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Title: Evaluations of land cover risk factors for canine leptospirosis: 94 cases (2002 to 2009).

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Abstract

Associations of land cover/land use variables and the presence of dogs in urban vs. rural address locations were evaluated retrospectively as potential risk factors for canine leptospirosis in Kansas and Nebraska using Geographic Information Systems (GIS). The sample included 94 dogs positive for leptospirosis predominantly based on a positive polymerase chain reaction test for leptospires in urine, isolation of leptospires on urine culture, a single reciprocal serum titer of 12,800 or greater, or a four-fold rise in reciprocal serum titers over a 2 to 4 week period; and 185 dogs negative for leptospirosis based on a negative polymerase chain reaction test and reciprocal serum titers less than 400. Land cover features from 2001 National Land Cover Dataset and 2001 Kansas Gap Analysis Program datasets around geocoded addresses of case/control locations were extracted using 2500 meter buffers, and the presence of dogs' address locations within urban vs. rural areas were estimated in GIS. Multivariate logistic models were used to determine the risk of different land cover variables and address locations to dogs. Medium intensity urban areas (OR = 1.805, 95% C.I = 1.396, 2.334), urban areas in general (OR = 2.021, 95% C.I. = 1.360, 3.003), and having urban address locations (OR = 3.732, 95% C.I. = 1.935, 7.196 entire study region), were significant risk factors for canine leptospirosis. Dogs regardless of age, sex and breed that live in urban areas are at higher risk of leptospirosis and vaccination should be considered.

Key words: Leptospirosis; Canine; Remote Sensing; Geographic Information Systems; Land cover/Land use

1. Introduction

Leptospirosis, a worldwide zoonotic disease commonly found in dogs, swine and cattle has been attributed to more than 200 pathogenic serovars from the genus *Leptospira*, although in any one geographic area the disease is typically limited to a few serovars (Greene, 2006). Although dogs serve as the maintenance host for serovar Canicola, most infections documented in dogs over the last 20 years in the United States are from serovars Grippotyphosa, Pomona, and Bratislava (Birnbaum et al., 1998; Ward et al., 2004; Greene, 2006; Ghneim et al., 2007). The spirochetes survive in various domestic and wildlife maintenance hosts, such as rodents and other small mammals. Susceptible dogs could be exposed to leptospires in the environment from an infected host's urine or contaminated water or moist soil, where the bacteria may survive for several months. Exposure to infection could occur when the dogs are out for recreation, during free range movement, and/or when contacting infected peridomestic wildlife or other wildlife vectors that visit urban areas for foraging (Levett, 2001). Leptospira serovars are typically maintained in and transmitted by peridomestic wildlife hosts and dogs may serve as sentinels of leptospirosis for the human population (Greene, 2006).

Previous studies suggest that different components of the physical environment surrounding a dog's home could indicate potential risks for canine leptospirosis. Urban areas (Alton et al., 2009), cultivated agricultural land (Kuriakose et al., 2008), water bodies and wetland areas (Ghneim et al., 2007), forest and wooded areas (Zhang, 1988; Nuti et al., 1993), periurban areas closer to wooded areas (Ward et al., 2004) and the act of working in flooded agricultural field and forests (Sharma et al., 2006; Kawaguchi et al., 2008) are significantly associated with canine and human leptospirosis status. These and several other land cover/land

use (henceforth referred to as land cover) areas are of concern due to the potential for such areas to act as habitats for infected wildlife vectors such as opossums, skunks, raccoons and rats.

Identifying associations of canine leptospirosis status with specific land cover types can be useful for mapping potential vector habitats, assessing vector habitat quality and improving our understanding on epidemiological effects of anthropogenic activities like intensive agriculture, urbanization and deforestation that lead to vector habitat loss and fragmentation. Effective preventive strategies for canine and human leptospirosis incidence can then be devised based upon such understanding. High quality (multi-temporal, high resolution) land cover datasets that could aid spatial epidemiological studies are becoming increasingly available in the public domain and have been used in combination with Geographic Information Systems (GIS) in developing strategies for prevention and control of human and animal disease systems (Meade et al., 1988).

The objective of this study was to evaluate dogs' urban vs. rural address locations and different land cover types from two disparate land cover datasets, within 2500 meters as potential risk factors for canine leptospirosis in Kansas and Nebraska.

2. Materials and Methods

2.1. Case Selection

Medical records of all dogs from Kansas and Nebraska that had urine polymerase chain reaction (PCR) testing for leptospirosis performed at the Kansas State Veterinary Diagnostic Laboratory (KSVDL) between February 2002 and December 2009 were retrospectively reviewed. When available, additional test results were included, specifically the results of leptospiral serology and urine culture for leptospirosis. A case was defined by a positive urine

PCR or a negative urine PCR and any one of the following: isolation of leptospires on urine culture, a single reciprocal serum titer ≥12,800, or a four-fold rise in the reciprocal convalescent serum titer. Dogs were deemed controls if the urine PCR was negative and reciprocal serum titers were <400.

2.2. Molecular diagnostic testing

Urine samples for PCR were handled for DNA isolation as previously reported (Harkin et al., 2003a). DNA samples were subjected to the semi-nested, pathogenic Leptospira PCR assay described by Woo et. al., (1997) that amplifies a conserved region of the 23S rDNA, with minor modifications. A unique Taqman probe was incorporated to distinguish pathogenic Leptospira from saprophytic serovars. This test has been commercially available through the KSVDL since 2002.

