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Modeling the distribution and abundance of the non-native parasite, canine heartworm, in California coyotes

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We applied a habitat-modeling approach using logistic regression to predict the distribution and abundance of canine heartworm (*Dirofilaria immitis*) in coyotes (*Canis latrans*) throughout California. Heartworm is an arthropod-borne parasite of considerable economic and ecological importance. In California, coyotes serve as the primary sylvatic maintenance host and represent a useful sentinel for this parasite. To develop the model, we used a large collection of coyote blood specimens and carcasses collected from spatially broad, yet nonrandom, locations in California. Survey data were useful in refining previous coarser models that predicted uniformly high prevalence of heartworm throughout the coastal and Sierra-Nevada foothills, by indicating variability within this broadly defined plant-climate zone. Due to the non-random nature and large spatial scale of our data-set, we restricted variables to those thought to be most generally important. Modeling indicated that woodlands with a relatively dense canopy, suitable breeding and host-seeking habitat of the western treehole mosquito (*Ochlerotatus sierrensis*), were a good predictor of heartworm prevalence. Within this habitat, prevalence increased with precipitation, which likely affected mosquito abundance. The distribution of heartworm was limited to areas with average cumulative temperatures high enough to enable larval development of heartworms within their mosquito vectors. The prediction accuracy of our model was supported by goodness-of-fit tests, cross-validation tests and external validation tests. The model provided a useful guide to the relative risk of heartworm exposure in California, although the resolution was necessarily coarse and prevalence estimates related to risk in an ordinal manner only.

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Landscape epidemiologists have traditionally attempted to understand ecological factors favoring transmission of parasites by first using statistical methods to determine whether infected hosts are heterogeneously distributed across the landscape and then, if heterogeneity is detected, describing the landscape features associated with clusters (Moore and Carpenter 1999). Ecologists often have addressed the reverse problem of inferring spatial distributions of plants and animals (usually non-parasitic species) from knowledge of habitat associations, e.g. via general linear models (Guisan and Zimmerman 2000). The traditional epidemiological procedure has the advantage that reasonable inferences can be made even when the parasite is expanding its range because inferences are based only on clusters and not their absences. However, where parasite populations

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have reached relatively stable spatial distributions, the ecological procedure, which also makes use of information on absence of parasites, offers a potentially more powerful approach to mapping exposure risk and for investigating causal hypotheses linking physiographic variables and parasite transmission (Hess et al. 2002). The ecological approach has recently been adopted for some arthropod-borne parasites, but has generally focused on vector habitat as the sole index of transmission risk (Ribeiro et al. 1996, Hay et al. 1998). While modeling vector distribution and abundance is sufficient to predict transmission risk for certain parasites, additional complexities in the lifecycle of many parasites may warrant the inclusion of additional variables (Kitron 2000, Hess et al. 2002). In this study, we used a habitat-modeling approach to investigate the distribution of canine heartworm (*Dirofilaria immitis*), a mosquito-vectored parasite, in California. We considered variables affecting both vector abundance and larval heartworm development. Our primary goals were to test hypotheses about relationships between physiographic variables and heartworm prevalence and use this information to create a risk map for heartworm in California coyotes.

Canine heartworm is a geographically invasive parasite of significance to some domestic pets, wildlife populations and to a lesser extent humans (Boreham 1988, Rawlings and Calvert 1995, Sacks and Blejwas 2000). Heartworm has expanded its range considerably worldwide over the past century, including the recent spread westward in North America to California (Lok 1988). Autochthonous cases of heartworm were first recognized in California, USA, in the Sierra-Nevada foothills in the late 1960s (McGreevy et al. 1970), and subsequently have been detected in many parts of California (Sacks 1998, Theis and Stevens 1998). Surveys for heartworm in California coyotes (*Canis latrans*) were conducted in the 1970s and early 1980s to determine geographic patterns of prevalence (Garcia and Voigt 1990). At the time these surveys were conducted, the spread of heartworm in California was still in progress (Sacks and Caswell-Chen 2003). Heartworm prevalence at present is relatively stable throughout California (Sacks and Caswell-Chen 2003), and a static habitat-modeling approach to defining its distribution is therefore appropriate.

Much is known about abiotic and biotic factors affecting heartworm transmission, including vector species and their habitat requirements and effects of ambient temperatures on larval heartworm development (Powell and Hogue 1979, Otto and Jachowski 1981, Scoles 1999, Sacks et al. 2003). These factors have been considered individually, so a multivariable approach to model-building should increase our understanding of the relative importance of physiographic variables and their interactions in determining spatial patterns of heartworm prevalence. For example, development of larval heartworms to the infective stage (L3) in their ectothermic vectors requires that ambient temperatures exceed 14°C and it occurs more rapidly with increasing temperatures (Fortin and Slocombe 1981, Slocombe et al. 1990). While low temperatures clearly constrain transmission (Knight and Lok 1996, Sacks et al. 2003), little is known about the quanitative relationship between temperature and transmission at temperatures above the transmission threshold. On the one hand, transmission might be expected to increase with increasing temperatures. However, mosquito host-seeking activity increases with increasing temperature up to a point but declines thereafter (Lee 1971, Otto and Jachowski 1981). Furthermore, mosquito longevity decreases as temperature increases (Reisen et al. 1983). Thus, the functional form of the relationship between ambient temperatures and heartworm transmission among coyotes depends on the relative strengths of these pathways as well as interactions with other variables such as humidity. Exactly how the factors affecting heartworm transmission interrelate in nature depends partly on their ranges of variability, which is region-specific. Assessing such relationships in one region, however, can provide general insights that are useful in constructing general hypotheses applicable to other regions.

