino County, which reports one of the highest Lyme borreliosis incidences in California (Fritz and Vugia, 2001). Furthermore, analysis of the model residuals revealed that if spatial autocorrelation occurs within this system, it is at a smaller spatial scale than the one for which this study was conducted. Based on only 2 predictive variables, the model yielded an overall accuracy of 85%, with 90% of sites with an elevated density of infected nymphs predicted as elevated risk by the model. While this is believed to be an accurate assessment of model performance, there is a need for future sampling in independent locations throughout the county to evaluate model performance at sites not included in our model build set.

Although the included variables provided an accurate model that could be extrapolated across the county, it is often more practical to assess personal risk based on variables that are easily identified on the ground (R.J. Eisen et al., 2006b). To that end, we compared density of infected nymphs among woodland types and showed that risk is typically higher in hardwood or hardwood-conifer dominated woodlands compared with redwood or pine-dominated coniferous woodlands. This finding is consistent with previous studies from Mendocino County (L. Eisen et al., 2002; R.J. Eisen et al., 2003) showing questing activity begins earlier in warmer areas and that the density of host-seeking *I. pacificus* nymphs consistently begins to decline when mean maximum daily temperatures exceed 21–23°C; this phenomenon often leads to a shorter, but higher peak nymphal density. Indeed, the median maximum temperature from March to August in hardwood and hardwood-conifer woodlands was 24°C, whereas that in all other examined woodland types was significantly lower at approximately 21°C.

For several reasons, we were unable to compare the spatial distribution of areas predicted to have elevated acarological risk to the spatial pattern for incidence of human Lyme borreliosis in Mendocino County, as we did in a previous study at the scale of the state of California (R.J. Eisen et al., 2006a). First, few endemic cases are reported annually from Mendocino County (e.g., range of 4–10 cases per year from 2000 to 2003; unpublished data provided by C.L. Fritz of the California Department of Health Services). This results in very low case counts, even if disease case data are aggregated over long time periods, per sub-county administrative unit for which population is known and disease incidence can be calculated. Such low sample sizes

often lead to spurious results. Second, although case counts were accessible at the zip-code scale, in several instances the case address corresponded with the location of a post office box rather than the actual residence, which then may be located in a different zip code. Third, within Mendocino County, there are only 26 zip-code areas and 29 census tracts which often are quite large and contain distinct clusters of human population within a small portion of the boundary unit. This makes it difficult to spatially link risk habitat with occurrence of human cases at the sub-county scale.

Implications for prevention and control of Lyme borreliosis

One benefit of acarological risk measures, compared with risk measures based on epidemiologic data, is the ability to generate risk assessments that are independent of a human population base, which allows for extrapolation onto public lands (R.J. Eisen and L. Eisen, 2008). In fact, we present here the first quantification from the western United States for percentages of areas with elevated acarological risk of exposure to *B. burgdorferi*-infected vector ticks falling on publicly accessible versus privately owned lands (6% and 94%, respectively, for Mendocino County). In the specific case of Mendocino County, public access lands most commonly include redwood habitats to the west and pine-dominated areas to the far northeast. These habitats typically do not have elevated acarological risk and therefore recreational activities taking place on public access lands present minimal risk for exposure to *B. burgdorferi* in Mendocino County. Hardwood or hardwood-conifer woodlands, which harbor both deer (key hosts for *I. pacificus* adults) and important *B. burgdorferi* reservoirs such as the western gray squirrel and commonly have elevated acarological risk, occur predominantly in the central portion of the county where private lands dominate and public access lands are rare. These private lands in central Mendocino County thus present ample opportunities for peridomestic exposure to *B. burgdorferi*.

