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Ecology of leptospirosis: environmental risk factors for domestic animals in the state of Georgia, USA

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DEDICATORIA

A mi padre y a mi madre.

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RESUMEN

La leptospirosis es un enfermedad zoonótica de distribución mundial que afecta a todas las especies de mamíferos. La transmisión ambiental de esta enfermedad y las relaciones complejas de transmisión entre especies demuestran que existen múltiples factores en la predicción de prevalencia y riesgo de infección. En este estudio se analizaron muestras de suero sanguíneo entregadas al Laboratorio de Diagnóstico Veterinario de la Universidad de Georgia para estimar la prevalencia específica de especies, la frecuencia de los serovares implicados e identificar los posibles factores de riesgo individuales y ambientales asociados a leptospirosis en animales domésticos en el estado de Georgia. Los datos incluyeron información para perros, gatos, caballos, cerdos domésticos y ferales analizados desde mayo de 2012 a mayo de 2013. La seroprevalencia general fue estimada (40.3% prevalencia; 95% CI 37.0 to 43.7, n=863) y variaciones significativas se encontraron al comparar la prevalencia específica de cada especie. El serovar infectivo más abundante en caballos y cerdos fue bratislava, mientras que en perros los más comunes fueron icterohaemorrhagiae seguido por canicola. En general, no se encontró gran variación en la prevalencia estacional, sin embargo existió un mayor número de entrega de muestras durante el otoño y verano. Factores de riesgo que incluían edad, sexo, cobertura de suelo, clima y diversidad de mamíferos fueron estimados por cociente de probabilidades relativas en análisis univariados y los factores significativos fueron incluidos en la selección de modelos logísticos. Un modelo de regresión logística fue obtenido y determinó que la especie, la edad, la estación y la cobertura de suelo con bosque son los factores más significativos para predecir un resultado seropositivo de leptospirosis en una muestra.

ABSTRACT

Leptospirosis is a globally distributed zoonotic disease affecting almost all mammal species. Environmental transmission and complex cross-species interactions have shown that multiple factors are involved in predicting prevalence and risk of infection. In the present study, serum samples submitted to the University of Georgia Veterinary Diagnostics Laboratory were used to estimate species-specific prevalence, serovar-specific frequencies and identify possible environmental and individual risk factors for leptospirosis in domestic animals of the state of Georgia. Data included records for dogs, cats, horses, domestic and feral pigs from May 2012 to May 2013. An overall seroprevalence was estimated (40.3% prevalence; 95% CI 37.0 to 43.7, n=863) and significant variations were found when comparing species-specific prevalence. The most common infecting serovar associated to horses and pigs was bratislava, whereas dogs were more likely to be infected by icterohaemorrhagiae or canicola. Overall seasonal prevalence did not show a great variation however, serum submissions were highest during the fall and the summer. Risk factors including age, sex, land cover, climate and mammal species richness were estimated by odds ratios in univariate analysis and significant factors were included in posterior model selection. A logistic regression model was obtained which determined specie, age, season and forest land cover as the most significant predictors of a seropositive outcome for leptospirosis.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Leptospirosis is a globally important zoonotic disease caused by spirochete bacteria of the genus *Leptospira* (Bharti et al., 2003; Levett, 2004). Considered a neglected tropical disease, leptospirosis is usually associated to occupational risks including field work and veterinary care (Bharti et al., 2003; Levett, 2001; Trevejo et al., 1998), however it has also been linked to water-associated recreational activities with various outbreaks reported in the past decade (Brockmann et al., 2010; C. Lau, Smythe, & Weinstein, 2010; Morgan et al., 2002; Sejvar et al., 2003; Stern et al., 2010). As of January 2013, leptospirosis has been restated as a nationally notifiable disease by the CDC in the United States (CDC, 2014). The global burden for leptospirosis is highly underestimated at half a million cases per year for human beings whereas information of incidence for animal species is extremely limited (Hartskeerl, Collares-Pereira, & Ellis, 2011).

Pathogenic *Leptospira* spp. can cause a systemic infection, which leads to renal colonization and persistent urine shedding of spirochetes to the environment (Bharti et al., 2003). The wide range of symptoms associated with leptospirosis make it difficult to recognize and therefore its reported incidence is severely biased (Hartskeerl et al., 2011; Levett, 2001). Human infection is often non specific with fever, myalgia and headaches which can progress to more serious conditions including, but not limited to jaundice, renal and hepatic dysfunction, pulmonary hemorrhage and death (Trevejo et al., 1998). In the case of dogs the disease is usually associated with fever and jaundice as well, but also affecting the digestive system and causing intravascular disseminated coagulation, renal failure and hemorrhages (Bolin, 1996). Leptospirosis infection in horses usually remains asymptomatic, but recurrent uveitis is a common post infection consequence; symptomatic individuals show similar symptoms as those described for other species (Rohrbach, Ward,

Hendrix, Cawrse-Foss, & Moyers, 2005; Verma, Stevenson, & Adler, 2013). In the case of cattle, pigs and goats, leptospirosis is responsible for great reproductive losses and it is fatal for some individuals due to septicemia and nephritis (Atherstone, Picozzi, & Kalema-Zikusoka, 2014; Grooms, 2006).