Because coyotes are ubiquitous in California and densities do not vary greatly among rural areas (Shivik 1995, Sacks et al. 1999b, Riley et al. 2003, B. Mitchell, unpubl.), the primary determinants of heartworm distribution in rural California coyotes probably involve heartworms and vectors. In addition to conducive temperatures, transmission also requires the presence of suitable mosquito vectors. Although California supports >50 species of mosquito, only a small number of these species can transmit heartworms (Bohart and Washino 1978). A few species can be locally important vectors (Corselli and Platzer 1982, Walters 1996), but the western treehole mosquito (*Ochlorotatus sierrensis*) is the primary heartworm vector in California as a whole, due in part to its broad distribution in continuous tracts of habitat (Weinmann and Garcia 1974). Transmission also should correlate positively with the density of suitable vectors. Mosquitoes, in general, require water for reproduction and their abundance is often positively associated with precipitation (Theis et al. 1999). Abundance of the western treehole mosquito is especially responsive to rainfall and annual heartworm transmission has been observed to correlate over time with rainfall and with abundance of western treehole mosquitoes (Sacks et al. 2003). Hence, heartworm abundance also may be positively correlated over space with average precipitation. To develop a general model, we limited candidate explanatory variables to the three we felt should be most widely applicable: temperature, precipitation and suitable habitat for the western treehole mosquito. Although the coyote blood specimens used in this study were collected and banked from areas of California encompassing a variety of environments, the locations sampled were highly clustered, occurring primarily on livestock ranches or grazing allotments, where coyotes were routinely removed for depredation control purposes (i.e., unrelated to research). Thus, it was especially important to restrict the model to variables most biologically relevant.

Materials and methods

Data-sets

Coyote blood specimens (n = 1,409) and carcasses (n = 294) were collected by United States Department of Agriculture/Wildlife Services and Santa Clara Vector Control District specialists. Coyotes were killed as part of livestock protection, public safety, or public health control programs and not for this study per se. Blood specimens were collected during 1994–2000 on filter paper strips, allowed to dry, mailed to the California Department of Health Services for their plague surveillance program and then supplied to us. Blood was eluted and frozen at $-20°C$ until testing using the DiroChek ELISA (Synbiotics Corporation, San Diego, CA, USA.) as described by Sacks et al. (2002). The ELISA was found to be highly sensitive (85%) and specific (96%) as used on filter paper blood specimens of coyotes (Sacks et al. 2002). Coyote carcasses were assessed for heartworm infection directly by necropsy (Sacks and Caswell-Chen 2003).

Descriptions of distances and directions from various landmarks (e.g. towns, highways, rivers) were recorded by trappers in the field for specimen locations and translated to spatial coordinates by us. Where they have been studied in California, coyotes have territories approximately 5 km^2 and transient coyotes use substantially larger areas (Shivik 1995, Sacks et al. 1999b, Riley et al. 2003, B. Mitchell, unpubl.). Therefore, the precision of the recorded specimen locations was deemed adequate relative to the scale of coyote home ranges.

For temperature, a continuous geographic coverage developed by Sacks (2002) that was expressed in cumulative annual heartworm-development-unit (HDU) degree-days (Fortin and Slocombe 1981, Slocombe et al. 1990) was used. The HDU values were grouped into the following categories: <0, 0–445, 445–1000 and then classes of 500 from 1000–1500 through 3500–4000 and >4000. Where HDU <0, the average daily temperature never exceeded the heartworm development threshold. The 445-HDU limit corresponded on average to that necessary for heartworm to develop to the infective stage in 30 days (B. N. Sacks, unpubl.), the conventionally accepted maximum development time necessary for transmission (Knight and Lok 1996). The first category implied that transmission was impossible and the second category implied that transmission was unlikely.

For vegetation data, a continuous coverage available from the California Gap Analysis Project (http://www.biogeog.ucsb.edu/projects/gap/gap_home.html) was used. Western treehole mosquitoes occur to some extent in many woodland and forested areas but are most abundant for the longest periods in denser hardwood stands that retain humidity during summer (Bohart and Washino 1978, Bennett 1980, Woodward et al. 2003). Although these mosquitoes occur in redwood (*Sequoia sempervirens*) forests (Strickland 1969), it is unclear to what extent. Therefore, our designation of western treehole mosquito habitat was restricted to the following types as detailed by Mayer and Laudenslayer (1988) for the California Wildlife-Habitat Relationships database: Coastal Oak Woodland, Montane Hardwood-Conifer, Montane Hardwood and Valley Oak Woodland. Although western treehole mosquitoes breed in blue oak woodlands, the mosquitoes migrate from these sparse woodlands shortly after emergence and are not abundant there for most of the summer (Woodward et al. 2003, B. N. Sacks, unpubl.). Distance from suitable habitat was classified into the following categories: 0 (i.e., within habitat), 0–1 km, 1–2 km, 2–3 km, 3–4 km, >4 km.

