Hotspots of canine leptospirosis in the United States of America

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Abstract

Leptospirosis is a widespread zoonotic disease that causes hepatic and renal disease in dogs and human beings. The incidence of leptospirosis in dogs in the USA appears to be increasing. This study used 14 years of canine leptospirosis testing data across 3109 counties in the USA to analyze environmental and socio-economic correlates with rates of infection and to produce a map of locations of increased risk for canine leptospirosis. Boosted regression trees were used to identify the probability of a dog testing positive for leptospirosis based on microscopic agglutination test (MAT) results, and environmental and socio-economic data. The Midwest, East and Southwest were more likely to yield positive tests for leptospirosis, although specific counties in Appalachia had some of the highest predicted probabilities. Location (suburban areas or areas with deciduous forest) and climate (precipitation and temperature) were predictors for positive MAT results for leptospirosis, although the precise direction and strength of the effects was difficult to interpret. Wide geographic variation in predicted risk was identified. This risk mapping approach may provide opportunities for improved diagnosis, control and prevention of leptospirosis in dogs.

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Introduction

Leptospirosis is a common and widespread zoonotic disease, with reservoirs in domestic and wild animals (Waitkins, 1985; Bharti et al., 2003; Nelson and Couto, 2003; Heymann, 2008; Costa et al., 2015). The disease is caused by spirochaetae bacteria belonging to the genus Leptospira, which infect a range of mammals, including humans, livestock (e.g. cattle, pigs and goats) and companion animals (e.g. dogs and horses) (Nelson and Couto, 2003; Heymann, 2008). Infection is typically transmitted through direct contact of oral or nasal mucosa, or broken skin, with contaminated urine or water, and dogs are at risk of infection from drinking contaminated water (Nelson and Couto, 2003; Heymann, 2008). Leptospirosis can cause severe clinical disease in dogs, including acute hepatic and/or renal failure. It can also produce a chronic carrier status, presenting as idiopathic polyuria/polydipsia, which may not be preceded by severe hepatic or renal disease (Ward, 2002b; Nelson and Couto, 2003).

Canine leptospirosis has been reported in the USA for more than 100 years (Bolin, 1996) and the prevalence of leptospirosis is reported to be increasing; the rate increased by 1.2 cases/100,000 dogs/year from 1983 to 1998 (Ward et al., 2002). The national proportion of positive microscopic agglutination test (MAT) results increased from 8.7% to 12% from 2002 to 2004 (Glickman et al., 2006; Moore et al., 2006). Clusters of cases of canine leptospirosis have been detected in Texas, California and the upper Midwest, suggesting that, whilst leptospirosis is ubiquitous across the USA, some areas are disproportionately affected (Ward, 2002a; Gautam et al., 2010; Hennebelle et al., 2013). Environmental and socio-economic factors are thought to explain the distribution and transmission of leptospirosis (Gubler et al., 2001; Sehgal, 2006; Reis et al., 2008). Flooding has been linked to outbreaks of leptospirosis (Reis et al., 2008). The risk of leptospirosis is also related to land cover (e.g. evergreen forests, percentage of wetlands and public open spaces) and proximity to forests (Tangkanakul et al., 2000; Ward et al., 2004; Ahern et al., 2005; Chheim et al., 2007; Alton et al., 2009; Raghavan et al., 2011, 2012a, 2012b, 2013). To date, there has been no systematic analysis of the distribution of leptospirosis in the USA and risk factors associated with the occurrence of the disease.

In the current study, we used novel statistical approaches to analyze large sets of leptospirosis test data. We tested for the correlation of positive results with a wide range of hypothesized drivers and used the results to identify ‘hotspots’ of leptospirosis (areas of relative higher probability for leptospirosis cases) and the factors that are likely associated with them. Based on the ecology and epidemiology of leptospirosis, we hypothesized that abiotic factors, such as precipitation and temperature, as well as anthropic activities, measured as change in land use cover, can be...
used to detect areas of higher prevalence (i.e. hotspots) of canine leptospirosis in the USA.

Materials and methods

Literature review

Literature related to the prevalence of canine leptospirosis and associated factors was reviewed to identify variables for selection and to assist model building. PubMed and ISI Web of Knowledge data bases were searched for the terms ‘leptospirosis OR Leptospira’ AND (dog OR dogs OR canine OR Canis familiaris). Articles used were limited to those published from 2000 onwards that were published in English, French or Spanish that focused on North America (Canada, USA and Mexico); most data were from post-2000, although some publications included earlier data, extending as far back as 1970.

