Spatially heterogeneous land cover/land use and climatic risk factors of tick-borne feline cytauxzoonosis

Ram K. Raghavan, Kelli Almes, Douglas G. Goodin, John A. Harrington, Paul W. Stackhouse Jr.

Published Version Information

Citation: Raghavan, R. K., Almes, K., Goodin, D. G., Harrington, J. A., & Stackhouse, P. W., Jr. (2014). Spatially heterogeneous land cover/land use and climatic risk factors of tick-borne feline cytauxzoonosis. Vector-Borne and Zoonotic Diseases, 14(7), 486-495.

Digital Object Identifier (DOI): doi:10.1089/vbz.2013.1496

Publisher's Link: http://online.liebertpub.com/doi/abs/10.1089/vbz.2013.1496

Spatially Heterogeneous Land Cover/Land Use and Climatic Risk Factors of Tick-Borne Feline Cytauxzoonosis.

Ram K. Raghavan[1*], Kelli Almes[1], Douglas G. Goodin[2] John A. Harrington[2] Paul W. Stackhouse Jr.[3]

[1] Kansas State Veterinary Diagnostic Laboratory/Department of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, Kansas, USA.
[2] Department of Geography, Kansas State University, Manhattan, Kansas, USA
[3] NASA Langley Research Center, Hampton, Virginia USA.

Abstract

Background: Feline cytauxzoonosis is a highly fatal tick-borne disease caused by a hemoparasitic protozoan, *Cytauxzoon felis*. This disease is a leading cause of mortality for cats in the Midwestern United States, and no vaccine or effective treatment options exist. Prevention based on knowledge of risk factors is therefore vital. Associations of different environmental factors, including recent climate were evaluated as potential risk factors for cytauxzoonosis using Geographic Information Systems (GIS).

Methods: There were 69 cases determined to be positive for cytauxzoonosis based upon positive identification of *C. felis* within blood film examinations, tissue impression smears, or histopathologic examination of tissues. Negative controls totaling 123 were selected from feline cases that had a history of fever, malaise, icterus, and anorexia but lack of *C. felis* within blood films, impression smears, or histopathologic examination of tissues. Additional criteria to rule out *C. felis* among controls were the presence of regenerative anemia, cytologic examination of blood marrow or lymph node aspirate, other causative agent diagnosed, or survival of 25 days or greater after testing. Potential environmental determinants were derived from publicly available sources, viz., US Department of Agriculture (soil attributes), US Geological Survey (land-cover/landscape, landscape metrics), and NASA (climate). Candidate variables were screened using univariate logistic models with a liberal p-value (0.2), and associations with cytauxzoonosis were modeled using a global multivariate logistic model ($p < 0.05$). Spatial heterogeneity among significant variables in the study region was modeled using a geographically weighted regression (GWR) approach.

Results: Total Edge Contrast Index (TECI), grassland-coverage, humidity conditions recorded during the 9th week prior to case arrival, and an interaction variable, diurnal temperature range percent mixed forest area were significant risk factors for cytauxzoonosis in the study region. TECI and grassland areas exhibited significant regional differences in their effects on cytauxzoonosis outcome, whereas others were uniform.

Conclusions: Land-cover areas favorable for tick habitats and climatic conditions that favor the tick life cycle are strong risk factors for feline cytauxzoonosis. Spatial heterogeneity and interaction effects between landcover and climatic variables may reveal new information when evaluating risk factors for vector-borne diseases.

Keywords: Cytauxzoonosis – Feline – Geographical Information Systems (GIS) – Geographically Weighted Regression (GWR) – Multivariate logistic – Climate – Humidity – Diurnal Temperature Range (DTR) – NASA.

Introduction

Cytauxzoonosis is a commonly diagnosed tick-borne disease among domestic cats in the Midwestern United States and a leading reason for feline mortality. This disease is caused by a hemoparasitic protozoan *Cytauxzoon felis*, which has been isolated from several members of the felid family. Bobcats are the reservoir hosts and could remain nonsymptomatic carriers after recovering from acute illness. Ticks that feed blood from bobcats or other wild felids could later transmit the disease to domestic cats.

Amblyomma americanum (lone star tick) is a known tick vector for cytauxzoonosis (Reichard et al. 2009), and *Dermacentor variabilis* (American dog tick) has been shown capable of transmitting the protozoa under experimental conditions (Blouin et al.1984). Cytauxzoonosis has a rapid disease course with high morbidity and high mortality, and most infections result in a disease state. Clinical symptoms can be noticed within 23 weeks. Currently, there is no vaccine available for this disease, and treatment options are very limited, which usually leads to fatal results in most infections. Prevention mainly relies on understanding and avoiding different risk factors, many of which could be found in a cats living environment.

Cytauxzoonosis has been reported primarily from the south-central and southeastern parts of the United States and it is particularly a concern in the quad-state region covering Kansas, Missouri, Oklahoma, and Arkansas where relatively high numbers of infections are diagnosed each year. Using ecological niche models, Mueller et al. (2013) reported that the potential distribution of *C. felis* is likely to expand in the region; however, this study did not include areas in Kansas. Reichard et al. (2009) identified several environmental risk factors for cytauxzoonosis in a study that enrolled infected cats from Oklahoma. The risk factors identified in that study primarily included having residences in areas that are suitable for ticks, for instance, wooded areas and living in proximity to natural, unmanaged landscapes. Environmental risk factors for vector-borne diseases are subject to changes with geographic areas due to the natural differences in the landscape and climatic conditions.

Occurrences of tick-borne and other diseases among domestic companion animals are very often correlated with certain land-cover areas (Reichard et al. 2009, Raghavan et al. 2011), as well as prior climatic and pet owner socioeconomic conditions (Colwell et al. 2011; Raghavan et al. 2013a). However, the kinds of landscape features and climatic parameters associated with different diseases could vary based on their tick vectors. Other influential factors that have often shown to be associated with tick-borne and wildlife diseases are the different landscape metrics, such as habitat fragmentation and patch density surrounding a pet owners residence (Uuema et al. 2009, Halos et al. 2010).