Disease transmission can occur through direct contact with urine from an infected animal or indirectly through contaminated soil or water (Adler & de la Peña Moctezuma, 2010) making leptospirosis a disease of environmental transmission. Portals of entry include skin abrasions, mucous membranes or conjunctiva and ingestion (Levett, 2001). Leptospiral survival in the environment may be attributed to cell aggregation mechanisms and biofilm formation (Ristow et al., 2008; Trueba, Zapata, Madrid, Cullen, & Haake, 2004), therefore contributing to its persistence and infection risk by floods. Even though it has been reported worldwide, leptospirosis is more common in a tropical setting due to the favorable conditions for its transmission, which include warmer temperature and higher humidity (Bharti et al., 2003; Levett, 2001). Outbreaks and risks have been associated to heavy rains, flooding, solid waste accumulation and proximity to urban slums (Barcellos & Sabroza, 2001; Ko, Galvão Reis, Ribeiro Dourado, Johnson, & Riley, 1999; Morgan et al., 2002; Reis et al., 2008)

Leptospirosis has been found in almost all mammalian species, including marine mammals, and is present in all continents except Antarctica (Adler & de la Peña Moctezuma, 2010; Atherstone et al., 2014). Even though not completely exclusive, *Leptospira* serovars have shown significant host preference and adaptation, establishing known reservoir hosts (Ko, Goarant, & Picardeau, 2009). These reservoir or maintenance hosts are responsible for continuing the enzootic disease transmission cycle and are defined as species in which the infection is endemic and transmitted through direct contact

between animals. Maintenance hosts are infected very young and the prevalence of chronic infection within the population increases with age (Levett, 2001). Typical serovar and reservoir hosts associations include pigs and cattle with serovar pomona, horses that harbor serovar bratislava, dogs with serovar canicola, raccoons with grippotyphosa and rats can harbor serovar icterohaemorrhagiae (Adler & de la Peña Moctezuma, 2010; André-Fontaine, 2006; Bernard, 1993; Bharti et al., 2003; Grooms, 2006). Animals may be maintenance hosts for certain serovars and incidental hosts for other serovars, which can cause infection and lead to severe or fatal outcomes (Levett, 2004).

Disease dynamics associated to leptospirosis transmission and maintenance in the environment is complex and highly dependent on the geographical area considered. Given that leptospirosis is transmitted environmentally and numerous mammalian species act as hosts, it has been proposed that human leptospirosis incidence is highly linked to ecosystem disruption and terrestrial mammal diversity (Derne, Fearnley, Lau, Paynter, & Weinstein, 2011). Previous reports have associated leptospirosis outbreaks with flooding (Ko et al., 1999; Reis et al., 2008); furthermore, extreme weather events and global climate change are expected to increase overall disease burden (C. L. Lau, Smythe, Craig, & Weinstein, 2010). Spatial clustering and environmental risk factors such as land cover, temperature and rainfall have been evaluated for leptospirosis in dogs (Gautam, Guptill, Wu, Potter, & Moore, 2010; Raghavan, Brenner, Higgins, Van der Merwe, & Harkin, 2011; Ward, Guptill, & Wu, 2004) but information regarding other domestic species is highly limited.

JUSTIFICATION

Leptospirosis is considered a neglected tropical disease that has been severely under-diagnosed having major impacts over human health (Abela-Ridder, Sikkema, & Hartskeerl, 2010). The World Health Organization considers leptospirosis as one of the most widespread zoonoses but consistent data on its prevalence is limited (World Health Organization, 2003). Available information on the burden of leptospirosis in domestic animal populations is scarce, and its effect on wildlife is basically unknown (Hartskeerl et al., 2011). Previous studies have analyzed potential risk factors for leptospirosis in humans, including lack of sanitation, rat populations, socioeconomic status and proximity to urban slums (Reis et al., 2008). In the case of domestic animals, prevalence and risk factors for dogs have been assessed in the United States and Canada, determining age, behavior, rainfall, seasonality, land cover and density of dairy cattle as potential factors affecting incidence (Raghavan et al., 2011; Ward, Glickman, & Guptill, 2002; Ward, Guptill, & Wu, 2004; Ward, 2002). However, information regarding risk factors for other domestic or wildlife animal species is limited (Hartskeerl et al., 2011).

Close contact to environmental and wildlife sources pose a high disease risk, however several factors such as the size of the infecting inoculum, infecting serovars and the host's immune response determine the outcome of the disease (McBride, Athanazio, Reis, & Ko, 2005). Due to the large number of variables included in the epidemiology of this disease, the development of public health strategies to control, prevent and lower disease incidence in humans and animals depends on the knowledge of *Leptospira* survival, and transmission dynamics both in the environment and among its animal hosts.

It has been proposed that a higher terrestrial mammalian diversity has a bio regulatory effect on leptospirosis incidence through mechanisms such as the dilution effect

(Derne et al., 2011). Additionally, recent studies have associated incidence to impermeable surfaces in urban areas and flooding events (C. L. Lau et al., 2010). The zoological and environmental implications for leptospirosis transmission suggest this disease exemplifies a quantifiable link between ecosystem functioning and public health (Derne et al., 2011). Therefore the importance of developing a more comprehensive understanding of large-scale and landscape level patterns of serovar variation, mammal species richness, land cover and seasonality as factors for leptospirosis incidence is a pressing need (Derne et al., 2011; C. L. Lau et al., 2010; Vinetz et al., 2005).

Through the analyses of existing datasets the aim of this work is to establish the relative roles of mammal species richness, land cover, seasonality and host species to explain the variability in prevalence and serovar diversity for domestic and livestock animal species in the state of Georgia, United States.