Data sources and explanatory variables

We obtained serological canine leptospirosis MAT results in the USA through an agreement with IDEXX Laboratories. A total of 87,355 tests were available in the proprietary database; however, vaccine history and whether samples were submitted for paired MATs were unknown. All MAT results for tests conducted from 2000 to 2014 at IDEXX Laboratories were included. The sensitivity of MAT has been estimated to be 50–67% at 1:400 dilution and 22–67% at 1:800 dilution, while the specificity ranges from 69–93% at 1:400 dilution and 69–100% for 1:800 dilution in dogs with confirmed leptospirosis, and dogs with clinical and laboratory indicators for leptospirosis, but where ultimately leptospirosis was ruled out (Miller et al., 2008). MATs performed at the same laboratories on samples from specific pathogen free dogs were 100% specific at both 1:400 and 1:800 dilutions (Miller et al., 2008). Although the sensitivity and specificity of both titers were similar, a cut-off of ≥1:800 dilution was selected for this study to limit the effect of unknown vaccination status; this was also the cut-off value used by IDEXX for diagnostic purposes. All serovars were included in our analysis without distinction. All data was anonymized and geo-referenced at USA zip code level.

To control the effect of dog population size on the number of samples submitted for testing, we estimated the dog population at county level. We weighted total state level dog population data (American Veterinary Medical Association, 2012) by county level human population data, assuming the proportion of dog owners is uniform within a state’s counties.

Variables for the spatial analyses were compiled in four main categories: (1) climate; (2) land cover type; (3) ecology; and (4) socio-economic status. Results from the literature review were used to provide information for selection of variables. Climatic variables included precipitation and temperature across the USA over a 30 year period (1981–2010) (Hijmans et al., 2005). Land cover type and percentage cover were collected from the 2011 National Land Cover Database (Jen et al., 2013). The mean number of rodent species per county distributed across the USA was used as an ecological indicator of host diversity under the assumption that higher diversity increases the likelihood of transmission of leptospirosis, in the absence of viable data on the number of rodents in a region (which is also likely to fluctuate widely over time).

Household income and percentage of residents with a Bachelor’s degree or higher were used as socio-economic variables. All variables were aggregated at county level using US Census 2010 county boundaries. Since the number of requested tests varied highly among counties, we determined the percentage of positive tests by county and used this proportion for all our analyses.

Modeling

Predictive models were constructed using boosted regression trees (BRTs) (Leathwick et al., 2006) with internal cross-validation, previously used to model species distributions (Leathwick et al., 2006; De'ath, 2007; Pritman et al., 2005) and disease ranges (Hay et al., 2013). Boosted regression trees fit an ensemble of regression trees to data in a stepwise fashion, up-weighting poorly predicted data points at each step and monitoring predictive accuracy to prevent over-fitting. Compared to traditional statistical techniques, they predict patterns in complex data sets accurately and are robust for use on data sets with many interacting variables or non-linear relationships; no p values, regression coefficients or confidence intervals are reported (Cutler et al., 2007; Elith et al., 2008).

The BRT used 31 county level variables for climate and precipitation, dog ownership, landscape composition and rodent diversity. Data analysis was performed using R (Jorge et al., 2015) and the R program dismo (R package, version 1.0–12). The predicted probability per county was mapped using ArcGIS version 10.2 (Environmental Systems Research Institute).

Results

Literature review

Five hundred articles were identified in the initial search, 474 of which were excluded on the basis of geography and relevance (e.g. randomized control trials, vaccine studies, laboratory diagnostic development, case studies and studies examining acute renal failure). We identified 26 peer reviewed publications with detailed testing data (see Appendix; Supplementary material). The sample size in these articles ranged from 32 (Martin et al., 2014) to over 1 million dogs (Ward et al., 2002). We identified a range of climatic, land-cover and socio-economic factors associated with canine leptospirosis (Tangkanakul et al., 2000; Ward et al., 2004; Ahern et al., 2005; Chneim et al., 2007; Alton et al., 2009; Raghavan et al., 2011, 2012a, 2012b, 2013) and included these variables in the model when data was available (Table S1; see Appendix; Supplementary material).

