Received: 18 July 2020 | Accepted: 27 January 2021

DOI: 10.1111/jvim.16060

STANDARD ARTICLE

Journal of Veterinary Internal Medicine Open Access

ACVIM American College of Veterinary Internal Medicine

# Investigation of spatio-temporal clusters of positive leptospirosis polymerase chain reaction test results in dogs in the United States, 2009 to 2016

Amanda M. Smith[1] | Jason W. Stull[1,3] | Michelle D. Evason[2,4] | J. Scott Weese[4] | Thomas E. Wittum[1] | Donald Szlosek[5] | Andréia Gonçalves Arruda[1]

[1]Department of Veterinary Preventive Medicine, College of Veterinary Medicine, The Ohio State University, Columbus, Ohio

[2]Department of Companion Animals, Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, Prince Edward Island, Canada

[3]Department of Health Management, Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, Prince Edward Island, Canada

[4]Department of Pathobiology, Ontario Veterinary College, Centre for Public Health and Zoonoses, University of Guelph, Guelph, Ontario, Canada

[5]IDEXX Laboratories, Inc, Westbrook, Maine

**Correspondence**
Amanda M. Smith, Department of Veterinary Preventive Medicine, College of Veterinary Medicine, The Ohio State University, Sisson Hall, 1920 Coffey Road, Columbus OH 43210, USA.
Email: smith.10344@osu.edu

## Abstract

**Background:** Leptospirosis is a zoonotic disease of concern and an investigation of recent spatio-temporal trends of leptospirosis in dogs in the United States is needed. *Leptospira* PCR testing has become increasingly used in veterinary clinical medicine and these data might provide information on recent trends of disease occurrence.

**Objectives:** To identify and describe clusters of PCR-positive *Leptospira* test results in dogs in the United States.

**Animals:** *Leptospira* real-time PCR test results from dogs (n = 40 118) in the United States from IDEXX Laboratories, Inc., between 2009 and 2016 were included in the analysis.

**Methods:** In this retrospective study, spatio-temporal clusters for a real-time PCR-positive test were identified using the space-time permutation scan statistic and the centroid of the zip code reported for each test. A maximum spatial window of 20% of the population at risk, and a maximum temporal window of 6 months were used.

**Results:** Seven statistically significant space-time clusters of *Leptospira* real-time PCR-positive test results were identified across the United States: 1 each located within the states of Arizona (2016), California (2014-2015), Florida (2010), South Carolina (2015), and 1 each located within the south-central region (2015), midwest region (2014), and northeast region (2011). Clusters ranged from 3 to 108 dogs and were identified during all years under study, except 2009, 2012, and 2013.

**Conclusions and Clinical Importance:** The spatial and temporal components of leptospirosis in dogs in this study are similar to those in previous work. However, clusters were identified in new areas, demonstrating the complex epidemiology of this disease.

**KEYWORDS**
disease clusters, epidemiology, infectious disease, United States

**Abbreviations:** MAT, microscopic agglutination test.

## 1 | INTRODUCTION

Leptospirosis is a potentially fatal zoonotic bacterial disease that affects humans and animals worldwide. There are many pathogenic *Leptospira* spp. serovars circulating globally, and serovars often have differing geographic spread, varying pathogenicity, and multiple maintenance hosts (including peridomestic wildlife and livestock species)—all of which might influence spatial trends.[1-3] Dogs acquire the bacteria through direct contact of mucous membranes or broken skin with infectious urine from maintenance hosts, or via contact with food, water or soil contaminated with infectious urine.[4,5] In dogs, leptospirosis has serious implications such as renal and hepatic failure, with the possibility of death.[6]

There is a seasonal increase in leptospirosis cases in dogs during the late summer and fall in northern temperate zones.[1,7] Leptospirosis is often associated with warm climates and exposure to water/high rainfall, as the bacteria survive well in water at an optimal environmental temperature of 30°C.[6] Although cases of leptospirosis in dogs have been diagnosed in all areas of the United States, there is clustering of leptospirosis cases in dogs from the west coast, midwest, northeast, and southwest.[1,8] Outbreaks have recently occurred in areas not previously associated with the disease.[9]

Over recent years, PCR *Leptospira* testing has become increasingly used in clinical veterinary medicine, often replacing or used in addition to the microscopic agglutination test (MAT). The PCR test has several benefits over MAT and other common clinical tests, including no interference from recent vaccination and greater sensitivity when predicting urine shedding compared to a single acute MAT.[6,10] The spatial and temporal clustering of leptospirosis seropositivity in dogs and cases in the United States between the years 1983 and 2010 is described.[1,7,11] These earlier studies were limited to specific geographic areas and utilized leptospirosis MAT results.[1,7,11] Different testing methodologies could provide different or additional results, therefore PCR-based analyses are important.

