# Neighborhood-level socioeconomic and urban land use risk factors of canine leptospirosis: 94 cases (2002–2009)

R. K. Raghavan, K. M. Brenner, J. J. Higgins, J. M. Shawn Hutchinson, K. R. Harkin



**Published Version Information**

**Citation**: Raghavan, R. K., Brenner, K. M., Higgins, J. J., Hutchinson, J. M. S., & Harkin, K. R. (2012). Neighborhood-level socioeconomic and urban land use risk factors of canine leptospirosis: 94 cases (2002–2009). Preventive Veterinary Medicine, 106(3-4), 324-331.



**Digital Object Identifier (DOI)**: doi:10.1016/j.prevetmed.2012.04.003

**Publisher's Link**: http://www.sciencedirect.com/science/article/pii/S0167587712001341

Title: Neighborhood-level socioeconomic and urban land use risk factors of canine leptospirosis. 94 cases (2002–2009).

R.K. Raghavan[a,*], K.M. Brenner[b], J.J. Higgins[c], J.M. Shawn Hutchinson[d], K.R. Harkin[b].

[a] Kansas State Veterinary Diagnostic Laboratory, College of Veterinary Medicine, Kansas State University, Manhattan, KS 66506–5701.

[b] Department of Clinical Sciences, College of Veterinary Medicine, Kansas State University, Manhattan, KS 66506–5701.

[c] Department of Statistics, College of Arts and Sciences, Kansas State University, Manhattan, KS 66506–5701.

[d] Department of Geography, College of Arts and Sciences, Kansas State University, Manhattan, KS 66506–5701.

[*] Corresponding author:

R.K. Raghavan

Kansas State Veterinary Diagnostic Laboratory

College of Veterinary Medicine

Manhattan, KS. 66506–5701

Tel.: 785 532 2492

Fax., 785 532 4481

E–mail address: rkraghavan@vet.k-state.edu

Abstract:

Associations of housing, population, and agriculture census variables, and presence near public places 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. Multivariable logistic regressions revealed different risk factors among different census units; however, houses lacking complete plumbing facilities [OR = 2.80, 95% C.I = 1.82, 4.32 (census unit, block group); OR = 1.36, 95% C.I. = 1.28, 1.45 (census tract); and, OR = 3.02, 95% C.I. = 2.60, 3.52 (county)]; and poverty status by age (18–64) [OR = 2.04, 95% C.I = 1.74, 2.39 (block group); OR = 1.53, 95% C.I. = 1.41, 1.67 (census tract); and, OR = 1.62, 95% C.I. = 1.50, 1.76 (county)] were consistent risk factors for all census units. Living within 2500 m of a university/college and parks/forests were also significantly associated with leptospirosis status in dogs. Dogs that live under these circumstances are at higher risk for leptospirosis and pet owners should consider vaccination.

Key words: Leptospirosis, Canine, Socio-economic status, Modifiable Areal Unit Problem (MAUP), Geographic Information Systems (GIS).

## 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 climates, 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 and Gauthier, 2003).

In studies that are mainly reported from South America the 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 Soares 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 other South

American countries 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, and 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 public or open land that provide recreational opportunities (Ghneim et al., 2007) and living within 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 use/land cover area surrounding up to 2500 m from dogs' residences were analyzed (Raghavan et al., 2011). 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 retrospective case-control 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, living within the proximity of certain public 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 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 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 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., 2003). 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 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; the client's street address at the time of sample submission, breed and sex.

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, 2011). The geographic coordinates for unmatched addresses (8%) were obtained using Google Earth software (version No: 5.2.1.1329) (Google Inc., Mountain View, CA). In all, geographic coordinates for 94 (out of 97) cases and 185 (out of 197) control data points in Kansas and Nebraska were obtained.

2.7. Host factors

Observations were grouped into five age groups < 1 y, 1 to 4 y, 4 to 7 y, 7 to 10 y and > 10 y; two sexes and 77 individual breeds, including mixed breeds and unknown or unspecified breeds were kept without grouping as a categorical variable.

2.8. Projection and data storage:

GIS datasets used in this study were projected (or re-projected from their original spatial reference) in to 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.

2.9. Census data:

U.S. Census 2000 data on population and housing were obtained in the form of Summary File 3 (SF–3) tables from the U.S. Census Bureau (U.S. Census Bureau, 2011). Identical census attribute information for Kansas and Nebraska were gathered at three geographic levels or 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. Each census category included several independent variables and they were evaluated separately in the study. 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.