Descriptive statistics

A total of 12,317/87,355 (14.1%) MAT tests performed by IDEXX in the USA from 2000 to 2014 were positive for leptospirosis. At least one MAT result was available for 1260/3109 (40.5%) counties in the contiguous USA (Fig. 1) from which 746/1260 (59.2%) counties had one or more positive tests. The number of samples submitted for MAT from a county ranged from 1 to 3761 (mean 62) and the maximum number of positive tests in one county was 670 (Cook County, Illinois).

Modeling

Values from predictive modeling were interpreted as the probability that a diagnostic test in a given county would be positive; for example, a probability of 0.14 indicates that there is a 14% chance that any given MAT will be positive. The Midwest, East and Southwest were more likely to yield positive tests for leptospirosis, although specific counties in Appalachia had some of the highest predicted probabilities (Table 1). The highest predicted probability observed was 0.37 in Webster County, West Virginia, whilst the lowest was 0.02 in Harney County, Oregon. The overall median predicted probability was 0.12. Eighty-four (2.7%) counties had predicted probabilities >0.2. The final model fitted 1200 trees; the cross-validation accuracy was 0.58, which is better than random chance, but closer to random chance than perfect accuracy. Hotspot maps of the relative risk of leptospirosis were produced from the BRT model for MAT results (Figs. 2 and 3). These show

<table>
<thead>
<tr>
<th>Rank</th>
<th>County</th>
<th>State</th>
<th>Predicted probability</th>
<th>Dogs expected to test positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Webster County</td>
<td>West Virginia</td>
<td>0.370</td>
<td>1/2.7</td>
</tr>
<tr>
<td>2</td>
<td>Marion County</td>
<td>Indiana</td>
<td>0.34</td>
<td>1/2.9</td>
</tr>
<tr>
<td>3</td>
<td>Harrisonburg City</td>
<td>Virginia</td>
<td>0.33</td>
<td>1/3.0</td>
</tr>
<tr>
<td>4</td>
<td>Staunton County</td>
<td>Virginia</td>
<td>0.33</td>
<td>1/3.0</td>
</tr>
<tr>
<td>5</td>
<td>Wayneboro City</td>
<td>Virginia</td>
<td>0.31</td>
<td>1/3.2</td>
</tr>
<tr>
<td>6</td>
<td>Adair County</td>
<td>Kentucky</td>
<td>0.31</td>
<td>1/3.2</td>
</tr>
<tr>
<td>7</td>
<td>Covington City</td>
<td>Virginia</td>
<td>0.30</td>
<td>1/3.3</td>
</tr>
<tr>
<td>8</td>
<td>Curry County</td>
<td>Oregon</td>
<td>0.30</td>
<td>1/3.3</td>
</tr>
<tr>
<td>9</td>
<td>Nicholas County</td>
<td>West Virginia</td>
<td>0.30</td>
<td>1/3.3</td>
</tr>
<tr>
<td>10</td>
<td>Bedford City</td>
<td>Virginia</td>
<td>0.29</td>
<td>1/3.4</td>
</tr>
</tbody>
</table>
Fig. 1. Percent of microscopic agglutination tests (MAT) results positive by county in the USA in 2000–2014. Darker colors indicate a greater proportion of positive tests. Counties with no data indicate that no MATs were submitted to the reference laboratory during the study period.

Fig. 2. Predicted probability of a positive microscopic agglutination test (MAT) result for canine leptospirosis in the continental USA. Predicted probabilities range from 0.023 to 0.371, indicating that approximately 1/3 dogs tested is expected to be positive for leptospirosis. Scale is green to red where green indicates lower probability and red indicates higher probability.
the probability by county that a given test will be positive for leptospirosis. Since the BRT model analyses complex interactions and non-linear relationships, the directionality and precise relationship between any individual variable and the outcome is too idiosyncratic and context dependent to be meaningful on its own; for example, there appears to be a slightly decreased probability of a positive test at higher ranges of precipitation in the coldest quarter of the year, conditional on other variables, and there appears to be a modest increase as the proportion of a county covered by low intensity developed land approaches 0.3. Instead, the overall predictions of a model for a county should be considered. Variables important to the model’s overall output included precipitation, temperature, deciduous forested land, and low density developed land (e.g. residential areas with houses built on large lots or areas with 20–49% of surface areas covered with impervious material) (Table 2).

