GEOSPATIAL ANALYSIS OF CANINE LEPTOSPIROSIS RISK FACTORS IN THE CENTRAL GREAT PLAINS REGION

by

RAM RAGHAVAN

M.S., KANSAS STATE UNIVERSITY, 2005

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Diagnostic Medicine/Pathobiology

College of Veterinary Medicine

KANSAS STATE UNIVERSITY

Manhattan, Kansas

2011

Abstract

Associations of land cover/land use, socio-economic and housing, and hydrological and soil-hydrological variables 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 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. Publicly available geographic datasets representing land cover/land use, socio-economic and housing characteristics, and hydrologic and soil hydrologic themes were analyzed along with geocoded addresses of case/control locations in GIS. Among different land cover/land use variables evaluated, urban areas (high and medium intensity urban areas and urban areas in general) and evergreen forests and forest/woodlands in general were significant risk factors. Among socio-economic and demographic determinants evaluated, houses lacking complete plumbing facilities, poverty status by age (18-64), and living within 2500 meters of a university/college or parks/forests were significant risk factors. Proximity to water features, hydrologic density and frequently flooded areas were identified as significant risk factors for canine leptospirosis among hydrologic and soil-hydrologic variables. Pet owners whose dogs live in such areas or under these circumstances should consider vaccination to prevent canine leptospirosis.

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Abstract

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Acknowledgments

This work would not have been possible without the extreme kindness of Dr. Ken Harkin and Dr. Karen Brenner who provided leptospirosis diagnostic data. I am forever indebted to Dr. Harkin for having always been there to provide help, directions and encouragements despite his busy schedule at the clinics. His endless passion for perfection in canine leptospirosis research and enthusiasm for GIS helped shape this study. I am also deeply grateful to Dr. T.G. Nagaraja for accommodating me as his student and for his guidance during some very difficult times, and Dr. Gary Anderson for his broad vision for GIS applications in Kansas State Veterinary Diagnostic Laboratory (KSVDL) which essentially formed a platform for this research.

My committee members Dr. Shawn Hutchinson and Dr. Keith Gido are the best one can have in their committee in the entire world. Their availability for consultation any time (sabbatical didn't matter), critical comments on my work, and keen interest in my success and constant encouragements helped me immensely throughout the project. I am very thankful to Dr. John Pickrell for sharing his broad knowledge in veterinary medicine and for his generosity in accepting to serve in my committee despite his retirement.

My sincere thanks to Dr. John Harrington (Department of Geography) and Dr. Walter Dodds (Division of Biology) for making it possible for me to complete this dissertation while working on NSF EPSCoR grant projects. Mr. Loren Wilson (K-State ISO) gave immense support in favor of this work during my employment with the Office of Mediated Education.

My dear wife, Akila and our dear daughter, Vasu will be quite relieved when I am finally done with the doctorate but they have been with me every step of the way in this effort. I thank them for their understanding, extreme patience, sacrifices, and encouragements.

Introduction

Leptospirosis is a bacterial zoonosis with a worldwide distribution (Levett, 2001). In man, symptomless infections are most common. For those that develop clinical disease, it can vary from a self-limiting febrile illness, a severe flu-like illness, or, in the most severe clinical cases, liver and kidney failure. The clinical symptoms of this disease in humans can include fever, headache, chills, muscle aches, anorexia, nausea, vomiting, jaundice, coughing or dyspnea, and sometimes a rash (CDC, 2011). The most commonly recognized form of the disease in dogs is acute renal failure, although other manifestations of the disease have been recognized. Signs of leptospirosis in dogs depend on and variety of host-pathogen interactions, including the age and immune status of the dog, environmental factors, and the serovar involved. Commonly recognized clinical symptoms in dogs include oculonasal discharge, anorexia, vomiting, abdominal pain, polyuria, diarrhea, lethargy, dyspnea, and fever (Greene, 2006). Leptospirosis is caused by long, thin, motile bacterial spirochetes that survive well in fresh water, soil and mud and are associated with reservoir or maintenance hosts such as dogs, cattle, pigs, and peridomestic wildlife such as opossums, raccoons, voles, and rats. For example, reservoir hosts may be infected and leptospiruric for months to years, making them an important public health risk.

There are over 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 40 years in the United States are from serovars Grippotyphosa, Pomona, and Bratislava (Bishop et al., 1979; Cole et al., 1982; Birnbaum et al., 1998; Ward et al., 2004; Greene 2006; Ghneim et al., 2007). Laboratory diagnosis of leptospirosis is traditionally done using

microscopic agglutination tests (MAT) and is considered as the gold standard at the current time. However, there can be numerous inaccuracies in the test and inconsistencies also arise due to differences in laboratory practices generally leading to a higher rate of false positive misclassification error. The Polymerase Chain Reaction test (PCR), an alternative method for laboratory diagnosis of leptospirosis is increasingly being adopted by laboratories worldwide that is more sensitive and leads to early detection of leptospires than seroconversion. The results of PCR diagnosis also results in fewer false positive cases for leptospirosis (Harkin et al., 2003a, 2003b; Merien, Hernandez et al., 2010).

Susceptible dogs could be exposed to leptospires in the environment from an infected host's urine or contaminated water, mud, 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 or supervised exercise, and/or when contacting infected peridomestic wildlife or other wildlife vectors that visit urban areas for foraging (Levett, 2001). The spirochetes enter the body through mucous membranes and abraded skin when they come in contact with contaminated surfaces. Leptospira serovars are typically maintained in and transmitted by peridomestic wildlife hosts and dogs that 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., 1997), 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 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.

Incidences of leptospirosis in man have been associated with socio-economic and demographic characteristics of a society such as lower income and literacy, as well as housing and increased population density. Poor sanitary conditions (Veras et al., 1985) and poor socio-economic conditions in urban slums (Veras et al., 1985; Reis et al., 2008) are commonly identified as risks for human leptospirosis. Martins Soares et al., (2010) explored several socio-economic and demographic characters of Sao Paulo, Brazil with historical human leptospirosis cases and found significant associations with lower average monthly income and literacy rate, as well as higher number of people living in a household, among other factors. Likewise, lower education, income, poorer housing types, and higher number of people living per household were risk factors for human leptospirosis in a different study from urban Recife in Brazil (Oliveira et al., 2009). Many of the socio-economic and housing characteristics differ in the U.S. compared to Brazil; however, a pet owner's education, age, income, population density and the housing characteristics of a neighborhood in which dogs reside are some factors that may have an impact on the health status due to the similarities in living conditions shared by pet animals.

Other factors that have a plausible influence on canine leptospirosis incidence include proximity to public or open land that provide recreational opportunities (Ghneim et al., 2007), proximity to newly urbanized areas (Ward et al., 2004), and agriculture and livestock related activities in the region (Ward et al., 2004). Urban areas were a significant risk factor for canine leptospirosis in Ontario, Canada (Alton et al., 2008). Much of the information related to land cover/land use, socio-economic, and demographic characteristics of a society and urban

characteristics such as locations of public places are available in the public domain in the form of geographic datasets. Associations of such potential risk factors with animal and human infectious diseases can be quantitatively evaluated using spatial analysis and geoprocessing capabilities of a Geographic Information System (GIS).

GIS is a combination of computer hardware, software, data and people that enables storage, retrieval, analysis and visualization of geographic data (Longley et al., 2005; Chang et al., 2003). Geographic data are those that can be referenced to a specific location on the earth surface (or earth's near surface) and are often referred to as geospatial data. Related sciences like cartography and to some extent spatial analysis could very well be centuries old as can be found in historical records and even seen in ancient cave drawings (Robinson et al., 1984) but the technology and science of GIS is relatively new which has grown alongside the improvements made in computing and information science since the early 1960s. Almost everything that happens, happens somewhere and knowing where something happens can be critically important (Longley et al., 2005). For this reason, GIS finds its use in many disparate disciplines ranging from sociology, medicine, engineering, business, transportation and risk estimation and management. The strength of GIS in research primarily lies in its ability to bring together data from multiple disciplines leading to synthesis of new information, and to a large extent depends upon its human component for its success.

John Snow in 1855 famously mapped the Soho cholera outbreak in London and noted that the addresses of the sick were clustered and the main source of water for them was from a single pump located in Broad Street, London. The pump handle was removed and soon afterwards the outbreak was contained. The use of GIS in veterinary, medical, and public health research today follows similar approaches only with more modern tools, computing capacity, and

fine scale, high resolution data. Developments in closely related disciplines such as remote sensing, geoprocessing, biostatistics, and data management have further enhanced the relevance, usefulness and application of GIS in medical research (Meade et al., 2005; Melnick, 2002).

Among the many applications of GIS in veterinary research it can be found widely used to identify environmental and socio-economic risk factors for various animal diseases, including canine leptospirosis. Ward et al., (2002, 2004) identified periurban areas closer to forest/woodland areas as significant risk factors for canine leptospirosis. Ghneim et al., (2008) studied the risk of land cover features at various distances from dogs' residences and found hydrologic density within 500 meters from dogs' homes and areas where standing water can be found, such as agricultural land, were risk factors for dogs. In a study where urban vs. rural addresses were differentiated using zip codes, Alton et al., (2009) found dogs living in urban areas were at significantly higher risk of leptospirosis than their rural counterparts. Many studies originating from South America have used GIS to identify poor sanitary conditions, population density, and income and housing characteristics as risk factors for human and canine leptospirosis (Reis et al., 2008; Martins-Soares et al., 2010; Oliveira et al., 2009).

Identification of risk factors for any disease provides managers, practitioners, and researchers an advanced warning about potential disease outbreaks, and is an important part of preventive veterinary medicine and one-health practice. Management and mitigation strategies, such as vaccination and quarantine, can be developed based on the knowledge and magnitude of risk factors for diseases. One common method for identifying risk factors for a disease is by retrospectively analyzing case-control data and different candidate variables that are likely to have an impact on the outcome of the disease event. This type of study design is known as a case-control study, where comparisons on the effects of independent variables are made between

groups of individuals that are positive for an event (disease), called 'cases', with those that are negative for an event (controls) (Dohoo et al., 2008). The case-control data is then analyzed using logistic regressions, a form of regression analysis that is used to predict the probability of occurrence of an event in the presence of certain covariate(s).