For precipitation, a continuous data layer representing average annual precipitation based on a 60-year period and 800 precipitation stations (http://www.gis.ca.gov/dataview.epl) was used.

General approach

Binomial infection status (i.e. ±) of individual coyotes (the sample units) was used in logistic regression analyses to construct a predictive model and prevalence (i.e. proportion of coyotes infected) in 50×50 km grid cells was used for diagnostics (see below). Using individual coyotes in logistic regression analyses was more powerful for estimating coefficients than using prevalence values in grid cells in an ordinary least squares regression. However, because our ultimate interest was in predicting prevalence at geographic locations, not classifying individual coyotes as infected or uninfected, comparisons of observed-to-predicted prevalence in grid cells were used to assess goodness-of-fit and prediction accuracy.

Model development

Variable selection for the logistic regression model followed Hosmer and Lemeshow (2000) and entailed 5 steps: (1) assessing significance of the three candidate independent variables via univariable logistic regression, (2) selecting a main-effects model initially assuming linearity of all relationships, (3) checking for nonlinearity in the logit using design variables (Hosmer and Lemeshow 2000), (4) refining the main effects model using second-order terms if necessary, and (5) assessing interaction terms. Step 3 effectively allowed us to assess linearity of variables while controlling for other variables. Comparisons between models were performed using the likelihood ratio test, which discounts for increasing numbers of parameters. To assess significance of independent variables in univariate and main-effects logistic regressions, alpha was set equal to 0.001. The value, 0.001, has been used previously instead of alpha = 0.05 to compensate for inflation of type I errors that may result from lack of independence in spatial data (Thomson et al. 1996).

Model diagnostics

Once the model was constructed, (1) residuals were tested for spatial autocorrelation, (2) goodness-of-fit was assessed, (3) the model was cross-validated, and (4) the model was tested via external validation. Moran's I and Geary's C tests were used to assess spatial autocorrelation (Lee and Wong 2001). Spatial autocorrelation tests were performed based on randomization and were weighted as the inverse of distance (Lee and Wong 2001).

Goodness-of-fit of the model was assessed in three ways. First, we used the Hosmer-Lemeshow test, which assessed goodness-of-fit based on 10 approximately equal-sized bins of data, classed according to their predicted prevalence (i.e. probability of infection, Hosmer and Lemeshow 2000). In this test, data are pooled without regard to spatial location, which could be relatively insensitive to specific locations where the model fit poorly. Therefore goodness-of-fit was also assessed based on 50 × 50 km grid cells (Sacks 2002). Observed and predicted numbers of positive and negative coyotes were compared in each grid cell using Bonferroni-adjusted chi-square goodness-of-fit tests (Krebs 1999) or binomial tests when expected numbers of infected coyotes were < 1 (Zar 1984). Overall goodness-of-fit was assessed by summing individual chi-square values (unadjusted) and degrees-of-freedom over all grid cells with > 1 expected infection. Finally, observed prevalence was regressed on predicted prevalence to produce an R^2 value. The Hosmer-Lemeshow and grid-cell based goodness-of-fit tests were useful in qualitatively testing the fit of models (overall or in certain locations) and the R^2 statistic provided a quantitative measure of how well the model fit.

Next, a cross-validation analysis was performed and jackknife confidence intervals calculated for model coefficients, similar to the analyses by Kramer et al. (2001). For each grid cell with ≥ 10 coyotes sampled, data were removed, then the model was parameterized with the remaining data and used to predict the prevalence in the omitted grid cell. Observed prevalence in the grid cell was then compared to that predicted by the model using these coefficients. Prediction accuracy was assessed for individual grid cells and overall based on observed vs predicted prevalence as described above for goodness-of-fit tests. The confidence intervals of coefficients were computed based on Student's t distribution and the standard deviations of the jackknifed coefficients.

The goodness-of-fit and cross-validation analyses addressed the problem of model interpolation. To address the problem of model extrapolation, an external validation test was conducted. After developing the model, additional specimens were collected (August 2001–June 2002) and tested from areas that were poorly represented in the initial sample. These sites included several locations where – as with the initial sample – coyotes were routinely removed (e.g. for livestock depredation) such that possible biases associated with demographic aspects of highly exploited populations present in the original sample also could have been present in the external validation sample. Therefore, serological specimens were also obtained from live coyotes in two radiotelemetry study sites where coyotes were not routinely removed and where mortality was low (Riley et al. 2003, B. Mitchell, unpubl.) relative to removal sites (Sacks et al. 1999a). For each site, prevalence was compared with predictions of the model using Bonferroni-adjusted chi-square goodness-of-fit or binomial tests as described above.

