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

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1 Title: Neighborhood-level socioeconomic and urban land use risk factors of canine leptospirosis.
2 94 cases (2002–2009).

3

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24 Abstract:

25 Associations of housing, population, and agriculture census variables, and presence near
26 public places were retrospectively evaluated as potential risk factors for canine leptospirosis
27 using Geographic Information Systems (GIS). The sample population included 94 dogs positive
28 for leptospirosis based on a positive polymerase chain reaction test for leptospires on urine,
29 isolation of leptospires on urine culture, a single reciprocal serum titer of 12,800 or greater, or a
30 four-fold rise in reciprocal serum titers over a 2 to 4 week period; and 185 dogs negative for
31 leptospirosis based on a negative polymerase chain reaction test and reciprocal serum titers less
32 than 400. Multivariable logistic regressions revealed different risk factors among different census
33 units; however, houses lacking complete plumbing facilities [OR = 2.80, 95% C.I = 1.82, 4.32
34 (census unit, block group); OR = 1.36, 95% C.I. = 1.28, 1.45 (census tract); and, OR = 3.02, 95%
35 C.I. = 2.60, 3.52 (county)]; and poverty status by age (18–64) [OR = 2.04, 95% C.I = 1.74, 2.39
36 (block group); OR = 1.53, 95% C.I. = 1.41, 1.67 (census tract); and, OR = 1.62, 95% C.I. = 1.50,
37 1.76 (county)] were consistent risk factors for all census units. Living within 2500 m of a
38 university/college and parks/forests were also significantly associated with leptospirosis status in
39 dogs. Dogs that live under these circumstances are at higher risk for leptospirosis and pet owners
40 should consider vaccination.

41

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

44

45

46

47 1. Introduction:

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

60 In studies that are mainly reported from South America the incidences of leptospirosis in
61 humans have been associated with socio-economic and demographic characteristics of a society
62 such as income, literacy, housing and population density (Veras et al., 1985; Everard et al., 1989;
63 Bakoss, 2007; Cruz et al., 2009). Martins Soares et al., (2010) explored several socio-economic
64 and demographic characteristics of Sao Paulo, Brazil with historical human leptospirosis cases
65 and found significant associations with average monthly income, literacy rate, and number of
66 people living in a household, among other factors. Likewise, education, income, housing type,
67 and number of people living per household were risk factors for human leptospirosis in a
68 different study from urban Recife in Brazil (Oliveira et al., 2009). Many of the measures of
69 socio-economic and housing conditions differ in the U.S. compared to Brazil and other South

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

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

79 Associations of socio-economic and demographic features to animal and human
80 infectious diseases can be quantitatively evaluated using spatial analysis and geoprocessing
81 capabilities of a Geographic Information System (GIS). In an earlier study, using GIS and
82 publicly available land cover datasets we found that urban areas in general and medium and high
83 density residential areas in particular are significant risk factors for leptospirosis when land
84 use/land cover area surrounding up to 2500 m from dogs' residences were analyzed (Raghavan et
85 al., 2011). However, variables representing specific socio-economic or demographic
86 characteristics of urban land use were not included in that study nor have they been analyzed in
87 other published literature.

88 The objectives of this retrospective case-control study were to investigate which urban
89 characteristics, specifically socio-economic and human demographic factors could be potential
90 risk factors for canine leptospirosis in Kansas and Nebraska. Further, living within the proximity
91 of certain public areas was also evaluated as potential risk factors for leptospirosis.

92

93 2. Materials and Methods:

94 2.1. Case selection:

95 The medical records of all dogs from Kansas and Nebraska that had urine polymerase
96 chain reaction (PCR) testing for leptospirosis performed at the Kansas State Veterinary
97 Diagnostic Laboratory (KSVDL) between February, 2002 and December, 2009 were
98 retrospectively reviewed. When available, additional test results were included, specifically the
99 results of leptospiral serology and urine culture for leptospirosis. A positive case was defined by
100 a positive urine PCR or a negative urine PCR and any one of the following: isolation of
101 leptospire on urine culture, a single reciprocal serum titer $\geq 12,800$, or a four-fold rise in the
102 reciprocal convalescent serum titer. Dogs were deemed negative controls if the urine PCR was
103 negative and reciprocal serum titers were < 400 .