METHODS

Data Source

Data used for analyses in this project was obtained from samples submitted for serological evaluations for leptospirosis to the University of Georgia Veterinary Diagnostic Laboratories at Tifton, Georgia under Dr. Sreekumari Rajeev. Serum samples were analyzed for domestic dogs, domestic cats, horses, domestic pigs and feral pigs. Even though the laboratory received some samples from other states, the analysis only included data from Georgia. Microscopic agglutination tests (MAT) were used to diagnose leptospirosis cases and serogroups tested included pomona, hardjo, grippotyphosa, icterohaemorrhagiae, canicola, bratislava and autumnalis. The MAT is the standard serological test used for leptospirosis diagnosis with an accepted minimum significant titer of 1:100 (OIE, 2008). In general, antibodies from natural exposure or vaccination are short lived, lasting between 1 and 3 months and having reciprocal titers of no more than 100 to 400 (Bolin, 1996). Based on Dr. Rajeev's personal experience, vaccination for leptospirosis in Georgia is not common and titers from serum samples submitted were most likely from clinical leptospirosis infection. Therefore, a cut off titer of 100 was assumed as an appropriate value to determine seroprevalence and conduct statistical analyses with reference in previous studies (Alton, Berke, Reid-Smith, Ojkic, & Prescott, 2009).

High quality land cover datasets were obtained through the Natural Resources Spatial Analysis Lab (NARSAL) at the University of Georgia, College of Agricultural and Environmental Sciences. The Georgia Land Use Trends (GLUT) Project uses LANDSAT data to generate GIS databases distinguishing among several landcover types. Specific

descriptions for land cover can be found through their website (NARSAL, n.d.). Data was obtained at the county level as a percentage of coverage. Based on previous studies relating land cover to leptospirosis risk, urban, agricultural, wetland and forest land use were considered in our analyses (Alton et al., 2009; Gautam et al., 2010; Raghavan et al., 2011). It has been proposed that terrestrial mammal biodiversity may have a protective effect over leptospirosis prevalence in humans (Derne et al., 2011), therefore the Georgia Gap database (Kramer et al., 2003) from the NARSAL was used to create a mammal species richness dataset for the state of Georgia at the county level and this information was also included in our analyses. Annual rainfall and mean temperature climate data for our study period was obtained from the Georgia Forestry Commission (Commission Georgia Forestry, n.d.).

Data Analysis

Laboratory prevalence of leptospirosis and its 95% confidence interval were calculated for each domestic species as number of positive cases divided by the total number of submissions for serologic testing for each species category. Each positive case was defined as having a reciprocal MAT titer ≥ 100 to one or more of the serovars tested and the possible infecting serovar was determined by the maximum titer. In cases for which equal maximum titer was recorded for more than one serovar, all serovars with that titer were considered as the possible infecting serovar for serovar-specific analyses, and therefore a submission could be counted more than once only for serovar analysis. Overall and species-specific seasonal prevalence was calculated in a similar manner, with total number of positives divided by the total number of laboratory submissions for each

particular season and calculating their 95% confidence intervals. A Chi-Squared test for equal proportions was used to determine significant differences among species prevalence.

Univariate logistic regression models were analyzed for all possible factors and odds ratios (OR) with 95% confidence intervals were calculated based on a profiled log-likelihood function. A likelihood ratio test was carried out for each model and a significant association in univariate analysis ($P < 0.2$) was used to include variables into a full model. Final model selection was made through backwards-stepwise logistic regression starting with the full model. Selection for the terms to be added or dropped from the model were based on Akaike's information criterion (AIC) algorithms through the step() function in R, which defines AIC as (-2 maximized log-likelihood +2 number of parameters). R Statistical software was used for all statistical analyses (R Core Team, 2013). Variables included land cover data provided at the county level as a percentage of cover for each land use type. Mammal species richness, mean annual rain and mean temperature were also obtained at the county level. All variables were log transformed to normalize data distributions prior to statistical analyses. For each laboratory submission age was classified under the categories ≤1 year, 11.-1.9 , 2-3.9, 4-6.9, 7-10, >10, and sex was classified as male or female. This classification was selected as reference in order to compare results with previous studies (Ward et al., 2002; Ward, Guptill, & Wu, 2004). Data with missing values were excluded from the analysis. Laboratory submissions from feral pigs were excluded from all logistic regression analysis due to the lack of information from their cases. Feral pigs are trapped and transported to two facilities in two different counties of Georgia, but no information is available from the place or time of capture. Likewise, due to their feral nature, the age of feral pigs can only be estimated as juveniles or adults.

RESULTS

From May 2012 to May 2013, a total of 863 serum samples were submitted for analyses to the University of Georgia Veterinary Diagnostic Laboratories at Tifton from 117 counties from the state (Figure 1). Overall, a total of 348 individuals (40.3% prevalence; 95% CI 37.0 to 43.7) had positive MAT results and prevalence varied among counties analyzed (Figure 2). Species-specific prevalence was also calculated, with horses having the highest prevalence and followed by feral pigs, domestic pigs, dogs and cats (Table 1). Prevalence among domestic animals was found to be significantly different between species ($X^2 = 82.1797, df = 4,\ p < 2.2 \times 10^{-16}$).

Serovar-specific distribution of leptospirosis showed that the most abundant infecting serovar varied among species (Table 2). Serovar bratislava accounted for the highest serovar-specific prevalence in horses, domestic and feral pigs whereas serovar icterohaemorrhagiae dominated prevalence in dogs, and autumnalis in cats (Figure 3).