Statistical details

For descriptive, goodness-of-fit, and cross-validation analyses, values of variables were calculated from the locations of data within grid cells rather than the continuous data in those grid cells. This was done to prevent biases due to heterogeneous sampling within grid cells. Distance to western treehole mosquito habitat and HDU were coded 1–6 and 1–10, respectively. All three explanatory variables were then standardized (i.e. so that average = 0, SD = 1) for multivariable analyses. Except for analyses involving development of the main-effects model (i.e. before adding second-order and interaction terms; Hosmer and Lemeshow 2000), for which alpha was set at 0.001, alpha was set at 0.05. Statistical analyses were performed in SYSTAT (version 9.0, SPSS Incorporated, Chicago, Illinois, USA).

Results

The locations of 1703 coyotes used to construct the model and heartworm prevalence in 50 × 50 km grid cells are presented in Fig. 1.

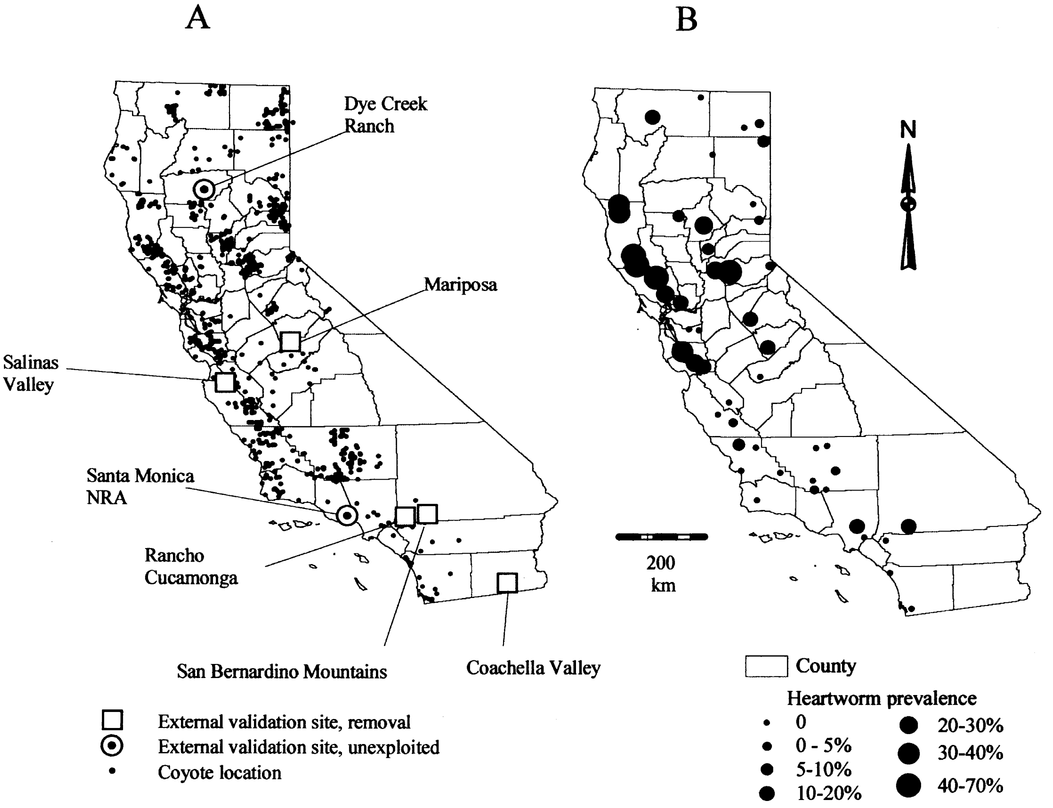

Fig. 1. Locations of 1703 coyotes used to construct the logistic regression model predicting heartworm prevalence and locations of external validation sites (n = 10–46 coyotes) (A) and prevalence as indicated by variable sized dots centered on the average coordinates of data contained within 50 × 50 km grid cells (B). Prevalence is only shown for grid cells with ≥ 10 coyotes, n = 1500 coyotes.

Model development

In univariable logistic regressions, all three variables (DIST, PRECIP, HDU) were highly significant at the 0.001 level (Table 1). Heartworm prevalence appeared nonlinearly related to HDU and precipitation, and linearly related to distance to *Oc. sierrensis* habitat (Fig. 2). However, multivariable analysis revealed that the shapes of some of these relationships reflected confounding by other variables. Analysis of design variables (Hosmer and Lemeshow 2000, Sacks 2002), which effectively controlled for confounding effects of other variables, led us to model HDU as a dichotomous variable separating the first quartile (HDU ≤ 1000) from the three upper quartiles and to model distance with a second-order term (Fig. 3). The second-order term for distance likely reflected our lumping of all distances > 4 km into a single "largest distance" class, which resulted in the influence of western tree hole mosquito habitat being on average disproportionately low in this category relative to the other 5 distance classes.

Table 1. Univariable logistic regressions for three independent variables as predictors of probability of heartworm infection.