An updated nationwide analysis of spatial and temporal clusters of positive leptospirosis test results in dogs using PCR test data would assess potential epidemiological changes of this disease based on current clinically used testing. The objective of this study was to identify and describe clusters of nationwide PCR positive *Leptospira* test results collected from client-owned dogs from IDEXX Laboratories Inc. across the United States and over several years.

## 2 | MATERIALS AND METHODS

### 2.1 | Data acquisition and descriptive analysis

*Leptospira* real-time PCR test results from blood, urine, or both sample types from dogs submitted by veterinarians in the United States from January 1, 2009 to December 31, 2016 were obtained from IDEXX Laboratories, Inc. Blood, urine, or both sample types were obtained from each dog and submitted to a commercial reference laboratory by a practicing veterinarian during the normal evaluation and monitoring of the dogs in his or her care. All samples were obtained with the consent of the pet owner. These samples were tested using the hemolysin adapted protein (hap-1) based *Leptospira* real-time PCR using default cycling conditions on a Roche LC480 instrument (Roche Applied Science, Indianapolis, Indiana) in the 384-well plate configuration. The assay is based on IDEXX's proprietary real-time PCR oligonucleotides (IDEXX Laboratories, Westbrook, Maine). DNA was extracted under standard protocols on a commercial platform (Corbett XTractor-Gene, Qiagen, Valencia, California). Hap-1 gene sequences were aligned and a region was selected for primer and hydrolysis probe design using Primer Express (Version 3.0, Applied Biosystems, Foster City, California), and real-time PCR was run with standard primer and probe concentrations using the Roche LightCycler 480 Probes Master mastermix (Version 3.0, Applied Biosystems). Using MAT as the gold standard, the sensitivity and specificity of the IDEXX hap-1 based *Leptospira* real-time PCR are 92% and 99%, respectively.[12]

Additional demographic information on the pet owner and veterinarian were not collected to ensure privacy. Data included sample submitting location (zip code), test date, and test result (positive or negative). As unique identifiers were not available, duplicate test result entries were identified based on identical dog signalment (i.e., date of birth, breed, sex), test submitting location (zip code) and test date (within 7 days). When test outcomes for a set of duplicate test result entries were the same, the most recent was retained and additional entries removed. If the test outcomes differed for a set of duplicate test result entries, all entries were removed. For descriptive purposes, illness event proportions and 95% confidence intervals were calculated for the study period and for each study year by dividing the number of illness events (positive *Leptospira* real-time PCR tests) by total number of tests. Stata 15 (StataCorp, College Station, Texas) was used for data management and descriptive analysis.

### 2.2 | Spatio-temporal cluster analysis

Data were collapsed to quantify the number of positive tests by date and zip code. Latitude and longitude coordinates were obtained for the centroids of all United States zip codes and these were matched with test result data by zip code.[13] Spatio-temporal clusters for a positive *Leptospira* real-time PCR test were identified using the space-time permutation scan statistic on SaTScan 9.6.[14] The space-time permutation model requires only case data (ie, positive test results) and utilizes overlapping cylinders to define the scanning window.[15] These overlapping cylinders are defined by a base matching the defined geographical area and a cylinder height corresponding to time. The model creates random permutations of the spatial and temporal attributes of each case in the data set.[15] A maximum spatial window of 20% of the population at risk, monthly time precision, and a maximum temporal window of 6 months were used.[1] The software also considered spatial and temporal windows less than the maximum parameters specified.

Clusters were identified and assessed by comparing the number of observed *Leptospira* real-time PCR positive test results with the number of expected positive test results within a scanning window,

where the null hypothesis was a random distribution of cases in space and time. The scan test was performed to detect high rates, with *P* values for the test statistics for identified clusters computed by Monte Carlo simulation of 999 replications under the null hypothesis. A *P*-value of <.05 was considered significant. Summary statistics including time period, radius, observed number of cases, expected number of cases, and the ratio of observed to expected cases were provided for each significant cluster. Significant clusters along with the total number of tests by zip code were mapped using ArcGIS version 10.2.2 (Environmental Systems Research Institute).

