Coarse-scale spatial and ecological analysis of tuberculosis in cattle: an investigation in Jalisco, Mexico

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Abstract. We have tested the hypothesis that coarse-scale environmental features are associated with spatial variation in bovine tuberculosis (BTB) prevalence, based on extensive sampling and testing of cattle in the state of Jalisco, Mexico. Ecological niche models were developed to summarize relationships between BTB occurrences and aspects of climate, topography and surface. Model predictions, however, reflected the distributions of dairy cattle *versus* beef cattle, and the non-random nature of sampling any cattle, but did not succeed in detecting environmental correlates at spatial resolutions of 1 km. Given that the tests employed seek any predictivity better than random expectations, making the finding of no environmental associations conservative, we conclude that BTB prevalence is independent of coarse-scale environmental features.

Keywords: ecological niche modeling, geographical information systems, tuberculosis, cattle, Mexico.

Introduction

Tuberculosis is highly prevalent in many developing countries. According to the World Health Organization, there are about 14 million human cases in the world, 80% of which are confined to developing countries (WHO, 2008). Although human tuberculosis is primarily caused by *Mycobacterium tuberculosis*, the etiological agent in cattle (*Mycobacterium bovis*) is also capable of causing disease in humans (Grange and Yates, 1994). Eradicating *M. bovis* from livestock is there-fore significant also for human health and welfare.

Bovine tuberculosis (BTB) is not randomly distrib-uted. Although the prevalence in beef cattle in Mexico is low (2.9%), it is considerably higher in dairy cattle (11.1%), largely owing to low participa-tion by dairy farmers in the national campaign for control and elimination of the disease (Díaz et al., 2004). The national BTB control campaign in Mexico focused on involving farmers by means of certification of farms as BTB-free after three consec-utive negative tuberculin test results. Despite these control measures, BTB transmission to cattle of ini-tially disease-free herds has frequently been reported (Feliciano Milián-Suazo, unpublished data). The sudden BTB outbreaks in certified BTB-free herds raise the question if *M. bovis* can survive undetected and that environmental factors are involved in the development of the infection into full-blown disease. This situation brings up the question of spatial rela-tionships between BTB occurrence and the ecological setting: a solid spatial and environmental under-

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standing of BTB distribution might facilitate the work of the national programme by identification of areas of higher or lower BTB transmission risk.

Ecological niche modeling (ENM) offers a new suite of tools and inferences for understanding broad spatial patterns in disease prevalence (Peterson, 2006a, 2007). These techniques, borrowed from the biodiversity community (Peterson, 2006b), relate known occurrences of species to raster geographical information system (GIS) maps summarising relevant features of ecological landscapes (e.g. climate, topography, soils and land cover). The result is a spatial, environmental model that summarises conditions under which the species is expected to be absent, *versus* potentially present (Araújo et al., 2005; Thuiller et al., 2005; Pearson et al., 2006). This type of model can be used to explore ecological characteristics of species or disease transmission phenomena (Peterson, 2006b), an application investigated in this contribution.

Although *M. bovis* is known to be an obligate intracellular pathogen, experimental results indicate that it can survive without this prerequisite, particularly under humid conditions (Williams and Hoy, 1930; Maddock, 1933; Genov, 1965). Indeed, recent reports confirm survival in farm environments outside the animal host, and that climatic conditions may influence survival rates (Duffield and Young, 1985; Young et al., 2005). Hence, the purpose of this study was to test the hypothesis that environmental characteristics might govern BTB transmission on spatial scales broader than microclimates and very local events: our central question is whether environmental correlates offer *any* significant explanatory power regarding BTB distribution in certain locals, in this case in Jalisco.