### 2.10. Agricultural census:

Agricultural census data for Kansas and Nebraska was obtained per county from the USDA National Agricultural Statistics Service (NASS) (USDA, 2011). Six county level agricultural census data were obtained from NASS in a tabular format, including the total number of cattle farms, total number of swine farms, the total number of dairy cattle, total number of beef cattle, the number of pigs and the number of hogs per county in year 2007.

### 2.11. Presence near public places:

Polygon areas representing ten different 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 m from dogs' homes in the study region were obtained from the US Census 2000 TIGER/Line dataset.

Buffered areas extending 2500 m from the boundaries of public places were created and cases/controls located completely outside (coded ‘0’) and within (coded ‘1’) the buffers were recorded independently for each public place type. Ten variables, representing location within 2500 m from every public place were thus derived.

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 and outside 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) or R Statistical Package 2.11.1 (R Core Development Team, 2011) when specified. During the exploratory spatial analysis of case/control locations in the study region clustering among cases and controls were evaluated using Cuzick-Edwards $K^{th}$ neighbor statistic (Cuzick and Edwards, 1990) within six major cities in the study region, including Manhattan, Wichita, Topeka and Kansas City in Kansas, and Omaha and Lincoln in Nebraska. Four neighbors were included in the analysis for cluster detection.

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 among all variables was done by fitting univariable logistic models and those variables with a *P*-value $\leq 0.1$ were selected for further analysis; however, care was taken not to remove variables that were deemed clinically relevant (Hosmer and Lemeshow, 2000; Ward et al., 2004; Raghavan et al., 2011). Multicollinearity was tested among screened variables by estimating the variable inflation factor (VIF) using the proc reg/tol vif option in SAS (SAS Institute Inc., Cary, NC). All variables with a VIF value of 10 or above were considered to indicate multicollinearity (Allison, 1999).

Multivariable logistic regression was conducted using screened variables in three separate steps with variables from each census unit at a time along with variables from other groups (agricultural census variables at county level and, location within 2500 m from public places). Observations for all census variables were kept in their original measurement units and were continuous. Observations for presence within 2500 m from public places were in categorical format scored as '0' if absent and '1' if present. Interaction terms were not included in the models.

Multivariable logistic models with events/trials operand were fit using the stepwise selection procedure in which a significance level, $P \leq 0.05$ was used for a variable to be retained and $P \geq 0.1$ to be removed from the model (SAS, 2011). Logistic models were ranked using Akaike Information Criterion (AIC) and the model with the lowest AIC value was deemed to be the best fitting model. Any confounding effect of host factors, age (< 1 y old as reference level), sex (female as reference level), and breed (unknown or unspecified as reference level) was estimated by adding them one at a time to the final logistic model, and a 10% or more change 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. Linearity assumption for logit in final models was assessed using Box-Tidwell test (Box and Tidwell, 1962). Model adequacy was tested using chi-squared goodness-of-fit test ($P$ <

0.05 indicated poor fit), and predictive ability measured by deriving the Area under Receiver's operator's characteristic (ROC) curve value.

Spatial autocorrelation if present in the case/control data could lead to the violation of underlying logistic regression assumptions (that the samples are independent and identically distributed) and will yield incorrect parameter estimates and error term. If the parameters in the multivariable model did not account for autocorrelation then the residuals of the model will 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 and Diggle, 2001; Ribeiro et al., 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.

Among 94 cases and 185 controls evaluated in this study, a majority had their physical addresses located in the city of Wichita [33.68%, 28.81% (case, control)] followed by Manhattan (13.82%, 19.45%), Lincoln (10.52%, 8.96%), Omaha (9.47%, 5.24%), Kansas City (6.31%, 4.62%) and Topeka (6.31%, 5.94%). All remaining cases (19.89%) and controls (26.98%) had rural addresses or they were from smaller cities in the study region.

Since there could be a bias in case reporting to hospitals from certain neighborhoods than others due to income differences, it was essential to verify if cases/controls showed any tendency to cluster in any of the major cities in the study region. However, no clustering was observed in any of the cities (where income levels among neighborhoods could vary). The Cuzick-Edwards estimates for case locations in Manhattan ($P$ = 0.19), Wichita ($P$ = 0.41), Topeka ($P$ = 0.24), Kansas City ($P$ = 0.28), Lincoln ($P$ = 0.31), and Omaha ($P$ = 0.47) did not indicate any clustering. Similarly, the Cuzick-Edwards estimates for control locations in Manhattan ($P$ = 0.05), Wichita ($P$ = 0.26), Topeka ($P$ = 0.36), Kansas City ($P$ = 0.19), Lincoln ($P$ = 0.18), and Omaha ($P$ = 0.22) did not indicate any clustering as well.