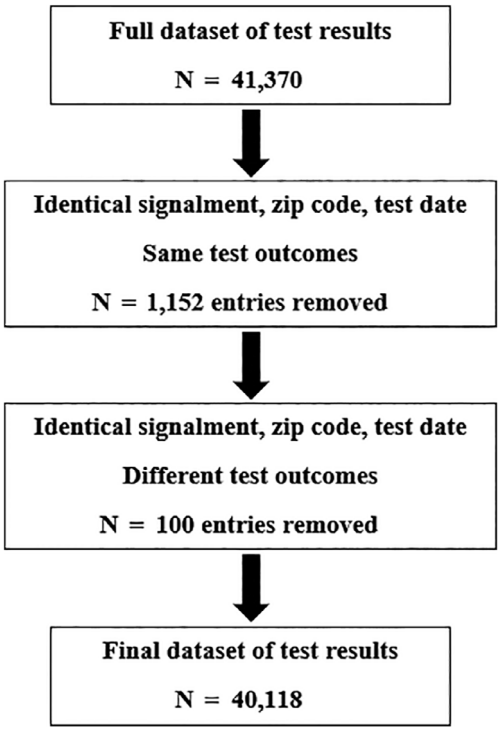


**FIGURE 1** Flowchart for the removal of duplicate test result entries for a dataset of *Leptospira* real-time PCR test results from dogs in the United States (2009-2016)

## 3 | RESULTS

A total of 41 370 test results were available from January 2009 through December 2016. Duplicate test result entries consisted of 1017 dogs where matching entries (concordant test results) were present; 1152 exact test entries were removed (range 1-116 duplicate entries removed per dog, median 1) (Figure 1). A total of 100 test results for 41 dogs with discordant test outcomes were likewise identified and removed (range 2-10 duplicate entries removed per dog, median 2). A total of 1252 duplicates were removed from the dataset due to duplicate entries, leaving 40 118 test results available for analysis.

Test results were available for every state and 5560 unique zip code tabulation areas (range 1 to 425 tests per zip code). Large urban areas had the greatest number of tests (e.g., New York City, Chicago, San Francisco, California). All states had at least 1 positive test except Utah, North Dakota, and Alaska. The *Leptospira* real-time PCR illness event proportion for dogs in the United States during the study period was 5.4% (2176/40118) and varied by year (Figure 2). Positive tests were representative of the examined spatial region (19.8% [1104/5560] of zip code tabulation areas had at least 1 positive test; range 1-40 positive tests per zip code) and time (every month of each year had at least 1 positive test except for April and June of 2009; range 1-92 positive tests per month of each year).

### 3.1 | Spatio-temporal cluster findings

Seven significant space-time clusters of positive *Leptospira* real-time PCR tests in dogs were identified (Table 1, Figure 3). Clusters ranged in size from 0 km (encompassing 1 zip code) to 517.5 km (encompassing 12 states and 351 zip codes) and occurred over numerous years within the study period examined (2010: 1 cluster; 2011: 1 cluster; 2014: 1 cluster; 2014/2015: 1 cluster; 2015: 2 clusters; 2016: 1 cluster). Given unique dates of birth, breed, and sex,