This study explored several candidate variables as potential risk factors for canine leptospirosis based upon previously published literature. The candidate variables were derived from broad thematic dataset groups that represented land cover/land use, neighborhood level socio-economic and demographic information, and hydrologic and soil-hydrologic information surrounding geocoded locations of cases and controls that were received at the Kansas State Veterinary Diagnostic Laboratory (KSVDL) between the years 2002 – 2009.

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Chapter 1 - Evaluations of land cover risk factors for canine leptospirosis: 94 cases (2002 to 2009).

Abstract

Associations of land cover and land use variables 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 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 incremental 500 meter buffers in GIS up to 5000 meters. Multivariate logistic models revealed different risk factors as the land cover area surrounding dogs' homes increased. High intensity urban areas (OR = 1.497, 95% C.I = 1.195, 1.876), medium intensity urban areas (OR = 1.870, 95% C.I = 1.446, 2.417) and urban areas in general (OR = 2.013, 95% C.I. = 1.355, 2.991), evergreen forests (OR = 1.704, 95% C.I. = 1.146, 2.531) and forest/woodlands in general (OR = 2.013, 95% C.I. = 1.551, 2.613) were identified as significant risk factors for canine leptospirosis from these two datasets within 5000 meters from dogs' residences.

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., 1997), 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 of the 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 can then be devised based upon such understanding. High quality land cover datasets that can 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).

Investigators using land cover or other spatial/environmental datasets for risk factor evaluations have used single (for example, Ward et al., 2004; Tassinari et al., 2008) or multiple buffer zones (spatial extents) in their analyses (for example, Nakhapakorn and Tripathi, 2004; Ghneim et al., 2007). Studies show that as the spatial scale and extent of study area changes the relative importance of parameters derived from the datasets and their predictive ability can change as well (Henderson-Sellers et al., 1985; Meentenmeyer and Box, 1987; Turner et al., 1989; Wu, 2004). The objective of this study was to evaluate land cover types from two disparate land cover datasets within regular incremental distances 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 KSUVDL since 2002.

2.3. Serological testing

The microscopic agglutination test was performed on all blood samples submitted to the KSU-VDL 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-solid EM media. All tubes were incubated at 30° C in an ambient atmosphere incubator and evaluated for evidence of growth weekly.

2.5. Demographic Information

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

2.6. Geocoding

Household addresses with information pertaining to house number, street, city, state and zip code were provided by clients at the time specimens for leptospirosis testing were submitted. Addresses were retrospectively verified for their accuracy either by using MapQuest (Map Quest. America Online, Denver, CO) or Google Maps (Google Inc., Mountain View, CA) and/or calling telephone numbers provided by clients. Geographic coordinates for these addresses were derived using a Geocode tool in ArcMap 9.3.1 software and US Census 2007 TIGER

(Topographically Integrated Geographic Encoding and Referencing system) shapefile with street level address information (US Census Bureau, 2010). The geographic coordinates for unmatched addresses were obtained using Google Earth software (version No: 5.2.1.1329) (Google Inc., Mountain View, CA). In all, geographic coordinates for 94 cases and 185 control data points in Kansas and Nebraska were obtained.

2.7. Projection and data storage

All GIS data used in this study were projected (or re-projected from their original spatial 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, 5 to 7 y, 8 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, 2010) (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 5000 meter polygon buffers and converted to polygon area features in ArcMap. Incremental buffers of 500 meters were then constructed around individual case/control locations that represented potential areas of influence up to a maximum distance of 5000 meters. The buffers were overlaid with NLCD data one incremental buffer at a time and the area of land cover types within each buffer was computed. The area of different land cover type within individual buffers was divided by the total area of the respective buffer to generate percent cover values. The process of quantifying cumulative land cover percentages was automated using a geoprocessing script written in the Python 2.4 scripting language.

Land cover percentages surrounding case/control locations at incremental distances were also derived using Kansas Gap Analysis Program (GAP) data (KS GAP, 2010) 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).

2.11. Statistical Analyses

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

The effect of season of arrival at the hospital (winter season as reference category) and host factors including age group (< 1 y as reference category), sex (female as reference category), and breed (ungrouped categorical variable) were analyzed individually by fitting bivariable logistic regressions.

Odds ratios (ORs) and their 95% confidence intervals derived using logistic regressions were used to determine the risks associated with land cover variables to dogs. Land cover variables extracted from NLCD and KS GAP datasets were grouped separately (Table 1) and analyzed independently in two separate steps. Observations of all land cover variables were kept in their original measurement units (percentages) in a continuous format. Land cover variables within 500 meter incremental distances were screened for their association with leptospirosis by fitting bivariable logistic regressions. Variables with a significance level of $P < 0.1$ were selected. A multicollinearity test was conducted among screened variables by estimating the variable inflation factor (VIF) (variables with a VIF > 10 were considered to indicate multicollinearity) (Dohoo et al., 2003). Multivariate stepwise logistic regression models were fitted using a significance level of $P \leq 0.05$ for variable entry and $P > 0.1$ for a variable to be removed from the model. All models were ranked using Akaike Information Criterion (AIC) value and the model with lowest AIC value was deemed to be the best fitting model. The model performance was measured using deviance chi-squared goodness-of-fit test ($P < 0.05$ indicates poor fit). Confounding effects of host factors, age group of dogs (< 1 year old as reference

category), sex (female as reference category), and breed on land cover variables were estimated by including them one at a time in the final logistic model. If such inclusion increased the coefficients of land cover variables by at least 10% or more then the adjusted ORs were recorded from those models. The bivariable screening, multivariate stepwise modeling and checks for host-factor confounding was repeated with variables within each buffer distance. A total of ten models for NLCD and KS GAP datasets each were derived.

3. Results

There were 94 dogs that were identified as cases based on a positive PCR (n=90 dogs), 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.

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, sex and breed were not significantly associated with leptospirosis status.

Results of the multivariate logistic regression with NLCD land cover variables (Table 2) indicated that dogs were at a significantly increased risk from land cover areas represented by developed high intensity areas at all areas of influence up to 2000 meters (OR 1.497, 95% C.I. = 1.119, 1.187) and were at increased risk from developed medium intensity land cover when the area of influence reached 2500 meters (OR = 1.866, 95% C.I. = 1.443, 2.412) and up to 3000

meters (OR = 1.870, 95% C.I. = 1.446, 2.417) surrounding their homes. Dogs were at a significantly higher risk from land cover areas represented by evergreen forests when the area of influence reached 3500 meters (OR = 1.645, 95% C.I. = 1.331, 2.033) and extended up to 5000 (OR = 1.704, 95% C.I. = 1.146, 2.531) meters surrounding their homes. No other NLCD land cover type was 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 land cover variables. The deviance goodness-of-fit test did not indicate serious model inadequacies at incremental distances.

Results of the multivariate logistic regression with Kansas GAP land cover variables (Table 3) indicated that dogs were at a significantly higher risk from land cover areas represented by urban areas for all areas of influence surrounding their homes up to 2500 meters (OR = 2.013, 95% C.I. = 1.355, 2.991), and were at a significantly higher risk from forest and woodland areas when the area of influence reached 3000 meters (OR = 1.873, 95% C.I. = 1.449, 2.422) and up to 5000 meters (OR = 2.013, 95% C.I. = 1.551, 2.613) surrounding their homes. No other Kansas GAP land cover variable was 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 land cover variables. The deviance goodness-of-fit test did not indicate serious model inadequacies at incremental distances.

4. Discussion

In this study, there was a seasonal prevalence of canine leptospirosis cases in Kansas and Nebraska, similar to the seasonal prevalence in North 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 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).

Using either the NLCD or KS GAP land cover data sources, urban areas within 2000 to 3000 meters were risk factors for leptospirosis status in dogs. This included both high intensity urban development (areas where people reside or work in high numbers, including apartment complexes, row houses, and commercial or industrial properties, and where impervious surfaces account for 80 to100 percent of the total cover (MRLC, 2010)), 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, 2010)), and urban areas in general in the 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.

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 in urban North America since there could be differences in socio-economic and housing characteristics, and urban planning in general, between the two regions.

Evergreen forests (areas dominated by trees generally over 5 meters tall comprising over 20% of total vegetation and more than 75% of the tree species maintain their leaves all year (MRLC, 2010)) and forest/woodland as a group (the eight different forest types and five woodland types in Kansas GAP were not individually significant) in the NLCD and KS GAP, respectively, were significant risk factors when the area of influence sizes ranged between 3000 to 5000 meters (3500 meters in NLCD). Similary, 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 the 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.

Another study similarly demonstrated that 43% of raccoons in a state park in Brown County, IL were seropositive for leptospirosis (Mitchell et al., 1999). In the Great Smoky Mountains National Park, 21% of white-tailed deer were seropositve for various leptospiral serovars (New et al., 1993). and several wildlife mammals were seropositive for leptospira serovars collected from an area where wildlife potentially interact with cattle (deFritas et al., 2010).

The identification of different risk factors with increasing the area of influence size in this study is potentially a reflection of the predominant land cover types found within the study region, whose proportions and overall composition change over increasing area. One possible explanation for the loss of significance of urban land cover types beyond the 3000 meter radius could be that the landscapes surrounding control locations contained higher percentages of urban land cover types beyond 3000 meters therefore minimizing their differences with urban features around cases at smaller distances. In addition, their differences within larger areas may not have been sufficient enough, or larger relative to the differences of forest types, for the multivariate logistic models to pick them as significant risk factors. A similar change in land cover proportions over increased distances could have led to the detection of significance for evergreen forests and forest/woodland areas at larger distances but not smaller distances.