Materials and methods

Data

The state of Jalisco in central-western Mexico is divided into eight political districts that vary in BTB prevalence (Table 1; Fig. 1). A key point is that BTB prevalence rates are markedly higher in districts with larger concentrations of dairy cattle (Milián-Suazo et al., 2000). In all, 87% of the dairy cattle population is concentrated in the northeastern part of the state, while the northern, southeastern, and western coastal zones hold mostly beef cattle (Milián-Suazo and Huitrón, 2001). Herd information and data regarding tuberculosis prevalence (i.e. proportion of reactors within herds) were obtained from the official registries of the Jalisco BTB campaign. A total of 2,400,000 records were available in the dataset, which correspond to 48,776 herds tested between 1995 and 2005. From those, a random sample of 2,286 herds were selected for the study.

Table 1. Herds and animals positive to the tuberculin test in selected farms by district in the state of Jalisco, Mexico, 1995-2005.

| District	Number of herds in the sample	Positive herds	Proportion of herds positive	Animals tested	Animals positive	Positive animals per district (%)
Coastal						
Tomatlán	283	4	0.01	8,969	17	0.2
El Grullo	142	10	0.07	2,889	10	0.4
Interior						
Zapopan	209	92	0.44	8,096	571	0.7
Lagos de Moreno	758	311	0.41	37,684	2,041	5.4
Ameca	133	6	0.05	3,283	13	0.4
La Barca	221	42	0.19	6,870	216	3.1
Cd. Guzmán	398	5	0.12	11,890	104	0.9
Colotlán	143	18	0.13	3,872	18	0.5
Total	2,287	488		83,553	2,990	
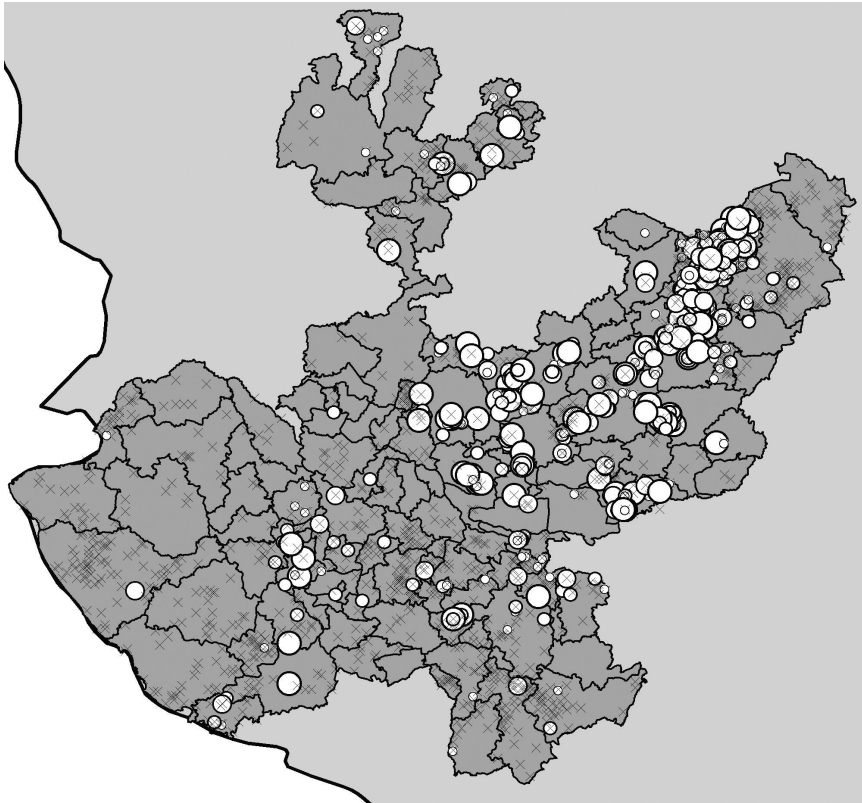
Fig. 1. Map of the Mexican state of Jalisco, showing municipal boundaries (thin outlines), as well as the limits of the north-eastern Jalisco region within which some analyses were focused (angular line and area to the north and east). Cattle herds sampled are shown by prevalence detected: X = zero prevalence, small circles = 6% prevalence, medium-sized circles = 6-12% prevalence, larger circles = 12-20% prevalence, largest circle >20% prevalence.