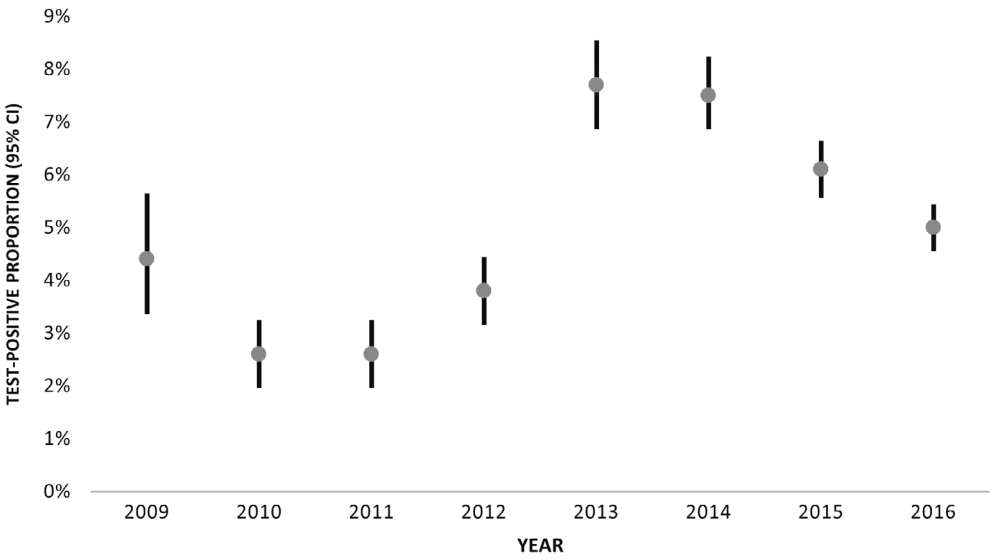


**FIGURE 2** Test-positive proportion and 95% confidence interval by year for *Leptospira* real-time PCR tests in the United States (2009-2016)

**TABLE 1** Attributes of significant clusters identified by the space–time permutation scan statistic for positive *Leptospira* real-time PCR tests in dogs in the United States (2009-2016)

| Cluster | Geographical area | Time period | Radius (km) | Observed number of positive tests | Expected number of positive tests | Observed/ expected ratio | *P* value |
|---|---|---|---|---|---|---|---|
| 1 | northern California | December 2014 to April 2015 | 151.9 | 26 | 3.9 | 6.6 | <.001 |
| 2 | central Arizona | November 2016 | 21.4 | 12 | 0.6 | 19.2 | <.001 |
| 3 | Michigan, southeast Wisconsin, northeast Illinois, north Indiana, northwest Ohio | June 2014 – November 2014 | 292.0 | 108 | 58.9 | 1.8 | <.001 |
| 4 | southeast Kansas, east Oklahoma, northeast Texas, north Louisiana, Arkansas, Missouri, south Illinois, southwest Indiana, west Kentucky, west Tennessee, northwest Alabama, Mississippi | March 2015 to July 2015 | 517.5 | 28 | 7.9 | 3.5 | .005 |
| 5 | southwest Florida | December 2010 | 0 | 3 | 0.01 | 310.9 | .015 |
| 6 | southeast South Carolina | August 2015 to September 2015 | 0 | 7 | 0.4 | 19.6 | .015 |
| 7 | east New York, Vermont, New Hampshire, Massachusetts. Connecticut, Rhode Island | October 2011 to December 2011 | 275.7 | 17 | 3.4 | 5.0 | .026 |