Seasonal distribution of cases showed a clear change in the number of positive cases among all species, with the fall and summer seasons having the greatest number of seropositive submissions for the study period (Figure 4). Fall season represented the most abundant number of positive laboratory samples for dogs, whereas the summer included most of the positive submissions for horses and domestic pigs; feral pigs dominated the winter season. Even though frequency distributions for seasonal seropositive submissions showed the fall and summer as the seasons with highest count, the overall seroprevalence calculated seasonally did not show a clear temporal distribution with a minimum seroprevalence in the fall at 36.7% and a maximum seroprevalence during the spring at 48.3% (

Table 3). Species-specific seroprevalence was calculated for available data, but heterogeneity in laboratory submissions resulted in wide confidence intervals and lack of data for certain species during specific seasons.

After removing records with missing data, logistic regression analysis was carried out for 564 submissions. Records from 110 out of the 159 counties in the state of Georgia, and observations from dogs (n=234), horses (n=123), cats (n=104) and domestic pigs (n=103) were included in the analysis. Significant associations were found between leptospirosis case status and factors such as animal specie, its age and sex (

Table 4). Environmental variables such as low intensity urban land cover, percentages of forest land cover and mean annual temperature were also significantly associated to a positive leptospirosis outcome (

Table 5).

Based on univariate logistic regression results, an initial full model was generated by including individual variables such as specie, age, sex, case season and environmental variables such as low intensity urban land cover, forest and mean annual temperature for the county. Stepwise logistic regression yielded a better-fit model including factors of specie, age, season and forest land cover for case-status outcome. Both models were compared based on AIC scores and likelihood ratio test with the last model having the lowest AIC score (Table 6).

DISCUSSION

Results from this study show significant differences in seroprevalence for leptospirosis among domestic animal species of the state of Georgia during the 2012 – 2013 study period. Although several seroprevalence surveys have been reported previously (Alton et al., 2009; André-Fontaine, 2006; Harland et al., 2012; O'Keefe, Jenner, Sandifer, Antony, & Williamson, 2002; Prescott et al., 2002; Scanziani et al., 2002) it is difficult to compare results with them given that different geographical areas are involved and there also exists variation in MAT cutoff titers used, serovars analyzed and animal species being studied. The MAT is the gold standard for leptospirosis diagnosis (OIE, 2008), however cutoff titers used in different studies range from ≥50 to ≥800 (Alton et al., 2009; O'Keefe et al., 2002; Prescott et al., 2002; Scanziani et al., 2002; Ward et al., 2002). Even though interpreting MAT results can be problematic due to cross-reactions, vaccine-induced antibodies, and previous infections, it is assumed that the highest titer corresponds to the infecting serovar and that a result greater than 1:100 can be considered as a seropositive case for epidemiological analysis (Bolin, 1996; OIE, 2008); therefore, a reciprocal titer ≥100 was used in this study. Differences in seroprevalence among domestic animal species could be explained by difference in their behavior and exposure given that leptospirosis is transmitted environmentally. Our data also shows that cats have a considerably lower seroprevalence, which coincides with results from other studies (Agunloye & Nash, 1996; André-Fontaine, 2006; Rodriguez et al., 2014) and can be attributed to both behavioral differences and a more acidic pH in urine that has a protective effect against leptospiral renal colonization. Even though leptospirosis in horses had been considered uncommon, recent data have found that leptospiral infection is actually widespread (Verma et al., 2013) and the results from our survey confirm that in the sample analyzed, horses had the highest

seroprevalence when compared to other animal species in the survey. Considering there are no leptospirosis vaccines for horses, seropositive animals in our survey are likely to have been infected with *Leptospira spp*. Finally, domestic and feral pigs from our data had similar seroprevalence values. This can imply that even though feral and domestic pigs have a different behavior and home range, the fact that they are the same species determines leptospirosis susceptibility.

Serovar analysis showed that *Leptospira interrogans* serovar bratislava was the most common infecting serovar for horses and pigs. It has been suggested that this serovar is actually host adapted to both species, for which they act as a reservoir and therefore is the most commonly associated with antibody response titers (Adler & de la Peña Moctezuma, 2010; Bernard, 1993; Ellis, McParland, Bryson, Thiermann, & Montgomery, 1986; Verma et al., 2013). Even though serovars associated to infection are variable and dependent on geographic location, our study shows that host-adapted associations in livestock animals are maintained in the state of Georgia. On the other hand, the most abundant serovars for dogs in our study were icterohaemorrhagiae followed by canicola and bratislava. Previous studies have reported serovar grippotyphosa in dogs as being the most abundant and usually linked to raccoon interactions (Ward, Guptill, Prahl, & Wu, 2004). However, it has been widely accepted that rats are the principal reservoir for serovar icterohaemorrhagiae and that serovar canicola is hosted in dogs (Adler & de la Peña Moctezuma, 2010). Considering both dogs and rats are most abundant near human settlements, this can lead to contact and disease transmission between both species and increase leptospirosis risk for dogs (Gautam et al., 2010; Ward et al., 2002; Ward, Guptill, & Wu, 2004). The most common infecting serovar in humans is also icterohaemorrhagiae, which leads to the possibility that the epidemiology of this serovar is highly associated to

urban settings (Levett, 2004; McBride et al., 2005; Reis et al., 2008). Nonetheless, other serovars have also been linked to human infection and so the importance of understanding the disease ecology, transmission and reservoirs of different leptospirosis serovars remains a pressing matter.