Climatic conditions play an important role in a ticks life cycle, which indirectly affects the prevalence and spatial distribution of the diseases they help transmit. While the individual effects (or main effects) of different environmental factors have been documented, knowledge of climate land-cover interactive effects on disease occurrences is generally lacking. In addition, influential factors affecting different disease occurrences over large spatial extents have been shown to change, with some risk factors being more important in some areas than others, a phenomenon referred to as spatial heterogeneity. Accounting for interaction effects among influential factors and spatial heterogeneity therefore are important when evaluating environmental risk factors for diseases. Increasing availability of high-resolution, remotely sensed land-cover datasets and climatic data coupled with spatial analytical methods facilitated by Geographic Information Systems (GIS) allows us to closely examine such relationships between disease status and environmental factors.

The objective of this study was to retrospectively verify the individual and interactive associations of different environmental and climatic factors with cytauxzoonosis cases received at Kansas State Veterinary Diagnostic Laboratory (KSVDL) between the years 2005–2012. Candidate environmental and climatic variables were derived from publicly available, high resolution US Geological Survey (USGS) and National Aeronautics and Space Administration (NASA) sources.

Materials and Methods

Case selection

The laboratory information management system of KSVDL was searched for any samples that were submitted as suspect for cytauxzoonosis or had a confirmed diagnosis from 2005 to 2012. A sample included whole blood samples or smears formicroscopic parasite screening or cats submitted for necropsy. A case was defined by positive detection of *C. felis* on a microscopic blood film examination, presence of schizonts within macrophages on impression smears from fresh tissue (lung, spleen, or lymph node) obtained at necropsy, or presence of schizonts within multiple organs on histopathology. Cats with a history of fever, malaise, icterus, and anorexia but no *C. felis* on blood film examination or schizonts within macrophages from fresh tissue or within multiple organs were considered as controls. Animals with only a blood film examination were included as controls only if they had additional findings to rule out *C. felis*, which included presence of regenerative anemia, cytologic examination of blood marrow or lymph node aspirate, other causative agent diagnosed, or survival of 25 days or greater post testing.

Host factors and time of case arrival

Cats were grouped based on their age (< 1 year, $1 - 3$years, $3 - 5$ years, > 5 years), sex (male, female, unknown), and home environment (indoor, outdoor, unknown) categories. Cases/ controls received at KSVDL were grouped based on the season they arrived at the diagnostic facility into four categories; fall (September to November), winter (December to February), spring (March to May), and summer (June to August).

Geocoding

Client-provided street level addresses at the time of case submissions were retrospectively verified for their accuracy using Google Maps (Google Inc., Mountain View, CA), and geographic coordinates were derived using a geocoding tool in ArcMap 10.1 software. The geographic coordinates for unmatched addresses were obtained using Google Earth software (v. 6.2.2.6613) (Google Inc., Mountain View, CA). In all, there were 69 cases (out of 77) and 123 controls (out of 164) forwhich precise point locations of households could be obtained. All geospatial datasets used in this study were projected (or reprojected from the original coordinate systems) in to the USA Contiguous Equal Area Conic Projection to preserve area measurements in the data. This coordinate system is based on the GeographicCoordinate System NorthAmerican 1983 Geographic Datum. All original, intermediate, and processed geospatial data were stored in a SQL Server/ArcSDE 10 Geodatabase.

Landscape metrics

The publicly available 2006 National Land Cover Dataset (NLCD) (Homer et al. 2007, Multi-Resolution Land Characteristics Consortium 2013) for the study region was obtained from the USGS in a raster grid format. Land-cover grids surrounding individual casecontrol locations were extracted from the raster dataset using 2500-meter polygon buffers, and converted to polygon area features in ArcMap. The choice of the 2500-meter distance was made based on our assumption that the most influential environmental factors for cytauxzoonosis operated within this distance considering the host and vector home ranges. The risk of Modifiable Areal Unit Problem (MAUP) when making such choices is discussed in Raghavan et al. (2013b). The area of different land-cover types within an individual buffer was divided by the total area to generate percent land-cover values. Different land-cover classes present in NLCD are shown in Table 1, and descriptions of different land-cover classes can be found from their source website (Multi-Resolution Land Characteristics Consortium 2013). In addition to deriving percent land-cover areas, the following landscape metrics were derived from the NLCD dataset surrounding casecontrol locations. Total Edge Contrast Index (TECI), calculated by

$$TECI = \left[\sum_{i=1}^{m} \sum_{k=i+1}^{m} e_{ik} d_{ik} \right]^{-E^*} \quad (1)$$

where $e_i k$ is the total length of edge between patch types i and k, E^* is the total length of edge in landscape, dik is the dissimilarity (edge contrast weight) between patches i and k. Patch richness (the number of patch types present in a landscape) and the largest patch index (LPI) were calculated by

$$LPI = \left[\sum_{j=1}^{n} a_{ij}\right]^{-A} \tag{2}$$

where a_{ij} is the area of patch ij and A is the total landscape area, were estimated using Fragstats 4.0 (McGarigal et al. 2012). TECI captures the percentage of all edge-lengths between land-cover types in NLCD, which essentially represents the adjacency between forested areas, mixed forest, grassland, built-up areas, and other land-cover types in this study. The choice of these pattern metrics was made based on our interest in identifying case associations with habitat fragmentation or any predilection for the presence of a particular patch in the surrounding landscape where cats had lived.

Climate

The Prediction of Worldwide Renewable Energy (POWER) web portal at the NASA Langley Research Center (Eckman and Stackhouse 2012) makes data available that includes daily estimates for various biologically relevant climate parameters (daily maximum, minimum and average daily temperatures, dew point, relative humidity, and precipitation) from the year 1983 to present day. NASA satellite and meteorological data products redistributed through POWER web tools are validated with surface-based solar and meteorological measurements to quantify uncertainties (White et al. 2008, 2011). POWER data were converted to raster layers covering the study region in ArcGIS, and the weekly mean estimates of maximum, minimum and average temperatures (°C), weekly mean diurnal temperature range (DTR) (difference between daily maximum and minimum temperature averaged over a 7-day period), precipitation (mm), and relative humidity (%) were derived from independent raster layers representing these climate parameters for up to 4 months prior to the dates on which cases were received at KSVDL. A representative value for each climatic parameter was derived by averaging weather parameter estimates to case control locations.

