CANINE LEPTOSPIROSIS IN THE UNITED STATES (2009-2016): USE OF PCR TESTING TO UNRAVEL COMPLEX SPATIAL, TEMPORAL, HUMAN- AND ANIMAL-LEVEL RISK FACTORS

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INTRODUCTION

Leptospirosis is a reemerging zoonotic disease of concern that threatens companion animal and human health. Spread through the urine of infected animals, leptospirosis can infect dogs in a variety of settings across the United States. Dogs exhibit a wide spectrum of clinical illness, with the possibility of death. Canine leptospirosis cases appear to be increasing in number in the United States, yet information on the epidemiology of the disease is lacking. Previous canine studies commonly used MAT test data, but due to greater sensitivity PCR testing is rapidly increasing. Evaluation of PCR data may provide greater insight into this complex disease.

Hypothesis: Test-positive prevalence of canine leptospirosis is significantly influenced by environmental and animal factors.

Objectives:

- Describe the recent temporal and spatial distribution of PCR-positive canine leptospirosis cases in the United States.
- Identify environmental, seasonal, dog- and human-level factors associated with canine leptospirosis.

MATERIALS AND METHODS

Data acquisition:

- Dataset from IDEXX Laboratories of canine leptospirosis PCR urine and blood tests submitted from January 2009 to December 2016 by US veterinary clinics. Data included veterinary clinic zip code, test date, dog demographics (breed, sex, date of birth), and test outcome. Data on human and environmental variables were acquired from publicly available databases.
- Extracted and cleaned data, removing duplicate entries (N=1,252) and coded missing data as appropriate. The final dataset contained 40,118 test entries and 14 variables were explored.

Risk factor analysis:

- Stata 15 was used for analysis.
- Univariable generalized mixed logistic regression models accounting for county and state performed for each variable to identify risk factors for a positive test. Variables with a p-value of <0.2 were eligible for the final model.
- A final multivariable generalized mixed logistic regression model accounting for county and state was built and confounding was assessed. Statistical significance based on a p-value of <0.05.

Data visualization:

- Calculated test-positive prevalence for each zip code, state, and region by year.
- ArcGIS to produce choropleth maps depicting test-positive prevalence by state and year.
- Line graphs to visualize regional and temporal changes in test-positive prevalence.

RESULTS

Risk factor analysis: Ten variables were statistically significant in univariable models (Table 1). Age, season, gender, precipitation, and palmer drought severity index were retained in the final multivariable model. Odds ratios, 95% confidence intervals, and p-values were similar to univariable results.

Table 1: Canine leptospirosis PCR tests in the United States, 2009-2016: univariable mixed logistic regression models accounting for county and state

Variable	Odds Ratio (95% CI)	p-value
Age (years)☆		**<0.001**
0-4	Reference	
5-7	0.716 (0.640, 0.801)	<0.001
8-10	0.486 (0.430, 0.550)	<0.001
> 10	0.345 (0.299, 0.398)	<0.001
Season ☆		**<0.001**
Spring (Mar, Apr, May)	Reference	
Summer (June, July, Aug)	1.36 (1.18, 1.56)	<0.001
Fall (Sept, Oct, Nov)	2.14 (1.86, 2.46)	<0.001
Winter (Dec, Jan, Feb)	1.68 (1.47, 1.91)	<0.001
Gender ☆		**<0.001**
Male	Reference	
Female	0.763 (0.699, 0.834)	
Region		**0.0024**
West	Reference	
Southwest	1.70 (1.06, 2.73)	0.026
Midwest	1.91 (1.43, 2.72)	<0.001
Southeast	1.23 (0.870, 1.74)	0.238
Northeast	1.39 (0.959, 2.00)	0.082
Urban Influence Code		**0.0113**
Non-urban	Reference	
Urban	1.31 (1.06, 1.61)	
Precipitation (inches)* ☆		**0.0003**
0 - 1	Reference	
1.01 - 3	1.33 (1.14, 1.56)	<0.001
3.01 - 5	1.44 (1.22, 1.70)	<0.001
> 5	1.37 (1.15, 1.64)	0.001
Temperature (°F)*		**0.0011**
0 - 40	Reference	
41 - 55	1.24 (1.07, 1.43)	0.003
56 - 70	1.05 (0.91, 1.21)	0.496
> 70	0.99 (0.85, 1.16)	0.914
Palmer Drought Severity Index* ☆		**<0.001**
≤ 0 (dry conditions)	Reference	
> 0 (wet conditions)	1.24 (1.12, 1.37)	
County Dog Density (dogs/sqmi)		**0.0054**
0-50	Reference	
51 - 150	1.17 (0.98, 1.39)	0.088
151 - 350	1.41 (1.15, 1.72)	0.001
> 350	1.32 (1.04, 1.64)	0.020
Year		**<0.001**
2009	Reference	
2010	0.568 (0.40, 0.80)	0.001
2011	0.566 (0.40, 0.80)	0.001
2012	0.857 (0.63, 1.17)	0.333
2013	1.81 (1.36, 2.40)	<0.001
2014	1.73 (1.31, 2.28)	<0.001
2015	1.35 (1.03, 1.78)	0.031
2016	1.11 (0.84, 1.46)	0.446