2.3. Serological testing

The microscopic agglutination test was performed on all blood samples submitted to the KSVDL for leptospiral serological testing. The test was performed for serovars Canicola, Bratislava, Pomona, Icterohemorrhagiae, Hardjo and Grippotyphosa.

2.4. Leptospiral culture

Urine culture was performed by inoculating 1-ml of urine obtained by cystocentesis immediately into 10-ml of liquid Ellinghausen-McCullough (EM) media, gently vortexing this inoculation and transferring 1-ml of this into another 10-ml of liquid EM media. One milliliter of each dilution (1:10 and 1:100) was then subsequently inoculated into separate 10 ml of semi-

105 solid EM media. All tubes were incubated at 30° C in an ambient atmosphere incubator and
106 evaluated for evidence of growth weekly.

107 2.5. Demographic Information

108 Medical records were reviewed to obtain the following information: the patient's age,
109 rounded up to the nearest month, at the time of sample submission; the date of sample
110 submission; and the client's street address at the time of sample submission.

111 2.6. Geocoding

112 Household addresses with information pertaining to house number, street, city, state and
113 zip code were provided by clients at the time specimens for leptospirosis testing were submitted.
114 Addresses were retrospectively verified for their accuracy either by using MapQuest (Map Quest.
115 America Online, Denver, CO) or Google Maps (Google Inc., Mountain View, CA) and/or calling
116 telephone numbers provided by clients. Geographic coordinates for these addresses were
117 derived using a Geocode tool in ArcMap 9.3.1 software and US Census 2007 TIGER
118 (Topographically Integrated Geographic Encoding and Referencing system) shapefile with street
119 level address information (US Census Bureau, 2011). The geographic coordinates for
120 unmatched addresses were obtained using Google Earth software (version No: 5.2.1.1329)
121 (Google Inc., Mountain View, CA). In all, geographic coordinates for 94 cases (out of 97) and
122 185 (out of 197) control data points in Kansas and Nebraska were obtained (Fig. 1).

123 2.7. Projection and data storage

124 All GIS data used in this study were projected (or re-projected from their original spatial
125 reference) in USA Contiguous Equal Area Conic Projection that is based on the Geographic

Coordinate System North American 1983 Geographic Datum. The choice of projection system was influenced by the types of spatial analyses performed as it was essential to maintain accurate area measurements of land cover types surrounding case/control locations. All original, intermediate and processed GIS data were stored in a SQL Server/ESRI ArcSDE 9.3.1 Geodatabase.

2.8. Season of arrival

Observations were grouped based on the seasons in which they arrived at the hospital in to four categories: spring (March to May), summer (June to August), fall (September to November), and winter (December to February).

2.9. Host factors

Observations were grouped into five age groups < 1 y, 1 to 4 y, 4 to 7 y, 7 to 10 y and > 10 y; two sexes and individual breeds were kept without grouping as a categorical variable.

2.10. Land cover variables

The publicly available 2001 National Land Cover Dataset (NLCD) (MRLC, 2011) (Homer et al., 2007; Wickham et al., 2010) for the study region was obtained from the United States Geological Survey (USGS) in a raster grid format. Land cover grids surrounding individual case/control locations were extracted from the raster dataset using 2500 meter polygon buffers, and converted to polygon area features in ArcMap. The area of different land cover type within individual buffer was divided by the total buffer area to generate percent land cover values.

Percentage land cover areas surrounding case/control locations within 2500 meter buffers were also derived using Kansas Gap Analysis Program (GAP) data (KARS, 2011) with case/control locations located completely within Kansas. Land cover information surrounding case/control locations within the State of Nebraska was publicly available in the form of a GAP dataset (NE GAP, 2010); however, a separate analysis with Nebraska data was not conducted due to concerns of potential over-fitting of logistic models with fewer cases (n = 27) and controls (n = 29) in relation to the total number of land cover variables (16).

The descriptions of different land cover types in NLCD and KS GAP can be found from their source websites, USGS (2010), and KARS (2011) respectively.

2.11. Urban vs. rural address location

Geographic boundary file of urban areas was obtained from the U.S. Census Bureau 2000 (U.S. Census Bureau, 2011). All cases/controls that were completely present within the urban boundaries were recorded as urban address locations and those outside were recorded as rural address locations. The U.S. Census Bureau classifies as “urban” all territory, population, and housing units located within an urbanized area (UA) or an urban cluster (UC). The UA and UC boundaries encompass densely settled territory, which consists of core census block groups or blocks that have a population density of at least 1,000 people per square mile and surrounding census blocks that have an overall density of at least 500 people per square mile. In some cases, less densely settled territory may be part of each UA or UC. The Census Bureau's classification of "rural" consists of all territory, population, and housing units located outside of UAs and UCs. (U.S. Census Bureau, 2011).

168 2.11. Statistical analyses

169 All statistical procedures were performed using the R Statistical Package 2.11.1 (R Core
170 Development Team, 2011), and all numerical data were originally stored and organized for
171 statistical analysis in Microsoft Excel 2010 (Microsoft Corporation, Redmond, WA).