Statistical analyses

Strengths of variable associations with cytauxzoonosis status in cats and geographical variability in risk factor influences were evaluated in three steps. First, the relevance of candidate variables to be used in modeling procedures was verified using univariate logistic regressions, and those with p 0.2 were selected for further analysis. Care was taken not to remove candidate variables that were deemed clinically relevant (Hosmer and

Lemeshow 1990). Multicollinearity among screened variables was tested by estimating the variance inflation factor (VIF) using the PROC REG/TOL VIF option in SAS (SAS Cary, NC) in which all variables with a VIF ≥ 10 were considered to indicate multicollinearity (Allison 1999). Observations for all land-cover, soil, and climate variables were kept in their original measurement units and were continuous. In addition to testing individual variable effects, significance of various second-level interaction effects on the response was also verified. In the second step, screened variables were selected as parameters for a global multivariate logistic model in a stepwise (both directions) procedure which takes the form,

$$ln\left[\frac{\hat{p}}{1-\hat{p}}\right] = \hat{\beta}_0 + \sum_k \hat{\beta}_k x_k + \epsilon_i \quad (3)$$

where $\hat{p}$ is the predicted value of response variable p, $\hat{\beta}_0$ the intercept coefficient, and $\hat{\beta}_k$ the coefficient for the explanatory variable x_k $(k = 1, .., n)$ and ei random error. Multivariate stepwise logistic regression models (global) were fitted using the significance level p = 0.05 for variable entry and ≥ 0.10 for a variable to be removed from the model. All models were ranked usingAkaike information criterion (AIC) value, and the model with lowest AIC value was deemed to be the best fitting model, which takes the form,

$$AIC_c = 2nlog_e(\hat{\sigma}) + nlog_e(2\pi) + n\{\frac{n + tr(S)}{n - 2 - tr(S)}\} \quad (4)$$

where $tr(S)$ is the trace of the hat matrix. The model performance was measured using deviance chi-squared goodness- of-fit test ($p \leq 0.05$ indicates poor fit). The predictive ability of the model was evaluated using the area under receiver operating characteristic (AUC) curve values. Odds ratios (OR) and 95% confidence intervals (CI) in the final model were used for interpreting risk factor associations with cytauxzoonosis status in cats. Potential confounding effects of host factors, age group of cats ($<$ 1 year old as reference category), sex (female as reference category), and home environment (outdoor as reference category) on predictor variables were estimated by including them one at a time in the final logistic model. If such inclusion changed the coefficients of explanatory variables by at least 10% or more, then the adjusted ORs were recorded from those models.

Variables retained in the final logistic model (global) were entered in a geographically weighted regression (GWR) model, which is a spatially explicit regression modeling approach for examining spatial nonstationarity of responses (disease outcomes in this study) by allowing model coefficients to vary continuously over space to represent local relationships. Because case status in this study was recorded in a binary format (0 = negative diagnosis for cytauxzoonosis and 1 = positive diagnosis), a logistic form of GWR was used, which incorporates a set of geographical locations to the models, taking the form

$$log[\frac{p_i}{1 - p_i}] = c + f_x(a_i) + \epsilon_i \quad (5)$$

where c is a constant, p_i is the probability (expectation) of a positive diagnosis, i, a_i is the determinant variable surrounding case–control location, f_x is a function enabling the regression parameter associated with a to vary smoothly over the study region, and e_i is random error. GWR estimates the parameters for each observation at location i using all observations with assigned weights through a weighting scheme according to spatial proximity, which is represented by Euclidean distances in this study. Nearer locations gain higher weights and vice versa. Two types of weighting functions are generally used–fixed and adaptive kernels. The latter ensures a certain number of nearest neighbors as local samples and better represents the degree of spatial heterogeneity (Fotheringham et al. 2002, Paez et al. 2002) and was the choice in this study. The adaptive kernel method is based on a bi-square distance decay function as follows (Fotheringham et al. 2002),

$$W_{si} = \begin{cases} \left[1 - (\frac{d_{si}}{d_{max}})^2\right]^2 d_{si} \leq d_{max} \\ 0 \quad otherwise \end{cases} \tag{6}$$

where, d_{max} is the maximum distance from the m^{th} farthest case–control location (m is the selected optimal number of neighboring points). The number of nearest neighbor points was chosen by AIC minimization method, which is preferable because it considers the possible variation in degrees of freedom among models centered on various observations (Fotheringham et al. 2002).

The logistic GWR generates a set of parameter estimates for the determinant variables at each casecontrol point location,which can be used to visually analyze spatial variations in the risk posed by determinant variables to cytauxzoonosis infection in cats. In addition, a pseudo t-statistic is also calculated to indicate the significance of the parameters, which is obtained by dividing the parameter estimates by their standard errors (Fotheringham and Brunsdon 2001). Parameter estimates and t-statistics were mapped in ArcGIS to reveal the spatial variations of risk by different determinant variables. Although these t-values cannot be interpreted in a formal statistical sense (Waller et al. 2007), they are often used as exploratory tools to highlight local areaswhere interesting relationships appear to be occurring. An interpolation method, the inverse distance weighted (IDW) algorithm, was employed to generate parameter estimate surfaces. IDW assumes that the predictive spatial surface is driven by local variations that are captured through the neighborhood (Watson and Philip 1985), and therefore was considered to be appropriate in the context of this study.

Results

Locations of cases enrolled in the study were found predominantly in the eastern half of Kansas and adjacent states (Fig. 1). This region receives relatively higher rainfall than the western portion of Kansas, roughly totaling 35–45 inches per annum compared with 15–20 inches in the west (Goodin et al. 2004). This region is also relatively more densely populated than western Kansas. Descriptive statistics for casecontrol host factor characteristics are provided in Table 2. Inclusion of indoor versus outdoor cats in the study did not alter model performance, indicating that cats are equally at risk from all risk factors identified in the study regardless of their home environment.

Among all the environmental and climatic variables screened with a liberal p value (0.2), eight were found to be significantly associated with the case status (Table 3), and were selected as candidate variables for multivariate logistic model (global). However, TECI, % grassland area within 2500 meters surrounding casecontrol locations (henceforth grassland area), relative humidity recorded during the 9th week prior to case arrival at the hospital (henceforth 9th week humidity), and a first-level interaction term, weekly mean DTR recorded 4 weeks prior to case arrival % mixed forest area (henceforth DTR mixed forest) were retained as significant variables at the final multivariate logistic model (global) (Table 4). The differences in statistical distribution of TECI, grassland area, and humidity conditions surrounding casecontrol locations are provided in Figure 2. The inclusion of host factors, age, sex, home environment, and time of arrival at hospital did not change the model parameter estimates by 10% or more. The chi-squared deviance goodness-of-fit test did not indicate model inadequacy ($p > 0.05$), and nonlinearity in logit was not noted. The AIC value of the final model was noted as 354, and the predictive ability of the model measured by AUC value was noted as 0.72.