For tick-borne diseases, prevention and control recommendations (Hayes and Piesman, 2003; Piesman and Eisen, 2008) can differ between elevated risk areas located on privately owned versus publicly accessible land, or between peridomestic settings and those used primarily for recreational activities. For example, simply avoiding elevated risk areas during peak nymphal questing periods may be a feasible approach to reduce recreational exposure on public lands, but is very difficult when people reside in homes surrounded by risk habitat. On the other hand, landscape modification or use of acaricides on vegetation to reduce tick abundance may be feasible control measures in peridomestic settings, but impractical in large recreational settings. Finally, it is worth emphasizing that some Lyme borreliosis prevention measures are practical in all situations. These include daily checks for and prompt removal of ticks after spending time in risk habitats, use of repellents, and wearing protective clothing (Hayes and Piesman, 2003; Piesman and Eisen, 2008).

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Fig. 1.

Dense woodlands in Mendocino County where elevated densities of *B. burgdorferi*-infected *I. pacificus* nymphs (>0.0627 infected nymphs per 100 m^2) are predicted to occur as shown in dark gray. Areas with low acarological risk are shown in light gray (dense woodlands predicted to have ≤ 0.0627 infected nymphs per 100 m^2) or white (non-dense woodland habitats where human risk of nymphal exposure is minimal). Sampling sites with elevated or low density of infected nymphs are shown as dark or light circles, respectively. The location of Mendocino County within California is shown in the figure inset.

Fig. 2. Overall prevalence of *B. burgdorferi* infection in *I. pacificus* nymphs by dense-woodland type. Figures in parentheses represent the numbers of nymphs tested.

Fig. 3.

Comparison of actual and model-predicted percentages of sites with elevated density of infected nymphs (>0.0627 infected nymphs per 100 m²) by dense-woodland type. Numbers of sampling sites included by woodland type are shown in parentheses.

Comparison of candidate models of elevated or low density of *B. burgdorferi*-infected *I. pacificus* nymphs in Mendocino County, California, U.S.A.

Table 1

Model	AIC ^a	Δ AIC ^b	AUC ^c	Sensitivity	Specificity	PPV ^d	NPV ^e	Variables ^f
1	49.15	7.74	84	92	67	74	90	Hardwood, Tmaxsum03, JulWet, NovNDVI, Inland
2	48.18	6.77	83	79	77	78	79	Hardwood, Tmaxsum03, JulWet, Inland
3	41.41	0	86	90	75	78	88	Ddew04, Tmaxsum03
4	42.60	1.19	88	90	69	75	87	Ddew04, Tmaxsum03, Inland, JulNDVI
5	43.41	2	89	85	77	79	83	Ddew04, Tmaxsum03, JulNDVI, NovBright
6	42.13	0.72	86	77	85	83	79	Ddew04, Tmaxsum03, Inland

^a AIC, Akaike Information criterion.

^b Δ AIC, AIC value for model-AIC value for most parsimonious model.

^c AUC, Area under the curve.

^d PPV, positive predictive value.

^e NPV, negative predictive value.

^f Hardwood, hardwood or hardwood-conifer vs. other woodland types; Tmaxsum03, average of monthly maximum temperatures from March to August 2003; JulWet, wetness derived from Landsat TM 5 July 14, 2002; NovNDVI, normalized difference vegetation index (NDVI) derived from Landsat TM 5 November 19, 2002; Inland, coastal or inland classification as defined previously (R.J. Eisen et al., 2006b); Ddew04, difference between maximum and minimum monthly average dew point for 2004; JulNDVI, NDVI derived from Landsat TM July 14, 2002; NovBright, brightness derived from Landsat TM 5 November 19, 2002.

Table 2

Parameter estimates for the multivariate logistic regression model selected as the best model of the probability of elevated density of *B. burgdorferi*-infected *I. pacificus* nymphs in Mendocino County, California, U.S.A.

Model variables	Parameter estimates		Likelihood ratio test		
	Estimate	S.E.	χ^2	d.f.	P
Ddew04	-2.18	0.67	10.43	1	0.0012
Tmaxsum03	1.10	0.25	19.79	1	<0.0001
intercept	-9.53	3.83	6.20	1	0.0128

S.E., standard error; d.f., degrees of freedom; Ddew04 = difference between maximum and minimum monthly average dew point for 2004; Tmaxsum03 = average of monthly maximum temperatures from March to August 2003.