It appears that when deriving risk factors from the surrounding physical environment it is critical to define an appropriate geographic area of potential influence from which candidate variables are derived since proportions of different variables (such as land cover) change with an increase in the spatial extent around case/control locations. Prior studies on environmental risk and exposure assessment have indicated that the predictive ability of different variables is sensitive to the choice of area of influence considered (Glickman, 1994; Chakraborty and Armstrong, 1997; Shepard et al., 1999). Using multiple areas of influence can overcome this

limitation to some extent but the determination of an area of influence could be based on an animal's behavior and/or its expected movement patterns, among other factors, deemed acceptable for the individual animal or disease system being studied. Other factors to consider are the prevalence and the role of other disease carrying vectors in the environment and their movement patterns during specific seasons. Analyzing several smaller incremental areas within the total area of influence could then be useful to identify associations with variables whose effects in the logistic models may change when the area of influence changes. An area of 2,500 meter radius was considered an appropriate size area of influence in this study based upon a number of assumptions. Primarily, this study assumed that dogs were confined to an area within a 2,500 meter radius of their primary residence and that unsupervised roaming was minimal. Furthermore, there was no evaluation of factors that relied upon the owners' subjective recollection, such as contact with wildlife or recent swimming activities, because this type of data could not be verified and there was concern for potential bias, particularly if the final diagnosis was leptospirosis.

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 the role of land cover 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. In a study conducted 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, 2010) and National Wetlands Inventory (NWI, 2010).

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 contrast 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 predominantly PCR-based, case selection process employed in this study, established a reliable research population. The positive case criteria set forth in this study (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).

Conclusions

High and medium intensity developed urban areas, evergreen forest in particular and forest/woodland areas in general are risk factors for canine leptospirosis in Kansas and Nebraska.

Acknowledgments

Support for this research was partly provided by Kansas State Veterinary Diagnostic Laboratory (KSVDL) and NSF (grant No. 0919466, Collaborative Research: EPSCoR RII Track 2 Oklahoma and Kansas: A cyber Commons for Ecological Forecasting). The authors thank Drs. Shawn Hutchinson, Department of Geography, and Keith Gido, Divison of Biology, Kansas State University, for reviewing an earlier version of the manuscript, and two anonymous reviewers for their valuable comments.

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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. Results of multivariate logistic models fit within incremental distances from dogs' residences for NLCD land cover features associated with leptospirosis status in the study region (n = 94 cases, 185 controls).

Distance	Land cover feature	Coefficient	S.E	*P*-Value	OR	95% C.I (low, high)
500	Developed, open space	0.822	0.737	0.078	2.275	0.536, 9.651
	Developed, high intensity	0.400	0.117	0.027	1.491	1.186, 1.876
1000	Developed, open space	0.819	0.760	0.079	2.268	0.511, 10.060
	Developed, high intensity	0.402	0.117	0.029	1.494	1.188, 1.880
	Pasture/hay	1.503	0.881	0.090	4.495	0.799, 25.273
1500	Developed, open space	0.796	0.651	0.077	2.216	0.620, 7.924
	Developed, high intensity	0.401	0.117	0.024	1.493	1.187, 1.878
	Pasture/hay	1.468	0.852	0.090	4.340	0.817, 23.055
2000	Developed, open space	0.790	0.634	0.078	2.203	0.635, 7.634
	Developed, high intensity	0.404	0.115	0.027	1.497	1.195, 1.876

	Developed, medium intensity	0.659	0.351	0.070	1.932	0.971, 3.845
	Pasture/hay	1.432	0.892	0.091	4.187	0.728, 24.054
2500	Developed, high intensity	0.409	0.244	0.611	1.505	0.933, 2.428
	Developed, medium intensity	0.624	0.131	0.016	1.866	1.443, 2.412
	Pasture/hay	1.433	0.891	0.091	4.191	0.730, 24.031
3000	Developed, medium intensity	0.626	0.131	0.014	1.870	1.446, 2.417
	Pasture/hay	1.430	0.887	0.095	4.178	0.734, 23.772
	Evergreen forest	0.455	0.254	0.082	1.576	0.958, 2.593
3500	Developed medium intensity	0.593	0.320	0.071	1.809	0.966, 3.387
	Evergreen forest	0.498	0.108	0.024	1.645	1.331, 2.033
4000	Developed medium intensity	0.588	0.311	0.075	1.800	0.978, 3.312
	Evergreen forest	0.526	0.109	0.022	1.692	1.366, 2.095
4500	Developed medium intensity	0.586	0.351	0.077	1.796	0.903, 3.574

	Evergreen forest	0.527	0.200	0.021	1.693	1.144, 2.506
5000	Developed medium intensity	0.588	0.350	0.077	1.800	0.906, 3.575
	Evergreen forest	0.533	0.202	0.020	1.704	1.146, 2.531

Observations of all land cover variables were in continuous format, and are percentage areas within incremental distances from dogs' residences.

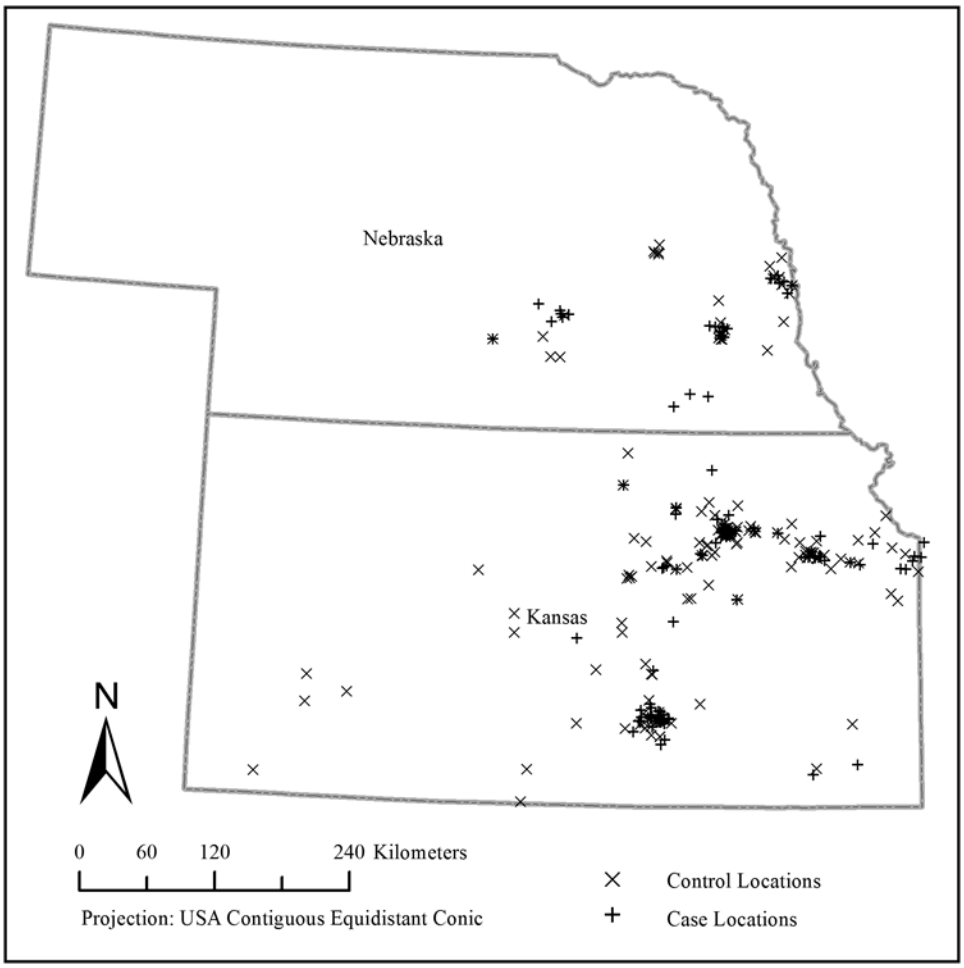
Table 3. Results of multivariate logistic models fit within incremental distances from dogs' residences for KS GAP land cover features associated with leptospirosis status in the study region (n = 68 cases, 156 controls).

Distance	Land cover feature	Coefficient	S.E	*P*-Value	OR	95% C.I (low, high)
500	Urban areas	0.711	0.201	0.015	2.036	1.373, 3.019
	Cultivated land	1.141	0.622	0.081	3.129	0.924, 10.592
1000	Urban areas	0.715	0.201	0.017	2.044	1.378, 3.031
	Prairie	1.811	0.983	0.090	6.116	0.890, 42.000
1500	Urban areas	0.723	0.201	0.012	2.060	1.389, 3.055
	Prairie	1.832	0.985	0.091	6.246	0.906, 43.060
2000	Urban areas	0.721	0.202	0.018	2.056	1.384, 3.055
	Prairie	1.835	0.997	0.091	6.265	0.887, 44.217
2500	Urban areas	0.700	0.202	0.026	2.013	1.355, 2.991
	Prairie	1.841	0.997	0.092	6.302	0.893, 44.483

	Shrubland	0.892	0.512	0.068	2.440	0.894, 6.656
3000	Forest/woodland	0.628	0.131	0.006	1.873	1.449, 2.422
	Prairie	1.841	1.102	0.094	6.302	0.726, 54.648
	Shrubland	0.911	0.512	0.068	2.486	0.911, 6.783
3500	Forest/woodland	0.698	0.132	0.006	2.009	1.551, 2.603
	Shrubland	0.915	0.554	0.071	2.496	0.842, 7.395
4000	Forest/woodland	0.698	0.131	0.004	2.010	1.555, 2.599
	Shrubland	0.917	0.560	0.070	2.501	0.834, 7.497
	Marsh	1.008	0.589	0.092	2.740	0.863, 8.692
4500	Forest/woodland	0.700	0.131	0.000	2.013	1.557, 2.603
	Shrubland	0.918	0.561	0.074	2.504	0.833, 7.519
5000	Forest/woodland	0.700	0.133	0.001	2.013	1.551, 2.613
	Shrubland	0.918	0.564	0.077	2.504	0.829, 7.564

Observations of all land cover variables were in continuous format, and are percentage areas within incremental distances from dogs' residences.

Figure 1. Case/control distribution in Kansas, Nebraska region.



Chapter 2 - Neighborhood-level socioeconomic and urban land use risk factors of canine leptospirosis. 94 cases (2002 to 2009).