Sample size was estimated considering overall 6% herd prevalence, and the goal of a 95% confidence level and 1% error. Probability-based proportional sampling, with stratification according to herd population by political district, was used to set final sample sizes. From these herds, a workable (but still sufficiently large) sample of animals was selected and the fine-resolution of spatial locations of herds was derived from measurements using a Geko 301 hand-held global positioning system (GPS) unit (Garmin, Ltd., Olathe, Kansas, USA).

Our spatial inferences were based on modeling ecological niches of species, here defined as the set of conditions under which a species is able to maintain stable populations without immigration subsidy (Grinnell, 1917, 1924). Niche modeling tech-

niques relate known occurrences (as geographic coordinates) to environmental variables (in the form of digital raster maps of relevant ecological parameters) to outline of the overall ecological dimensions of the ecological niche of the species. ENM algorithms search for non-random associations between occurrences of the species and raster geographical information system (GIS) maps of relevant environmental dimensions, which can be used to identify areas that fit the ecological requirements of the species (Soberón and Peterson, 2005).

In the present analysis, we based initial models on points at which any cow had been found to be tuberculosis-positive (i.e., prevalence >0%). Later analyses focused only in dairy cattle areas in northeastern Jalisco and only on high-prevalence (i.e. >20%) sites

as compared to zero-prevalence sites. The latter were restricted to zero-prevalence sites that were >1.41 km (= the corner-to-corner diagonal distance across a 1-km grid square) from any presence site to assure that no tuberculosis-positive cow had been detected anywhere within the same grid square.

We used 22 raster GIS datasets to summarize environmental variation across Jalisco: four aspects of topography (elevation, slope, aspect and a compound topographic index), all derived from the Hydro-1K digital elevation dataset (USGS, 2001); seven climate features (annual mean temperature, mean diurnal temperature range, maximum temperature of warmest month, minimum temperature of coldest month annual precipitation, precipitation of the wettest month, and precipitation of the driest month) all from the WorldClim dataset (Hijmans et al., 2005); and remotely-sensed normalized difference vegetation index (NDVI) data, representing monthly composites for February-December 1995 from the Advanced Very High Resolution Radiometer (AVHRR) sensor (UMD, 2001). The NDVI data were chosen to coincide with the initiation of prevalence data gathering, but they more than anything represent the typical trajectory of "greenness" through the course of a year. The spatial resolution of all environmental data sets was 1 km, so no re-sampling to different resolutions was necessary.

Ecological niche modeling (ENM)

Numerous approaches have been applied to the challenge to develop niche models using presence-only occurrence data (Elith et al., 2006). Among the many choices, two stand out, both based on evolutionary computing principles: the genetic algorithm for rule-set prediction (GARP) (Stockwell and Noble, 1992; Stockwell and Peters, 1999) and Maxent (Phillips et al., 2004, 2006), which uses maximum entropy probability fitting. GARP has seen the broadest application in such applications, whereas recent comparative evaluations of ENM algorithms have generally evaluated Maxent favourably (Elith et al., 2006). We used both

approaches throughout this study. However, as the results of the two approaches were qualitatively identical, we report only the GARP results.

GARP is an evolutionary-computing approach that uses inferential methods (logistic regression, range rules, negated range rules, atomic rules) to generate rules regarding distributions of species in ecological space, which are then "evolved" in an iterative process of rule selection, evaluation, testing and incorporation (or rejection). Testing is based on random subsets re-sampled from available occurrence data, separated from the outset into extrinsic training data (50% of points), and intrinsic training and testing data (25% each). The latter two suites of points are related to equal numbers of points randomly re-sampled from the set of points where the species has not been recorded ("pseudo-absences"). To avoid complications caused by different levels of prevalence, intrinsic training and test data and corresponding pseudo-absence data are re-sampled randomly with replacement to create equal-sized data sets with equal representation of presences and absences (1,250 points each).