*Weather data for month and year test performed

☆ Variable retained in final multivariable model

Data visualization:

- Overall test-positive prevalence was 5.4% with highest prevalence in the midwest (Figure 1).
- Prevalence peaked in 2013, and then decreased into 2016 (Figure 2).
- All states had at least one dog tested; all but Alaska, North Dakota, and Utah had at least one dog test positive.
- Test-positive prevalence peaked in the fall in the southeast, midwest, and northeast. It remained relatively constant across seasons in the west and southwest (Figure 3).

Figure 1: Canine leptospirosis state-level PCR test-positive prevalence across the United States, 2009-2016

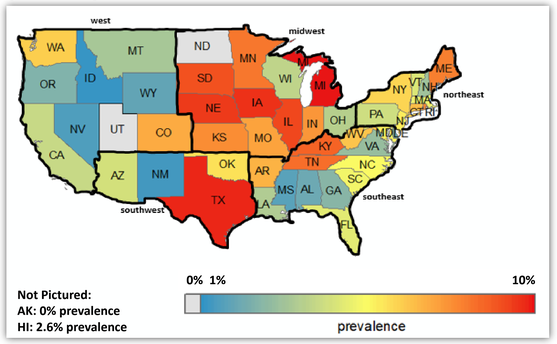


Figure 2: Annual canine leptospirosis PCR test-positive prevalence across the United States, 2009-2016

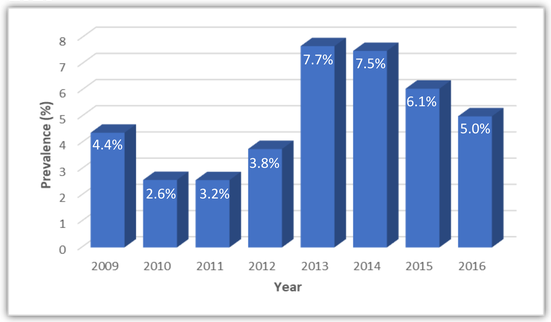
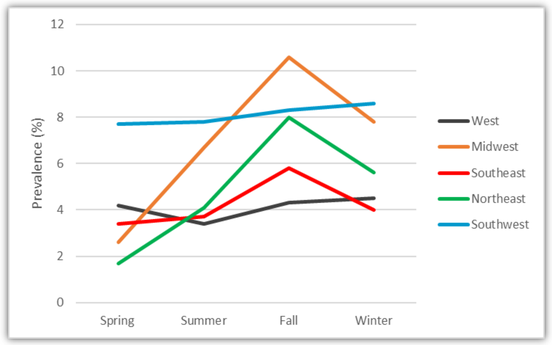


Figure 3: Seasonal changes in canine leptospirosis PCR test-positive prevalence by region in the United States, 2009-2016



CONCLUSIONS

- This study utilized PCR test data (sensitivity: 92%, specificity: 99%).[1] Previous studies have commonly utilized MAT test data, which is less sensitive (sensitivity: 22% – 67%).[2] Therefore, our conclusions differ from previous studies.
- Environmental and dog factors were implicated in the odds of a dog testing positive, aligning with our hypothesis.
- The west, midwest, southwest, and southeast regions have previously been identified as canine leptospirosis hot-spots.[3,4] In the current study, test-positive prevalence was highest in the midwest; interestingly, the west and southeast regions were not identified as high test-positive prevalence areas.
- As identified previously, weather factors, and gender were significant predictors for a positive canine leptospirosis PCR test.[3,5] Increased age has previously been found to be a risk factor, but in the current study younger dogs had higher odds of testing positive.[5,6]

Limitations: Although paired blood and urine samples from each dog were requested for testing, individual samples were tested (e.g., blood or urine). Any potential resulting classification errors or biases are unknown. Zip codes available were for the veterinary clinic where the dog was tested. It is unknown if this zip code differed from the dog's home zip code.