Seasonality as a risk factor for leptospirosis infection has been previously analyzed (Levett, 2001; Ward, Guptill, Prahl, et al., 2004; Ward, 2002). Due to environmental transmission, moderate temperatures and high humidity aid in *Leptospira spp.* survival, causing higher infection rates during the summer and fall in temperate geographical locations or during the rainy season in tropical areas (Bharti et al., 2003). Seasonal analysis of our data showed a higher number of laboratory submissions for testing during the summer and the fall. However when considering prevalence, no significant differences were found among seasons and the spring had the highest percentage for prevalence. Our data might have been subject to bias given the great heterogeneity of sampling. When analyzed by species, prevalence by season showed great variation as did the number of serum samples submitted for each species. Therefore, on future studies it would be advised to control for sample sizes throughout the year and among species in order to have unbiased data and be able to compare prevalence between species with a seasonal influence.

Individual risk factors were assessed for leptospirosis prevalence and in univariate analysis statistically significant associations between specie, age and season with prevalence were found. Previous studies have reported that age and sex are significant risk factors for leptospirosis in dogs, with age categories between 4 and 6.9 years and a male sex having the greatest risk (Ward, Guptill, Prahl, et al., 2004). No previous studies have assessed individual or environmental risk factors associated to leptospirosis in other

domestic or livestock animal species. Our data showed that odds of a seropositive result in horses was four times higher than that of dogs. Seropositivity in horses varies enormously depending on geographical location, with prevalence rates as low as 1.5% or as high as 71% (Ebani, Bertelloni, Pinzauti, & Cerri, 2012; Hamond et al., 2011). Results from our study show that for the sample analyzed, leptospirosis risk for horses is significantly high with prevalence rates of 69%, establishing Georgia as a high-risk location for leptospirosis in horses. Age was also found to be a significant risk factor when analyzing all species together, with the 7.0-9.9 years of age category as having the highest odds for being seropositive. However, it must be taken into consideration that the animal species analyzed have different lifespans and the heterogeneity of species sampling might have influenced the results. This does not mean that age as a factor in our analysis was biased, but it does imply that age categories may have different odds of being seropositive if analyzed by individual species.

Environmental risk factors in univariate logistic regressions showed urban and forest land cover along with mean annual temperature to have a significant association to seropositive outcomes, as has been reported in other studies (Gautam et al., 2010; Ward, Guptill, & Wu, 2004). Even though terrestrial mammal species richness had been previously suggested as having a protective effect over leptospirosis infection in humans (Derne et al., 2011), our results did not show any significant association between mammal species richness and seroprevalence in domestic animals. However, this factor should not be dismissed and the influence of wildlife over prevalence in domestic animals needs to be evaluated and analyzed in order to understand sylvatic cycles and wildlife reservoirs of leptospirosis. On the other hand, percentage of urban land cover and associated impermeable surfaces have been linked to higher prevalence rates in human leptospirosis

due to the increased risk of flooding in these areas (C. L. Lau et al., 2010). Additionally, urbanized areas with low sanitation have also been associated with leptospirosis in humans (Ko et al., 1999; Reis et al., 2008). Whether the associations found between prevalence and low urban land cover in our study can be attributed to low sanitation, flooding risk, higher animal density or interaction with rats is unknown. Therefore, the need for future studies that analyze these risk factors and can also include human prevalence is of great importance and a fundamental piece to understanding leptospirosis ecology and transmission.

Logistic regression models from our data included all significant factors from univariate analysis. A final model yielded specie, age, sex, season and forest land cover as the most significant predictors of a seropositive result. This model exemplifies the influence of both individual and environmental risk factors for leptospirosis infection in domestic animal species considered for our analysis. Given the high variability of leptospirosis prevalence rates between geographical locations, we can only extrapolate our results to the state of Georgia and the counties sampled. Even though our sample size was sufficiently large to do this type of analysis, high heterogeneity in the number of samples per animal species could have influenced the results, with horses and dogs having the largest number of serum samples in our data. Additional to this, the exclusion of feral pigs from multivariate analysis restricts our model to domestic animal species with limited home ranges. For future studies, it would be interesting to include capture site data of feral pigs to analyze any associations between environmental variables and a wild animal species. Furthermore, wildlife serosurveys should be performed in order to identify serovars present in this geographic location and compare them to the serovar makeup of the domestic animals analyzed. This would not only indicate possible wildlife-domestic

animal interactions and disease transmission, but it could also validate or reject the proposed hypotheses of mammal species richness having a protective effect through a dilution mechanism (Derne et al., 2011; Vinetz et al., 2005).

This study has showed that there are significant individual and environmental risk factors for leptospirosis in the sample analyzed for the state of Georgia. As a preliminary study it shows that even though different species have variable exposure and susceptibility to infection there are certain factors that increase the risk of leptospirosis asides from species alone. Being the most common bacterial zoonoses, leptospirosis has become a matter of global public health (Langston & Heuter, 2003; Vijayachari, Sugunan, & Shriram, 2008) and therefore the understanding of its transmission ecology is of great importance. Considering the environmental implications, host-serovar associations and the wide range of susceptible hosts, the ecology of leptospirosis is a fundamental piece in understanding factors associated to human risk of leptospirosis and the link with ecosystem functioning (Derne et al., 2011). This study has only analyzed environmental risk factors at a local scale with small sample size. However, developing large-scale understanding of transmission mechanisms is necessary in order to predict and develop effective control strategies for a constantly changing ecosystem subject to climate change, urbanization, decreasing biodiversity and land cover alterations.