Abstract

Associations of housing, population, and agriculture census variables, and presence near public places and newly urbanized areas were retrospectively evaluated as potential risk factors for canine leptospirosis using Geographic Information Systems (GIS). The sample population included 94 dogs positive for leptospirosis based on a positive polymerase chain reaction test for leptospires on 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. Multivariate logistic regressions revealed different risk factors among different census units; however, houses lacking complete plumbing facilities [OR = 2.880, 95% C.I = 1.867, 4.442 (block group); OR = 1.382, 95% C.I. = 1.301, 1.469 (tract); and, OR = 2.900, 95% C.I. = 2.489, 3.380 (county)]; and poverty status by age (18-64) [OR = 2.079, 95% C.I = 1.763, 2.451 (block group); OR = 1.717, 95% C.I. = 1.560, 1.890 (tract); and, OR = 1.717, 95% C.I. = 1.560, 1.890 (county)] were consistent risk factors for all areal units. Living within 2500 meters of a university/college (OR = 2.181, 95% C.I. = 1.520, 3.128) and parks/forests (OR = 1.531, 95% C.I. = 1.220, 5.126) were significant risk factors for dogs. Leptospirosis status and presence within newly urbanized areas and surrounding areas up to 1500 meters were strongly associated. Dogs that live in these conditions are at higher risk for leptospirosis and pet owners should consider vaccination.

1. Introduction

Leptospirosis is a worldwide zoonotic disease that can create disease in and be transmitted by rodents, small mammals, dogs, swine, and cattle, among others, and has been attributed to more than 200 pathogenic serovars from the genus Leptospira, although in any one geographic area disease is typically limited to a few serovars (Greene et al., 2006). Three basic epidemiological patterns of transmission are described for leptospirosis (Faine et al., 2000). The first, transmission to humans (and presumably dogs) in temperate climates occurs through direct contact with cattle and pigs. The second pattern is associated with tropical wet areas, but in contrast to the first involves many serovars and large numbers of reservoir species infecting humans and animals. The third pattern, which concerns urban environments and is of importance to humans and dogs, is typically associated with rodent transmission of limited serovars, although other peridomestic wildlife, such as raccoons and opossums may play a role (Feigin et al., 1973; Demers et al., 1985; Vinetz et al., 1996; Richardson et al., 2003).

Incidences of leptospirosis in humans have been associated with socio-economic and demographic characteristics of a society such as income, literacy, housing, and population density (Veras et al., 1985; Everard et al., 1989; Bakoss, 2007; Cruz et al., 2009). Martins et al., (2010) explored several socio-economic and demographic characteristics of Sao Paulo, Brazil with historical human leptospirosis cases and found significant associations with average monthly income, literacy rate, and number of people living in a household, among other factors. Likewise, education, income, housing type, and number of people living per household were risk factors for human leptospirosis in a different study from urban Recife in Brazil (Oliveira et al., 2009). Many of the measures of socio-economic and housing conditions differ in the U.S.

compared to Brazil and, to our knowledge no study has previously addressed the influence of pet owner socio-economic and demographic characteristics with canine leptospirosis in the U.S.

A pet owner's education, age, income and population density, and the housing characteristics of a neighborhood in which dogs reside are some factors that may have an impact on the health status due to the similarities in living conditions shared by pets and their owners. Other factors that may influence canine leptospirosis incidence in urban settings include proximity to the following: public or open land that provide recreational opportunities (Ghneim et al., 2007), newly urbanized areas (Ward et al., 2004), and agriculture and livestock related activities in the region (Ward et al., 2004).

Associations of socio-economic and demographic features to animal and human infectious diseases can be quantitatively evaluated using spatial analysis and geoprocessing capabilities of a Geographic Information System (GIS). In an earlier study, using GIS and publicly available land cover datasets we found that urban areas in general and medium and high density residential areas in particular are significant risk factors for leptospirosis when land cover area surrounding up to 3500 meters from dogs' residences were analyzed (Raghavan et al., in press). However, variables representing specific socio-economic or demographic characteristics of urban land use were not included in that study nor have they been analyzed in other published literature.

The objectives of this study were to investigate which urban characteristics, specifically socio-economic and human demographic factors could be potential risk factors for canine leptospirosis in Kansas and Nebraska. Further, the proximity to public land or open areas and proximity to newly urbanized areas was also evaluated as potential risk factors for leptospirosis.

2. Materials and Methods

2.1. Case Selection

The medical records of all dogs from Kansas and Nebraska that had urine polymerase chain reaction (PCR) testing for leptospirosis performed at the Kansas State University Veterinary Diagnostic Laboratory (KSUVDL) 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 positive 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 negative control 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 KSUVDL since 2002.

2.3. Serological testing

The microscopic agglutination test was performed on all blood samples submitted to the KSUVDL 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-solid EM media. All tubes were incubated at 30° C in an ambient atmosphere incubator and evaluated for evidence of growth weekly.

2.5. Demographic Information

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

2.6. Geocoding

Household addresses with information pertaining to house number, street, city, state and zip code were provided by clients at the time specimens for leptospirosis testing were submitted. Addresses were retrospectively verified for their accuracy either by using MapQuest (Map Quest. America Online, Denver, CO) or Google Maps (Google Inc., Mountain View, CA) and/or calling telephone numbers provided by clients. Geographic coordinates for these addresses were derived using a geocoding tool in ArcMap 9.3.1 software and US Census 2007 TIGER (Topographically Integrated Geographic Encoding and Referencing system) shapefile with street level address information (US Census Bureau, 2010). The geographic coordinates for unmatched addresses were obtained using Google Earth software (version No: 5.2.1.1329) (Google Inc., Mountain View, CA). In all, geographic coordinates for 94 cases and 185 control data points in Kansas and Nebraska were obtained.

2.7. Projection and data storage

GIS datasets used in this study were projected (or re-projected from their original spatial reference) into the USA Contiguous Equal Area and Equidistant Conic Projections, both of which were based on the Geographic Coordinate System North American 1983 Geographic Datum. All original, intermediate and processed GIS data were stored in a SQL Server/ESRI ArcSDE 9.3.1 Geodatabase.

2.8. Census data

U.S. Census 2000 data on population and housing was obtained in the form of Summary File 3 (SF-3) tables. Identical census attribute information for Kansas and Nebraska were gathered at three geographic levels or areal units (census units) at which census data were aggregated by the US Census Bureau: block groups (containing between 600 and 3,000 people within a county), census tracts (containing between 1,500 and 8,000 people intended to represent neighborhoods), and counties.

GIS data files for block groups, tracts and counties were obtained from the ESRI Street Map data based on US Census Bureau 2000 census information. From the Summary File -3 (SF-3) tables, 33 housing and 37 population related variables (Table 1) were extracted for each census unit by spatial query and joined to the census shapefiles using the common Federal Information Processing Standards (FIPS) codes. The geocoded addresses of cases/controls were overlaid in ArcMap with block group, census tract, and county shapefiles in three separate operations, and the number of cases/controls that were within census units were recorded separately using a spatial join procedure in ArcMap.

2.9. Agricultural census

Agricultural census data for Kansas and Nebraska was obtained per county from the USDA National Agricultural Statistics Service (NASS) (USDA, 2010). County level data for total number of cattle and swine farms, and inventory data of the total number of cattle (and beef and dairy cattle, separately), and the number of pigs and hogs per county in year 2007 were obtained from NASS in a tabular format. The county level agricultural census data were divided by the area of their respective counties in order to normalize for differences in county sizes over the study region. The numbers of cases/controls present within counties were recorded using spatial join procedure in ArcMap.

2.10. Presence around newly urbanized areas

Newly urbanized areas in the study region were derived by the extracting areas around cities that were newly added to the 2000 census using the extract tool in ArcMap. Shapefiles of 1990 city boundaries in the study region were only available for the cities of Wichita, Topeka, Lawrence, and Kansas City in Kansas, and Lincoln and Omaha in Nebraska. The 1990 city boundaries, and boundary files for the same cities for year 2000 were downloaded from the US Census Bureau and newly urbanized areas derived using the extract tool in ArcMap. Incremental buffers every 500 meters up to a maximum of 2500 meters surrounding the newly urbanized area was created using the buffer analysis tool in ArcMap and the number of cases/controls that were within these buffer regions and within the remaining city area were recorded.

2.11. Presence near public places

Polygon areas representing public places around cities, including golf courses, hospitals, industrial parks, primary/secondary schools, shopping centers, sports stadiums, and local, county, and state parks/forests and universities/colleges within 5000 meters from dogs' homes in the study region were obtained from the US Census 2000 TIGER/Line dataset. GIS layers representing storm drainage systems were obtained from the local governments for the cities of Manhattan, Topeka, Lawrence, Wichita, Omaha and Lincoln. Buffered areas extending 2500 meters from the boundaries of public places were created and cases/controls located completely within the buffers were recorded independently for each public place type.

2.12. Data organization and statistical analysis

All census data were originally stored in a Microsoft Access 2010 (Microsoft, Redmond, CA) database and later as ESRI shapefiles during spatial analysis. The number of cases/controls within incremental buffer distances from newly urbanized areas, and the distances to public places from cases/control locations were stored as ESRI shapefiles. All numerical data were stored in Microsoft Excel 2010 (Microsoft, Redmond, CA) prior to statistical analyses conducted using SAS software (SAS Institute, Cary, NC).

Odds ratios and 95% confidence intervals derived using logistic regressions were used to determine associations of canine leptospirosis status with independent variables. There were a total of 33 housing related variables and 37 population related variables at block group, census tract, and county levels; 6 agricultural census variables at county level; and 10 variables representing proximity to different public places. Variable screening and multivariate logistic regression modeling was conducted in separate steps for eight groups of independent variables, which were as follows: population variables at block group, census tract, and county levels;

housing variables at block group, census tract, and county levels; agricultural census variables at county level and location within 2500 meters from public places. Observations for all census variables were kept in their original measurement units and were continuous. Observations for presence within 2500 meters from public places were in categorical format, scored as '0' if absent and '1' if present.

Variable screening among all block level population variables was done by fitting bivariate logistic models and those variables with a *P*-value ≤ 0.1 were selected for further analysis. Multicollinearity was tested for by estimating the variable inflation factor (VIF) among covariates using the proc reg/tol vif option in SAS (SAS Institute Inc., Cary, NC). All variables with a VIF of 10 or above were considered to indicate multicolinearity (Allison, 1999). When possible, some of the correlated variables were combined together to form one indicator variable and in other cases variables were dropped from the analysis based on our judgment of their relevance to canine leptospirosis. Model fitting was repeated until all variables had a VIF value below 10.