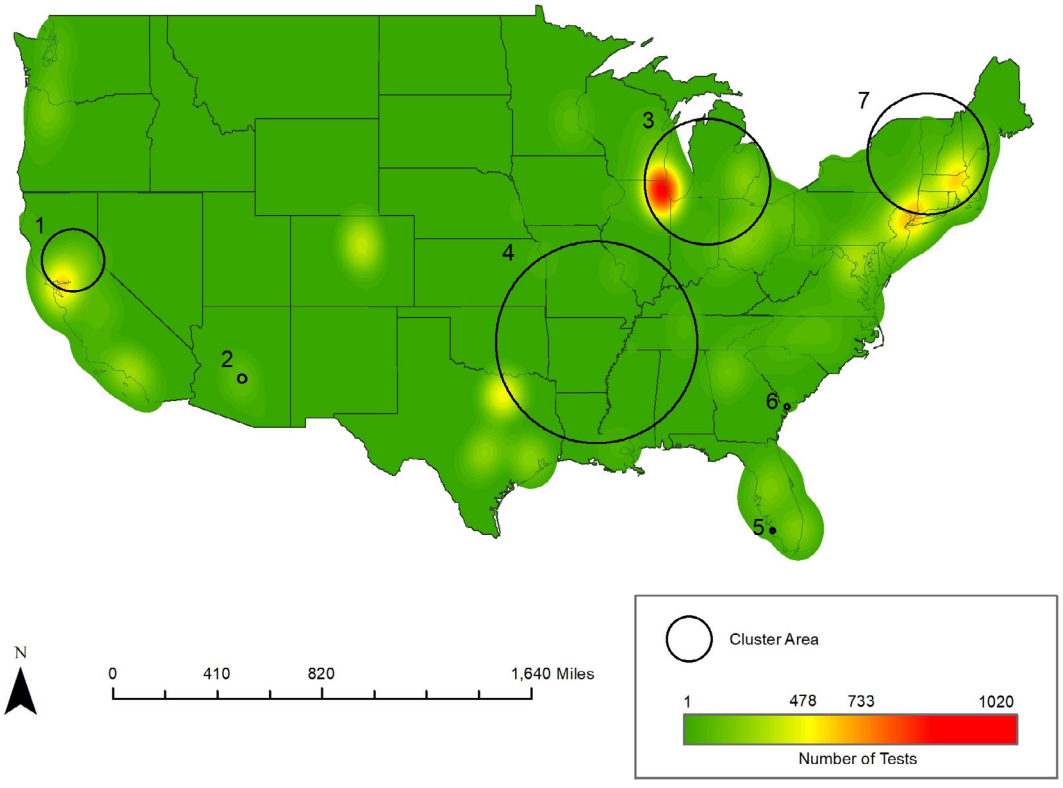


**FIGURE 3** Significant spatial cluster locations of positive *Leptospira* real-time PCR tests identified by the space–time permutation scan statistic and total number of tests in the United States (2009-2016). Circles represent the clusters (and numbers correspond to the cluster numbers in Table 1). Radii for Florida (Cluster 5) and South Carolina (Cluster 6) not drawn to scale. Orange and red areas represent greater test numbers in those geographical areas (see map key)

tests in clusters encompassing a single zip code (clusters #5 and #6) were assumed to have come from individual dogs.

## 4 | DISCUSSION

This study identified 7 significant space-time clusters for *Leptospira* real-time PCR testing in dogs in the United States from 2009 through 2016. Leptospirosis space-time clusters in dogs identified using PCR-positive test results have not been reported.

Clusters were identified across the United States, with the largest clusters encompassing several states in the upper midwest (cluster #3), south-central (#4), and northeast (#7). The finding of the clusters in the upper midwest, south-central, northeast, and northern California in our work was similar to those identified during prior time periods.[1,7] Four of the 7 significant clusters were identified during an 18-month window (June 2014-September 2015). This finding is perhaps unsurprising, as the years 2014 and 2015 had high test-positive proportions relative to the other years included in the study.

The cluster locations in Arizona (cluster #2), Florida (#5) and South Carolina (#6) are not identified locations for leptospirosis clusters in dogs. The Arizona cluster is consistent with the reported leptospirosis outbreak in dogs that occurred in 2016.[9] This demonstrates the assistance surveillance and spatial tools can provide during active and retrospective outbreak investigations. Leptospirosis in dogs is currently not a reportable disease in South Carolina. It is unknown if it was a reportable disease throughout the study period; and, as such, veterinarians or public health entities might not have been aware of multiple positive leptospirosis tests in dogs in a single South Carolina zip code over the 2-month period.[16] Required reporting of leptospirosis in dogs should be considered to improve jurisdictional awareness of disease occurrence and further protect veterinary and human health.

It is unknown what factors cause sporadic increases in leptospirosis in dogs and might influence clusters in known high-prevalence areas or during specific time periods. Potential factors include serovar, environmental, and sociodemographic variables influencing clusters.[1,7,11] Focal shifts in wildlife populations could further influence such clusters. Wildlife serve as the reservoir for several pathogenic leptospirosis serovars suggested to have circulated in dogs in reported cluster locations.[1] Reservoir wildlife species have high a prevalence of *Leptospira* shedding; 41% (n = 1704) of raccoons in the United States are positive for at least 1 *Leptospira* serovar, and *Leptospira* prevalence in urban rat populations is 12% in New York City and 65% in Baltimore.[17-19]

We also suspect there are interactions between reservoir hosts and their environment that increase the prevalence of this pathogen during certain times of the year. For example, mature rats have the highest prevalence of *Leptospira* shedding compared to juveniles, and the proportion of mature rats is highest in summer and fall.[20-22] In addition, periodic drivers such as new building, decreased funding for city rodent elimination programs, and changes in predator presence might alter the abundance and presence of reservoir species populations from year to year, as well as influence the overlap between dogs and infected reservoir hosts or their contaminated environments.