All variables retained in the multivariate logistic model (global) were entered as parameters in a multivariate logistic GWR model (local), which resulted in a substantial reduction in AIC value (ΔAIC $= 44$) compared to the global model. The difference between the local and global model AIC values was significant ($p < 0.05$) in an analysis of variance (ANOVA) F-test. The AUC value for local model was noted as 0.88, a substantial improvement in model sensitivity/specificity, indicating spatial heterogeneity in the effect of different explanatory variables on casecontrol occurrences in the region. No notable geographical variation in the influences of climate variables (humidity and DTR mixed forest) on casecontrol distribution could be seen; however, a strong positive relationship between TECI and casecontrol distribution was evident toward the southeastern region in a northwest to southeastern gradient (Fig. 3), and a positive relationship in the opposite direction between grassland areas and casecontrol distribution was present in a southeast to northwestern gradient (Fig. 4). A summary of GWR model parameters and their directions of association is presented in Table 5.

Discussion

This study used cases received at a diagnostic laboratory, geospatial analytical methods, and publicly available data sources for identifying novel environmental and climatic risk factors for cytauxzoonosis, enhancing the current ecoepidemiological understanding of this disease. All of the identified risk factors can be related to the role of *A. americanum* ticks in the region, whose control is essential for managing not only cytauxzoonosis but also other zoonotic diseases, including tularemia (Raghavan et al. 2013b) and human monocytic ehrlichiosis.

TECI, a measure of landscape fragmentation, is a risk factor for feline cytauxzoonosis in the south-central and southeastern portions of the study region bordering Oklahoma, Missouri, and Arkansas. Landscape fragmentation leads to more and smaller habitat patches, increased isolation among habitat patches, decreased complexity of patch shape, and higher proportions of edge habitats (Saunders et al. 2002), and studies have shown the risks associated with fragmented landscape for tick-borne diseases (e.g., Halos et al. 2010, Li et al. 2012). Fragmented landscapes can support habitats for wildlife carriers of *C. felis* and influence the abundance of small mammals, many of which are potential hosts for young and adult ticks. Suburban developments at the edges of forest/woodland areas also increase human and pet exposures to infected ticks.

In contrast to the geographic pattern of TECI risk to cats, grassland vegetation surrounding pet owner residences was a significant risk to cats in the north-central and northwestern areas in the study region. The spatial differences in the influences of these risk factors can have prevention/management implications and were identifiable thanks to the GWR modeling approach, which also improved the overall model predictive ability by applying local weights to the parameter estimates. The risk of higher grassland acreage surrounding homes has been identified as a significant risk for feline tularemia in Kansas (Raghavan et al. 2013b), one other tickborne disease that is also transmitted by *A. americanum* among other ticks. Habitats for *A. americanum* include grassland areas, although the wooded edges along fence lines in pastures and home backyards may also support their life cycle. Grasslands in the study region are less intensively maintained and are generally used only for grazing. Infection may be obtained from pathogen-carrying ticks while cats are outdoors, from pet owners, or from other pets in the household that return home after outdoor activities with infected ticks attached.

Humidity conditions recorded 9 weeks prior to case arrival is a significant risk factor for cytauxzoonosis in the study region. The 2500-meter areas surrounding case locations recorded relatively higher humidity conditions during the 9th week prior to case arrival compared to areas surrounding their control counterparts (Fig. 2), and significant differences could not be seen for other weeks. This finding is similar to Raghavan et al.

(2013b), wherein higher humidity conditions surrounding case locations were recorded during the 8th week prior to feline tularemia case arrivals from the same region. Brown et al. (2011) noticed elevated humidity (30-year average) to be associated with higher numbers of human tularemia cases in Missouri, and studies from other regions have shown similar associations as well (Estrada-Penà 2002, Diuk-Wasser et al. 2010). Although humidity has been adequately shown to play an important role in a ticks life cycle, any biophysical mechanisms that favor cytauxzoonosis incidences following higher humidity conditions in the landscape are not clear. The mechanistic basis for a humiditycytauxzoonosis connection is likely to involve multiple pathways and needs further investigations. Higher humidity conditions recorded during late spring and summermonths also coincide with higher human outdoor activities, which may indirectly increase their pet exposure to ticks.

When evaluating the effects of influential environmental factors, studies have typically treated past climate (or future climate-change scenarios) and land-cover effects on disease outcomes separately (Lindgren and Gustafson 2001, Jackson et al. 2006, Randolph 2010, Raghavan et al. 2011). However, microclimatic conditions that affect a ticks life cycle and perhaps its ability to sustain and later transmit different pathogens to hosts could be regulated by habitat type and other physical factors such as soil moisture and elevation (Randolph and Storey 1999). Studies that address climate land-cover interactions on disease outcomes can be rarely found. The significant interaction effect noted between DTR and mixed forest in the present study indicates a combined effect of climateland-cover on cytauxzoonosis outcome.

Another interaction term, humidity grassland was significant at the p = 0.2 level, but this variable was not retained in the final multivariate logistic (global) model. In simple terms, the interaction between these two factors indicate that the effect of DTR on the odds of diagnosing positive cytauxzoonosis cases varies with different values of percent of mixed forest area surrounding case locations, and vice versa.