Given the inherently stochastic nature of GARP, the algorithm produces alternative model solutions for the same input data, so we followed the filtering and consensus methods of Anderson et al. (2003) to choose optimal models from among alternatives. For each model, 100 replicate models were generated; the 20 models with the lowest omission error (measured as failure to predict points in the extrinsic testing dataset) were retained, and then the 10 models with the proportional area predicted closest to the median were selected. This "best subset" of multiple replicate models was summed for each grid square to obtain a map summarising the final predictions for the potential distribution of BTB.

Validating model predictions

Throughout this analysis we developed model validations schemes based on spatially independent subsets of available occurrence data. In general, both for Jalisco as a whole, as well as when limited to north-

east Jalisco, circles with 100 km diameters (the size arbitrarily chosen to optimize the presence of both high and low prevalence) were located in strategic regions, i.e. three areas selected by eye to cross zones with a prevalence >20% and zones with zero-prevalence herds to assure maximal diversity in the response variable. Occurrences from within the circles were omitted from model training, i.e. our predictions were based on all points *outside* of the circle in question, and then projected into each circle. Independent occurrences from within the circle were then overlaid onto the predictions and coincidence of predictions and independent test data tallied.

The hypothesis that coincidence between independent test points and predictions by the model was greater than that expected at random was assessed as follows. We assumed that random predictive success would follow the proportion of the total area of the test circle covered by the model prediction. We used a cumulative binomial probability distribution to calculate the probability of achieving the observed level of coincidence by chance, given a probability of success equal to the proportional area predicted present. It should be noted that this test is seeking any detectable predictive ability, and may not offer sufficiently elevated confidence to constitute a useful prediction (M. Turrelli, personal communication). However, given the negative result obtained, it becomes a very sensitive test for ecological correlates.

Finally, to confirm lack of predictivity, we compared environmental conditions of "cattle presence" *versus* "cattle absence" and "infected" *versus* "non-infected" cattle using Wilcoxon tests. In the former comparisons, we contrasted cattle sample points (without regard to BTB status) with values of environmental parameters at 1,000 randomly placed points across northeastern Jalisco. In the latter comparisons, we contrasted environmental parameters associated with BTB-positive and BTB-negative herds.

Results

Tests of the ENM ability to anticipate distributions of independent test occurrence points repre-

senting detections of tuberculosis-positive cattle across all of Jalisco were developed based on three 100 km circles (Fig. 2). In all three cases, predictions within the test circles were statistically significantly more coincident with independent test data than random expectations (all P <0.001; Table 2). Nonetheless, in spite of this apparent indication of "predictivity" of BTB distributions, we noted that areas of high prediction coincided with areas in which dairy cattle are most frequent, suggesting that our models might simply be reconstructing distributions of dairy cattle, as opposed to anything specific to areas of high BTB prevalence. As a result, given that the northeastern portion of the state represented a clear concentration of BTB-positive cattle, and given the contrasting regional distributions of dairy and beef cattle, we focused further tests in northeastern Jalisco to see if niche models could discriminate BTB-present sites from BTB-absent sites within dairy cattle areas.

Within northeastern Jalisco, again, we used three 100 km circles to test model predictivity into regions from which no input data participated in model development (Fig. 2). Once again, the modelled predictions were statistically significantly better than random expectations (all P <0.001; Table 2). However, the BTB presence-based model predicted points of zero BTB prevalence just as well, or better than, as it did BTB presences (also P <0.001). Predictions based only on high-prevalence sites were not statistically significantly predictive of distributions of BTB-positive cattle. In this sense, our results suggest that the BTB presence-based models are only predicting the non-random nature of dairy cattle distributions or cattle sampling for BTB within northeastern Jalisco, rather than detecting unique environmental features of BTB-infected dairy cattle distributions.