104

105 2.2. Molecular diagnostic testing:

106 Urine samples for PCR were handled for DNA isolation as previously reported (Harkin et
107 al., 2003). DNA samples were subjected to the semi-nested, pathogenic *Leptospira* PCR assay
108 described by Woo et. al., (1997) that amplifies a conserved region of the 23S rDNA, with minor
109 modifications. A unique Taqman probe was incorporated to distinguish pathogenic *Leptospira*
110 from saprophytic serovars. This test has been commercially available through the KSVDL since
111 2002.

112

113 2.3. Serological testing:

114 The microscopic agglutination test was performed on all blood samples submitted to the
115 KSVDL for leptospiral serological testing. The test was performed for serovars Canicola,

116 Bratislava, Pomona, Icterohemorrhagiae, Hardjo, and Grippytyphosa.

117

118 2.4. Leptospiral culture:

119 Urine culture was performed by inoculating 1 ml of urine obtained by cystocentesis
120 immediately into 10 ml of liquid Ellinghausen-McCullough (EM) media, gently vortexing this
121 inoculation and transferring 1 ml of this into another 10 ml of liquid EM media. One milliliter of
122 each dilution (1:10 and 1:100) was then subsequently inoculated into separate 10 ml of semi-
123 solid EM media. All tubes were incubated at 30° C in an ambient atmosphere incubator and
124 evaluated for evidence of growth weekly.

125

126 2.5. Demographic information:

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

130

131 2.6. Geocoding:

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

139 information (US Census Bureau, 2011). The geographic coordinates for unmatched addresses
140 (8%) were obtained using Google Earth software (version No: 5.2.1.1329) (Google Inc.,
141 Mountain View, CA). In all, geographic coordinates for 94 (out of 97) cases and 185 (out of 197)
142 control data points in Kansas and Nebraska were obtained.

143

144 2.7. Host factors

145 Observations were grouped into five age groups < 1 y, 1 to 4 y, 4 to 7 y, 7 to 10 y and >
146 10 y; two sexes and 77 individual breeds, including mixed breeds and unknown or unspecified
147 breeds were kept without grouping as a categorical variable.

148

149 2.8. Projection and data storage:

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

155

156 2.9. Census data:

157 U.S. Census 2000 data on population and housing were obtained in the form of
158 Summary File 3 (SF-3) tables from the U.S. Census Bureau (U.S. Census Bureau, 2011).
159 Identical census attribute information for Kansas and Nebraska were gathered at three
160 geographic levels or census units at which census data were aggregated by the US Census
161 Bureau: block groups (containing between 600 and 3,000 people within a county), census tracts

162 (containing between 1,500 and 8,000 people intended to represent neighborhoods), and counties.
163 GIS data files for block groups, tracts and counties were obtained from the ESRI Street
164 Map data based on US Census Bureau 2000 census information. From the Summary File –3 (SF–
165 3) tables, 33 housing and 37 population related variables (Table 1) were extracted for each
166 census unit by spatial query and joined to the census shapefiles using the common Federal
167 Information Processing Standards (FIPS) codes. Each census category included several
168 independent variables and they were evaluated separately in the study. The geocoded addresses
169 of cases/controls were overlaid in ArcMap with block group, census tract, and county shapefiles
170 in three separate operations, and the number of cases/controls that were within census units were
171 recorded separately using a spatial join procedure in ArcMap.

172

173 2.10. Agricultural census:

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

179

180 2.11. Presence near public places:

181 Polygon areas representing ten different public places around cities, including golf
182 courses, hospitals, industrial parks, primary/secondary schools, shopping centers, sports
183 stadiums, and local, county, and state parks/forests, and universities/colleges within 5000 m from
184 dogs' homes in the study region were obtained from the US Census 2000 TIGER/Line dataset.

185 Buffered areas extending 2500 m from the boundaries of public places were created and
186 cases/controls located completely outside (coded '0') and within (coded '1') the buffers were
187 recorded independently for each public place type. Ten variables, representing location within
188 2500 m from every public place were thus derived.