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ANNEX 1 – TABLES

Table 1. Seroprevalence by species of serum samples evaluated at the University of Georgia Veterinary Diagnostic Laboratories. A reciprocal MAT titer ≥100 used to indicate seropositivity.

Specie	No. tested	No. seropositive	Seroprevalence (%)	95% CI
Horses	155	107	69	61.0-76.1
Feral Pigs	159	75	47.2	39.3-55.2
Domestic Pigs	160	59	36.9	29.5-44.9
Dogs	278	90	32.4	27.0-38.3
Cats	111	17	15.3	9.4-23.7

Table 2. Serovar-specific distribution of positive serum samples analyzed at the University of Georgia, Veterinary Diagnostic Laboratories. Maximum titer was used to determine infecting serovar.

	Horses	Feral Pigs	Domestic Pigs	Dogs	Cats	Total
Serovar bratislava	**72**	**50**	**46**	**18**	**1**	**187**
Serovar icterohaemorrhagiae	**25**	**9**	**9**	**51**	**1**	**95**
Serovar autumnalis	**16**	**8**	**8**	**16**	**12**	**60**
Serovar canicola	**16**	**9**	**4**	**26**	**1**	**56**
Serovar pomona	**5**	**4**	**2**	**7**	**2**	**20**
Serovar hardjo	**2**	**8**	**2**	**4**	**4**	**20**
Serovar grippotyphosa	**3**	**1**	**2**	**5**	**0**	**11**

Table 3. Overall and species-specific seasonal seroprevalence for all samples submitted to the University of Georgia, Veterinary Diagnostic Laboratories for the 2012-2013 study period. All species included for calculations in bold.

Season		No. Tested	No. Seropositive	Seroprevalence (%)	95% CI
Fall		**324**	**119**	**36.7**	**31.5-42.3**
	Horses	42	31	73.8	57.7-85.6
	Dogs	187	73	39.0	21.3-46.5
	Domestic Pigs	41	10	24.4	12.9-40.6
	Cats	54	5	9.3	3.5-21.1
Spring		**87**	**42**	**48.3**	**37.5-59.2**
	Dogs	8	8	100	59.8-100
	Horses	11	8	72.7	39.3-92.7
	Domestic Pigs	18	13	72.2	46.4-89.3
	Feral Pigs	36	13	36.1	21.3-53.8
	Cats	14	0	0	0-26.8
Summer		**329**	**134**	**40.7**	**35.4-46.3**
	Horses	100	67	67.0	56.8-75.9
	Feral Pigs	51	23	45.1	31.4-59.5
	Domestic Pigs	101	36	35.6	26.5-45.9
	Dogs	8	73	11.0	5.2-21.0
	Cats	0	4	0	0-60.4
Winter		**123**	**53**	**43.1**	**34.3-52.3**
	Feral Pigs	72	39	54.2	42.1-65.8
	Horses	2	1	50.0	9.5-90.5
	Cats	39	12	30.8	17.5-47.7
	Dogs	10	1	10.0	0.5-45.9

Table 4. Risk factors for leptospirosis among domestic animal serum samples submitted to University of Georgia, Veterinary Diagnostic Laboratories for the 2012-2013 study period.

	Likelihood ratio test					
Factor	**χ2**	**Df**	**P value**	**OR**	**95% CI**	
Specie	81.330	3	1.59E-17			
			Dogs	1.000	NA	NA
			Horses	4.327	2.716	7.012
			Cats	0.336	0.183	0.591
			Domestic Pigs	0.811	0.492	1.320
Age	37.643	5	4.45E-07			
			<1	1.000	NA	NA
			1-1.9	4.798	2.007	11.675
			2-3.9	3.444	1.579	7.637
			4-6.9	4.552	2.312	9.300
			7-9.9	6.055	3.154	12.134
			>10	4.067	2.283	7.626
Sex	2.170	1	0.140684			
			Female	1.000	NA	NA
			Male	1.290	0.919	1.810
Season	11.442	3	0.0096			
			Fall	1.000	NA	NA
			Spring	2.351	1.240	4.523
			Summer	1.436	0.984	2.096
			Winter	0.715	0.357	1.363

Table 5. Association (Odds ratio [OR]) between a leptospirosis diagnosis and environmental factors from the county from which the serum sample was received. Significant associations (P<0.2) are highlighted.

Environmental Variable	OR	95% CI		P value
Low intensity urban	1.291	0.907	1.839	0.1549
High intensity urban	1.042	0.894	1.214	0.5938
Forest	1.611	0.986	2.651	0.0572
Pastures and Crops	0.884	0.691	1.131	0.3235
Wetland	0.917	0.762	1.105	0.3620
Mammal species richness	2.035	0.033	121.660	0.7340
Mean annual rain	0.310	0.016	5.806	0.4351
Mean annual temperature	0.010	0.000	2.966	0.1119

Table 6. Model comparisson between the full initial model including significant associations from univariate analysis and the final model obtained through backwards stepwise regression.