There were differences in the number and types of significant housing and population variables identified in logistic models fit with covariates from different census units (Tables 2–4). When block group level housing and population variables were analyzed along with agricultural census and public places variables, the housing related variables significantly associated with leptospirosis status in the logistic model were; the total number of structures built during the years (1940–1949) and the number of households lacking complete plumbing facilities (houses lacking hot and cold piped water, a flush toilet, and a bathtub or shower). Significant population related covariates associated with leptospirosis status in the logistic model were 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). Presence within 2500 m from university/college campuses and park/forest areas were significantly associated with leptospirosis status in dogs (Table 2).

When census tract level housing and population variables were analyzed along with agricultural census and public places variables, the only housing related covariate significantly associated with leptospirosis status in the logistic model was the number of households lacking complete plumbing facilities, and the only population related covariate significantly associated with leptospirosis status in the logistic model was poverty status in 1999 by age (18–64). Presence within 2500 m from university/college campuses and park/forest areas were significantly associated with leptospirosis status in dogs (Table 3).

Using county level housing and population variables along with agricultural census and public places variables, the housing related covariates significantly associated with leptospirosis status in the logistic model were the number of households lacking complete plumbing facilities and the number of owner occupied homes. The only population related covariate significantly associated with leptospirosis status in the logistic model was poverty status in 1999 by age (18–64) (Table 4). Presence within 2500 m from university/college campuses was marginally significant, and park/forest areas were significantly associated with leptospirosis status in dogs.

Two 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 multivariable logistic model. 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, and non-linearity in logit and residual autocorrelation was absent. Confounding effects of age, breed, and sex were not noted for any models.

## 4. Discussion:

The lack of clustering of cases and controls in any of the major cities in the study region indicate a lack of sample bias for low income vs. high income neighborhoods in the study population. In addition, the issue of referral bias is usually encountered in case-control studies with the diagnostic laboratories receiving relatively higher numbers of cases from immediate neighboring areas due to proximity and familiarity with the facility. However, the referrals in this study originated from all major cities and rural areas in the study region, and in addition, 25.6% of the study population included dogs that were diagnosed by the primary care veterinarian outside KSVDL. The number of days that the dogs lived in their owner's household was not provided to us during case submissions. For the purposes of this study, it was assumed that the dogs spent most of their lives in their owners' households except for those times spent outside during recreation and/or supervised exercise.

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/areal units (Fig. 1). It has been well documented that the choice of areal unit could affect the strength and significance of statistical associations and renders 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 census variables in multivariable logistic models at different areal levels (block group, census tract, and county) 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 variables associated with canine leptospirosis status 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.

Among all public lands within an area covering 5 km from 2000 census city boundaries, proximity to colleges/university campuses and state parks/forests were significantly associated with leptospirosis status (when analyzed along with county level census data, the significance value of college/university campus was slightly over $\alpha = 0.05$). 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 and there is potential for water run-off, flooding and overflow from streams nearby. Therefore, the risk of public places such as college/universities and similar 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., 2006). 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, college/university campuses 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 and whose income levels are typically low, 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 m 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, and likely other regions in the world as well. Pet owners living under such neighborhood characteristics and treating veterinarians should consider vaccination for their dogs in order to prevent leptospirosis.

Acknowledgements:

Support for this research was partly provided by Kansas State University Diagnostic Laboratory and NSF (grant No. 0919466, Collaborative Research: EPSCoR RII Track 2 Okhlahoma and Kansas: A cyber Commons for Ecological Forecasting). The authors thank Dr. Michael Ward, Faculty of Veterinary Science, University of Sydney, Dr. John Keller, Department of Regional and Community Planning, Kansas State University, Dr. Keith Gido, Division of Biology, Kansas State University, and two anonymous reviewers for their valuable suggestions to improve earlier versions of the manuscript.

References:


Allison, P.D., 1999. Logistic regression using the SAS system. Theory and application. SAS Institute Inc., Cary, NC.

Bakoss, P., Machacova, E., Jarekova, J., 2007. Results of surveillance of human leptospirosis. Epidemiologie Mikrobiologie Imunologie 56, 140–149.