Future Directions: Additional research using specific information (home location, vaccination and exposure histories, husbandry practices) on each dog is needed to further investigate canine leptospirosis. This study provided data for a current case-control study focused on urban canine leptospirosis in the city of Chicago.

ACKNOWLEDGEMENTS

The authors wish to thank IDEXX Laboratories for providing the canine data analyzed in this study. Funding for this project was provided by the AKC Canine Health Foundation.

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1. Leutenegger CM, Palaniappan R, Elsemore D, et al. Analytical sensitivity and specificity of a real-time PCR assay detecting pathogenic Leptospira in dogs based on the HAP-1 gene. ACVIM 2009.
2. Miller MD, Annis KM, Lappin MR, et al. Sensitivity and specificity of the microscopic agglutination test for the diagnosis of leptospirosis in dogs. ACVIM 2008.
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FURTHER INFORMATION

Disclosure: There is no conflict of interest related to this research.

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RECENT TRENDS OF CANINE LEPTOSPIROSIS IN THE UNITED STATES: SPATIAL, TEMPORAL, ENVIRONMENTAL AND ANIMAL-LEVEL RISK FACTORS

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Leptospirosis is a reemerging zoonotic disease of concern that threatens companion animal and human health. Spread through the urine of infected animals, *Leptospira* spp. can infect dogs in a variety of settings across the United States. It produces a wide spectrum of clinical illness, with the possibility of death. Canine leptospirosis cases appear to be increasing in number in the United States, yet information on the epidemiology of the disease is lacking.

Hypothesis: Test-positive prevalence of canine leptospirosis is significantly influenced by environmental and animal factors.

Objectives:

- Describe the recent temporal and spatial distribution of canine leptospirosis in the United States.
- Identify environmental, seasonal, dog- and human-level factors associated with canine leptospirosis.

MATERIALS AND METHODS

Data acquisition:

- Dataset from IDEXX Laboratories of canine leptospirosis PCR tests submitted from January 2015 to December 2016 by US veterinary clinics. Data included veterinary clinic postal code, test date, dog breed, sex, date of birth, and test result. Data on environmental variables were acquired from publicly available databases.
- Extracted and cleaned data, removing duplicate entries (N=644) and coded missing data as appropriate.

Risk factor analysis:

- Univariable generalized mixed logistic regression models accounting for county and state to identify risk factors for a positive test. Variables with a p-value of <0.2 were eligible for the final model. A final multivariable generalized mixed logistic regression model accounting for county and state was built.

Data visualization:

- Calculated test-positive prevalence for each postal code, state, and region by year.
- Choropleth maps depicting test-positive prevalence by state and year.
- Line graphs to visualize regional and temporal changes in test-positive prevalence.

RESULTS

- 18,727 test entries and 14 variables were explored in association with a positive test outcome: season, breed group, sex, mean regional temperature and precipitation by month, state percent inland and overall water, estimated state dog density, urban influence code, income, education, and percent of state population that fishes and hunts.

Risk factor analysis:

- Five variables were statistically significant in univariable models (Table 1).
- The final multivariable model contained 3 variables: **female sex**, **dog age**, and **increased precipitation.** Odds ratios and 95% confidence intervals were similar to univariable results (Table 1).

Table 1: Significant variables identified in univariable generalized mixed logistic regression models predicting a positive canine leptospirosis test, accounting for county and state

Variable	Odds Ratio (95% CI)	p-value
Season		**<0.01**
Dec – Feb	Reference	
Mar – May	0.69 (0.56, 0.86)	<0.01
June – Aug	1.12 (0.93, 1.35)	0.229
Sept – Nov	1.38 (1.16, 1.65)	<0.01
Sex		**<0.01**
Male	Reference	
Female	0.77 (0.68, 0.88)	
Age (years)		**<0.01**
≤ 4	Reference	
5 – 7	0.72 (0.61, 0.85)	<0.01
8 – 10	0.45 (0.38, 0.54)	<0.01
≥ 11	0.26 (0.21, 0.33)	<0.01
Average regional temperature by month (°F)		**<0.01**
≤ 44	Reference	
45 – 58	1.50 (1.25, 1.82)	<0.01
59 – 70	1.11 (0.91, 1.35)	0.310
≥ 71	1.44 (1.18, 1.76)	<0.01
Average regional precipitation by month		**0.01**
< 4 inches	Reference	
≥ 4 inches	1.19 (1.03, 1.37)	