Variable	Lower 95% confidence limit of coefficient	Upper 95% confidence limit of coefficient	Odds ratio	P
DIST[a]	0.32	0.42	0.37	≪ 0.001
PRECIP[b]	1.98	2.54	2.24	≪ 0.001
HDU[c]	1.13	1.49	1.30	≪ 0.001

[a] Distance from western treehole mosquito habitat.
[b] Average annual precipitation.
[c] Cumulative annual heartworm development units (average daily temperatures above 14°C minus 14°C).

Spatial autocorrelation

Using 613 coyotes from north-coastal, central Sierra Nevada, and south San Francisco Bay regions of California, where heartworm was locally prevalent (Fig. 1), residuals from the model were spatially autocorrelated by Moran's I ($z = 2.66$, $P < 0.01$), but not by Geary's C ($z = 1.62$, $P > 0.10$). When this region was decomposed into three sub-regions, residuals in one of them (south San Francisco Bay) were significantly autocorrelated by Moran's I ($z = 2.49$, $P = 0.01$), and by Geary's C ($z = 2.71$, $P < 0.01$), but neither of the other two sub-regions showed significant autocorrelation in residuals based on either Moran's I ($z = 0.65$, -0.79, $P > 0.10$, northwestern, central Sierra Nevada, respectively) or Geary's C ($z = 0.12$, 0.44, $P > 0.10$).

Goodness-of-fit

Goodness-of-fit of the model was supported by both the Hosmer-Lemeshow test ($C_8 = 7.04$, $P = 0.53$) and the grid cell-based chi-square test ($\chi^2_{49} = 59.7$, $P = 0.14$). The model accounted for most of the variance in the observed prevalence of heartworm among grid cells ($R^2 = 0.86$). Of the 49 grid cells, only one had an infection rate that differed significantly from the expected one based on the model. This grid cell was composed largely of coyotes trapped at the urban edge in the South San Francisco Bay area. The model predicted that 33 of 137 coyotes (24%) should have been heartworm-positive compared to 53 coyotes (39%) observed to be heartworm-positive ($\chi^2_1 = 15.7$, $P \ll 0.001$).

Cross-validation prediction error and jackknife confidence intervals

The chi-square test based on all 49 cross-validated grid cells indicated a significant difference between predicted and observed prevalence ($\chi^2_{49} = 79.6$, $P < 0.01$). However, this difference was due to a single significant grid cell, the south San Francisco Bay grid cell referred to above. The cross-validation model predicted that 27 of 137 coyotes (19%) in the deviant south San Francisco Bay grid cell should have been heartworm-positive ($\chi^2_1 = 32.3$, $P \ll 0.001$). None of the other 48 grid cells differed significantly from predictions. When the deviant south San Francisco Bay area grid cell was removed, the chi-square test based on the remaining 48 grid cells indicated no significant difference between predicted and observed prevalence ($\chi^2_{48} = 47.3$, $P = 0.50$). Even with the deviant grid cell included, cross-validation predictions were similarly close to observed values as the model based on all data ($R^2 = 0.84$).

Prediction error of the model was generally low (95% CI = -16–13%). Jackknife confidence intervals for model coefficients were narrow and did not include zero (Table 2), indicating that increasing numbers of grid cells alone would not qualitatively change the model.

External validation

A total of 172 coyotes was sampled for external validation of the model. Of these, 116 were from southern California, which was, in general, poorly sampled in the initial data-set. Observed prevalence did not differ significantly from predictions in 6 out of 7 sites (Table 3). In the Coachella Valley, 2 of 18 coyotes tested positive for heartworm, which was significantly greater than the predicted prevalence of $< 0.01\%$.

Discussion

Through our heartworm survey and the model based on the survey data, we were able to improve on existing knowledge of the distribution and abundance of heartworm in California, including an improved understanding of the habitat relationships most critical to the parasite. We produced a map of heartworm prevalence in coyotes throughout California, which serves also as a qualitative map of relative risk of exposure to mosquitoes with infective heartworm larvae applicable to domestic and wild animals and, to a lesser extent, humans. The accuracy of the map was supported by goodness-of-fit tests, cross-validation tests, and external validation tests. Below we discuss our key biological findings and uses and limitations of our model.

Distribution and abundance of heartworm in California

Before the present study, the geographic pattern of heartworm risk in northern and central California was investigated using domestic dogs and was ultimately described in terms of broadly defined plant-climate zones (Theis and Stevens 1998). Our survey results supported the primary findings of that model, that the highest prevalence, in general, was in the foothills. However, we also found places where the coarseness of the previous model was misleading. For example, some foothill regions, such as the inner South Coast Range foothills (San Benito, southeastern Monterey Counties), had relatively low prevalence of heartworm. By basing our model on a small number of variables known individually to be important to heartworm transmission, we were able to construct a more precise and accurate map of heartworm relative risk for coyotes in California.