Barcellos, C., Sabroza, P.C., 2000. Socio-environmental determinants of the leptospirosis outbreak of 1996 in western Rio de Janeiro: a geographical approach. International Journal of Environmental Health Research 10, 301–313.

Batista, C.S.A., Alves, C.J., Azevedo, S.S., Vasconcellos, S.A., Morais, Z.M., Clementino, I.J., Alves, F.A.L., Lima, F.S., Neto, J.O.A., 2005. Seroprevalence and risk factors for leptospirosis in dogs from Campina Grande, State of Paraiba, Brazil. Arquivo Brasileiro de Medicina Veterinaria e Zootecnia 57, 179–185.

Box, G.E.P., Tidwell, P.W., 1962. Transformation of the independent variables. Technometrics 4, 531-550.

Cruz, L.S., Vargas, R., Lopes, A.A., 2009. Leptospirosis: a worldwide resurgent zoonosis and important cause of acute renal failure and death in developing nations. Ethnicity & Disease 19, 37–41.

Cuzick, J., Edwards, R. Spatial clustering for inhomogeneous populations. Journal of the Royal Statistical Society Series B 52, 73–104.

Demers, R.Y., Frank, R., Demers, P., Clay, M., 1985. Leptospiral exposure in Detroit rodent control workers. American Journal of Public Health 75, 1090–1091.

Diez Roux, A. V., 2000. Multilevel analysis in public health research. Annual Review of Public Health 21, 171–192.

Everard, C.O.R., Hayes, R.J., Edwards, C.N., 1989. Leptospiral infection in school-children from Trinidad and Barbados. Epidemiology and Infection 103, 143–156.

Faine, S., Adler, B., Bolin, C., Perolat, P., 2000. Leptospira and leptospirosis. 2nd edition. MediSci, Melbourne.

Feigin, R.D., Lober, L.A.J., Anderson, D., Pickering, L., 1973. Human leptospirosis from immunized dogs. Annals of Internal Medicine 79, 777–785.

Fotheringham, S., 1989. Scale independent spatial analysis. In: Goodchild, M.F., Gopal, S., (Eds.), The accuracy of spatial databases. 1994Taylor and Francis, London, pp. 221–228.

Gaynor, K., Katz, A.R., Park, S.Y., Nakata, M., Clark, T.A., Effler, P.V., 2007. Leptospirosis on Oahu: An outbreak associated with flooding of a University Campus. American Journal of Tropical Medicine and Hygiene 76, 882–885.

Ghneim, G.S., Viers, J.H., Chomel, B.B., Kass, P.H., Descollonges, D.A., Johnson, M.L., 2007. Use of a case-control study and geographic information systems to determine environmental and demographic risk factors for canine leptospirosis. Veterinary Research 38, 37–50.

Greene, C.E., 2006. Laboratory diagnosis of canine leptospirosis and babesiosis. 24th Annual ACVIM Forum, Louisville, Kentucky, USA, 31 May-3 June, 2006., 490–491.

Harkin, K.R., Roshto, Y.M., Sullivan, J.T., 2003. Clinical application of a polymerase chain reaction assay for diagnosis of leptospirosis in dogs. Journal of the American Veterinary Medical Association 222, 1224–1229.

Hosmer, D.W., Lemeshow, S., 2000. Model-building strategies and methods for logistic regression. In: Applied Logistic Regression. Second Edition. Eds: Hosmer, D.W., Lemeshow, S. pp: 91-142. John Wiley & Sons, New York.

Karande, S., Kulkarni, H., Kulkarni, M., De, A., Varaiya, A., 2002. Leptospirosis in children in Mumbai slums. Indian Journal of Pediatrics 69, 855–858.

Kawaguchi, L., Sengkeopraseuth, B., Tsuyuoka, R., Koizumi, N., Akashi, H., Vongphrachanh, P., Watanabe, H., Aoyama, A., 2008. Seroprevalence of leptospirosis and risk factor analysis in flood-prone rural areas in Lao PDR. American Journal of Tropical Medicine and Hygiene 78, 957–961.

Krojgaard, L.H., Villumsen, S., Markussen, M.D.K., Jensen, J.S., Leirs, H., Heiberg, A.C., 2009. High prevalence of Leptospira spp. in sewer rats (*Rattus norvegicus*). Epidemiology and Infection 137, 1586–1592.

Liverpool, J., Francis, S., Liverpool, C.E., Dean, G.T., Mendez, D.D., 2008. Leptospirosis: case reports of an outbreak in Guyana. Annals of Tropical Medicine & Parasitology 102, 239–245.