Data visualization:

- Overall test-positive prevalence across the United States was 5.5%; Texas (10% prevalence), Illinois (8.5%), Nebraska (8.2%), Iowa and West Virginia (each 8.1%) had the highest prevalence.
- In 2015 (Figure 1), the highest prevalence was found in the Midwest and South-central regions. Prevalence shifted in 2016 (Figure 2), with a noticeable increase in Arizona and Minnesota, and a decrease in the lower Midwest. Alaska and Hawaii are not pictured (no cases either year, except for 3.5% prevalence in Hawaii in 2016).
- Temporal and regional differences in test-positive prevalence were identified (Figure 3).

Figure 1: Canine leptospirosis test-positive prevalence across the United States in 2015

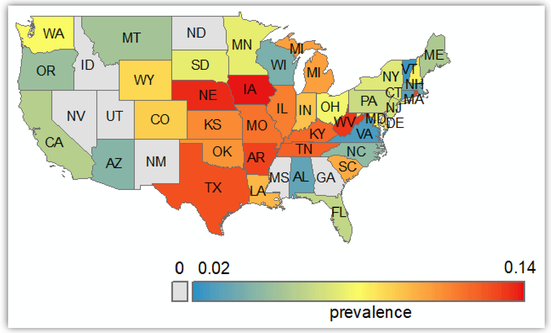


Figure 2: Canine leptospirosis test-positive prevalence across the United States in 2016

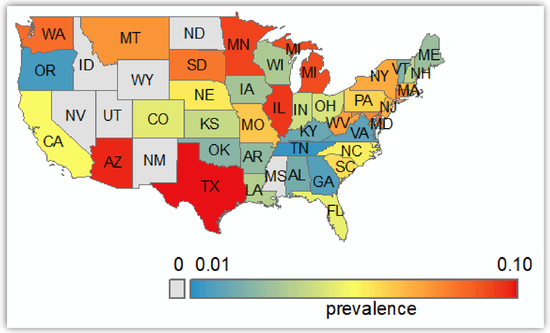
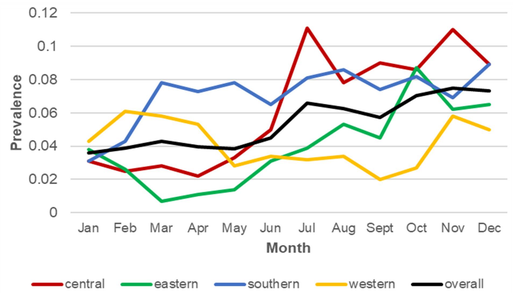


Figure 3: Temporal and regional canine leptospirosis test-positive prevalence for 2015 and 2016 combined



CONCLUSIONS

- This study utilized PCR test data (sensitivity: 92%, specificity: 99%).[1] Previous studies have commonly utilized MAT test data, which is less sensitive (sensitivity: 22% – 67%)[2] and vaccination can make MAT results difficult to interpret, thus our conclusions differ from previous studies.
- The Western, Midwest, and South-central regions have previously been identified as canine leptospirosis hot-spots.[3,4] In the current study, over both years, test-positive prevalence was highest in the Midwest and south-central regions; interestingly, the Western region was not identified as a high test-positive prevalence area.
- As identified previously, increased precipitation and temperatures were both significant predictors for a positive canine leptospirosis PCR test.[3]
- State-based prevalence varied between states and time (2015 vs. 2016). This illustrates how the distribution of cases can rapidly change over space and time.
- Only environmental and dog factors were implicated in the odds of a dog testing positive, aligning with our hypothesis. It is important to note that not all variables of interest were available for analysis due to limited information on each entry, and additional variables were limited to publicly available data.

Future Directions: Additional research is needed to investigate canine leptospirosis to identify modifiable risk factors (e.g., vaccination). Targeted education and prevention efforts at clients with dogs at risk are needed to decrease prevalence in the canine population and prevent possible transmission to humans. The spatial and temporal differences identified in this study can guide the location and timing of prevention campaigns.

ACKNOWLEDGEMENTS

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FURTHER INFORMATION

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