The spatio-temporal changes in temperature, precipitation, and humidity that are expected to occur under different climate- change scenarios will affect the biology and ecology of vectors and intermediate hosts and consequently the risk of disease transmission (Githeko et al. 2000). Diurnal temperature range has been suggested as an index of climate change (Karl et al. 1991, Braganza et al. 2004), and DTR has been decreasing since the 1950s due to increasing daily minimum temperature (T_{min}) at a faster rate than the daily maximum temperature (T_{max}), and also due to Tmin decreasing at a slower rate than T_{max}. For most parts of the United States, trends show that T_{max} have remained constant or have increased only slightly, but T_{min} values have increased at a faster rate (Karl et al. 1991, 1993). Host-seeking behavior of ticks (Randolph and Storey 1999) and the survival of parasites they carry are strongly influenced by DTR (Ochanda

2006). Any such effect on the vector *A. americanum*, or the parasites they carry, such as *C. felis*, has not been reported before and new investigations will help us understand the mechanical basis of such association. Humidity and DTR are correlated because higher humidity conditions reduce the suns ability to heat the boundary layer (roughly 2 km of the lowest atmosphere), which narrows temperature differences in a given day. We kept both of these variables in the multivariate model because the interpretation for interaction terms are made differently from main-effect interpretations. Unlike the direct effect of humidity, DTR mixed forest points to a more complex problem; i.e., how do climate and the physical environment interact in influencing the outcome of a disease? One plausible scenario could be that ticks in areas with a certain percentage of mixed forest are more likely to transmit *C. felis* when DTR conditions are within a certain range but not others. Identifying associations between climatic factors and disease outcomes is often challenging due to other confounding factors (Patz et al. 2003), but such knowledge is vital for quantifying any role that climate change may be playing toward the amplification and/or spatial expansion of disease incidences. Tick-borne diseases may share similar climate constraints due to the broad role climate plays in the thermoregulation of vector growth, as well as in tick reproduction and survival. Therefore, the identification of humidity and for the first time the combined DTR mixed forest effects on cytauxzoonosis potentially has implication in our broader efforts to understand the linkage between climate change and tick-borne disease. Whether there is consistency in such effects across diverse vector populations and geographic region needs to be studied.

Conclusions

TECI, a measure of habitat fragmentation, and higher grassland acreage surrounding pet owner residences are risk factors, with some regional variability, for feline cytauxzoonosis. Humidity conditions recorded 9 weeks prior to case arrival and the combined effect of diurnal temperature range recorded during the 4th week prior to case arrival and higher mixed forest acreage surrounding residences are strong predictors for cytauxzoonosis throughout the region. The identification of climate variable associations with cytauxzoonosis in this study is significant in the context of climate change impacts on tick-borne diseases. *A. americanum* is a growing concern in the study region due its potential to transmit many zoonotic and animal diseases. Studies on the biology, distribution, and ecology of important tick species in the region are generally lacking and are warranted.

Acknowledgments

This study was supported by the Kansas State Veterinary Diagnostic Laboratory (KSVDL). We thank James M. Hoell, SSAI at the NASA Langley Research Center, for his excellent help in providing methods to more easily access the POWER data used in the analyses. The NASA Langley Research Center POWER Project is funded through the NASA Earth Science Directorate Applied Science Program.

Author Disclousure Statement

No competing financial interests exist.

References

Allison PD. Logistic Regression Using SAS: Theory and Application. SAS Institute, 2012.

Blouin EF, Kocan AA, Glenn BL, Kocan KM, et al. Transmission of *Cytauxzoon felis* Kier, 1979 from bobcats, *Felis rufus* (Schreber), to domestic cats by *Dermacentor variabilis* (Say). J Wildlife Dis 1984; 20:241–242.

Braganza K, Karoly DJ, Arblaster JM. Diurnal temperature range as an index of global climate change during the twentieth century. Geophys Res Lett 2004; 31.

Brown HE, Yates KF, Dietrich G, MacMillan K, et al. An acarologic survey and *Amblyomma americanum* distribution map with implications for tularemia risk in Missouri. Am J Trop Med Hyg 2011; 84:411–419.

Colwell DD, Dantas-Torres F, Otranto D. Vector-borne parasitic zoonoses: Emerging scenarios and new perspectives. Vet Parasitol 2011; 182:14–21.

Diuk-Wasser MA, Vourch G, Cislo P, Hoen AG, et al. Field and climate-based model for predicting the density of hostseeking nymphal *Ixodes scapularis*, an important vector of tick-borne disease agents in the eastern United States. Global Ecol Biogeogr 2010; 19:504–514.

Eckman RS, Stackhouse PW, Jr. CEOS contributions to informing energy management and policy decision making using space-based Earth observations. Appl Energy 2012; 90:206–210.

Estrada-Penà A,Ayllon N, de la Fuente J. Impact of climate trends on tick-borne pathogen transmission. Front Physiol 2012; 3:64. Fotheringham AS, Brunsdon CM. Spatial variations in school performance: A local analysis using geographically weighted regression. Geograph Environ Modeling 2001; 5:43–66.

Fotheringham AS, Brunsdon CC, Charlton M. Geographically Weighted Regression: The Analysis of Spatially Varying Relationships. Chichester, UK: John Wiley & Sons Ltd., 2002.

Githeko AK, Lindsay SW, Confalonieri UE, Patz JA. Climate change and vector-borne diseases: A regional analysis. Bull World Health Org 2000; 78:1136–1147.

Goodin DG, Mitchel JE, Knapp MC, Bivens RL. Climate and weather of Kansas. An Introduction. 2004. Available at www.k-state.edu/ksclimate/documents/kgsed.pdf

Halos L, Bord S, Cotte V, Gasqui P, et al. Ecological factors characterizing the prevalence of bacterial tick-borne pathogens in *Ixodes ricinus* ticks in pastures and woodlands. Appl Environ Microbiol 2010; 76:4413–4420.

Homer C, Dewitz J, Fry J, Coan M, et al. Completion of the 2001 National Land Cover Database for the conterminous United States. Photogrammetric Engineering and Remote Sensing 2007; 73:337–341.

Hosmer DW, Lemeshow S, Sturdivant RX. Model-building strategies and methods for logistic regression. In: Applied Logistic Regression, 3rd ed. Hoboken, NJ: John Wiley & Sons, Inc., 1990:91–142.

Jackson LE, Hilborn ED, Thomas JC. Towards landscape design guidelines for reducing Lyme disease risk. Int J Epidemiol 2006; 35:315–322.

Karl TR, Kukla G, Razuvayev VN, Changery MJ, et al. Global warming–evidence for asymmetric diurnal temperaturechange. Geophys Res Lett 1991; 18:2253–2256.

Karl TR, Jones PD, Knight RW, Kukla G, et al. A new perspective on recent global warming–asymmetric trends of daily maximum and minimum temperature. Bull Am Meteorolog Soc 1993; 74:1007–1023.