**Discussion**

This analysis has produced the first comprehensive predictive risk map for canine leptospirosis in the contiguous USA and statistical

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Percentage relative influence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deciduous forest</td>
<td>Land cover</td>
<td>10.9</td>
</tr>
<tr>
<td>Precipitation (coldest quarter mean)</td>
<td>Bioclimate</td>
<td>9.0</td>
</tr>
<tr>
<td>Scrubland and shrub land</td>
<td>Land cover</td>
<td>6.30</td>
</tr>
<tr>
<td>Developed (low intensity)</td>
<td>Land cover</td>
<td>5.7</td>
</tr>
<tr>
<td>Temperature (mean)</td>
<td>Bioclimate</td>
<td>5.0</td>
</tr>
</tbody>
</table>

*Greater relative influence indicates greater contribution to model results.*
support for some previously hypothesized risk factors. The variation in canine leptospirosis risk in specific counties and regions of the USA appears to be mainly influenced by environmental and land use factors. Our model suggests that landscape and environmental factors, specifically low density developed land (e.g. residential areas with houses built on large lots or areas with 20–49% of surface areas covered with impervious material), deciduous forested land, precipitation and temperature, are useful in predicting MAT test results. However, as stated above, the model’s predictions for individual variables are too idiosyncratic and context dependent to be broadly applicable or simply summarized.

Whilst boosted regression trees allow for the building of complex models with multiple variables, one of the major drawbacks of this approach is that the individual effects of any one specific variable are not easily interpretable. Given the complexity of our model and the number of variables included (e.g. multiple land cover variables), we could identify important variables, but could not describe the precise relationship between one variable and the risk of leptospirosis. Despite the complexity, the analysis allowed us to indicate how much influence a particular variable might have on the model prediction (Table 2).

The mechanistic relationship between different vegetation types and the risk of leptospirosis is unclear and may be a reflection that rural regions provide more opportunity for rodent reservoirs to transmit leptospirosis to dogs. Deciduous forest cover, which contributed most to the predictive power of the model, has not been proposed previously as a risk factor for canine leptospirosis. It is likely that deciduous forest is a proxy for other conditions favorable for leptospirosis transmission, such as specific precipitation regimes, higher rodent density and specific dog behavior.

One drawback of our model is that it does not account for flooding; therefore, it is possible that variables representing precipitation could explain some of the leptospirosis risk attributed to flooding in the literature (Ahern et al., 2005; Raghavan et al., 2012a). Our finding of the risk for leptospirosis in sparsely residential areas (lower density developed land) aligns with the findings of previous studies (Ward et al., 2004; Gnehm et al., 2007; Raghavan et al., 2011), whilst only one study has cited rural areas as a risk for leptospirosis (Alton et al., 2009). Lower density developed land provides a combination of impervious surfaces that can concentrate rain water run-off and acts as a good habitat for reservoir animals, such as rodents and raccoons, hence simulating the effects of ‘flooding’ without requiring a specific level of annual rainfall.

Many of the counties with the highest overall predicted risk are in Appalachia (i.e. West Virginia, Eastern Kentucky and Western Virginia). The Appalachian Mountains receive high annual precipitation and contain predominantly deciduous forests. Clusters of leptospirosis cases have been identified in Illinois and Michigan in other studies (Ward, 2002a; Gautam et al., 2010) and coincide with the results of our modeling. Significant spatial clusters were observed throughout the USA, including in Texas, California, the greater Chicago area and some regions of the upper Midwest (Ward, 2002a; Gautam et al., 2010; Hennebelle et al., 2013). This increased risk may be explained by the proximity to the Great Lakes and the moderately high annual precipitation in these areas.

In addition, we identified several counties in Kansas and Nebraska at higher risk for leptospirosis, despite the absence of available testing data for many counties in those states. The presence of leptospirosis in these states is supported by the literature. Raghavan et al. (2011) Raghavan et al. (2012a), Raghavan et al. (2012b) examined specific risk factors (hydrologic, environmental and socio-economic factors) for canine leptospirosis in Kansas and Nebraska. In the study of Harkin et al. (2003), 41/500 (8.2%) dogs were shedding leptospires.

Our analytical approach has some important limitations. Firstly, due to a lack of available testing data, some areas of the contiguous USA (i.e. the upper Midwest and parts of the Southeast) were poorly represented in our testing data. Whilst the use of predictive methods allows us to make predictions even when data are lacking, the inclusion of data from these areas would increase predictive accuracy. Additional analyses using testing data from these areas would provide a means to refine and externally validate our results.