The main limitation of our study, which is similar to that of previous studies, was that the data were acquired from a single commercial laboratory.[1,11] This likely contributed to some areas of the United States being underrepresented, and the absence of these areas might have influenced the spatial locations of the clusters. There could also be selection bias, as real-time PCR is not the only available diagnostic test and toward the end of our data cohort, an additional in-clinic antibody test became available. An additional limitation is that the zip codes utilized in this study were for the sample submitting facility and not necessarily the dog's home location. However, we expect most dogs lived, and were likely exposed to *Leptospira* spp., near the sample submitting facility. As such, we expect this to have had minimal impact on our findings as we used a moderate maximum spatial window during the analysis. In addition, it is unknown to what extend a positive test in this study correlated with clinical disease. Therefore, the clusters identified are clusters of positive tests and not necessarily clusters of leptospirosis clinical disease. It also was unknown what prompted testing for each dog - a dog could have been tested if presenting with clinical disease or a clinician might take a different approach and decide to test based on a recent positive leptospirosis diagnosis in a dog. There are likely a variety of biases playing a role in testing and the makeup of the data set used.

Additionally, as the space-time permutation model only accounts for positive tests, there is a chance some clusters are influenced by a larger volume of testing in an area (ie, urban center) during specific times. Another potential limitation was that it was unknown if blood, urine, or paired blood and urine samples were submitted for each test. In dogs, blood samples are generally positive by PCR-based testing first in disease course, followed by urine samples.[23] Therefore, the samples submitted could have altered test sensitivity and the number of positive tests included in the analysis. Another limitation was no unique dog identifier was available. A conservative approach in removing duplicates was taken to ensure all positive results in the dataset were unquestionably positive and to minimize falsely identifying a cluster.

We observed clusters similar in space and time to those identified in previous work, and it appears that the spatial and temporal components of this disease in dogs have not drastically changed in the United States since the early 2000s. As leptospirosis in dogs is a disease influenced by many factors that can change over time (e.g., circulating serovars, host populations, vaccination), it is encouraging that much appears to remain the same. Although this study did not identify anything new, it provides an update on the status of leptospirosis in dogs in the United States. Future work should focus on collecting dog-level (e.g., exposure histories, vaccination) and clinical data (e.g., infecting serovar, clinical signs) to further investigate the epidemiology of leptospirosis clusters in both high- and low-prevalence areas in order to elucidate effective prevention strategies.

### ACKNOWLEDGMENT

Funding provided by the American Kennel Club Canine Health Foundation, grant 2380-A. The American Kennel Club Canine Health Foundation did not play a role in the design of the study or collection, analysis, and interpretation of data, or in writing the manuscript.

## CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

## OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

## INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Authors declare no IACUC or other approval was needed.

## HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

## ORCID

*Amanda M. Smith* https://orcid.org/0000-0003-0403-6548
*Andréia Gonçalves Arruda* https://orcid.org/0000-0001-7040-9686