172 The effect of season of arrival at the hospital (winter season as reference category) and
173 host factors including age group (< 1 y as reference category), sex (female as reference
174 category), and breed (dogs were not grouped into any general breed categories and unknown or
175 unspecified was used as reference category) were analyzed individually by fitting bivariate
176 logistic regressions.

177 Odds ratios (ORs) and their 95% confidence intervals derived using logistic regressions
178 were used to determine the risks associated with explanatory variables to leptospirosis status in
179 dogs. Land cover variables extracted from NLCD and KS GAP datasets were grouped
180 separately (Table 1) and analyzed independently in two separate steps. Observations of all land
181 cover variables were kept in their original measurement units (percentage) in a continuous
182 format. Presence within urban vs. rural areas were in binary format and rural locations was used
183 as reference category in the logistic models. Land cover variables within 2500 meter buffer area
184 and presence of dogs' addresses within urban vs. rural areas were screened for their association
185 with leptospirosis by fitting bivariate logistic regressions, and variables with a significance level
186 of $P < 0.1$ were selected. A multicollinearity test was conducted among all screened variables
187 by estimating the variance inflation factor (VIF) (variables with a VIF > 10 were considered to
188 indicate multicollinearity) (Dohoo et al., 2003). Presence of dogs' addresses within urban areas
189 was analyzed along with NLCD land cover variables for the entire study region and with KS
190 GAP land covers variables for those case/controls within Kansas. Multivariate stepwise logistic

191 regression models were fitted using a significance level, $P = 0.05$ for variable entry and $P > 0.10$
192 for a variable to be removed from the model. All models were ranked using Akaike Information
193 Criterion (AIC) value and the model with lowest AIC value was deemed to be the best fitting
194 model. The model performance was measured using deviance chi-squared goodness-of-fit test
195 ($P < 0.05$ indicates poor fit) and the predictive ability of the model was evaluated using the area
196 under Receiver Operating Characteristic (ROC) curve values. Confounding effects of host
197 factors, age group of dogs (< 1 year old as reference category), sex (female as reference
198 category), and breed (unknown or unspecified as reference category) on predictor variables were
199 estimated by including them one at a time in the final logistic model. If such inclusion increased
200 the coefficients of explanatory variables by at least 10% or more then the adjusted ORs were
201 recorded from those models.

202 Spatial autocorrelation if present in the case/control data could lead to the violation of
203 underlying logistic regression assumptions (samples are independent and identically distributed)
204 and will yield incorrect parameter estimates and error term. If the parameters in the multivariate
205 model did not account for autocorrelation then the residuals of the model will reveal
206 autocorrelation and need to be verified (Robinson, 2000). A monte-carlo test based on the
207 empirical variogram of residuals and their spatial envelopes (generated by permutations of data
208 values across spatial locations) was used to check for spatial autocorrelation using the geoR
209 library of R Statistical Package 2.11.1 (Ribeiro et al., 2001; 2003).

210 3. Results

211 There were 94 dogs that were identified as cases based on a positive PCR (n=90 dogs),
212 isolation of leptospires from the urine (n=1), a single reciprocal titer ≥ 12,800 (n=2), or a four-

fold rise in serum reciprocal titers (n=1). Of the dogs that were PCR positive, serology was not performed in 22 dogs, 7 dogs had a negative acute titer with no convalescent titer performed, and 61 dogs had concurrent elevated titers to one or more serovar. There were 185 control dogs that had a negative PCR and a reciprocal serum titer of < 400. The demographic characteristics of case, control dogs enrolled in this study are shown in Table 2. Box plots of percentage area occupied by different NLCD and KS GAP land cover variables within 2500 meters around case/control locations are presented in Fig. 2.

Dogs that arrived at the hospital during fall months (September to November) had higher odds (OR = 2.649, 95% C.I. = 1.040, 5.720) of being diagnosed as positive for leptospirosis status, and no other season showed significant association. Dogs' age group (P = 0.147), sex (P = 2.227) and breed (P = 1.210) were not significantly associated with leptospirosis status.

There were 81 cases and 115 controls (out of 94 cases and 185 controls) present completely within urban boundaries in the entire study region, and 56 cases and 90 controls (out of 67 cases and 156 controls) present completely within urban boundaries in Kansas. Results of the multivariate logistic regression with NLCD land cover variables and address location (Table 2) indicated that dogs were at a significantly increased risk from land cover areas represented by developed medium intensity urban areas within 2500 meters from dogs' homes (OR = 1.866, 95% C.I. = 1.443, 2.412) and urban address location (OR = 3.346, 95% C.I. = 1.662, 6.737). Results of the multivariate logistic regression with Kansas GAP land cover variables and address location (Table 3) indicated that dogs were at a significantly higher risk from land cover areas represented by urban areas surrounding their homes up to 2500 meters (OR = 2.013, 95% C.I. = 1.355, 2.991) and urban address location (OR = 3.732, 95% C.I. = 1.935, 7.196).

No other NLCD or Kansas GAP land cover variable were found to significantly improve the model fit when added to individual models. Host factor effects of age, gender and breed did not improve the estimates of explanatory variables; and the deviance goodness-of-fit test did not indicate serious model inadequacies. Residual autocorrelation in the final models was not noted and the area under ROC curve value was 0.79 and 0.82 for NLCD and KS GAP models respectively.

