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

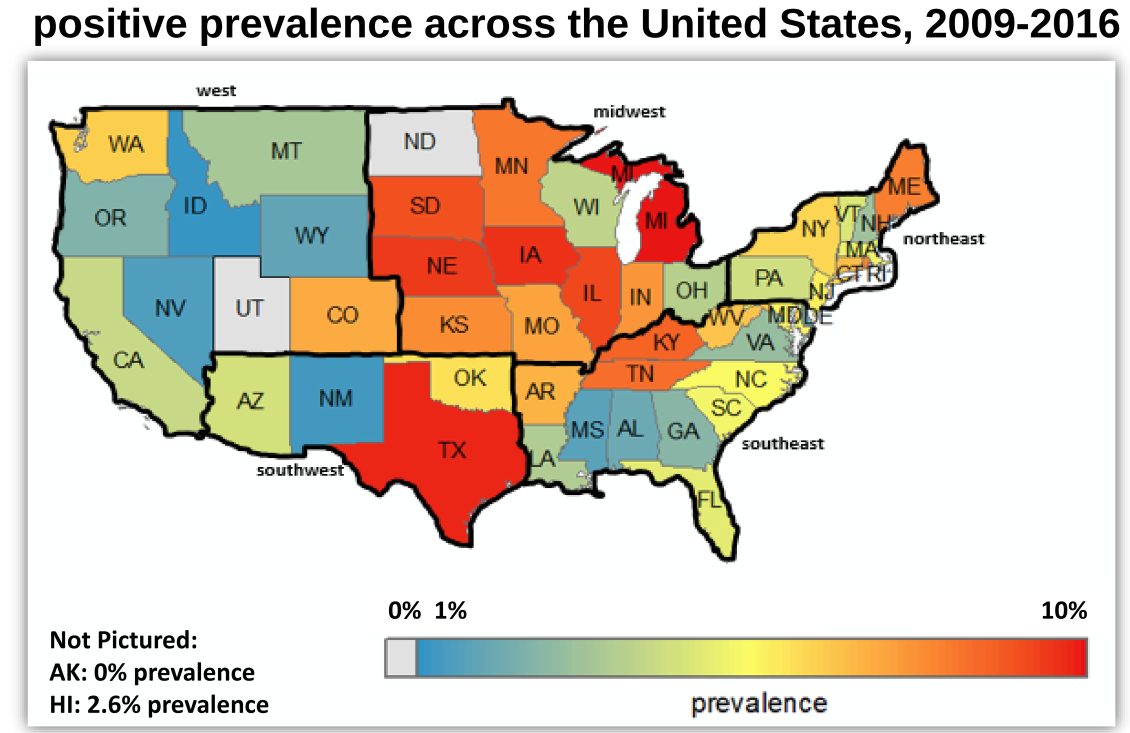


Figure 2: Annual canine leptospirosis PCR test-positive prevalence across the United States, 2009-2016