## REFERENCES

1. Gautam R, Guptill LF, Wu CC, Potter A, Moore GE. Spatial and spatio-temporal clustering of overall and serovar-specific Leptospira microscopic agglutination test (MAT) seropositivity among dogs in the United States from 2000 through 2007. *Prev Vet Med*. 2010;96(1–2):122-131. https://doi.org/10.1016/j.prevetmed.2010.05.017.
2. Goldstein RE, Lin RC, Langston CE, Scrivani PV, Erb HN, Barr SC. Influence of infecting serogroup on clinical features of leptospirosis in dogs. *J Vet Intern Med*. 2006;20(3):489-494.
3. Langston CE, Heuter KJ. Leptospirosis. A re-emerging zoonotic disease. *Vet Clin North Am Small Anim Pract*. 2003;33(4):791-807.
4. Van de Maele I, Claus A, Haesebrouck F, Daminet S. Leptospirosis in dogs: a review with emphasis on clinical aspects. *Vet Rec*. 2008;163(14):409-413.
5. Levett PN. Leptospirosis. *Clin Microbiol Rev*. 2001;14(2):296-326. https://doi.org/10.1128/CMR.14.2.296-326.2001.
6. Sykes JE, Hartmann K, Lunn KF, Moore GE, Stoddard RA, Goldstein RE. 2010 ACVIM small Animal consensus statement on leptospirosis: diagnosis, epidemiology, treatment, and prevention. *J Vet Intern Med*. 2011;25(1):1-13. https://doi.org/10.1111/j.1939-1676.2010.0654.x.
7. Ward MP. Clustering of reported cases of leptospirosis among dogs in the United States and Canada. *Prev Vet Med*. 2002;56(3):215-226. https://doi.org/10.1016/S0167-5877(02)00160-5.
8. White AM, Zambrana-Torrelio C, Allen T, et al. Hotspots of canine leptospirosis in The United States of America. *Vet J*. 2017;222:29-35. https://doi.org/10.1016/j.tvjl.2017.02.009.
9. Guagliardo SAJ, Iverson SA, Reynolds L, et al. Despite high-risk exposures, no evidence of zoonotic transmission during a canine outbreak of leptospirosis. *Zoonoses Public Health*. 2019;66:7-231. https://doi.org/10.1111/zph.12557.
10. Harkin KR, Roshto YM, Sullivan JT, Purvis TJ, Chengappa MM. Comparison of polymerase chain reaction assay, bacteriologic culture, and serologic testing in assessment of prevalence of urinary shedding of leptospires in dogs. *J Am Vet Med Assoc*. 2003;222(9):1230-1233.
11. Hennebelle JH, Sykes JE, Carpenter TE, Foley J. Spatial and temporal patterns of Leptospira infection in dogs from northern California: 67 cases (2001–2010). *J Am Vet Med Assoc*. 2013;242(7):941-947.
12. 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. *J Vet Intern Med*. 2009;23:772 [abstract].
13. The United States Census Bureau. National Zip Code Tabulation Areas, 2010. 2018. https://www2.census.gov/geo/docs/maps-data/data/gazetteer/ (Accessed October 1, 2018)
14. Kulldorff M. and Information Management Services, Inc. SaTScan™ v9.6: Software for the spatial and space-time scan statistics. Published online 2018. www.satscan.org
15. Kulldorff M, Heffernan R, Hartman J, Assunção R, Mostashari F. A Space–time permutation scan statistic for disease outbreak detection. Blower SM, ed *PLoS Med* 2005;2(3):e59. doi:https://doi.org/10.1371/journal.pmed.0020059
16. Clemson Animal Health Programs. Reportable Diseases. 2019. https://www.clemson.edu/public/lph/ahp/reportable-diseases/index.html (Accessed August 7, 2019)
17. Pedersen K, Anderson TD, Maison RM, et al. Leptospira antibodies detected in wildlife in the USA and the US Virgin Islands. *J Wildl Dis*. 2018;54(3):450-459. https://doi.org/10.7589/2017-10-269.
18. Firth C, Bhat M, Firth MA, et al. Detection of zoonotic pathogens and characterization of novel viruses carried by commensal *Rattus norvegicus* in New York City. *MBio*. 2014;5(5):e01933-e01914. https://doi.org/10.1128/mBio.01933-14.
19. Easterbrook JD, Kaplan JB, Vanasco NB, et al. A survey of zoonotic pathogens carried by Norway rats in Baltimore, Maryland, USA. *Epidemiol Infect*. 2007;135(7):1192-1199. https://doi.org/10.1017/S0950268806007746.
20. Krøjgaard LH, Villumsen S, Markussen MDK, Jensen JS, Leirs H, Heiberg A-C. High prevalence of Leptospira spp. in sewer rats (*Rattus norvegicus*). *Epidemiol Infect*. 2009;137(11):1586-1592. https://doi.org/10.1017/S0950268809002647.
21. Vanasco NB, Sequeira MD, Sequeira G, Tarabla HD. Associations between leptospiral infection and seropositivity in rodents and environmental characteristics in Argentina. *Prev Vet Med*. 2003;60(3):227-235.
22. Himsworth CG, Jardine CM, Parsons KL, Feng AYT, Patrick DM. The characteristics of wild rat (*Rattus* spp.) populations from an Inner-City neighborhood with a focus on factors critical to the understanding of rat-associated zoonoses. Schneider BS, ed. *PLoS One* 2014;9(3):e91654. doi:https://doi.org/10.1371/journal.pone.0091654
23. Greenlee JJ, Alt DP, Bolin CA, Zuerner RL, Andreasen CB. Experimental canine leptospirosis caused by *Leptospira interrogans* serovars Pomona and Bratislava. *Am J Vet Res*. 2005;66(10):1816-1822.

**How to cite this article:** Smith AM, Stull JW, Evason MD, et al. Investigation of spatio-temporal clusters of positive leptospirosis polymerase chain reaction test results in dogs in the United States, 2009 to 2016. *J Vet Intern Med*. 2021;35:1355–1360. https://doi.org/10.1111/jvim.16060