4. Discussion

In this study, there was a seasonal prevalence of canine leptospirosis cases in Kansas and Nebraska, similar to the seasonal prevalence in N. America reported by others (Ward, 2004; Alton et al., 2009) with an increase in leptospirosis cases during the fall. The seasonal trend could be related to plausible higher prevalence of leptospira serovars in the urban abiotic environment and/or among wildlife vectors following rainfall events during fall and the preceding summer (Ward, 2002).

Vaccination status for dogs included in the study was not available. There are some concerns that vaccinations may not completely prevent shedding; however, studies show that vaccines do prevent renal colonization and urinary shedding of leptospires to a great extent (Harkin et al., 2003b; Minke et al., 2009). Vaccination, however, would not prevent infection from non-vaccinal serovars, but has been shown to almost completely eliminate clinical disease, renal colonization, leptospiruria, and death following extreme challenge in the laboratory setting (Schreiber et al., 2005; Minke et al., 2009).

Using either the NLCD or KS GAP land cover data sources, urban areas within 2500 meters were risk factors for leptospirosis status in dogs. Other reports have used different buffer

sizes in their evaluation of canine leptospirosis associations with land cover/environmental variables: for example, 1000 meters (Ward et al., 2004); and 500, 2000, 5000 and 10,000 meters (Ghneim et al., 2007). Our choice of buffer size was roughly guided by the amount of area that a healthy dog could potentially cover in a day during leashed or supervised exercise and also the potential home ranges of wild mammals such as raccoons (Rosatte et al., 2006), opossums (Sunquist et al., 1987) and skunks (Weissinger et al., 2009) that at times carry leptospira. The urban areas identified as risk factors in this study included medium intensity urban development (a mixture of constructed materials and vegetation with impervious surfaces accounting for 50-79 percent of the total cover, most commonly single-family housing units (MRLC, 2011)), and urban areas in general in KS GAP dataset (only one urban land cover class was presented in KS GAP dataset). Streams that are commonly found in urban areas are prone to flash floods after rainfall events because of impervious surfaces, and flooding has been previously identified as a significant risk for canine leptospirosis (Ward et al., 2004; Park et al., 2006; Gaynor et al., 2007; Liverpool et al., 2008). Temporary pools of stagnant water that form after rainfall/flood events along pedestrian side-walks, recreational areas and other similar urban areas could potentially contribute to higher leptospira transmission in urban settings as well.

Similar to the identification of urban address location as a risk factor in this study, in a study where urban and rural areas were distinguished using zip code information, Alton et al., (2009) found urban areas of Ontario, Canada to be a significant risk factor for dogs compared to the rural areas. The role of different urban wildlife populations as maintenance hosts of leptospirosis have also been widely reported in the literature (Tomich, 1979; Lindenbaum and Eylan, 1982; Vanasco et al., 2003; Tucunduva et al., 2007; Koizumi et al., 2009; Krojgaard et al.,

2009), and it is possible that the risk of leptospirosis in dogs residing in urban areas is due to a high concentration of urban wildlife and subsequently higher risk of transmission.

Socio-economic characteristics of urban areas such as human population density, poverty status, and the number of people living in a household have been identified as risk factors for leptospirosis in Brazil (Oliveira et al., 2009; Martins Soares et al., 2010); however, further studies are essential to verify if similar risk factors exist for dogs in urban North America since there could be differences in socio-economic and housing characteristics and urban planning, in general, between the two regions.

The risk of urban areas to dogs could also be due to infected wildlife mammals visiting urban back yards for foraging and/or migratory behavior or due to dogs contracting leptospirosis from wildlife when they are out for recreation. Ward et al., (2004) reported that living within 1000 meters of woodland areas was a significant risk factor for leptospirosis in dogs. Likewise, reports from tropical climates indicate that humans living in proximity of forested or woodland areas, and those who work in forests, are at higher odds of contracting leptospirosis (Hogerzeil et al., 1986; Zhang et al., 1988; Sharma et al., 2006). Serologic surveys among wildlife mammals show the common prevalence of leptospires among them. In the Posavina forests in Croatia, Margaletic et al., (2002) isolated 17 different strains of leptospires in three rodent species, and identified positive leptospiral antibody titers in several small rodent species. Likewise, in a serological survey conducted among raccoons within forested areas in Indiana, Raizman et al., (2009) recorded a 47% seropositive rate for leptospiral titers among raccoons. Several wildlife mammals were seropositive for leptospira serovars collected from an area where wildlife potentially interact with cattle (de Fritas et al., 2010).

Two disparate land cover datasets derived based upon different remote sensing images and methodologies were used in this study to cover satisfactory temporal and spatial resolution and remarkably similar land cover types were identified as risk factors from each of these datasets. However, the scope of this study was limited to quantifying potential risk of different land cover variables alone. Further studies are necessary to determine associations of specific factors for leptospirosis survival and spread within urban settings such as proximity to public areas, human demographics, and socio-economic characteristics within urban boundaries. Surface water collected from urban environments had higher concentrations of pathogenic leptospires than samples from rural areas (Ganoza et al., 2010). In a case-control study conducted using canine population in Northern California, Ghneim et al., (2007) found significant correlation between positive leptospirosis cases and hydrographic density within 500 meters from dogs' homes. However, land cover areas representing bodies of water in both datasets in this study were not significantly associated with leptospirosis at any distance. Apart from variations that may arise due to the differences in climate in these geographically distinct regions, it is likely that the land cover datasets used here may not be adequate to identify associations with water bodies. Land cover datasets are derived from satellite images taken with a primary focus on classifying ground cover data based on spectral reflectance, and many streams and bodies of water could be underrepresented in them. Also, many of the smaller size water bodies that likely provide an optimal environment for leptospira survival may have gone undetected in such relatively coarse scale images. Further studies are essential to quantify leptospirosis association with bodies of water using datasets specifically created for capturing hydrologic features such as the National Hydrography Dataset (USGS, 2011) and National Wetlands Inventory (NWI, 2011).