189

190 2.12. Data organization and statistical analysis:

191 All census data were originally stored in a Microsoft Access 2010 (Microsoft, Redmond,
192 CA) database and later as ESRI shapefiles during spatial analysis. The number of cases/controls
193 within and outside newly urbanized areas, and the distances to public places from cases/control
194 locations were stored as ESRI shapefiles. All numerical data were stored in Microsoft Excel
195 2010 (Microsoft, Redmond, CA) prior to statistical analyses conducted using SAS software (SAS
196 Institute, Cary, NC) or R Statistical Package 2.11.1 (R Core Development Team, 2011) when
197 specified. During the exploratory spatial analysis of case/control locations in the study region
198 clustering among cases and controls were evaluated using Cuzick-Edwards K^{th} neighbor statistic
199 (Cuzick and Edwards, 1990) within six major cities in the study region, including Manhattan,
200 Wichita, Topeka and Kansas City in Kansas, and Omaha and Lincoln in Nebraska. Four
201 neighbors were included in the analysis for cluster detection.

202 Odds ratios and 95% confidence intervals derived using logistic regressions were used to
203 determine associations of canine leptospirosis status with independent variables. There were a
204 total of 33 housing related variables and 37 population related variables at block group, census
205 tract, and county levels; 6 agricultural census variables at county level, and 10 variables
206 representing proximity to different public places. Variable screening among all variables was
207 done by fitting univariable logistic models and those variables with a P -value ≤ 0.1 were selected

208 for further analysis; however, care was taken not to remove variables that were deemed clinically
209 relevant (Hosmer and Lemeshow, 2000; Ward et al., 2004; Raghavan et al., 2011).

210 Multicollinearity was tested among screened variables by estimating the variable inflation factor
211 (VIF) using the proc reg/tol vif option in SAS (SAS Institute Inc., Cary, NC). All variables with a
212 VIF value of 10 or above were considered to indicate multicollinearity (Allison, 1999).

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

220 Multivariable logistic models with events/trials operand were fit using the stepwise
221 selection procedure in which a significance level, $P \leq 0.05$ was used for a variable to be retained
222 and $P \geq 0.1$ to be removed from the model (SAS, 2011). Logistic models were ranked using
223 Akaike Information Criterion (AIC) and the model with the lowest AIC value was deemed to be
224 the best fitting model. Any confounding effect of host factors, age (< 1 y old as reference level),
225 sex (female as reference level), and breed (unknown or unspecified as reference level) was
226 estimated by adding them one at a time to the final logistic model, and a 10% or more change in
227 coefficient values of independent variables were considered to indicate confounding due to that
228 particular factor, in which case adjusted odds ratios and their 95% confidence intervals were
229 recorded. Linearity assumption for logit in final models was assessed using Box-Tidwell test
230 (Box and Tidwell, 1962). Model adequacy was tested using chi-squared goodness-of-fit test ($P <$

231 0.05 indicated poor fit), and predictive ability measured by deriving the Area under Receiver's
232 operator's characteristic (ROC) curve value.

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

241

242 3. Results:

243 There were 94 dogs that were identified as cases based on a positive PCR ($n = 90$ dogs),
244 isolation of leptospires from the urine ($n = 1$), a single reciprocal titer $\geq 12,800$ ($n = 2$), or a four-
245 fold rise in serum reciprocal titers ($n = 1$). Of the dogs that were PCR positive, serology was not
246 performed in 22 dogs, 7 dogs had a negative acute titer with no convalescent titer performed, and
247 61 dogs had concurrent elevated titers to one or more serovar. There were 185 control dogs that
248 had a negative PCR and a reciprocal serum titer of < 400 .

249 Among 94 cases and 185 controls evaluated in this study, a majority had their physical
250 addresses located in the city of Wichita [33.68%, 28.81% (case, control)] followed by Manhattan
251 (13.82%, 19.45%), Lincoln (10.52%, 8.96%), Omaha (9.47%, 5.24%), Kansas City (6.31%,
252 4.62%) and Topeka (6.31%, 5.94%). All remaining cases (19.89%) and controls (26.98%) had
253 rural addresses or they were from smaller cities in the study region.