Multivariate logistic models were fit with the remaining variables using the stepwise selection procedure with a higher significance level, $P \leq 0.05$ for a variable to be retained and $P = 0.1$ to be removed from the model. To prevent over-contribution of covariate attribute values from some census units in the models, the event/trial option was used in proc logistic, where "event" was the total number of positive cases and "trial" was the sum of all cases and controls within a census unit. Logistic models were ranked using Akaike Information Criterion (AIC) and the model with the lowest AIC value deemed to be the best fitting model. Any confounding effect of host factor (age, sex, and breed) was estimated by adding them one at a time to the final logistic model, and a 10% or more increase in coefficient values of independent variables were

considered to indicate confounding due to that particular factor, in which case adjusted odds ratios and their 95% confidence intervals were recorded. Model adequacy was tested using chi-squared goodness-of-fit test ($P < 0.05$ indicated poor fit). Screening, modeling, and checks for confounding effects were repeated for the other covariate groups.

Cochran-Mantel-Haenszel (CMH) test in proc freq procedure was used to test leptospirosis association with newly urbanized areas found in different cities in the study region. Cities were used as stratum. The numbers of cases/controls that were completely within 500 meters of newly urbanized areas were compared to the numbers of cases/controls that were within the city boundaries. The procedure was repeated with 500 meter incremental distances from newly urbanized areas up to 2500 meters. CMH statistics and Breslow-Day homogeneity test for odds ratios were estimated in the procedure.

3. Results

There were differences in the number and types of significant housing and population variables identified in logistic models fit with covariates aggregated in different areal units (Tables 2-4). Using block groups, the housing related covariates significantly associated with leptospirosis status in the logistic model were: the year structures built (1940-1949) (the total number of structures built during the years 1940-1949) (OR = 2.252, 95% C.I. = 1.510, 3.359), the number of households lacking complete plumbing facilities (OR = 2.880, 95% C.I. = 1.867, 4.442) and the number of owner occupied homes (OR = 0.714, 95% C.I. = 0.528, 0.966). Significant population related covariates associated with leptospirosis status in the logistic model were total population (OR = 1.755, 95% C.I. = 1.229, 2.508), average family size (OR = 1.403, 95% C.I. = 1.162, 1.694), and poverty status in 1999 by age (18-64) (number of individuals in

the age group 18-64 that were below poverty line the year 1999) (OR = 2.079, 95% C.I. = 1.763, 2.451) (Table 1).

Using census tracts as areal units, the only housing related covariate significantly associated with leptospirosis status in the logistic model was the number of households lacking complete plumbing facilities (OR = 1.382, 95% C.I. = 1.301, 1.469). The population related covariates significantly associated with leptospirosis status in the logistic model were poverty status in 1999 by age (18-64) (OR = 1.717, 95% C.I. = 1.560, 1.890), and poverty status in 1999 by age (65-74) (number of individuals in the age group 65-74 that were below poverty line the year 1999) (OR = 1.299, 95% C.I. = 1.023, 1.650) (Table 2).

Using counties as areal units, the housing related covariates significantly associated with leptospirosis status in the logistic model were the number of households lacking complete plumbing facilities (OR = 2.900, 95% C.I. = 2.489, 3.380) and the number of owner occupied homes (OR = 0.788, 95% C.I. = 1.596, 1.912). Population related covariates significantly associated with leptospirosis status in the logistic model were average family size (OR = 1.588, 95% C.I. = 1.524, 1.655) and poverty status in 1999 by age (18-64) (OR = 1.717, 95% C.I. = 1.560, 1.890) (Table 3).

Among county level agricultural census variables, the density of cattle farms, and the number of beef cattle per county were significantly ($P < 0.1$) associated with leptospirosis status but were not significant in the multivariate logistic model. Presence within 2500 meters of a college/university (OR = 2.181, 95% C.I. = 1.520, 3.128) and state park/forest (OR = 2.501, 95% C.I. = 1.220, 5.126) were the two significant covariates associated with leptospirosis status among all public places (Table 5).

For all models described above, no other covariates were found to be significant and/or found to improve the model fit when added. The chi-square deviance goodness of fit test did not indicate any model inadequacy. Confounding effects of age, breed, and sex were not noted.

The Cochran-Mantel-Haenszel test indicated a strong association between leptospirosis status and presence within, and around, newly urbanized areas up to a distance of 1500 meters when adjusted for different cities (Table 6). The non-significant *P*-values in the Breslow-Day homogeneity test for odds ratios indicated that there was a significant association between leptospirosis status and presence within and around newly urbanized areas, up to distances of 1500 meters surrounding newly urbanized areas in the study region. There was no significant association at buffer distances of 2000 and 2500 meters surrounding newly urbanized areas (Table 6).

4. Discussion

Demographic and socio-economic data collected by the U.S. Census Bureau and other agencies are highly relevant to public health and epidemiological research. However, such data are most commonly aggregated at the level of administrative boundaries or census units. It has been well documented that the choice of areal unit affects the strength and significance of statistical associations and rendering the results difficult to compare with other studies. This is known as the Modifiable Areal Unit Problem (MAUP) (Openshaw, 1984; Unwin, 1996). Currently there are no solutions to fully overcome the effects of MAUP and related methodological issues have not yet been adequately addressed. Recommendations have been made to minimize MAUP effects in statistical inference by analyzing the aggregated covariates in hierarchical levels of areal units from the finest spatial resolution possible to a coarser

resolution and to verify consistent model results (Fotheringham, 1989; Ratcliffe and McCullagh, 1999; Diez-Roux, 2000). Three hierarchical levels of census units commonly used in epidemiological studies were used in this study for identical housing and population covariates.

There were differences in the significant variables in multivariate logistic models at different areal levels likely due to MAUP; however, the number of households that lack plumbing facilities and the number of individuals in the 18-64 year age group that are below poverty line were consistent risk factors in all areal units. These and other housing and population related covariates associated with canine leptospirosis status in this study at independent areal units are indicative of lower pet-owner socio-economic conditions and lower housing standards, which are likely related. The findings reported here are similar to some of the risk factors reported in studies from Brazil (Oliveira et al., 2009; Barcellos et al., 2000; Veras et al., 1985) where more canine and human leptospirosis cases were shown to originate from poorer neighborhoods. As in this study, the vaccination status of dogs included in the studies originating from Brazil are not clear but dogs could be at higher risk in such urban environments due to pet owners failing to vaccinate their dogs and/or higher prevalence of leptospirosis in the environment due to substandard housing and other neighborhood conditions.

Dogs living within and up to 1500 meters of newly urbanized areas were significantly associated with leptospirosis status, similar to a previous report using 36 cases (Ward et al., 2004) in Indiana, U.S., where higher leptospirosis incidence was noticed among dogs that lived within 1000 meters from boundaries of cities that were classified rural in 1990 census but were urban in 2000 census. Visitations by infected wild mammal vectors were suspected as a source of leptospirosis transmission to healthy dogs in that study. Newly urbanized areas are often closer to forest/wooded areas and, sometimes, cultivated agricultural fields which are commonly

identified as risk factors for contracting leptospirosis. In addition, peridomestic wildlife vectors in these areas could play a role in disease transmission as well (Guerra, 2009). When dogs whose geocoded locations were beyond 1500 meters (2000 and 2500 meters) were added to the case/control pool there was no association with leptospirosis status, likely due to reduction in differences between case/control numbers at increased areas.

Among all public lands within an area covering 5 kilometers from 2000 census city boundaries, proximity to colleges/universities and state parks/forests were significantly associated with leptospirosis status. Land use areas representing parks/forests and college/universities are similar in that they provide ample open spaces for canine recreation and are places where high dog-to-dog and wild mammal contact could occur. However, parks/forests are relatively well drained areas compared to college/universities that have built up areas such as parking-lots and pavements with potential for water run-off, flooding, and overflow from streams nearby. Therefore, the risk of public places such as college/universities and similar anthropogenic environments may be due to flooding events.

An outbreak in human leptospirosis in a university campus was reported after flooding and embankment overflow within the campus (Gaynor et al., 2007), and one human case of leptospirosis was diagnosed after a similar flood event on another university campus (Park et al., 1998). Precipitation and flooding have been associated with increased leptospirosis incidence (Kawaguchi et al., 2008; Ward et al., 2004; Liverpool et al., 2008) and flood-prone or frequently flooded areas are risk factors for human and canine leptospirosis (Morshed et al., 1994; Karande et al., 2002; Batista et al., 2005). In addition, colleges/universities in the study region are generally found in high density neighborhoods where housing is relatively older and the resident

population comprise higher number of students that likely change year to year, factors which could play a role in higher transmission rates.

Proximity to open sewer and public waste disposal sites has been associated with human leptospirosis from other countries (Oliveira et al., 2009; Krojgaard et al., 2009; Sarkar et al., 2002). In the U.S., open sewer systems are not permitted by legislation unless they are within treatment plants. Public waste disposal sites and landfills in the study region were located beyond 5000 meters from any case/control location and away from the city boundaries; therefore, geographic features representing such areas were not included in the analysis. Proximity to storm water drainage systems in the study region, some of which are open to the environment was not associated with leptospirosis status. It is possible that the open storm water drainage systems in the study region are free of leptospira, inaccessible for direct contact, or the peridomestic animal movement around these areas could be minimal.

5. Conclusion

Poverty status among people in 18-64 year age group, houses that lack plumbing facilities, and proximity to public parks, college/universities, and newly urbanized areas are risk factors for canine leptospirosis in Kansas and Nebraska.

Acknowledgements

Support for this research was partly provided by Kansas State Diagnostic Laboratory (KSVDL) and NSF (grant No. 0919466, Collaborative Research: EPSCoR RII Track 2 Okhlahoma and Kansas: A cyber Commons for Ecological Forecasting). The authors thank Dr. Keller, Department of Regional and Community Planning, Kansas State University for valuable suggestions, and Drs. Shawn Hutchinson, Department of Geography, and Keith Gido, Division of Biology, Kansas State University, for reviewing an earlier version of the manuscript.