As with Alton et al., (2009) this study did not find any association between dog's age group and leptospirosis status. These findings, however, are in contrary to two other studies that identified discordant age groups at risk, 4.0 to 6.9 years (Ward et al., 2004) and <1 year and 8 years or older (Ghneim et al., 2007). The differences observed in these studies could be related to the case selection methodologies used. The authors believe that the relatively higher number of cases enrolled in the study and predominantly PCR-based, case selection process employed established a reliable research population. In comparison to two other studies that evaluated associations of land cover and other environmental variables with canine leptospirosis, the current study had 94 cases and 185 controls enrolled, whereas Ward et al., (2004) (36 cases, 138 controls) and Ghneim et al., (2007) (30 cases, 36 controls) had fewer cases and controls. The positivity criteria set in this study for cases (a positive PCR result, a four-fold increase in convalescent titers, a single reciprocal titer equal to or greater than 12,800, or a positive culture) eliminated false positive cases associated with vaccine titers. Reciprocal titers as high as 3,200 have been identified in vaccinated, healthy dogs, and this fact, in addition to the unknown vaccine status of patients in this study, guided the establishment of the minimum single reciprocal titer cut-off at 12,800, a four-fold increase over 3200 (Harkin et al., 2003a). Other studies have established that a PCR positive result, in isolation, confirms the presence of pathogenic serovars and a diagnosis of leptospirosis (Harkin et al., 2003a; Geisen et al., 2007). Furthermore, the sensitivity of this methodology is such that early detection of leptospirosis infection can be achieved prior to seroconversion (Merien et al., 1995; Harkin et al., 2003b; Hernandez-Rodriguez et al., 2011).

Conclusions

Medium intensity developed urban areas, and urban areas in general are risk factors for canine leptospirosis despite dogs' age, sex and breed. Pet owners living in these types of areas and treating veterinarians should consider vaccinating their dogs to prevent leptospirosis. This study follows many previous studies that have used GIS and remotely sensed datasets for identifying important risk factors for zoonotic diseases, further adding to the evidence of their relevance to preventive veterinary medicine research.

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Table 1

Land cover types found in NLCD, and Kansas GAP datasets. Items in italics within parentheses were grouped to represent broader land cover types whose names are in bold letters. Years represent the time period during which satellite images of land cover were captured for creating the data set, including multiple images within a year. Resolution indicates the fineness of ground data as captured by a satellite image, shorter resolution meaning higher clarity; and, spatial scale indicates the scale for which interpretations are appropriate.

Land cover/land use dataset	Land cover/land use types
NLCD (source: MRLC (2010), years: 1992 – 2001, resolution: 30 m, 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.
Kansas GAP (source: KARS (2010), years, 1995-2000, resolution: 15 m, spatial scale: 1:100,000)	**Forest/woodland** (*maple - basswood forest, oak - hickory forest, post oak - blackjack oak forest, pecan floodplain forest, ash - elm - hackberry floodplain forest, cottonwood, floodplain forest, mixed oak floodplain forest, evergreen*

forest, disturbed land, bur oak floodplain woodland, mixed oak ravine woodland, post oak - blackjack oak woodland, cottonwood floodplain woodland, deciduous woodland), **shrubland** (*sandsage shrubland, willow shrubland, salt cedar or tamarisk shrubland*), **prairie** (*tallgrass prairie, sand prairie, western wheatgrass prairie, mixed prairie, alkali sacaton prairie, shortgrass prairie, salt marsh/prairie, low or wet prairie*), **marsh** (*freshwater marsh, bulrush marsh, cattail marsh, weedy marsh*), conservation reserve program, cultivated land, water, urban areas.

Table 2

Case-control characteristics enrolled in the study

	Number (%) of	
	Controls	Cases
Age (yr):		
< 1	21 (11.41)	15 (15.95)
1 – 4	28 (15.21)	12 (12.76)
4 – 7	33 (17.93)	14 (14.89)
7 – 10	68 (36.95)	32 (34.04)
> 10	34 (18.47)	21 (22.34)
Sex:		
Male	83 (42.78)	42 (44.68)
Female	101 (52.06)	52 (55.31)
Season of arrival:		
Spring	41 (22.16)	18 (19.14)
Summer	55 (29.72)	24 (25.53)
Fall	37 (20.00)	32 (34.04)

Winter	52 (28.10)	20 (21.27)

Table 3

Results of multivariate logistic regressions for canine leptospirosis status with NLCD (n = 94 cases, 185 controls) and KS GAP (n = 68 cases, 156 controls) derived land cover variables within 2500 meters from dogs' residences and their urban vs. rural address locations.