Martins Soares, T.S., Dias de Oliveira Latorre, M.d.R., Laporta, G.Z., Buzzar, M.R., 2010. Spatial and seasonal analysis on leptospirosis in the municipality of Sao Paulo, Southeastern Brazil, 1998 to 2006. Revista de Saude Publica 44, 283-291.

Morshed, M.G., Konishi, H., Terada, Y., Arimitsu, Y., Nakazawa, T., 1994. Seroprevalence fo leptospirosis in rural flood prone district of Bangladesh. Epidemiology and Infection 112, 527–531.

Oliveira, D.S.C., Guimaraes, M.J.B., Portugal, J.L., Medeiros, Z., 2009. The socio-demographic, environmental and reservoir factors associated with leptospirosis in an urban area of north-eastern Brazil. Annals of Tropical Medicine and Parasitology 103, 149–157.

Openshaw, S., 1984. The modifiable areal unit problem, Concepts and Techniques in Modern Geography No. 28, Geo Books, Norwich.

Park, S.Y., Effler, P.V., Nakata, M., Sasaki, D., Katz, A.R., Clark, T.A., Gaynor, K., 2006. Leptospirosis after flooding of a university campus - Hawaii, 2004. Morbidity and Mortality Weekly Report 55, 125–127.

R Core Development Team, 2011. R: A language and environment for statistical computing, reference index version 2.11.1. R Foundation for Statistical Computing, Vienna, Austria.

Raghavan, R., Brenner, K., Higgins, J., Van der Merwe, D., Harkin, K.R., 2011. Evaluations of land cover risk factors for canine leptospirosis: 94 cases (2002–2009). Preventive Veterinary Medicine 101, 241–249.

Ratcliffe, J. H., McCullagh, M. J., 1999. Hotbeds of crime and the search for spatial accuracy

Geographical Systems 1, 385–398.

Ribeiro, P.J., Christensen, O.F., Diggle, P.J., 2003. geoR and geoRglm: Software for model based geostatistics. In: Hornik, K., Leisch, F., Zeileis, A. (Eds.), 3rd International Workshop on Distributed Statistical Computing (DSC 2003), Vienna, Austria.

Ribeiro, P.J., Diggle, P.J., 2001. geoR: a package for geostatistical analysis., R-News. Vienna, pp. 15–18.

Richardson, D.J., Gauthier, J.L., 2003. A serosurvey of leptospirosis in Connecticut peridomestic wildlife. Vector-Borne and Zoonotic Diseases 3, 187–193.

Robinson, T.P., 2000. Spatial statistics and geographical information systems in epidemiology and public health. Advances in Parasitology 47, 81–128.

SAS, 2011. SAS/STAT(R) 9.2 User's Guide. Second Edition. http://support.sas.com/documentation/cdl/en/statug/63033/HTML/default/viewer.htm#statug_logistic_sect010.htm

Sarkar, U., Nascimento, S.F., Barbosa, R., Martins, R., Nuevo, H., Kalafanos, I., Grunstein, I., Flannery, B., Dias, J., Riley, L.W., Reis, M.G., Ko, A.I., 2002. Population-based case-control investigation of risk factors for leptospirosis during an urban epidemic. American Journal of Tropical Medicine and Hygiene 66, 605–610.

USDA, 2011. National Agricultural Statistics Service. http://www.nass.usda.gov/

U.S. Census Bureau, 2011. U.S. Census Bureau, State and County Quick facts.

http://quickfacts.census.gov/qfd/meta/long_58575.htm

Unwin, D. J., 1996. GIS, spatial analysis and spatial statistics, Progress in Human Geography 20, 540–441.

Veras, F.M.F., Rouquayrol, M.Z., Gomes, I.L.D.P., 1985. Epidemiological study of the leptospirosis cases observed in Fortaleza, Brazil during the epidemic of . Revista de Medicina da Universidade Federal do Ceara 25, 55–62.

Vinetz, J.M., Glass, G.E., Flexner, C.E., Mueller, P., Kaslow, D.C., 1996. Sporadic urban leptospirosis. Annals of Internal Medicine 125, 794–798.

Ward, M.P., Guptill, L.F., Wu, C.C., 2004. Evaluation of environmental risk factors for leptospirosis in dogs: 36 cases (1997–2002). Journal of the American Veterinary Medical Association 225, 72–77.