254 Since there could be a bias in case reporting to hospitals from certain neighborhoods than
255 others due to income differences, it was essential to verify if cases/controls showed any tendency
256 to cluster in any of the major cities in the study region. However, no clustering was observed in
257 any of the cities (where income levels among neighborhoods could vary). The Cuzick-Edwards
258 estimates for case locations in Manhattan ($P = 0.19$), Wichita ($P = 0.41$), Topeka ($P = 0.24$),
259 Kansas City ($P = 0.28$), Lincoln ($P = 0.31$), and Omaha ($P = 0.47$) did not indicate any
260 clustering. Similarly, the Cuzick-Edwards estimates for control locations in Manhattan ($P =$
261 0.05), Wichita ($P = 0.26$), Topeka ($P = 0.36$), Kansas City ($P = 0.19$), Lincoln ($P = 0.18$), and
262 Omaha ($P = 0.22$) did not indicate any clustering as well.

263 There were differences in the number and types of significant housing and population
264 variables identified in logistic models fit with covariates from different census units (Tables 2–
265 4). When block group level housing and population variables were analyzed along with
266 agricultural census and public places variables, the housing related variables significantly
267 associated with leptospirosis status in the logistic model were; the total number of structures built
268 during the years (1940–1949) and the number of households lacking complete plumbing
269 facilities (houses lacking hot and cold piped water, a flush toilet, and a bathtub or shower).
270 Significant population related covariates associated with leptospirosis status in the logistic model
271 were poverty status in 1999 by age (18–64) (number of individuals in the age group 18–64 that
272 were below poverty line the year 1999). Presence within 2500 m from university/college
273 campuses and park/forest areas were significantly associated with leptospirosis status in dogs
274 (Table 2).

275 When census tract level housing and population variables were analyzed along with
276 agricultural census and public places variables, the only housing related covariate significantly

277 associated with leptospirosis status in the logistic model was the number of households lacking
278 complete plumbing facilities, and the only population related covariate significantly associated
279 with leptospirosis status in the logistic model was poverty status in 1999 by age (18–64).
280 Presence within 2500 m from university/college campuses and park/forest areas were
281 significantly associated with leptospirosis status in dogs (Table 3).

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

289 Two agricultural census variables (the density of cattle farms, and the number of beef
290 cattle per county) were significantly ($P < 0.1$) associated with leptospirosis status but were not
291 significant in the multivariable logistic model. For all models described above, no other
292 covariates were found to be significant and/or found to improve the model fit when added. The
293 chi-square deviance goodness of fit test did not indicate any model inadequacy, and non-linearity
294 in logit and residual autocorrelation was absent. Confounding effects of age, breed, and sex were
295 not noted for any models.

296 4. Discussion:

297 The lack of clustering of cases and controls in any of the major cities in the study region
298 indicate a lack of sample bias for low income vs. high income neighborhoods in the study
299 population. In addition, the issue of referral bias is usually encountered in case-control studies

300 with the diagnostic laboratories receiving relatively higher numbers of cases from immediate
301 neighboring areas due to proximity and familiarity with the facility. However, the referrals in this
302 study originated from all major cities and rural areas in the study region, and in addition, 25.6%
303 of the study population included dogs that were diagnosed by the primary care veterinarian
304 outside KSVDL. The number of days that the dogs lived in their owner's household was not
305 provided to us during case submissions. For the purposes of this study, it was assumed that the
306 dogs spent most of their lives in their owners' households except for those times spent outside
307 during recreation and/or supervised exercise.