Dataset	Variable	Coefficient	S.E	*P*-Value	OR	95% C.I (low, high)
NLCD	Developed, high intensity	0.402	0.244	0.631	1.496	0.927, 2.413
	Developed, medium intensity	0.591	0.131	0.018	1.805	1.396, 2.334*
	Pasture/hay	1.433	0.891	0.099	4.010	0.699, 22.996
	Urban location	1.333	0.335	0.002	3.732	1.935, 7.196*
	Rural location	reference category				
KS GAP	Urban areas	0.704	0.202	0.021	2.021	1.360, 3.003*
	Prairie	1.811	0.997	0.092	6.116	0.866, 43.168
	Shrubland	0.888	0.512	0.071	2.430	0.890, 6.629

Urban location	1.208	0.357	0.001	3.346	1.662, 6.737*
Rural location	reference category				

* Significantly associated ($P < 0.05$) with leptospirosis status. Barren land ($P < 0.170$) and woody wetlands ($P < 0.131$) from NLCD and forest/woodland ($P < 0.128$) were excluded from the multivariate model during stepwise procedure.

Observations of all land cover variables were in continuous format, and are percentage land cover areas surrounding dogs' residences within 2500 meters. Area under ROC (Receiver Operation Characteristic) curve = 0.79 and 0.82 for NLCD and KS GAP models respectively.

Figure

Fig. 1. Case/control distribution in the study region.