		Likelihood ratio test		
Factors in model formula	AIC	χ2	Df	P value
Specie, Sex, Age, Season Low Intensity Urban Forest Mean annual temperature	654.4	140.4524	15	1.87E-22
Specie, Age, Season, Forest	645.11	138.7237	12	1.08E-23

ANNEX 2 – FIGURES

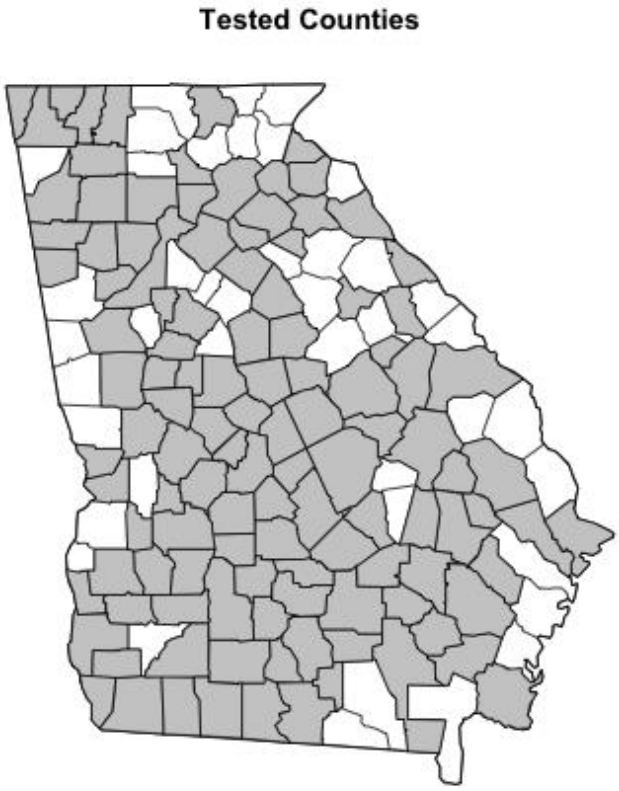

Figure 1. Subdivision by counties of the state of Georgia. Counties from which serum samples were received are shown in gray.

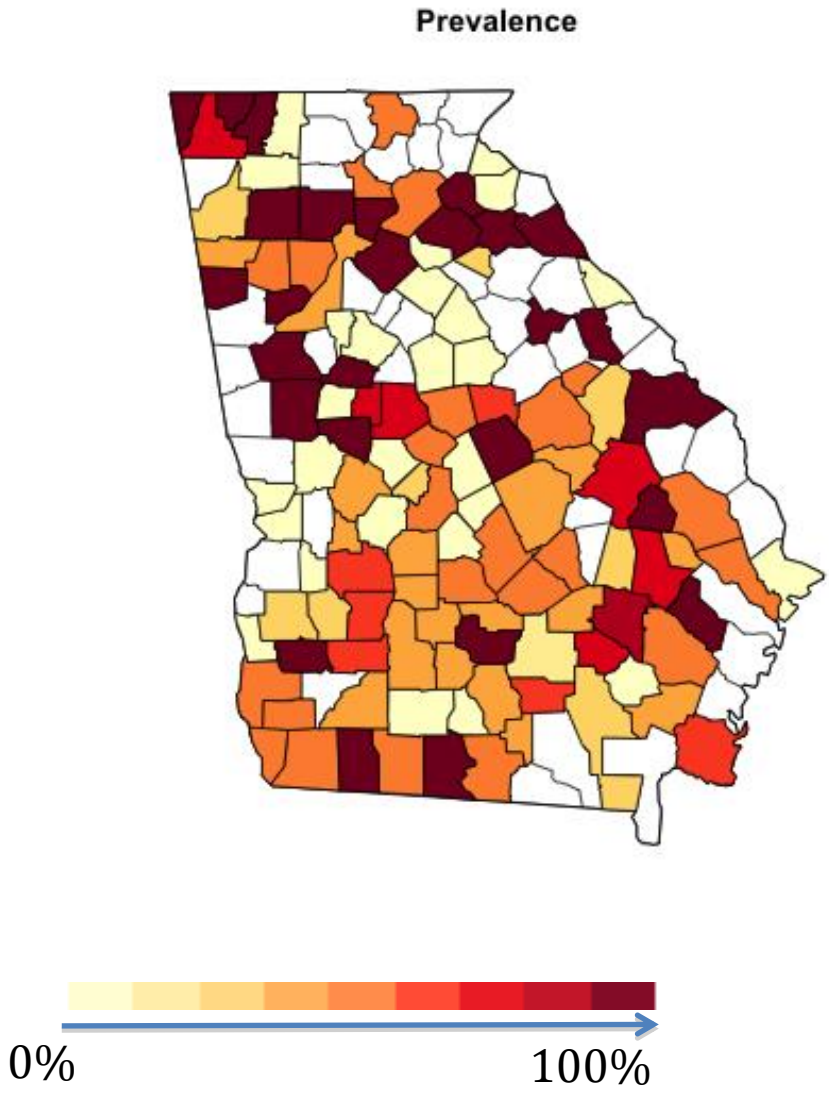

Figure 2. Prevalence heat map for analyzed counties in the state of Georgia for which darker colors represent higher overall prevalence in each county.