Secondly, we used a titer threshold of ≥1:800 to select positive tests for our model. This cut-off threshold is expected to identify more exposures, whilst minimizing the risk of including positive titers due to vaccination. When vaccinated dogs were monitored for 1 year post-vaccination, some dogs developed titers ≥1:800 by weeks 7–15, whereas by weeks 29–52 none of the dogs had titers ≥1:400 and only a small percentage had titers ≥1:100 (Martin et al., 2014). Our literature review indicated that MAT titer thresholds for considering a test to be positive ranged from ≥1:100 to ≥1:3,200. The 2010 American College of Veterinary Internal Medicine (ACVIM) Small Animal Consensus Statement on Leptospirosis (Sykes et al., 2011) states that there is a lack of consensus as to which titer level should be used to determine that a test is negative for leptospirosis and also that single positive titers must be considered with clinical signs and a paired titer, since even a titer ≥1:800 does not confirm a diagnosis of leptospirosis. We followed the recommendation of the diagnostic laboratory (positive titer ≥1:800), which fits with our desire to exclude animals vaccinated within the past year and to have enough confidence in the likelihood of exposure to proceed with the epidemiological analysis.

Given the relatively low vaccination rates for canine leptospirosis in the USA (<4% of dogs in 2011) (American Veterinary Medical Association, 2012), we do not expect this to have a major impact on the results (Klaassen et al., 2003). Although vaccine history and whether samples were submitted for paired MATs were unknown, given the overall size of the data set, this was considered unlikely to have an impact on results.

Data were aggregated at county level due to data availability and to estimate leptospirosis risk in an easily interpretable manner to aid veterinarians in describing risks to owners. County lines are often arbitrary and may have changed during the course of our study. Using an arbitrary unit of area (county) may have augmented some of the observed effects. This would be likely to have the greatest impact in the Western USA, where the counties are large.

Our analysis provided some counterintuitive results. Several counties with high-predicted values were found in areas where clusters of leptospirosis had not previously been identified. In some of these counties, the available data did not have a high proportion of positive tests, indicating differences between the predicted values and existing leptospirosis clusters (Figs. 2 and 3) (Gautam et al., 2010; Hennebelle et al., 2013). Counties with low risk sometimes adjoin counties at high risk; for example, in Virginia, small counties with high risk are sometimes entirely surrounded by larger counties with lower risk. This may be due to smaller counties being population centers or due to artifacts in the data. These specific counties should be examined further to determine whether the low level of reported leptospirosis is due to lack of data, lack of testing or better vaccination coverage.

Areas identified by our model as higher risk areas differed from the overall distribution of proportion of available positive tests throughout the USA (Figs. 1 and 2), indicating that this method provides utility compared to analyzing historical testing data alone. Identifying the county level risk for leptospirosis can contribute to determining where to implement prevention and control measures, testing and vaccination.

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Leptospirosis is a disease of increasing concern for both people and dogs. Identified canine leptospirosis incidences in the USA have ranged from 0.04% in a study of hospital prevalence from 1970–1998 across the USA, to as high as 29% in a study examining tests submitted to the veterinary diagnostic lab in Illinois from 1996 to 2003. This variation makes it important to identify risk areas for this disease (Ward et al., 2002; Boutillier et al., 2003). The US Centers for Disease Control and Prevention recently reinstated leptospirosis in human beings as a nationally notifiable disease; in 2014, there were 21 human cases of leptospirosis (Centers for Disease Control, 2015). Dogs play an important role as potential indicators of areas with high endemicity for leptospirosis and, although infrequent, zoonotic transmission of leptospirosis from dogs to human beings can occur. Thus, recognizing and preventing canine leptospirosis has implications for human health as well as dogs.

Conclusions

Our model can be used to characterize the risk of canine leptospirosis across the USA and can be applied to improve delivery of veterinary services, including diagnostics and vaccination, and to identify areas for increased research and surveillance efforts. Canine leptospirosis remains an important disease, widely distributed in the USA, with varying levels of disease risk. Recognizing and identifying the areas most at risk will help to provide improved veterinary care and control of this disease.

Conflict of interest statement

Funds for this project were provided by Zoetis, which currently markets a vaccine against canine leptospirosis. Andrea Wright and Eileen Ball are employees of Zoetis. None of the other authors of this paper have a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

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Appendix: Supplementary material

Supplementary data to this article can be found online at doi:10.1016/j.tvjl.2017.02.009.

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