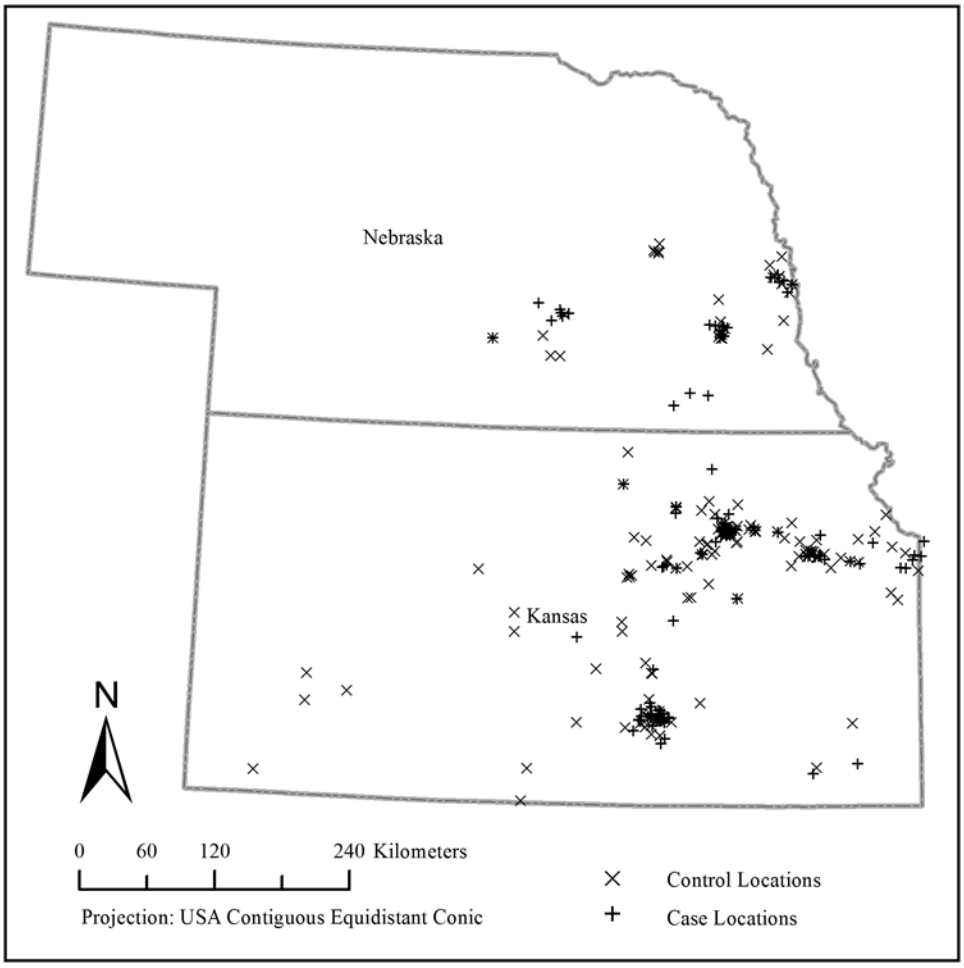
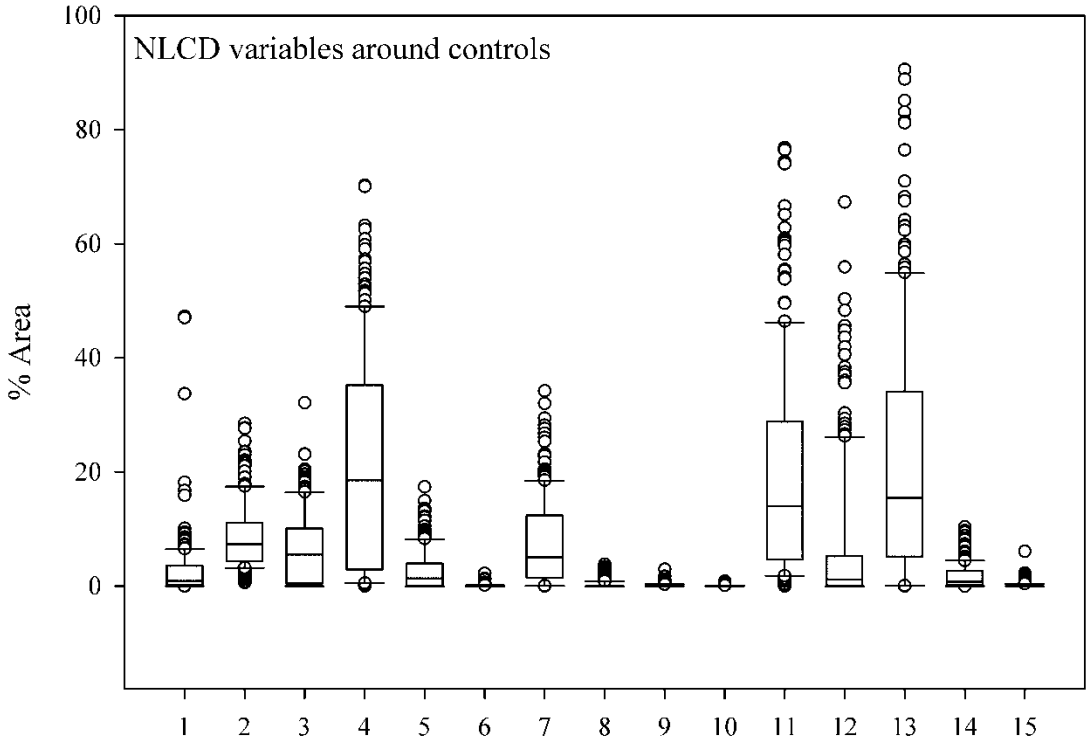
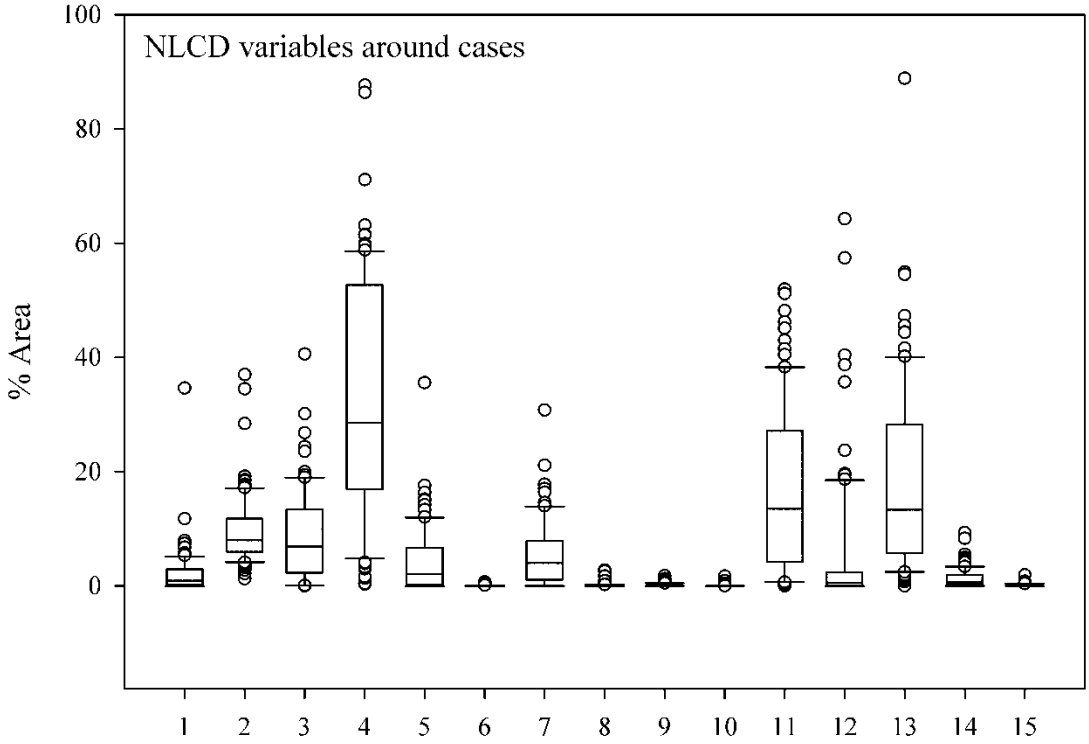
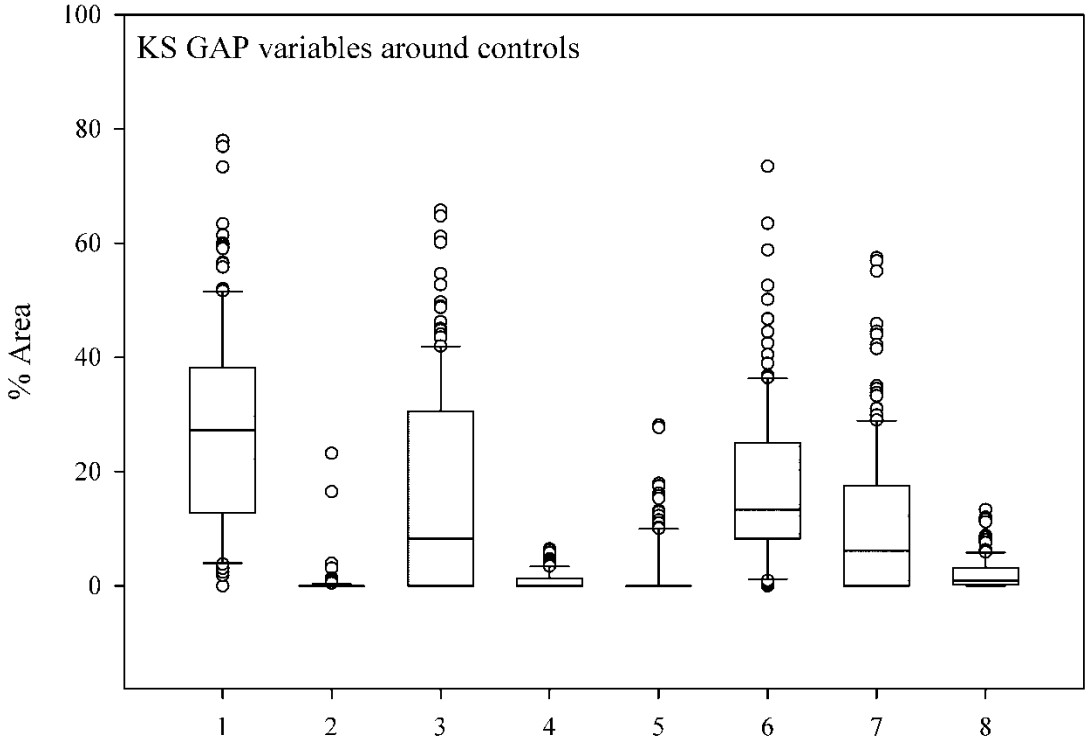
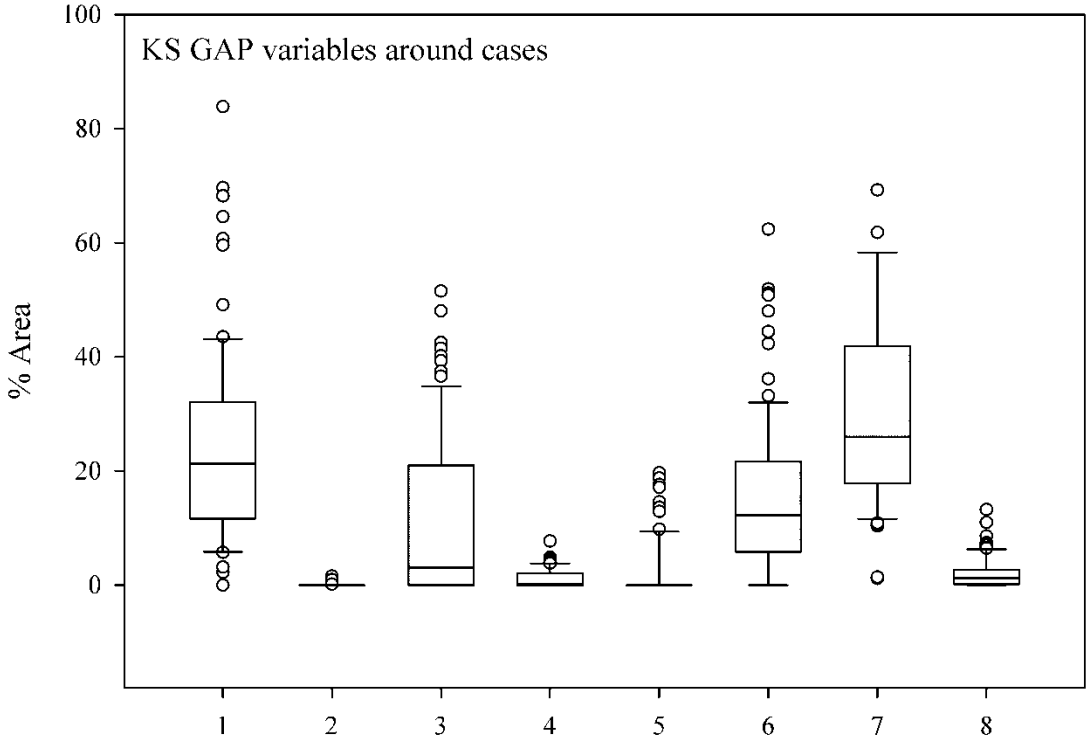


Figure 2. Boxplots of percentage area occupied by NLCD and Kansas GAP land cover variables within 2500 m of case/control locations in the study region.






KS GAP variables around controls
% Area
100
80
60
40
20
0
1
2
3
4
5
6
7
8


KS GAP variables around cases
% Area
100
80
60
40
20
0
1
2
3
4
5
6
7
8

Numbers on the x-axis of box plots of NLCD variables represent: 1. open water, 2. developed - open space, 3. developed - low intensity, 4. developed - medium intensity, 5. developed - high intensity, 6. barren land, 7. deciduous forest, 8. evergreen forest, 9. mixed forest, 10. scrub/shrub, 11. grassland/herbaceous, 12. pasture/hay, 13. cultivated crops, 14. woody wetlands, 15. emergent herbaceous wetland land cover types.

Numbers on the x-axis of box plots of Kansas GAP variables represent: 1. forest/woodland, 2. shrubland, 3. prairie, 4. marsh, 5. conservation reserve program, 6. cultivated land, 7. water and 8. urban area land cover types.