The model also yielded insights about how causal variables acted relative to one another and in concert to determine heartworm risk at the statewide scale. Precipitation and proximity to western treehole mosquito habitat were most predictive of heartworm prevalence consistent with the hypotheses that the western treehole mosquito is the primary vector for heartworm in California (Weinmann and Garcia 1974) and that its abundance is positively related to precipitation (Sacks et al. 2003). Areas of western treehole mosquito habitat with higher rainfall correspond well with the "Sierran insect province" (i.e. a geographic area representing the

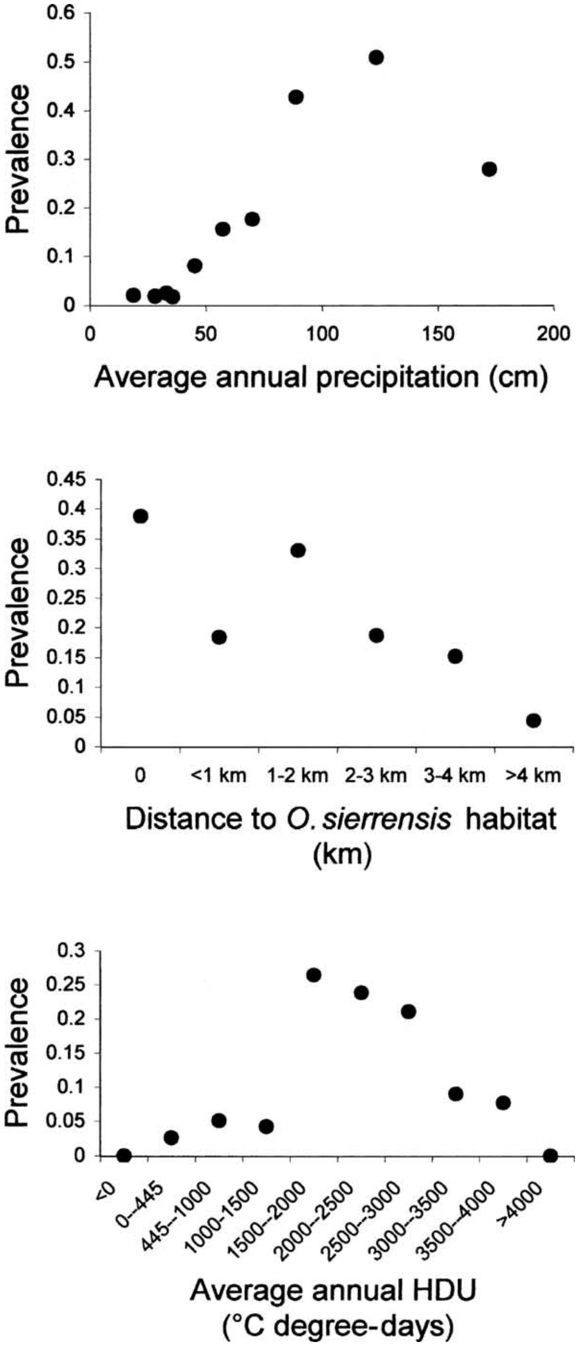

Fig. 2. Relationships between prevalence of heartworm in coyotes with precipitation, distance to vector habitat and temperature. Precipitation was graphed by plotting the prevalence in approximate deciles of precipitation on average precipitation within the decile.

range of many insects; Powell and Hogue 1979), suggesting the possibility that other potential vectors play a role. There has only been a small number of mosquito species implicated as important heartworm vectors in California. Of these, only one, *Anopheles punctipennis*, has a range similar to that of the western treehole mosquito (Bohart and Washino 1978). In at least one North Coast Range location, the western treehole mosquito was a far more important vector than *A. punctipennis* (Sacks et al. 2003).

Although temperature (i.e. HDU) appeared less important than precipitation and proximity to western treehole mosquito habitat as geographic determinants of heartworm prevalence, it clearly played a role in limiting the distribution to areas where temperature was sufficiently high for heartworm larvae to develop within the vector in time to be transmitted. The univariate relationship between temperature and prevalence appeared parabolic, but the reduced prevalence at higher temperatures was explained at least as much by the lower precipitation in the hotter areas (e.g. deserts). Previously, Sacks et al. (2003) determined that heartworm transmission in a northern California location was seasonally limited by temperature consistent with the HDU model. Findings in the present study suggest also that temperature limits heartworm transmission geographically in California.

Our finding of significant interaction between precipitation and HDU also made sense. While prevalence had a positive relationship with precipitation where temperatures were sufficiently high to allow transmission, prevalence was consistently low where temperatures were typically below the transmission threshold, regardless of precipitation. This finding is consistent with the role of temperature as a constraint on transmission.

We detected no relationship between HDU and heartworm prevalence when HDU was sufficient for transmission (Sacks 2002). Aspects of higher temperatures that inhibit transmission such as lower host-seeking activity and shorter life span of vectors (Lee 1971, Bennett 1980, Otto and Jachowski 1981) probably countered the more rapid larval development of heartworms associated with higher temperatures (Fortin and Slocombe 1981).