308 Demographic and socio-economic data collected by the U.S. Census Bureau and other
309 agencies are highly relevant to public health and epidemiological research. However, such data
310 are most commonly aggregated at the level of administrative boundaries or census/areal units
311 (Fig. 1). It has been well documented that the choice of areal unit could affect the strength and
312 significance of statistical associations and renders the results difficult to compare with other
313 studies. This is known as the Modifiable Areal Unit Problem (MAUP) (Openshaw, 1984; Unwin,
314 1996). Currently there are no solutions to fully overcome the effects of MAUP and related
315 methodological issues have not yet been adequately addressed. Recommendations have been
316 made to minimize MAUP effects in statistical inference by analyzing the aggregated covariates
317 in hierarchical levels of areal units from the finest spatial resolution possible to a coarser
318 resolution and to verify consistent model results (Fotheringham, 1989; Ratcliffe and McCullagh,
319 1999; Diez Roux, 2000). Three hierarchical levels of census units commonly used in
320 epidemiological studies were used in this study for identical housing and population covariates.

321 There were differences in the significant census variables in multivariable logistic models
322 at different areal levels (block group, census tract, and county) likely due to MAUP; however,

323 the number of households that lack plumbing facilities and the number of individuals in the 18–
324 64 year age group that are below poverty line were consistent risk factors in all areal units. These
325 and other housing and population related variables associated with canine leptospirosis status at
326 independent areal units are indicative of lower pet-owner socio-economic conditions and lower
327 housing standards, which are likely related. The findings reported here are similar to some of the
328 risk factors reported in studies from Brazil (Oliveira et al., 2009; Barcellos et al., 2000; Veras et
329 al., 1985) where more canine and human leptospirosis cases were shown to originate from poorer
330 neighborhoods. As in this study, the vaccination status of dogs included in the studies originating
331 from Brazil are not clear but dogs could be at higher risk in such urban environments due to pet
332 owners failing to vaccinate their dogs and/or higher prevalence of leptospirosis in the
333 environment due to substandard housing and other neighborhood conditions.

334 Among all public lands within an area covering 5 km from 2000 census city boundaries,
335 proximity to colleges/university campuses and state parks/forests were significantly associated
336 with leptospirosis status (when analyzed along with county level census data, the significance
337 value of college/university campus was slightly over $\alpha = 0.05$). Land use areas representing
338 parks/forests and college/universities are similar in that they provide ample open spaces for
339 canine recreation and are places where high dog-to-dog and wild mammal contact could occur.
340 However, parks/forests are relatively well drained areas compared to college/universities that
341 have built up areas such as parking-lots and pavements and there is potential for water run-off,
342 flooding and overflow from streams nearby. Therefore, the risk of public places such as
343 college/universities and similar environments may be due to flooding events.

344 An outbreak in human leptospirosis in a university campus was reported after flooding
345 and embankment overflow within the campus (Gaynor et al., 2007), and one human case of

346 leptospirosis was diagnosed after a similar flood event on another university campus (Park et al.,
347 2006). Precipitation and flooding have been associated with increased leptospirosis incidence
348 (Kawaguchi et al., 2008; Ward et al., 2004; Liverpool et al., 2008) and flood-prone or frequently
349 flooded areas are risk factors for human and canine leptospirosis (Morshed et al., 1994; Karande
350 et al., 2002; Batista et al., 2005). In addition, college/university campuses in the study region are
351 generally found in high density neighborhoods where housing is relatively older and the resident
352 population comprise higher number of students that likely change year to year and whose income
353 levels are typically low, factors which could play a role in higher transmission rates.

354 Proximity to open sewer and public waste disposal sites has been associated with human
355 leptospirosis from other countries (Oliveira et al., 2009; Krojgaard et al., 2009; Sarkar et al.,
356 2002). In the U.S., open sewer systems are not permitted by legislation unless they are within
357 treatment plants. Public waste disposal sites and landfills in the study region were located
358 beyond 5000 m from any case/control location and away from the city boundaries; therefore,
359 geographic features representing such areas were not included in the analysis. Proximity to storm
360 water drainage systems in the study region, some of which are open to the environment was not
361 associated with leptospirosis status. It is possible that the open storm water drainage systems in
362 the study region are free of leptospira, inaccessible for direct contact, or the peridomestic animal
363 movement around these areas could be minimal.

364 5. Conclusion:

365 Poverty status among people in 18–64 year age group, houses that lack plumbing
366 facilities, and proximity to public parks, college/universities, and newly urbanized areas are risk
367 factors for canine leptospirosis in Kansas and Nebraska, and likely other regions in the world as
368 well. Pet owners living under such neighborhood characteristics and treating veterinarians should

369 consider vaccination for their dogs in order to prevent leptospirosis.