Finally, outside of the niche modeling framework, we compared the simple environmental characteristics of points of known high BTB prevalence *versus* those of known zero prevalence (Figs. 3 and 4), and of "cattle presence" *versus* "cattle absence" based on 1,000 randomly placed points (representing absence) using Wilcoxon tests. In the latter compar-
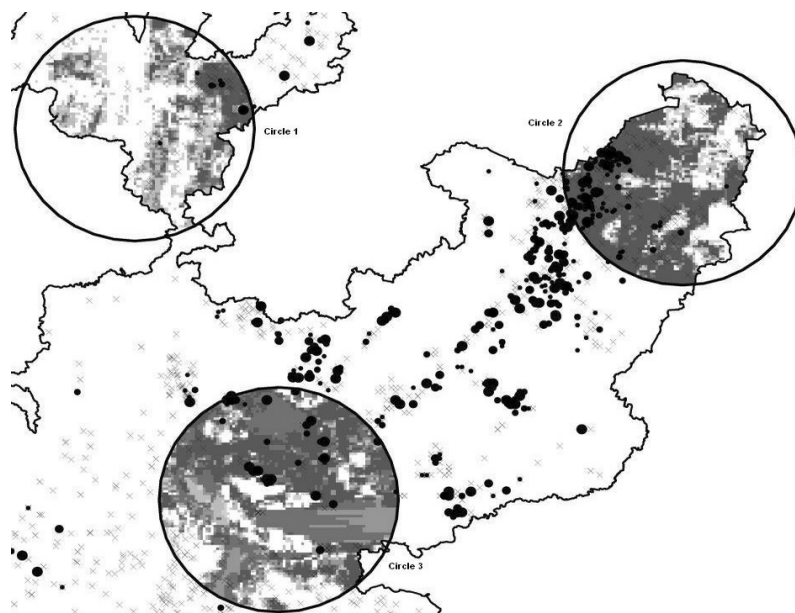
Fig. 2. Details of three areas in Jalisco, Mexico, from which spatial subsets of available occurrence data were excluded (sequentially) from model construction to provide an independent test of model prediction. Each circle is 100 km in diameter; shown inside each are the model predictions developed based on points outside the circle, with the independent test points from within that circle overlaid (results summarized in Table 2). Circle sizes reflect prevalence, as in Figure 1. Shading inside circles indicates best-subsets GARP model agreement in predicting presence: white areas = 5 of 5 models predict absence, darkest gray = 5 of 5 models predict potential presence.

ison (cattle presence *versus* cattle absence), we observed significant differences (P <0.05) in 14 out of 22 environmental variables. In the former case, comparing infected *versus* non-infected cattle herds, only four out of 22 variables (maximum temperature of warmest month, annual precipitation, precipitation of wettest month, precipitation of driest month) showed significant (P <0.05) dif-

ferences. Hence, these results suggest that environmental conditions are more closely related to cattle presence than to high BTB prevalence. Distances to the nearest road showed statistically significant differences in both the comparison of "cattle presence" *versus* "cattle absence" (P <0.001) and in the comparison of infected *versus* non-infected herds (P = 0.019).

Table 2. Summary of tests carried out regarding the ability of ENMs to anticipate distributions of independent test occurrence points representing distributions of tuberculosis-positive cattle in Jalisco and in a restricted area of northeastern Jalisco, based on the regions shown in Figure 3. "--" indicates probabilities above the threshold of α = 0.05.