Uses and limitations of the model

The primary limitation of our model was in its resolution. In general, there is an unavoidable trade-off between how well a model predicts transmission risk on a fine scale over a small area and how well it predicts transmission risk over a large area (Kitron 2000). In this study, we were concerned with the coarse-grain distribution of heartworm in coyotes throughout the state of California. For this reason, as well as those mentioned above (i.e. robustness to non-random sampling), we chose variables likely to be generally important to coyote exposure. A consequence of this decision was that we

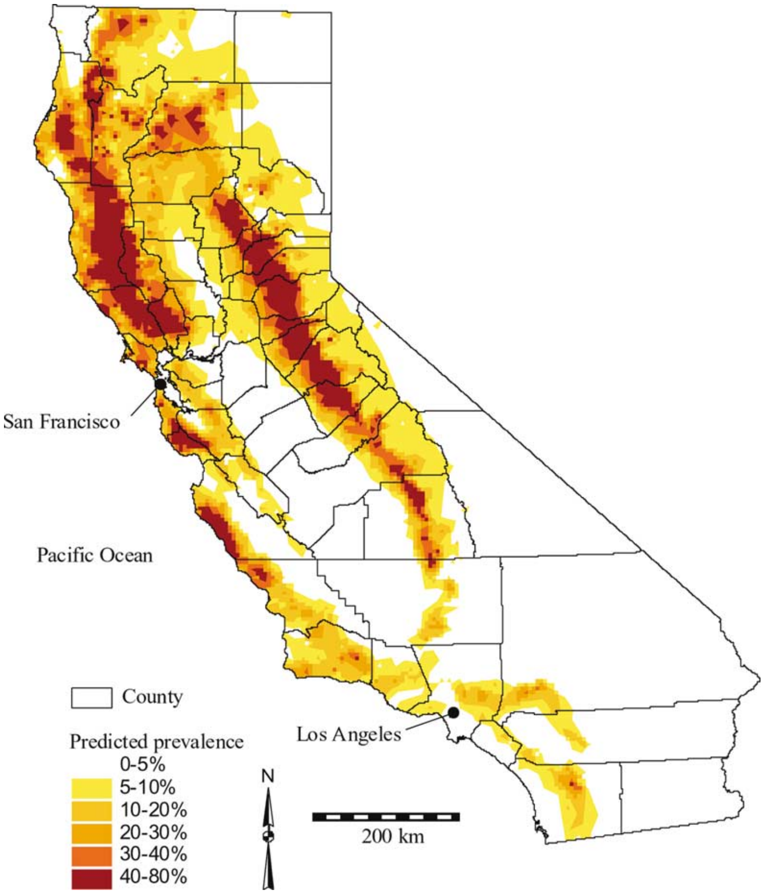

Fig. 3. Predicted heartworm prevalence in coyotes throughout California. Predicted Prevalence = $-2.42+1.03(\text{dichotHDU})+0.48(\text{PRECIP})-0.59(\text{DIST})-0.13(\text{PRECIP2})-0.36(\text{DIST2})+1.28(\text{dichotHDU}\times\text{PRECIP})-0.32(\text{dichotHDU}\times\text{PRECIP2})$. The image is based on 1-km pixels. Coefficients of terms involving PRECIP and DIST referred to standardized values (i.e. with $\bar{x}=0$ and SD = 1) based on the unstandardized $\bar{x}\pm\text{SD}=57.8\pm32.5$ cm and 4.33 ± 2.08 km, respectively.

ignored variables that might be important locally. For example, it is known that *Anopheles freeborni* is an important vector in some locations in the Sacramento Valley (Walters 1996), and are most abundant where rice fields and cattle are found in close proximity (Wood et al. 1991). It is also likely that foci exist due to riparian- and ditch-breeding mosquitoes such as *Aedes vexans* in the Sacramento Valley, Coachella Valley and along the Arizona border (Corselli and Platzer 1982, Walters 1996). Indeed, in our external validation survey, we observed seropositive coyotes in the Coachella Valley and Sacks (2002) found one seropositive coyote (total n = 5) along the Arizona border, both places where the model predicted prevalence < 0.01%. It is important to note, however, that the prevalence in these exceptional sites was still relatively low compared to the higher-prevalence sites associated with western treehole mosquito habitat.

Table 2. Jackknife confidence intervals for coefficients in final model.

	Low 95% confidence limit	High 95% confidence limit
CONSTANT	– 2.614	– 2.241
DichotHDU	0.931	1.132
PRECIP	0.397	0.567
DIST	– 0.713	– 0.461
PRECIP 2	– 0.191	– 0.060
DIST2	– 0.497	– 0.204
dichotHDU × PRECIP	1.168	1.391
dichotHDU × PRECIP 2	– 0.388	– 0.248

In addition, heartworm exposure risk in urban areas is probably influenced by densities of susceptible and/or microfilaremic dogs, which varies considerably (Walters 1996, Theis and Stevens 1998). Our model, which did not include effects of variable canid densities, therefore reflected exposure risk in rural California, where the coyote, with its relatively uniform density, represents the primary maintenance host. Interestingly, the one significant error of our model (39% observed vs 24% predicted) occurred in an urban area, where dogs could have increased the reservoir potential. However, another possible explanation for this discrepancy is systematic sampling bias. Coyotes here were removed only when

Table 3. Observed versus predicted heartworm prevalence in 7 external validation sites (shown in Fig. 1).