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Table 4. Population and housing variables evaluated in the study

Census category	Independent variables*
Housing	
Housing Units	Total housing units.
Urban and rural	Urban, rural, farm, nonfarm.
Tenure	Owner occupied, renter occupied.
Race of householder	White alone, Black or African American alone, American Indian and Alaska Native alone, Asian alone, Native Hawaiian and Other Pacific Islander alone, some other race alone, two or more races.
Household size	1-person, 2-person, 3-person, 4-person, 5-person, 6-person, 7-or-more person household.
Median number of rooms	Median number of rooms.
Year structure built	Built 1999 to March 2000, 1995 to 1998, 1990 to 1994, 1980 to 1989, 1970 to 1979, 1960 to 1969, 1950 to 1949, 1940 to 1949, Built 1939 or earlier.
Plumbing facilities	Complete plumbing facilities, lacking complete plumbing facilities.
Population	
Population	Total population.

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Family size	Average family size
Urban and rural	Urban, rural, farm, nonfarm.
Race	White alone, Black or African American alone, American Indian and Alaska Native alone, Asian alone, Native Hawaiian and Other Pacific Islander alone, some other race alone, two or more races.
Household income in 1999	Less than $10,000, $10,000 to $14,999, and thirteen other variables representing $49,999 incremental income thereof up to $199,999, and $200,000 or more.
Poverty status in 1999 by Age	Under 5 years, 5 years, 6 to 11 years, 12 to 17 years, 18 to 64 years, 65 to 74 years, 75 years and over.

* Observations for all the independent variables are counts, in continuous form, and recorded per areal unit (block group, tract or county).

Table 5. Results of multivariate logistic models ($P < 0.05$) with block group level housing and population factors associated with canine leptospirosis status in the study region (n = 94 cases, 185 controls).

Covariates	Estimate	S.E	OR	95% C.I	*P*-value
Housing:					
Year structures built (1940-1949)	0.812	0.204	2.252	1.510–3.359	0.001*
Lacking complete plumbing facilities	1.058	0.221	2.880	1.867–4.442	0.000*
6-person household	0.178	0.440	1.089	0.908–1.572	0.062
Owner occupied	-0.336	0.154	0.714	0.528–0.966	0.003*
Population:					
Poverty status in 1999 by age (18-64)	0.732	0.084	2.079	1.763–2.451	0.008*
Average family size	0.267	0.190	1.306	0.899–1.895	0.082
Two or more races	-0.522	0.343	0.593	0.302–1.162	0.065
Household income (25,000 – 29,999)	0.133	0.087	1.142	0.963–1.354	0.059
Household income (30,000 – 34,999)	-0.216	0.111	0.805	0.648–1.001	0.072

C.I. – Confidence interval.

* Significantly ($P < 0.05$) associated with leptospirosis status.

Table 6. Results of multivariate logistic models ($P < 0.05$) with census tract level housing and population factors associated with canine leptospirosis status in the study region (n = 94 cases, 185 controls).

Covariates	Estimate	S.E	OR	95% C.I.	*P*-value
Housing:					
Year structures built (1940-1949)	0.211	0.186	1.234	0.857–1.778	0.081
Built 1939 or earlier	0.142	0.098	1.152	0.951–1.396	0.063
Lacking complete plumbing facilities	0.324	0.031	1.382	1.301–1.469	0.041*
6-person household	0.211	0.146	1.234	0.927–1.644	0.057
Population:					
Poverty status in 1999 by age (18-64)	0.541	0.049	1.717	1.560–1.890	0.027*
Poverty status in 1999 by age (65-74)	0.262	0.122	1.299	1.023–1.650	0.039*
Household income (30,000 – 34,999)	-0.421	0.284	1.523	0.376–1.145	0.053

C.I. – Confidence interval.

* Significantly ($P < 0.05$) associated with leptospirosis status.

Table 7. Results of multivariate logistic models ($P < 0.05$) with county level housing and population factors associated with canine leptospirosis status in the study region (n = 94 cases, 185 controls).

Covariates	Estimate	S.E	OR	95% C.I.	*P*-value
Housing:					
Median number of rooms	0.613	0.351	1.844	0.926–3.669	0.071
Lacking complete plumbing facilities	1.065	0.078	2.900	2.489–3.380	0.002*
Owner occupied	-0.238	0.081	0.788	0.672–0.923	0.038*
Population:					
Average family size	0.463	0.021	1.588	1.524–1.655	0.011*
Poverty status by age (18-64)	0.541	0.049	1.717	1.560–1.890	0.027*
Household income (30,000 – 34,999)	1.059	0.666	2.883	0.781–10.63	0.093

C.I. – Confidence interval.

* Significantly ($P < 0.05$) associated with leptospirosis status.

Table 8. Results of multivariate regression ($P < 0.05$) with public places present within 2500 meters and canine leptospirosis status.

Covariates	Estimate	S.E.	OR	95% C.I.	*P*-value
Presence with 2500 meters:					
College/University	0.780	0.184	2.181	1.520–3.128	0.028*
Parks/Forest	0.917	0.366	2.501	1.220–5.126	0.039*
Industrial Parks	0.426	0.358	1.531	0.759–3.088	0.712

C.I. – Confidence interval.

* Significantly ($P < 0.05$) associated with leptospirosis status.

Table 9. Cochran-Mantel-Haenszel (CMH) and Breslow-Day test statistics for association between leptospirosis status and newly urbanized areas in various cities in the study region.

Buffer distance	CMH*	*P*-value	Breslow-Day	*P*-value
0	4.586	0.032	5.976	0.200
500	4.005	0.045	1.814	0.769
1000	6.598	0.010	1.794	0.773
1500	4.614	0.031	3.328	0.649
2000	0.617	0.431	2.561	0.767
2500	0.680	0.409	4.752	0.769

* CMH general association value controlling for city.

Chapter 3 - Evaluation of hydrologic risk factors for canine leptospirosis: 94 cases (2002 to 2009).

Abstract

Associations of hydrologic and soil-hydrologic variables 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 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. Hydrologic variables were derived from National Hydrographic Dataset Plus (NHD Plus), National Flood Hazard Layer (NFHL), and National Wetlands Inventory (NWI), and soil-hydrologic variables from Soil Survey Geographic database (SSURGO) around geocoded addresses of case/control locations. Multivariate logistic models were used to determine the risk of different land cover variables and address locations to dogs. Proximity to water features (O.R. = 0.828 95% C.I. = 0.795, 0.863), hydrologic density (O.R. = 2.809, 95% C.I. = 1.588, 4.969) and frequently flooded areas (OR = 4.051, 95% C.I. = 2.172, 7.555) within 2500 meters surrounding case/control locations were significant risk factors for canine leptospirosis. Necessary precaution including vaccination is recommended for dogs that live in these circumstances.

1. Introduction

Leptospirosis, an important worldwide zoonotic disease, has been suggested as a re-emerging zoonotic disease in dogs in North America (Alton, et al., 2009), although it is likely more accurate to state that the disease is endemic with fluctuations in yearly prevalence potentially associated with climate variability. Concern has also been raised about a change in prevalent serovars, although there is evidence that this serological shift is not as significant as often implied and may be more a function of the population of dogs studied. Evidence of serovars Grippotyphosa and Pomona infections in dogs in North America dates back to the 1970s and 1980s (Bishop et al., 1979; Birnbaum et al., 1998; Cole et al., 1982). Factors contributing to the perceived re-emergence or yearly fluctuations in prevalence of this disease are not completely understood but living within urban areas (Alton et al., 2009) and suburban or newly urbanized areas (Ward et al., 2004; Sykes et al., 2011), flooding events (Ward et al., 2002, 2004), contact with infected wild and peridomestic vectors (Ward et al., 2004), and less than ideal socio-economic conditions in areas where dogs reside (Reiss et al., 2008; Martins-Soares et al., 2010) are some factors that are commonly reported to play a role.

Leptospires survive best in moist soil that has a slightly alkaline or neutral pH and can thrive for long periods of time (3-4 weeks) in surface water (Smith and Turner, 1961; Burtaccio et al., 2001; Vanasco et al., 2003). Leptospirosis outbreaks in dogs primarily occur due to exposure to water contaminated with urine of an infected animal, although direct exposure to urine from small rodents, raccoons, opossums, and skunks may also be sources of infection. In both dogs and humans, exposure to an open body of water is commonly described as a source of risk for leptospirosis (Haake et al., 2002; Levett, 2001; Morgan et al., 2002). Using Geographic Information Systems (GIS) Ghneim et al. (2007) showed that hydrographic density was higher

within a 500 m radius of an infected dog's home in Northern California, and areas that were suspected to hold stagnant water such as cultivated agricultural fields and wetlands were significantly correlated with canine leptospirosis cases in the same study. Agricultural fields with stagnant water were a risk for farm workers (Sharma et al., 2006) and proximity to rivers and streams were suspected to be risk factors for human leptospirosis in Sao Palo, Brazil (Martins Soares et al., 2010).

Proximity to and the type of water body, density of hydrographic features such as streams, ponds/lakes, and wetland areas around dogs' homes, and soil hydrologic characteristics such as drainage and flooding and ponding frequency of an area are some environmental characteristics that could be used to evaluate the risk of hydrologic features in the environment for canine leptospirosis. GIS can be used to spatially analyze the significance of such environmental factors to human and animal diseases (Meade et al., 1988) and has been effectively used in the past for identifying important risk factors for canine leptospirosis (Ward et al., 2002; 2004).

The objective of the study was to analyze publicly available geographic databases using GIS to identify hydrologic 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-solid EM media. All tubes were incubated at 30° C in an ambient atmosphere incubator and evaluated for evidence of growth weekly.

2.5. Demographic Information

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

2.6. Geocoding

Household addresses with information pertaining to house number, street, city, state and zip code were provided by clients at the time specimens for leptospirosis testing were submitted. Addresses were retrospectively verified for their accuracy either by using MapQuest (Map Quest. America Online, Denver, CO) or Google Maps (Google Inc., Mountain View, CA) and/or calling telephone numbers provided by clients. Geographic coordinates for these addresses were derived using a Geocode tool in ArcMap 9.3.1 software and US Census 2007 TIGER (Topographically Integrated Geographic Encoding and Referencing system) shapefile with street level address information (US Census Bureau, 2010). The geographic coordinates for unmatched addresses were obtained using Google Earth software (version No: 5.2.1.1329) (Google Inc., Mountain View, CA). In all, geographic coordinates for 94 cases and 185 control data points in Kansas and Nebraska were obtained.