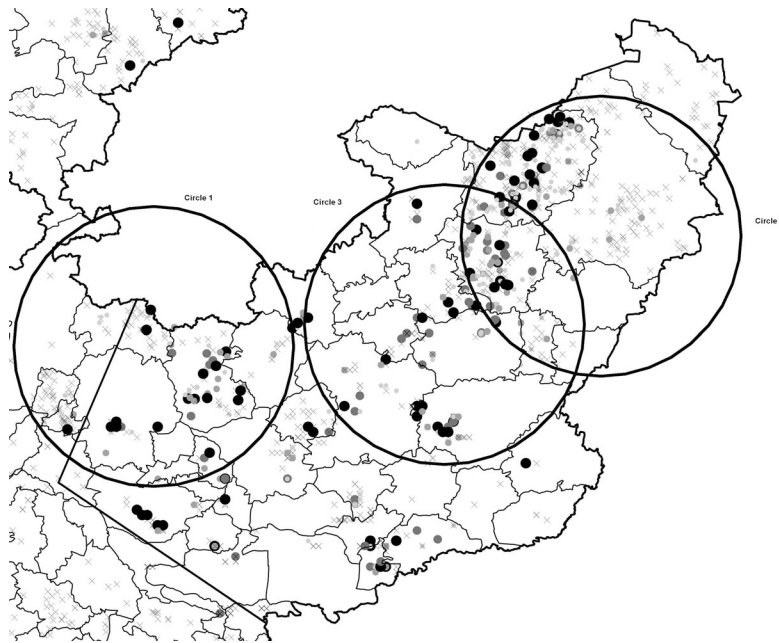| Test	Presences correctly predicted	Total number of presences available	Proportion of area predicted presence	P
All Jalisco				
Circle 1	4	7	0.114	<0.001
Circle 2	108	108	0.565	<0.001
Circle 3	25	47	0.298	<0.001
Northeastern Jalisco				
Circle 1, prevalence >0%	9	54	0.163	--
Circle 2, prevalence >0%	108	228	0.497	--
Circle 3, prevalence >0%	160	191	0.729	<0.001
Circle 3, prevalence >20%	26	31	0.768	--

Fig. 3. Summary and illustration of tests carried out in northeastern Jalisco, Mexico, showing the three test circles (100 km diameter) and the distribution of sampling points and their respective prevalence (circle sizes reflect prevalence, as in Fig. 1).

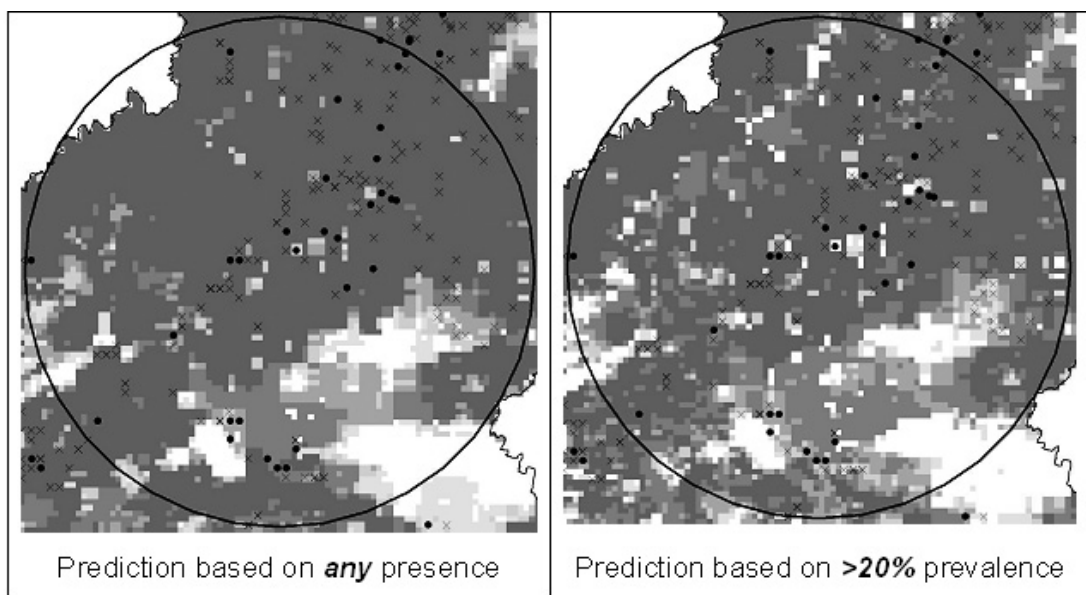Prediction based on **any** presence   Prediction based on **>20%** prevalence

Fig. 4. Example results (circle 3, Fig. 3) from tests carried out regarding prediction of BTB cases, as contrasted with absences (zero prevalence) in northeastern Jalisco, Mexico. Each circle is 100 km in diameter, and shown inside each are the model predictions developed based on points *outside* the circle, with the independent test points from within that circle overlaid (X = zero prevalence, • >20% prevalence). Shading inside circles indicates best-subsets GARP model agreement in predicting presence: white = 5 of 5 models predict absence, darkest gray = 5 of 5 models predict potential presence.