Li S, Hartemink N, Speybroeck N, Vanwambeke SO. Consequences of landscape fragmentation on Lyme disease risk: A cellular automata approach. Plos One 2012; 7:e39612.

Lindgren E, Gustafson R. Tick-borne encephalitis in Sweden and climate change. Lancet 2001; 358:16–18.

McGarigal K, Cushman SA, Ene E. FRAGSTATS v4: Spatial Pattern Analysis Program for Categorical and Continuous Maps. 2012. Available at www.umass.edu/landeco/research/ fragstats/fragstats.html

Multi-Resolution Land Characteristics Consortium (MRLC).

National Land Cover Database. 2013. Available at www.mrlc.gov

Tables

Table 1. Land cover types found in the National Land Cover Database (NLCD)

Land cover land use data	Land cover types
NLCD (source, Multi-Resolution Land Characteristics Consortium [MRLC] 2011; years: 1992-2001; resolution: 30 meters, spatial scale 1:100,000)	Open water, developed–open space, developed–low intensity, developed–medium intensity, developed–high intensity, barren land, deciduous forest, evergreen forest, mixed forest, scrub/shrub, grassland/herbaceous, pasture/hay, cultivated crops, woody wetlands, emergent herbaceous wetland.

Table 2. Case–Control characteristics enrolled in the study.

	Number (%) of	
	Cases	Controls
Season of arrival		
Spring	13 (18.84)	21 (23.57)
Summer	22 (31.88)	46 (41.46)
Fall	28 (40.57)	47 (39.02)
Winter	6 (8.69)	9 (9.75)
Age (year)		
< 1	34 (44.92)	47 (41.46)
1–3	22 (17.39)	24 (20.32)
3–5	5 (15.21)	18 (17.88)
> 5	6 (6.52)	11 (13.82)
Unknown	9 (21.73)	23 (20.32)
Sex		
Male	31 (44.92)	51 (30.89)
Female	26 (37.68)	48 (28.45)
Unknown	12 (17.39)	24 (18.69)
Living environment		
Indoor	21(30.43)	44(39.02)
Outdoor	33 (47.82)	68 (61.78)
Unknown	15 (21.73)	11 (13.00)

Table 3. Results of Bivariate Logistic Regression Models for Feline Cytauxzoonosis Status with Geospatial Variables in the Study Region ($p < 0.2$, n = 69 Cases, 122 Controls)

Variable[a]	Estimate	OR	p	95% CI
Total Edge Contrast Index	1.85	6.37	0.00	5.25, 7.72
Grassland	0.89	2.44	0.00	1.60, 3.71
Mixed forest	1.25	3.50	0.08	1.12, 10.92
Medium intensity urban areas	0.25	1.29	0.11	0.89, 1.87
Humidity (9th week)	0.88	2.42	0.00	2.04, 2.87
Humidity (9th week) $*$ grassland	1.15	3.17	0.09	1.09, 9.15
DTR $*$ Total Edge Contrast Index	1.75	5.76	0.16	1.06, 31.08
DTR $*$ mixed forest	0.98	2.67	0.01	2.26, 3.15

Table 4. Results of Multivariate Logistic Regression Models for Feline Cytauxzoonosis Status with Geospatial Variables in the Study Region (p ¡ 0.05, n = 69 cases, 122 Controls)

Variable	Estimate	SE	OR	p	95% CI
Total Edge Contrast Index	1.63	0.09	5.13	0.00	4.24, 6.22
Grassland	0.88	0.21	2.42	0.03	1.59, 3.69
Humidity (9th week)	0.91	0.08	2.49	0.00	2.10, 2.95
Humidity (9th week) $*$ grassland	1.16	0.61	3.19	0.09	0.96, 10.18[a]
DTR$*$ mixed forest	1.15	0.23	3.18	0.00	2.01, 5.03

Table 5. Summary of Multivariate Geographically Weighted Regression (GWR) Model and Directions of Co-Variate Relationships Evaluated in the Study

	Significantly related case/control locations		
	$p < 0.05$	% Positive	% Negative
Total Edge Contrast Index	61%	27.8	33.2
Grassland	68%	29.4	38.6
Humidity (9 weeks prior)	57%	19.8	37.2
DTR $*$ mixed forest	76%	37.6	38.4

Figures

Figure captions:

Fig. 1. Case–control locations in the study region.

Fig. 2. Distribution of percentage Total Edge Contrast Index, grassland vegetation, and relative humidity surrounding case–control locations in the study region.

Fig. 3. Interpolated (inverse distance weights) parameter estimate surface and t-values of Total Edge Contrast Index association with case–control location in the study region (n = case 69, control = 123). Color images available online at www.liebertpub.com/vbz

Fig. 4. Interpolated (inverse distance weights) parameter estimate surface and t-values of percentage grassland area association with casecontrol location in the study region (n = case 69, control 123). Color images available online at www.liebertpub.com/vbz

Figures

Fig. 1.