2.7. Projection and data storage

All GIS data used in this study were projected (or re-projected from their original spatial reference) in to the USA Contiguous Equidistant 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 distance measurements between case/control locations and environmental variables. All original, intermediate and processed GIS data were stored in a SQL Server/ESRI ArcSDE 9.3.1 Geodatabase.

2.8. Proximity to hydrological features and hydrologic density

Water-bodies and stream flowline data are available in public domain for the study region from National Hydrographic Dataset (NHD) Plus (Horizon Systems Corporation, Herndon, VA). NHD Plus is a value-added product developed using the NHD created by the US Geological Survey (USGS) and the US Environmental Protection Agency's (EPA) Office of Water (NHD, 2011). The USGS used a higher resolution (1:24,000) Digital Line Graph enabling the inclusion of fairly small sized water bodies into the dataset (USGS, 2010). The water-bodies dataset included polygon area features representing lakes/ponds, reservoirs, swamps and marshes, and a class of unspecified water bodies. The NHD Plus flowline data had line features including canals, ducts, and streams/rivers.

The two water feature types that were used in this study (water bodies and flowlines) are represented by different geometries and therefore they were originally presented in two disparate datasets. To estimate proximity (the distance from case/control locations to their closest water feature), and to quantify hydrologic density (the length of shorelines of all water features present within 2500 meters from cases/controls), polylines representing flowlines were converted to

polygons by applying a 0.1 meter flat-end buffer. The converted polylines were then merged with the polygon water body data using the merge tool in ArcGIS to generate a unified data layer representing all water features in the region.

Proximity and hydrologic density were estimated based on the information provided in the NHD Plus dataset on Strahler stream orders. Strahler stream ordering is a method used to classify stream segments (Strahler, 1952) based on the number of tributaries upstream, starting with classification of streams without any tributaries as first order streams, a segment downstream from the confluence of two first order streams as second order stream and so on. The following six variables were derived from NHD Plus dataset: proximity to nearest water feature with all streams included in the dataset; type of the nearest water feature with all streams included in the dataset; proximity to nearest water feature excluding streams with Strahler stream orders 'null' and '1' in the dataset; type of the nearest water feature with Strahler stream orders 'null' and '1' in the dataset removed; density of water features (shoreline length of all water features within 2500 meters from geocoded locations of cases/controls) with all streams included; and, density of water features excluding streams with Strahler stream orders 'null' and '1', in the dataset.

Estimates of flowlines that were originally represented in the dataset as line features were halved for hydrologic density estimation. The presence/absence of water in the NHD Plus streams and water bodies at specific times of a year could not be reliably verified from the available data. The distances to nearest water feature were recorded in meters and hydrologic density was recorded in percentages and these variables were kept in continuous form. The type of nearest water feature and Strahler stream order were in categorical form.

2.10. Exposure to flood hazard areas

The latest National Flood Hazard Layer (NFHL) available in public domain from the Federal Emergency Management Agency (FEMA) was used to analyze potential risk due to exposure to flood hazard areas. NFHL is the standard dataset used by the U.S. federal and state agencies for flood management, mitigation, and insurance activities and is derived based on the Digital Flood Insurance Rate Map (DFIRM) databases and Letters of Map Revision (LOMRs). Areas of flood risk in NFHL are classified into three classes as areas that are prone to 1% annual chance flood event, 0.2% annual chance flood event, and areas of minimal flood risk. The 1% and 0.2% annual chance flood event indicates area boundaries of a flood that has a 1% or 0.2% chance of being equaled or exceeded in any given year (NFHL, 2011). These areas are also generally known as the 100 or 500 year flood plains, respectively.

Thousand meter buffer areas surrounding 1% and 0.2% annual chance flood event areas were constructed in separate operations and the numbers of cases/controls within the buffer (exposed) and outside (unexposed) were recorded for comparison in logistic regressions.

2.11. Proximity to wetland areas

Geographic dataset of wetland areas for the study region is available from the National Wetlands Inventory (NWI, 2011) of U.S. Department of Fish and Wildlife Services. At the time this study was being conducted not all of wetland areas for the Kansas portion of the study region were available from the NWI. Therefore, only cases/controls that had wetland inventory data present within 2500 meter buffer areas surrounding their geocoded locations were included in the analysis (58 cases, 147 controls).

Two variables were derived, the distance to the closest wetland area boundary from geocoded case/control locations (estimated in meters), and wetland density (total perimeter length of wetland areas within 2500 meters divided by the buffer areas). Observations for all variables were in continuous format.

2.12. Soil-hydrologic characteristics

Soil-hydrologic data pertaining to flooding frequency, ponding frequency, and drainage class of soils surrounding cases/controls were obtained from the State Survey Geographic Database (SSURGO) dataset from the (United States Department of Agriculture, National Resources Conservation Service (USDA NRCS) (SSURGO, 2011). In the SSURGO dataset soil-hydrologic attributes are recorded per polygon area called 'map unit area'. Flooding frequency of a map unit refers to the annual probability of a flood event expressed as one of five classes: frequent, occasional, rare, very rare, and none. Ponding frequency is the percentage of the map unit that is subject to water being ponded on the top surface, expressed as one of four classes: 0-14%, 15-49%, 50-74% or 75-100%. The drainage class of a map unit refers to the frequency and duration of wet periods, referred to as one of six classes: excessively drained, somewhat excessively drained, moderately well drained, well drained, poorly drained, and somewhat poorly drained (SSURGO, 2011). The map unit areas are assigned any one of the many classes of a soil-hydrologic attribute at a time.

Percentage map unit areas within 2500 meters from case/control locations (total map unit area divided by area of buffer) occupied by different classes of flood frequency, ponding frequency, and drainage class were estimated in three separate steps in ArcGIS. There were five variables for flooding frequency (one each for the percentage area occupied by each flood

frequency class), four variables for ponding frequency, and six soil drainage class variables. All variables were kept in their original form as continuous variables and were percentages.

2.13. Statistical analyses

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

Odds ratios and their 95% confidence intervals derived using logistic regressions were used to determine risk of hydrologic variables to dogs. Hydrologic variables derived from the three thematic datasets (NHD Plus, NFHS and NWI) were screened for association with leptospirosis status by fitting individual bivariate logistic regressions, and variables with $P < 0.1$ were selected for further analysis. A multicollinearity test was conducted among all screened variables by estimating the variance inflation factor (VIF) (variables with a VIF > 10 were considered to indicate multicollinearity) (Dohoo et al., 2003). Multivariate stepwise logistic regression models were fitted using a significance level, $P \leq 0.05$ for variable entry and $P > 0.10$ for a variable to be removed from the model. All models were ranked using Akaike Information Criterion (AIC) value and the model with lowest AIC value was deemed to be the best fitting model. The model performance was measured using deviance chi-squared goodness-of-fit test ($P < 0.05$ indicates poor fit) and the predictive ability of the model was evaluated using the area under Receiver Operating Characteristic (ROC) curve value. Confounding effects of host factors, age group of dogs (< 1 year old as reference category), sex (female as reference category), and breed (unknown or unspecified as reference category) on predictor variables were estimated by including them one at a time in the final logistic model. If such inclusion increased

the coefficients of explanatory variables by at least 10% or more then the adjusted ORs were recorded from those models.

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

3. Results

There were 94 dogs that were identified as cases based on a positive PCR (n=90 dogs), 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 median distance to nearest water feature with all Strahler stream orders in the dataset was 373 meters for cases and 389 meters for controls. When Strahler stream order 'null' was removed, the median distance to nearest water feature was 455, and 466 meters for cases and controls. The distance between cases and controls to their nearest water feature was 664 and 922 meters when Strahler strem orders 'null' and '1' were removed.

Many hydrological and soil-hydrological variables were associated with canine leptospirosis status at $P < 0.1$ significance level (Table 10) but only proximity to water features (excluding streams with Strahler stream orders 'null', '1') (O.R. = 0.828 95% C.I. = 0.795, 0.8630), hydrologic density (excluding streams with Strahler stream orders 'null', '1') (O.R. = 2.809, 95% C.I. = 1.588, 4.969) and flooding frequency (frequent) of soil map unit areas (henceforth frequently flooded areas) within 2500 meters surrounding case/control locations (OR = 4.051, 95% C.I. = 2.172, 7.555) were retained as significant variables ($P < 0.05$) at the end of multivariate logistic regression (Table 11). No other variables evaluated in the study 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 independent 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 for the final model was noted as 0.78.

4. Discussion

The purpose of this study was to evaluate various hydrologic and soil-hydrologic features in Kansas and Nebraska as potential risk factors for leptospirosis in dogs. Leptospirial transmission is usually mediated through urine-contaminated water, although direct transmission also occurs, so that proximity, distribution and density of water can be considered as an important factor in the spread of disease. Leptospiral spirochetes can survive on surface water and also in moist soil, such as riverbanks and channels, whereas their survival in cold and dry environments is poor (Andre-Fontaine, 2006). Since the survival of spirochetes in water and soil is limited, however, reservoir hosts that maintain the infection in an environment are important for re-inoculation of water bodies. These contaminated water bodies then become important

sources of infection for man and their pets, particularly dogs, and leptospirosis becomes an occupational (agricultural, sewage and meat processing plant workers) and recreational hazard (boating, triathlon) (Padre et al., 1988; CDC, 1998; Morgan, et al., 2002; Meites et al., 2004; Narita, et al., 2005). Not surprisingly, this study identified three water-related risk factors for canine leptospirosis: proximity to water features, hydrographic density, and frequently flooded areas.

As in this study, proximity to water features have been identified as risk factors for other disease systems such as canine blastomycosis where studies have repeatedly shown that cases often occur within 400 meters of a water body (Baumgardner et al., 1995; Arceneaux et al., 1998; Baumgardner et al., 2005; Chen et al., 2008). Many of these studies suspect dogs contract the fungal spores (*Blastomyces dermatitidis*) when they are near water features during recreation or supervised exercise. A similar transmission could occur in the case of leptospira to dogs in areas where wet soil or standing water is present near water features. This, in addition to the movement of infected wildlife reservoirs that live in the vicinity of water features, may be reasons why proximity to water features is an important risk factor for dogs.