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531 Table 1. Population and housing variables from US Census Bureau SF-3 data evaluated in the
 532 study.

533	Census category	Independent variables*
534	<i>Housing</i>	
535	Housing Units	Total housing units.
536	Urban and rural	Urban, rural, farm, nonfarm.
537	Tenure	Owner occupied, renter occupied.
538	Race of householder	White alone, Black or African American alone, American
539		Indian and Alaska Native alone, Asian alone, Native
540		Hawaiian and Other Pacific Islander alone, some other race
541		alone, two or more races.
542	Household size	1-person, 2-person, 3-person, 4-person, 5-person, 6-
543		person, 7-or-more person household.
544	Median number of rooms	Median number of rooms.
545	Year structure built	Built 1999 to March 2000, 1995 to 1998, 1990 to 1994,
546		1980 to 1989, 1970 to 1979, 1960 to 1969, 1950 to 1949,
547		1940 to 1949, Built 1939 or earlier.
548	Plumbing facilities	Complete plumbing facilities, lacking complete plumbing
549		facilities.

550 *Population*

551 Continued next page.,

552	Population	Total population.
553	Family size	Average family size
554	Urban and rural	Urban, rural, farm, nonfarm.
555	Race	White alone, Black or African American alone, American
556		Indian and Alaska Native alone, Asian alone, Native
557		Hawaiian and Other Pacific Islander alone, some other race
558		alone, two or more races.
559	Household income in 1999	Less than \$10,000, \$10,000 to \$14,999, and thirteen other
560		variables representing \$49,999 incremental income thereof
561		up to \$199,999, and \$200,000 or more.
562	Poverty status in 1999 by Age	Under 5 years, 5 years, 6 to 11 years, 12 to 17 years, 18 to
563		64 years, 65 to 74 years, 75 years and over.

564 * Observations for all the independent variables are counts, in continuous form, and recorded per
565 areal unit (block group, tract or county). Each census category included several independent
566 variables and they were evaluated separately in the study (for example, seven independent
567 variables for the census category, Poverty status in 1999 by Age were evaluated).

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

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

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577	Covariates	Estimate	S.E	OR	95% C.I	P-value	
578	Year structures built (1940–1949)	0.80	0.20	2.22	1.50, 3.30	0.00*	
579	Lacking complete plumbing facilities		1.03	0.22	2.80	1.82, 4.32	0.00*
580	Household income (30,000–34,999)	0.12	0.08	1.13	0.95, 1.34	0.07	
581	6–person household	0.11	0.44	1.11	0.47, 2.64	0.09	
582	Poverty status in 1999 by age (18–64)	0.71	0.08	2.04	1.74, 2.39	0.00*	
583	University/college	0.39	0.17	1.49	1.05, 2.11	0.04*	
584	Park/forest	0.86	0.36	2.37	1.17, 4.82	0.02*	

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

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

587 Area under ROC curve value = 0.71.

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

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

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596	Covariates	Estimate	S.E	OR	95% C.I.	P-value
597	6-person household	0.18	0.14	1.20	0.90, 1.59	0.06
598	Lacking complete plumbing facilities		0.31	0.03	1.36	1.28, 1.45 0.04*
599	Poverty status in 1999 by age (18–64)	0.43	0.04	1.53	1.41, 1.67	0.02*
600	Poverty status in 1999 by age (65–74)	0.21	0.12	1.24	0.96, 1.59	0.07
601	University/college	0.46	0.18	1.58	1.11, 2.26	0.03*
602	Park/forest	0.76	0.36	2.15	1.06, 4.36	0.02*

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

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

605 Area under ROC curve value = 0.71.

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611 Table. 4. Results of multivariable logistic models ($P < 0.05$) with county level housing and

612 population variables along with variables of agricultural census and public places associated with

613 canine leptospirosis status in the study region (n = 94 cases, 185 controls).

614

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

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

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

624 Area under ROC curve value = 0.67.

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

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