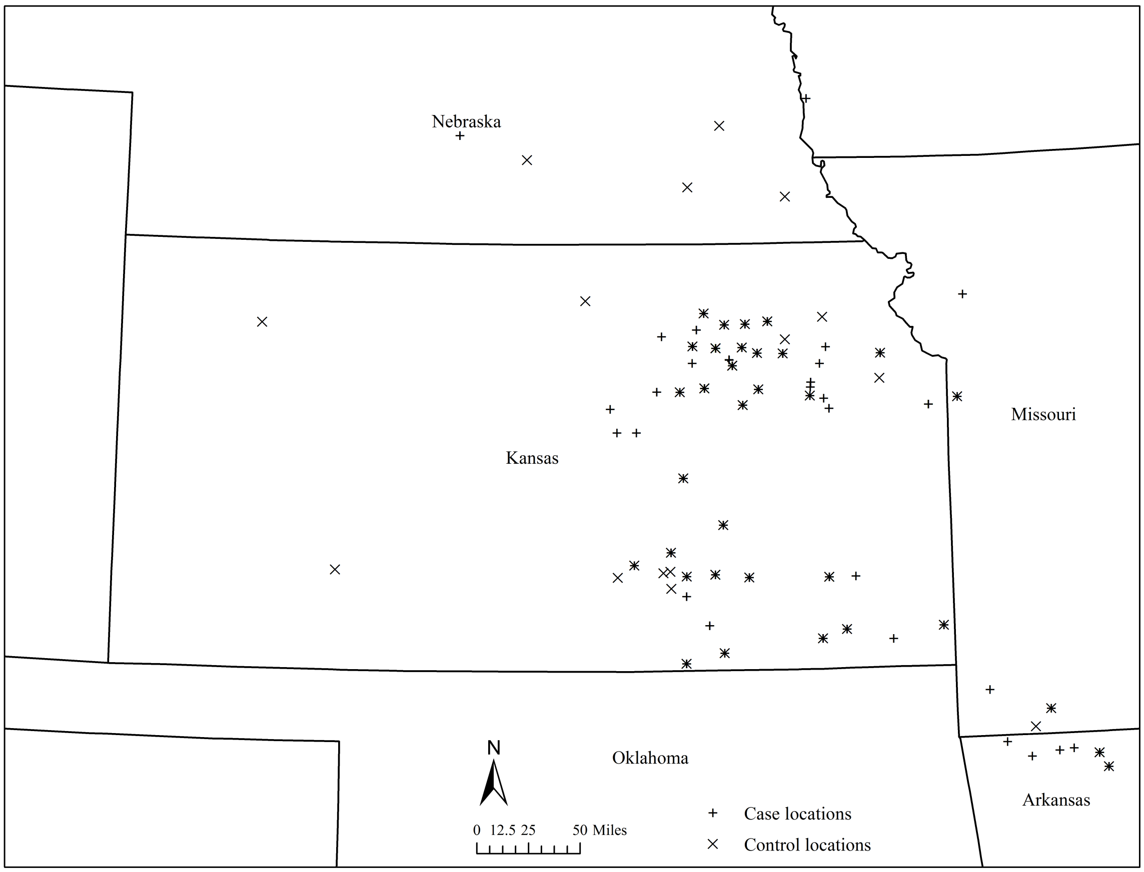
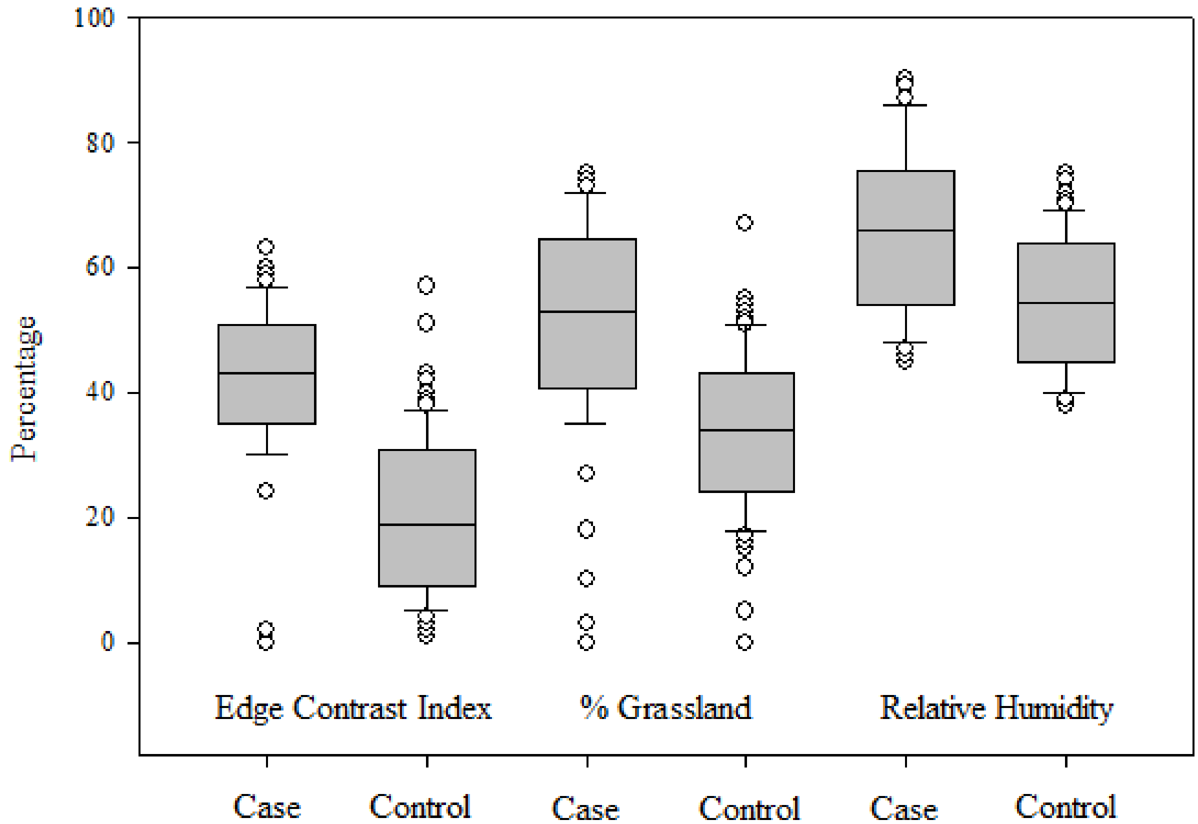