Discussion

Influences of external environmental conditions on the survival of *M. bovis* have been reported earlier. For example, *M. bovis* exposed in open pasture remained viable and virulent for at least seven weeks in the English summer (Maddock, 1933). *M. bovis* mixed artificially with cow faeces and exposed on pasture land survived at least 35 weeks in the winter, 28 weeks in autumn, and up to 14 weeks in the summer, in southern England; survival time increased when protected from direct sunlight (Williams and Hoy, 1930). In northern Europe, *M. bovis* mixed with organic matter survived 22-47 weeks when exposed to sunlight at 24-34°C, but up to 104 weeks if buried 5 cm below the surface of shaded soil and 52 weeks if at a depth of 1 cm (Genov, 1965). Vaccine strain *M. bovis* (Bacillus Calmette-Guérin; BCG) remains viable in soil for >15 months and significant levels of *M. bovis* DNA and RNA persist in the field, suggesting presence of viable cells as an environmental reservoir for infection, a potential risk to cattle (Young et al., 2005).

The reports cited, however, focus on the micro-environments presented within particular farms rather than on broader-scale ecological settings. At some spatial extent, such broader-scale influences probably do indeed exist – we doubt that *M. bovis* would be transmitted particularly effectively in more extreme environments such as the Arctic or the driest deserts. Still, our results suggest quite clearly that, at the scale of Jalisco, or the northeastern portion of Jalisco, coarse-scale environmental influences are not discernable for BTB prevalence. Rather, major factors affecting BTB prevalence appear to be more related to the spatial distribution of dairy cattle herds, and perhaps to particular conditions of cattle management.

Prevalence of BTB is much higher in dairy than in beef cattle, owing to at least two reasons: management practices and food supply. In the study area, dairy cattle maintained in very intensive production conditions, with large numbers of animals kept per unit area, which increases the chances of disease transmission by close contact between animals. Also, dairy farms are located close to urban areas, mostly with temperate weather, where food supply is available. Such weather conditions can increase the chances of survival for *Mycobacterium*. Beef cattle, on the contrary, are raised in dry coastal areas, with few cattle per unit area, and lots of sunshine, conditions that reduce disease transmission and survival of *Mycobacterium*. We were unable to relate BTB prevalence with macro-environmental conditions in the present study; however, we did not analyze micro-environments, where factors such as humidity, temperature, or season influence prevalence of the disease.

Our analysis of coarse resolution BTB prevalence correlates is certainly complicated by micro-scale factors. That is, particular management practices in certain herds may affect prevalence dramatically, creating broad background variation from which the coarse-resolution spatial environmental variation that we sought would have to be discerned. We failed to detect coarse-scale environmental correlates against the background of other possible factors acting on different spatial and temporal scales. However, we report our results as a benchmark for other such studies that may use more refined or more sensitive techniques.

More generally, in previous disease applications of ENM techniques (Peterson, 2006a), the focus has been on detecting environmental correlates of geographic distributions of species and successfully predicting distributions of disease transmission phenomena. In this case, however, we applied several quite-sensitive approaches to detecting such niche dimensions and making such predictions, but failed to demonstrate significant associations. Moreover, this suite of tests addresses the hypothesis of any nonrandom similarity of the two niches (in this case, infected *versus* non-infected cattle herds), however subtle (M. Turelli, personal communication). As such, our negative result represents a conservative test, and coarse-scale environmental correlates of BTB transmission appear to be lacking.

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