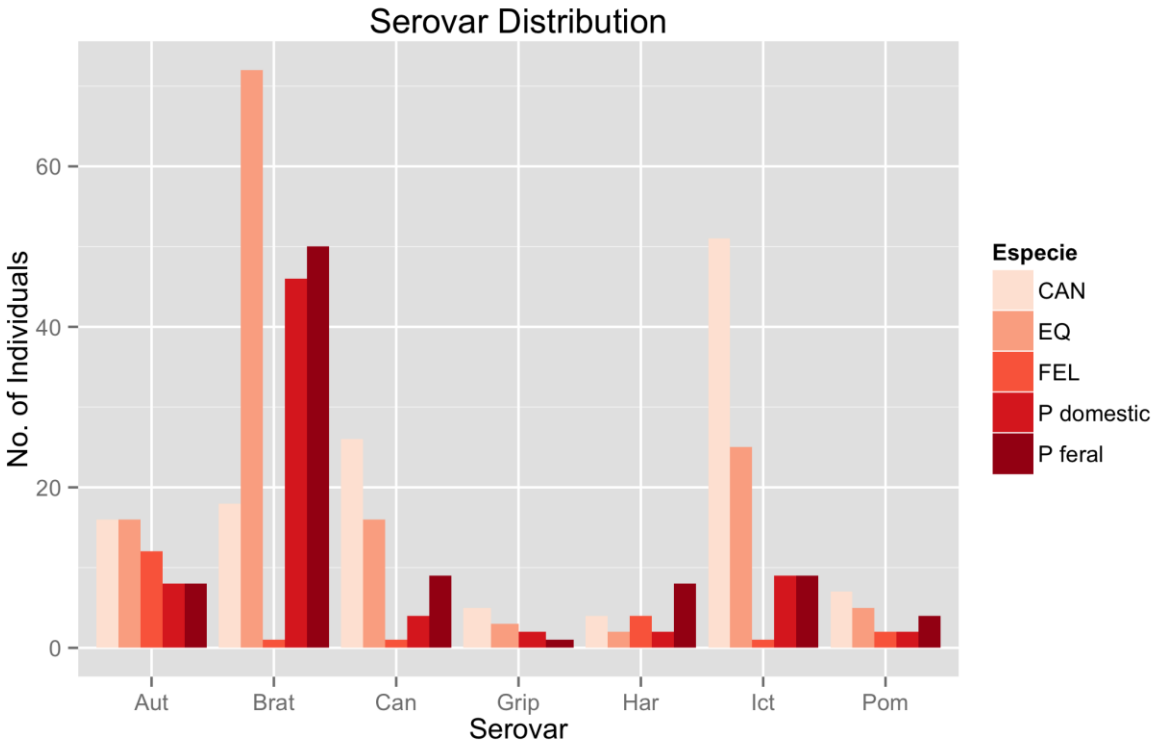

Figure 3. Distribution of infecting serovars among domestic species for which serum samples were submitted.

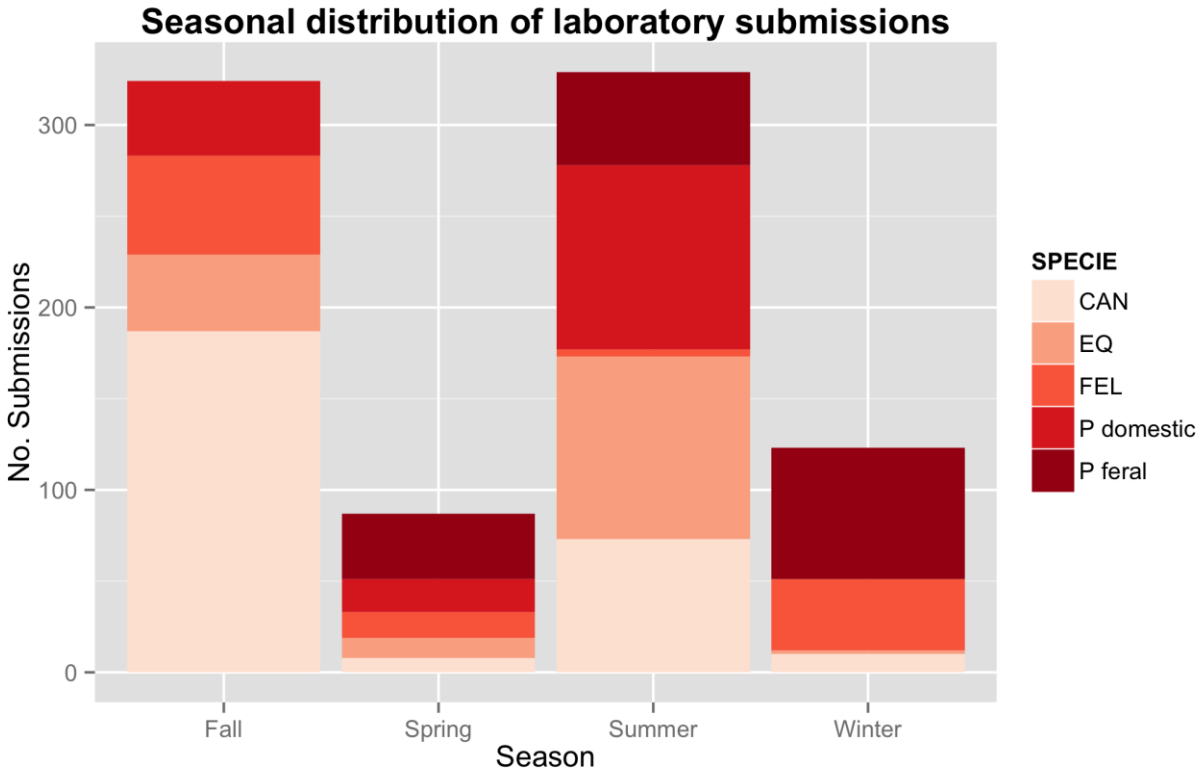

Figure 4. Seasonal submission of samples analyzed at The University of Georgia, Veterinary Diagnostic Laboratories during the 2012-2013 study period.