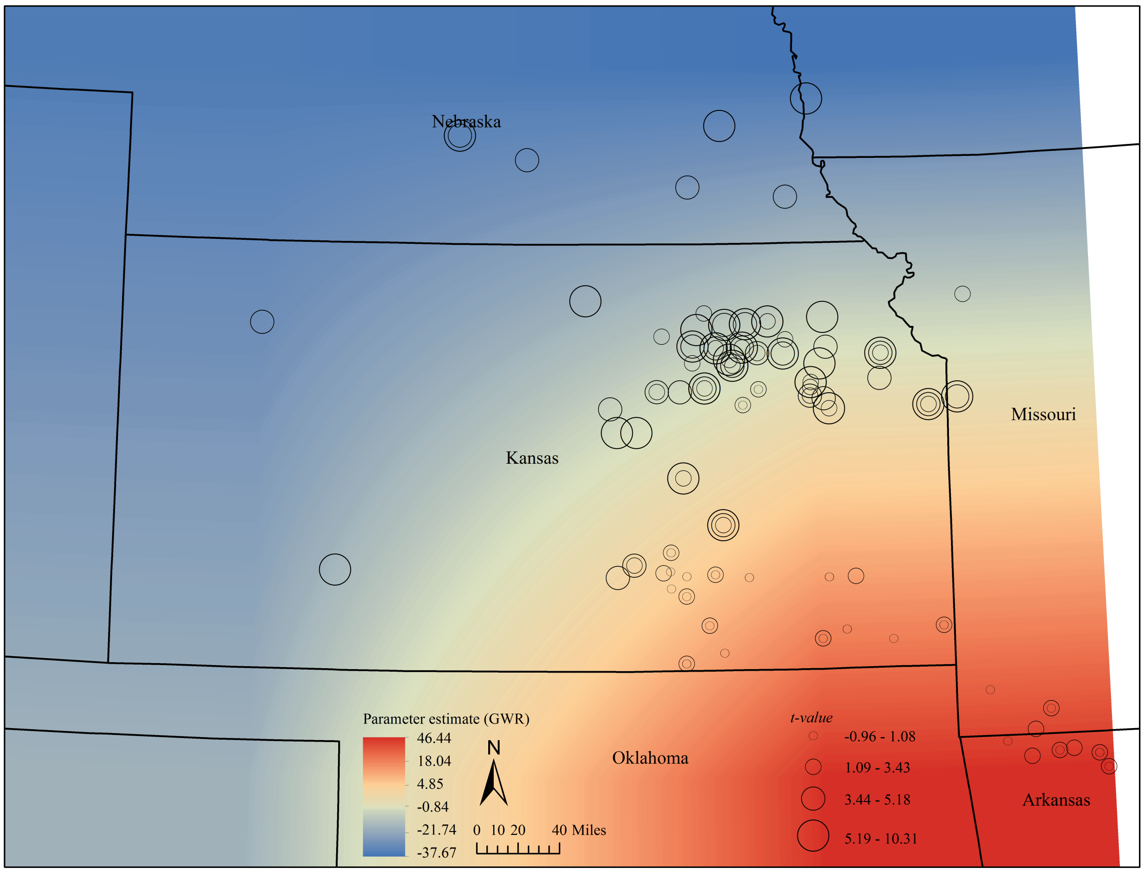
Fig. 2.



100
80
60
40
20
0
Percentage
Edge Contrast Index
% Grassland
Relative Humidity
Case
Control
Case
Control
Case
Control

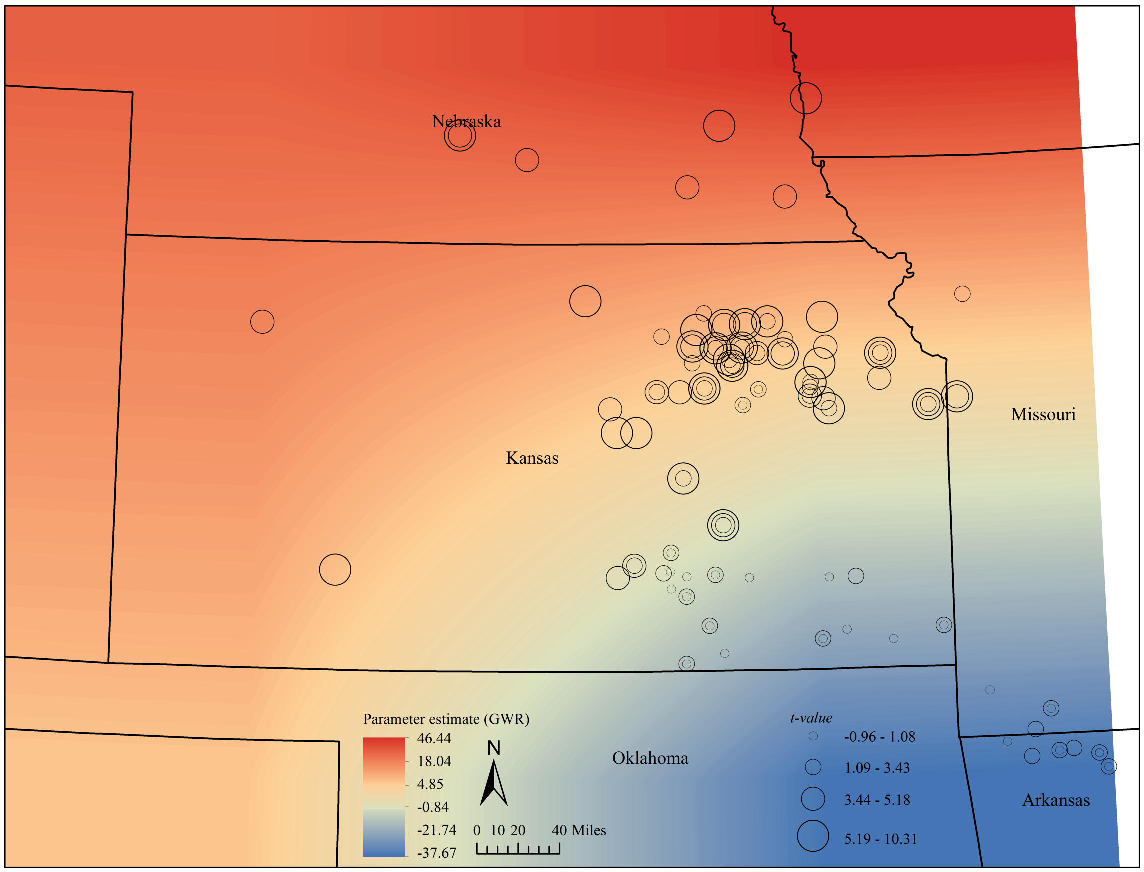
527

Fig. 3.


Nebraska
Kansas
Missouri
Oklahoma
Arkansas
Parameter estimate (GWR)
46.44
18.04
4.85
-0.84
-21.74
-37.67
N
0 10 20 40 Miles
t-value
-0.96 - 1.08
1.09 - 3.43
3.44 - 5.18
5.19 - 10.31

528

Fig. 4.


Nebraska
Kansas
Missouri
Oklahoma
Arkansas
Parameter estimate (GWR)
46.44
18.04
4.85
-0.84
-21.74
-37.67
N
0 10 20 40 Miles
t-value
-0.96 - 1.08
1.09 - 3.43
3.44 - 5.18
5.19 - 10.31

529