Site	Longitude	Latitude	n	Predicted prevalence (%)	Observed prevalence (%)	χ_1^2
Salinas Valley	– 121.46	36.57	10	5	0	–[b]
Dye Creek Ranch[c]	– 122.01	40.08	34	11	6	0.80
Mariposa	– 119.99	37.33	12	17	0	2.40
Coachella Valley	– 115.33	32.86	18	0	11	–[b]**
San Bernardino Mountains[a]	– 117.00	34.16	24	18	25	0.71
Rancho Cucamonga	– 117.49	34.12	22	7	9	0.11
Santa Monica Mountain NRA[c]	– 118.75	34.16	46	3	2	0.11

Note: unless otherwise noted, differences were nonsignificant ($P > 0.50$).
**Bonferroni-adjusted $P < 0.01$.
[a]includes Big Bear, Lake Arrowhead, Oak Glen, Redlands.
[b]binomial test used because expected number of cases < 1.
[c]site of radiotelemetry study.

they entered urban areas, although they undoubtedly spent most of their time in the adjacent habitat, which was good western treehole mosquito habitat. If so, the model would have consistently underestimated the probability of infection in these coyotes. This explanation is supported by the observation that model residuals in this area were significantly spatially autocorrelated.

The model provides only qualitative index of relative risk for exposure to heartworm in rural California. There are several reasons why the model cannot be used to quantify relative risk. First, prevalence is non-linearly related to transmission intensity; transmission intensity or probability of becoming infected in a given time period can increase indefinitely (at least in principle), whereas prevalence cannot increase beyond 100%. Second, even the estimates of prevalence cannot be taken literally. Because the prevalence estimates were based largely on serological tests with imperfect sensitivity and specificity, the estimates were slightly biased (Salman and Gardner 2000, Sacks et al. 2002). This is especially important in areas where the actual prevalence was near zero. We would be expected to record a seroprevalence of 4% due solely to false-positive cases (Sacks et al. 2002). Third, prevalence for coyotes is different than in other species in the same area. For example, dogs tend to have lower infection risk than coyotes in the same area due to differences in exposure and susceptibility (Theis and Stevens 1998). Further, some of the factors affecting dogs, such as use of prophylaxis, likely vary geographically.

Finally, although the model apparently performed well in most of California, it probably was less accurate in high mountainous areas. Our model predicted 5–10% prevalence in coyotes from these areas, which seems unlikely based on temperature and previous surveys in coyotes (Acevedo and Theis 1982). Temperature was incorporated into our model as a dichotomous variable, where the low-temperature group included areas where cumulative HDU was and was not conducive to transmission. The choice to pool HDU categories this way was based on statistical rather than biological considerations. Because the high mountainous areas with the lowest temperature were underrepresented in the low-temperature category (Sacks 2002), the prevalence was overestimated in these lowest-temperature areas.

Spatial autocorrelation and use of non-random data

Because our data were spatially non-random, we were initially concerned about type I errors due to autocorrelation in the dependent variable not explained by the model and about poor performance of the model in less-well sampled parts of the state, e.g. due to autocorrelation in independent variables. However, diagnostics indicated that both potential problems were minimized. For example, the lack of spatial autocorrelation in model residuals in most areas (i.e. excluding the south San Francisco Bay area) suggested that the model was sufficient to explain autocorrelation in the dependent variable. The general accuracy of the model was confirmed by validation analyses. In particular, the accuracy in the external validation sites, which included sampling of areas that differed in several ways (e.g. exploitation level of coyotes, presence of livestock, etc.) from those used to create the model, suggested that the model was even useful in parts of the state that were poorly sampled in this study. Indeed, in an additional diagnostic procedure, we found no relationship between prediction accuracy and the degree to which particular combinations of variables were represented in the data (Sacks 2002). Thus, by paying attention to biological realism (e.g. avoiding phenomenological trend-surface methods) and restricting variables to those known to be generally important (at least in isolation), we effectively applied a powerful method of statistical inference despite severe violations of the random sampling assumption.

General conclusions

Our model was most useful in identifying areas of medium to high heartworm risk in California. Among areas for which the model indicated low prevalence, heartworm prevalence was underestimated in some because of locally important factors not accounted for in the model, and overestimated in others because of imperfect specificity of the serological test. Nevertheless, the use of the ecological or habitat modeling approach to assess distribution of heartworm in California was generally successful. As is the case for some other arthropod-borne parasites (Ribeiro et al. 1996, Hay et al. 1998), it appeared that habitat modeling of the vector alone would have been nearly sufficient to index heartworm distribution and abundance. However, we also included temperature, which apparently related to heartworm development and possibly vector longevity more so than to vector abundance. Our findings suggests that similar modeling approaches could be used in other areas to better assess geographic patterns of heartworm distribution as well as to better understand the ecological factors favoring transmission.

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