The finding that proximity to water features was a risk factor to dogs is in contrast to Ward et al., (2004) where case/control presence within 1000 meters from streams were not noted as a risk factor. The difference noted in these studies could be due to the type of water feature dataset used for analyzing the risk and differences in study region among other causes. Proximity to water features was not significant in the present study when distances from case/control locations were measured to all water features originally present in NHD Plus dataset. However, they were revealed as significant risk factor when streams whose Strahler

stream orders were 'null' and '1' were removed. Also, water bodies such as ponds and lakes were included in the proximity analysis here.

Frequently flooded areas within 2500 meter areas surrounding dogs' homes was noted as a risk factor for leptospirosis, although presence of dogs' residences within 0.1% chance flood event areas (100 yr. flood plains) was not a risk in this study. It is possible that the difference noted could be due to the differences in areas represented as frequently flooded in these two datasets: the map unit areas represented in SSURGO are in much finer scale and are based on finer spatial resolution data. Numerous studies have shown associations between human and canine leptospirosis with flood events and/or flooded areas (Vanasco et al., 2003; Kawaguchi et al., 2008; Liverpool et al., 2008), and it is likely that these risks are due to increased leptospira prevalence in frequently flooded areas in the days following rainfall and flooding events and higher rate of transmission from frequently flooded areas to dogs either due to dogs contracting them directly or indirectly from infected wildlife and peridomestic vectors (Ward et al., 2004). Frequently flooded areas within urban boundaries may include impervious surfaces and stream banks and are especially important in the context of leptospirosis transmission due to higher density of dogs and peridomestic wildlife that live there. Urban and semi-urban areas have been reported as risk factors for dogs (Ward et al., 2004; Alton et al., 2009; Sykes et al., 2011), and Ganoza et al., (2010) noted higher concentrations of pathogenic leptospira spirochetes in water samples collected from Amazonian urban surface waters compared with rural samples.

Hydrologic density within 2500 meters from dogs' homes was a significant risk in this study similar to the study by Ghneim et al., (2008) where hydrologic density within 500 meters surrounding dogs' residences in northern California were significantly associated with leptospirosis. Hydrologic density was not a risk factor beyond 500 meters in that study. Both

studies have used similar data sources, NHD Plus here and NHD in Ghneim et al., (2008); however, minor streams were removed from the dataset in this study and water bodies' data were included. In this study, shoreline length was used in estimating hydrologic density instead of area of water features, as in the study by Ghneim et al., (2008), since shorelines represent relatively precise account of the available access to water for dogs. Use of shoreline length also allowed the incorporation of flowline data in the analysis whose area measurements are otherwise unavailable in public datasets and are difficult to estimate because of natural fluctuations in streams size and direction over time. This, in addition to differences in geographic locations of the studies and case selection methods, may have contributed to the differences noted. Increased hydrologic density surrounding dogs' residences may also be indicative of more areas with frequent flood risk.

Other reports have used different buffer sizes in their evaluation of canine leptospirosis associations with 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.

Proximity to water feature and hydrologic density were not significant when the NHD Plus dataset included all streams in the original dataset possibly due to the small stream segments acting as statistical noise. Flowline data in NHD are created using several overlapping 30 meter Digital Elevation Models (DEMs). Due to the relatively high resolution of the dataset and choices that are typically made during the stream derivation process many low lying areas on the

earth's surface are classified as streams which may not exist on ground. There was a concern that many could be false representations of streams (Fig. 2), especially the smaller line features present in the dataset. In addition, there was no ground-truth data available for NHD Plus that verified the actual presence or accuracy of the smaller streams. Therefore, the analyses in this study were based on Strahler stream order information.

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 environmental variables with canine leptospirosis including similar hydrological features, 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).

5. Conclusions

Proximity to water features, hydrologic density, and frequently flooded areas are risk factors for canine leptospirosis.

Acknowledgements

Support for this research was partly provided by Kansas State Veterinary Diagnostic Laboratory (KSVDL) and NSF (grant No. 0919466, Collaborative Research: EPSCoR RII Track 2 Oklahoma and Kansas: A cyber Commons for Ecological Forecasting). The authors thank Drs. Shawn Hutchinson, Department of Geography, and Keith Gido, Division of Biology, Kansas State University, for reviewing an earlier version of the manuscript.

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Table 10. Significant ($P < 0.1$) variables associated with canine leptospirosis status in the study region.

Variable	Coefficient	S.E.	O.R.	*P*-value	95% C.I.
NHD Plus:					
Proximity to nearest water feature -	-0.280	0.044	0.810	0.027	0.743, 0.880
(excluding streams with Strahler stream orders ‘null’, ‘1’)					
Type of nearest body of water -	2.113	0.916	8.273	0.091	1.373, 49.817
(excluding streams with Strahler stream orders ‘null’, ‘1’)					
Unknown	reference category				
Hydrologic density -	1.041	0.291	2.832	0.027	1.601, 5.009
(excluding streams with Strahler stream orders ‘null’, ‘1’)					
NFHL:					
Presence within 0.1 chance flood event boundary	-0.921	0.666	0.398	0.074	0.107, 1.468
NWI:					

14	Proximity to nearest wetland boundary	-0.821	0.666	0.439	0.074	0.119, 1.623
15	SSURGO:					
16	Frequently flooded areas	1.217	0.318	3.377	0.021	1.810, 6.298

17 Observations of all variables were kept in a continuous format except type of water feature (excluding streams with Strahler stream
18 orders 'null', '1') which was categorical.

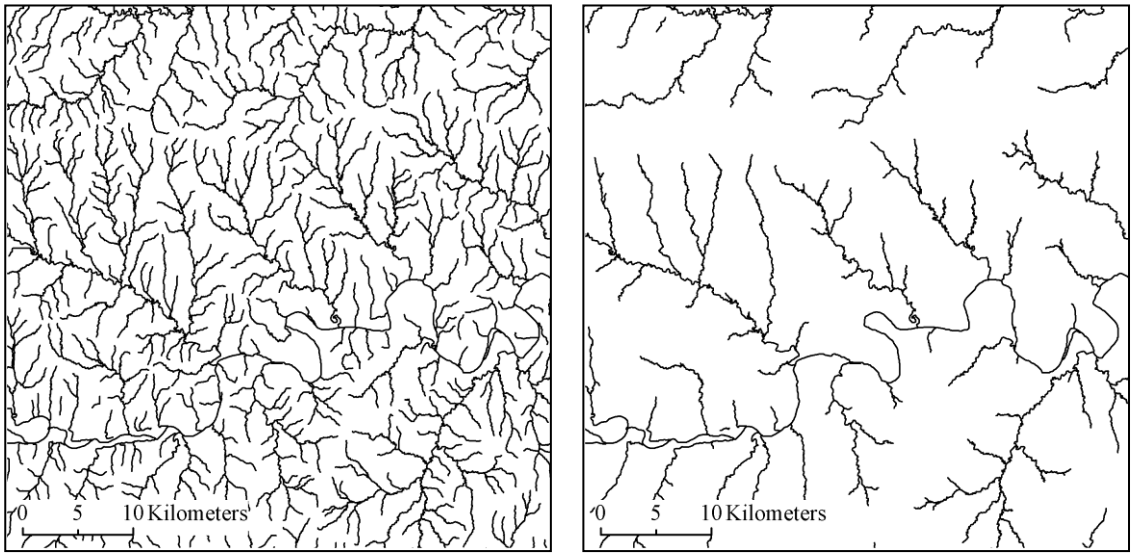
Table 11. Results of multivariate logistic regression for association of hydrological variables with canine leptospirosis status ($P < 0.05$) in the study region.

Variable	Coefficient	S.E.	O.R.	*P*-value	95% C.I.
Proximity to nearest water feature - (excluding streams with Strahler stream orders 'null', '1')	-0.188	0.021	0.828	0.033	0.795, 0.863*
Hydrologic density - (excluding streams with Strahler stream orders 'null', '1')	1.033	0.291	2.809	0.008	1.588, 4.969 *
Proximity to nearest wetland boundary	-0.788	0.666	0.454	0.088	0.123, 1.677
Frequently flooded areas	1.399	0.318	4.051	0.029	2.172, 7.555*

* Significantly associated ($P < 0.05$) with leptospirosis status.

Observations of all variables were in continuous format, and are distances measured in meters for proximity variables and percentages for hydrologic density (total water feature length divided by 2500 meter buffer area) and frequently flooded areas (total map unit areas classified as flood frequency (frequent) within 2500 meter buffer area). Area under ROC (Receiver Operation Characteristic) curve for the model was noted as 0.88.

Figure 2. A map representation of NHD Plus stream network data in Riley County, KS. The picture on left frame shows stream network of all Strahler stream orders, and the picture on right frame shows stream network without Strahler stream orders labeled ‘null’ and ‘1’ in the dataset.



Conclusions

Several risk factors for canine leptospirosis have been identified in this study, many of which have not been previously reported and some that are consistent with findings in other studies. High intensity urban areas, medium intensity urban areas and urban areas in general; evergreen forests and forest/woodlands in general;, houses lacking complete plumbing facilities and poverty status by age (18-64); living within 2500 meters of a university/college or, and parks/forests; and proximity to water features, hydrologic density, and frequently flooded areas were all identified as significant risk factors for canine leptospirosis. Dogs living in such areas or circumstances should be vaccinated to prevent and control canine leptospirosis.

GIS is a powerful and effective tool for identifying canine leptospirosis risk factors. This study shows that the identification of risk factors from the surrounding environment for diseases such as canine leptospirosis could be influenced by factors such as changing the spatial extent of the study region, Modifiable Areal Unit Problem (MAUP), and data quality. Careful evaluation of the area of influence for a given disease, evaluation of the strength of association of variables at multiple spatial scales, and careful examination of publicly available data and their further manipulation could help overcome these issues.

All of the risk factors identified in this study are based upon global logistic models covering the entire Central Great Plains region. However, it is likely for some of the risk factors to be more important in some areas than others. Geographically weighted regression could be used to identify specific drivers of the disease at specific areas in the study region.