Woo, T.H.S., Patel, B.K.C., Smythe, L.D., Symonds, M.L., Norris, M.A., Dohnt, M.F., 1997. Identification of pathogenic Leptospira genospecies by continuous monitoring of fluorogenic hybridization probes during rapid-cycle PCR. Journal of Clinical Microbiology 35, 3140–3146.

Table 1. Population and housing variables from US Census Bureau SF–3 data 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* | |

Continued next page.,

| | |
|---|---|
| Population | Total population. |
| 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). Each census category included several independent variables and they were evaluated separately in the study (for example, seven independent variables for the census category, Poverty status in 1999 by Age were evaluated).

Definitions of different census variables can be found from their source (U.S. Census Bureau) website at: http://www.census.gov/main/www/glossary.html

Table 2. Results of multivariable logistic models ($P < 0.05$) with block group level housing and population variables along with variables of agricultural census and public places associated with canine leptospirosis status in the study region (n = 94 cases, 185 controls).

| Covariates | Estimate | S.E | OR | 95% C.I | *P*-value |
|---|---|---|---|---|---|
| Year structures built (1940–1949) | 0.80 | 0.20 | 2.22 | 1.50, 3.30 | 0.00* |
| Lacking complete plumbing facilities | 1.03 | 0.22 | 2.80 | 1.82, 4.32 | 0.00* |
| Household income (30,000–34,999) | 0.12 | 0.08 | 1.13 | 0.95, 1.34 | 0.07 |
| 6–person household | 0.11 | 0.44 | 1.11 | 0.47, 2.64 | 0.09 |
| Poverty status in 1999 by age (18–64) | 0.71 | 0.08 | 2.04 | 1.74, 2.39 | 0.00* |
| University/college | 0.39 | 0.17 | 1.49 | 1.05, 2.11 | 0.04* |
| Park/forest | 0.86 | 0.36 | 2.37 | 1.17, 4.82 | 0.02* |

C.I. – Confidence interval (low, high).

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

Area under ROC curve value = 0.71.

Table 3. Results of multivariable logistic models ($P < 0.05$) with census tract level housing and

population variables along with variables of agricultural census and public places associated with canine leptospirosis status in the study region (n = 94 cases, 185 controls).

| Covariates | Estimate | S.E | OR | 95% C.I. | *P*-value |
|---|---|---|---|---|---|
| 6–person household | 0.18 | 0.14 | 1.20 | 0.90, 1.59 | 0.06 |
| Lacking complete plumbing facilities | 0.31 | 0.03 | 1.36 | 1.28, 1.45 | 0.04* |
| Poverty status in 1999 by age (18–64) | 0.43 | 0.04 | 1.53 | 1.41, 1.67 | 0.02* |
| Poverty status in 1999 by age (65–74) | 0.21 | 0.12 | 1.24 | 0.96, 1.59 | 0.07 |
| University/college | 0.46 | 0.18 | 1.58 | 1.11, 2.26 | 0.03* |
| Park/forest | 0.76 | 0.36 | 2.15 | 1.06, 4.36 | 0.02* |

C.I. – Confidence interval (low, high).

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

Area under ROC curve value = 0.71.

Table. 4. Results of multivariable logistic models ($P < 0.05$) with county level housing and population variables along with variables of agricultural census and public places associated with canine leptospirosis status in the study region (n = 94 cases, 185 controls).

| Covariates | Estimate | S.E | OR | 95% C.I. | *P*-value |
|---|---|---|---|---|---|
| Lacking complete plumbing facilities | 1.10 | 0.07 | 3.02 | 2.60, 3.52 | 0.00* |
| Owner occupied | -0.19 | 0.08 | 0.82 | 0.69, 0.96 | 0.03* |
| Poverty status in 1999 by age (18–64) | 0.48 | 0.04 | 1.62 | 1.50, 1.76 | 0.02* |
| Household income (30,000–34,999) | 0.97 | 0.66 | 2.64 | 0.72, 9.67 | 0.07 |
| University/college | 0.35 | 0.18 | 1.42 | 0.99, 2.03 | 0.05 |
| Park/forest | 0.82 | 0.36 | 2.27 | 1.12, 4.61 | 0.03* |

C.I. – Confidence interval (low, high).

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

Area under ROC curve value = 0.67.

Fig. 1.
Distribution of case/control locations in counties of Kansas and Nebraska. Case locations were distributed in 25 counties and control locations in 43 counties in the study region. Of the block groups and census tracts (not shown in the map), cases and controls were distributed within 129 and 149 block groups respectively; and, within 90 and 103 census tract units respectively.