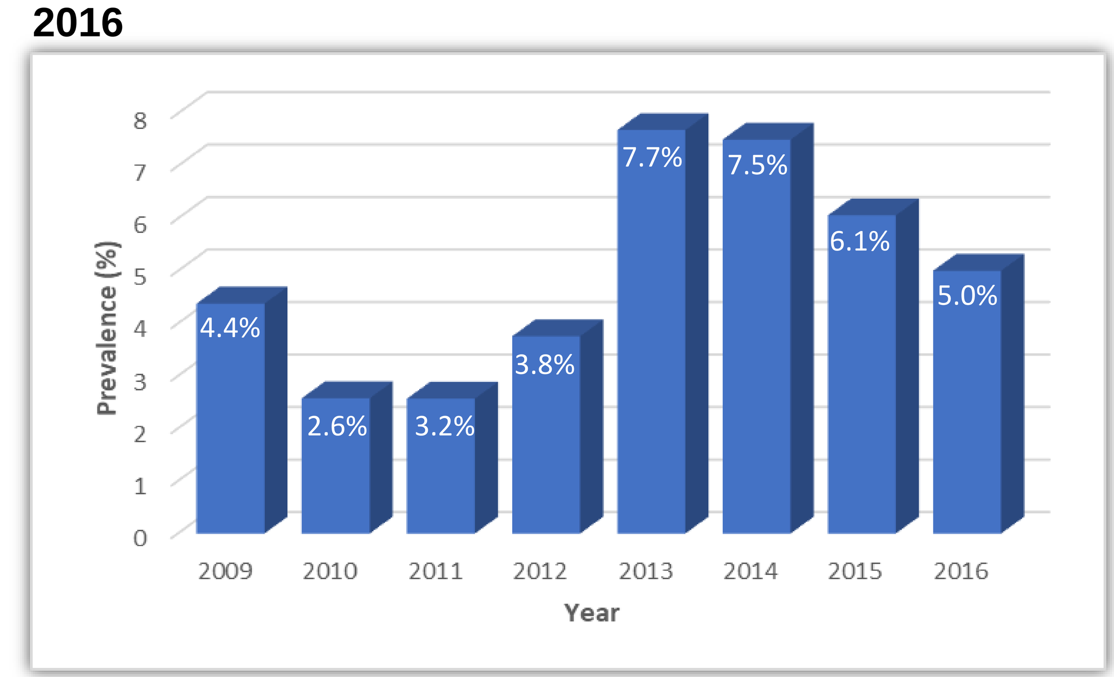
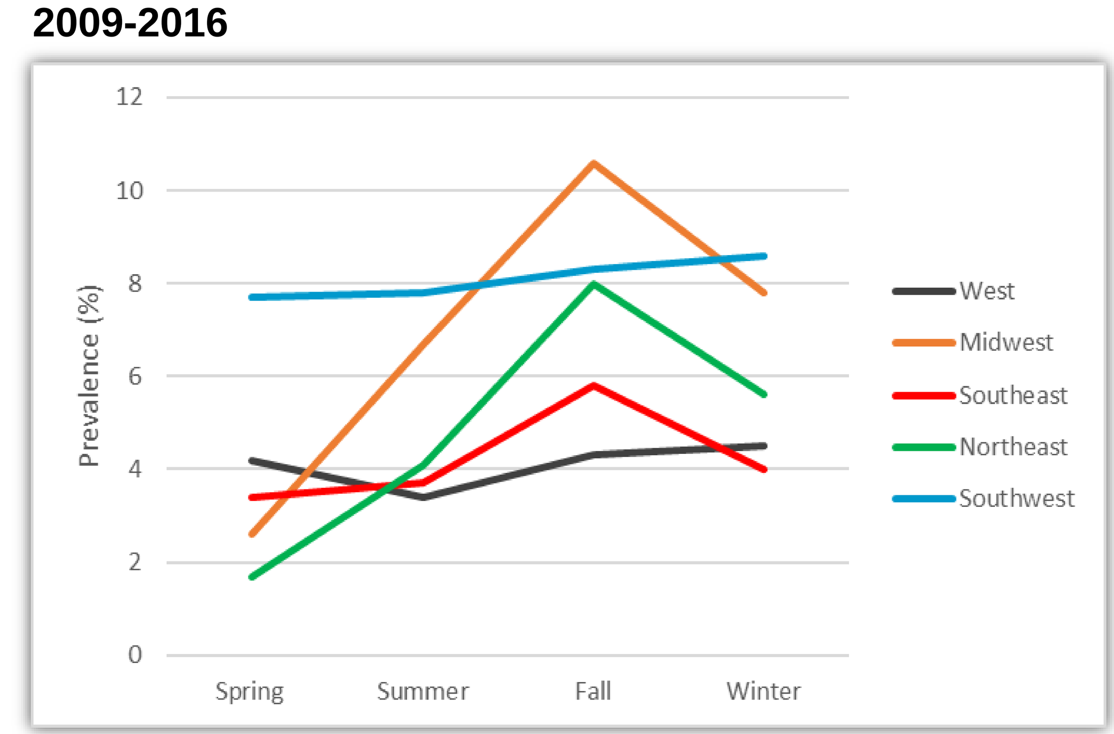


Figure 3: Seasonal changes in canine leptospirosis PCR test-positive prevalence by region in the United States, 2009-2016



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.

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REFERENCES

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.
3. White AM, Zambrana-Torrelio C, Allen T, et al. Hotspots of canine leptospirosis in the United States of America. The Veterinary Journal 2017; 222:29-35.
4. Moore GE, Guptill LF, Glickman NW, et al. Canine leptospirosis, United States, 2002-2004. Emerging Infectious Diseases 2006; 12(3):501-503.
5. Ward MP, Glickman LT, Guptill LF. Prevalence of and risk factors for leptospirosis among dogs in the United States and Canada: 677 Cases (1970–1998). Journal of the American Veterinary Medical Association 2002; 220(1):53–58.
6. Hennebelle JH, Skyes JE, Foley J. Risk factors associated with leptospirosis in dogs from northern California: 2001–2010. Vector-Borne and Zoonotic Diseases 2014; 14(10): 733–39.

FURTHER INFORMATION

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

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