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Regional and Temporal Variations of *Leptospira* Seropositivity in Dogs in the United States, 2000–2010

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Background: Previous studies have reported a seasonal increased risk for leptospirosis, but there is no consistent seasonality reported across regions in the United States.

Objectives: To evaluate and compare seasonal patterns in seropositivity for leptospirosis in dogs for 4 US regions (northeast [NE], midwest [MW], south-central [SC], and California-southern west coast [CS]).

Animals: Forty four thousand nine hundred and sixteen canine serum samples submitted to a commercial laboratory for microscopic agglutination tests (MAT) from 2000 through 2010.

Methods: In this retrospective study, positive cases were defined as MAT titers ≥1 : 3,200 for at least one of 7 tested serovars. Four geographic regions were defined, and MAT results were included in regional analyses based on hospital zip-code. A seasonal-trend decomposition method for times series was utilized for the analysis. Monthly variation in the seropositive rate was evaluated using a seasonal cycle subseries plot and logistic regression.

Results: Two thousand and twelve of 44,916 (4.48%) samples were seropositive. Compared to seropositive rates for February, significantly higher monthly rates occurred during the 2nd half of the year in the MW (OR 3.92–6.35) and NE (OR 2.03–4.80) regions, and only in January (OR 2.34) and December (OR 1.74) in the SC region. Monthly seropositive rates indicative of seasonality were observed earlier in the calendar year for both CS and SC regions.

Conclusions and Clinical Importance: Seasonal patterns for seropositivity to leptospires differed by geographic region. Although risk of infection in dogs can occur year round, knowledge of seasonal trends can assist veterinarians in formulating differential diagnoses and evaluation of exposure risk.

Key words: Dogs; *Leptospira*; Microscopic agglutination tests; Seasonal cycle subseries plot; Seasonal-trend decomposition procedure based on loess; Seropositive.

Leptospirosis is a common zoonotic disease with worldwide distribution affecting many mammalian species.[1–4] It has long been recognized as a disease in dogs, with *Leptospira interrogans* serovars Canicola and Icterohaemorrhagiae being the major serovars contributing to infection in dogs.[5,6] Dogs usually become infected by contact with urine or water containing *Leptospira*.[6,7] Diagnosis is often made via serology and the microscopic agglutination test (MAT) is the most commonly used method for diagnosing infection.[8] The highest MAT titers are often considered indicative of the infective serogroup/serovar, but such interpretations can be erroneous because of cross-reactions between serogroups.

Outside of the United States, there is an increased incidence of leptospirosis in humans associated with high rainfall and flooding, and it is speculated that these conditions increase the chances of contact with leptospire-contaminated water.[2,9,10] Although such outbreaks are not common in the continental United States (US), this association with rainfall has been investigated in leptospirosis in domestic animals.[11–13]

Abbreviations:

CI	confidence interval
CS	California-southern west coast
MAT	microscopic agglutination test
MW	midwest
NE	northeast
OR	odds ratio
SC	south-central
STL	seasonal-trend decomposition procedure based on loess

In California, the number of cases in dogs seen at a university referral hospital correlated (71%) with the annual rainfall at a nearby metropolitan area.[11] Rainfall in the previous 3 months had a 41% correlation with leptospirosis in dogs in Indiana between 1983 and 1998.[12]

More commonly, leptospirosis in dogs in the United States is associated with season of year, with increased frequency of diagnoses in fall.[6,14,15] In New York City, the greatest number of cases in dogs occur between October and December.[16] In Washington State, seropositive rates in dogs are greatest in late summer and fall.[17] This time of year, however, does not always correlate with highest rainfall in many regions of the United States, nor do all US regions have the highest incidence in this season.[a]

Possible seasonal variations of seropositivity to *Leptospira* in dogs at various regions across the United States remain unclear. The main objective of this study was to evaluate the seasonal patterns of leptospirosis in dogs in 4 US regions (northeast [NE], midwest [MW], south-central [SC], and California-southern

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west coast [CS]) using MAT results from a single clinicopathologic data source.

Materials and Methods

The results of leptospirosis MATs for canine serum samples submitted between January 1, 2000, and December 31, 2010 were obtained from IDEXX Laboratories Inc. The MAT results for 7 serovars (Autumnalis, Bratislava, Canicola, Grippotyphosa, Hardjo, Icterohaemorrhagiae, and Pomona) were reported as the highest dilution of serum that agglutinated >50% of live leptospires. All MATs were performed at a single laboratory. The MAT results were reported using 2-fold serial dilutions of serum samples, beginning from 1 : 100. Test request date, veterinary hospital zip code, clinic ID, animal ID, and animal name were included in the dataset.

To reduce the number of seropositive animals associated with titers increased by vaccination, positive results were defined as MAT titers ≥1 : 3,200 for at least one of the tested serovars. Nine percent of the tested dogs had multiple entries in the dataset. Only 1 MAT result was used for each dog. Based on animal ID, the 1st hospital visit per dog was used in analysis except when a subsequent MAT documenting seroconversion was available in which case the latter result was used. The monthly seropositive rate (%) with 95% confidence interval (CI) was calculated by dividing the number of positive results by the total number of samples submitted for that month. The total dataset was first analyzed to investigate the overall trend and seasonality of seropositivity in the United States.

Four regions (state abbreviations shown) were then defined (northeast [NE]: ME, NH, VT, MA, RI, CT, NJ, NY, PA, and DE; midwest [MW]: KY, OH, IN, and MI; south-central [SC]: LA, AR, OK, and TX; California-southern west coast [CS]: CA) using zip codes of the submitting hospital (Fig 1). The NE, MW, and SC regions were determined using zip codes starting with 0, 4, and 7, respectively, whereas the CS region was defined using zip codes between 90001 and 96100. These regions were so defined to provide regions of similar latitude (MW and NE), different latitude (MW/NE versus SC), and different weather systems (eg, CS: Pacific-influenced; SC: Caribbean-influenced).

Monthly seropositive rates were calculated by region during the study period. A seasonal-trend decomposition procedure based on loess (STL) was utilized for the time series analysis, which decomposes the time series into 3 components: seasonal, trend, and remainder.[18] The STL procedure consists of a sequence of applications of the loess smoothing operations to identify data patterns that are not required to conform to mathematical polynomial equations. Annual (12 month) patterns as the seasonal component were first determined, then removed for smoothing to find the trend. The remainder component was the monthly residuals from the seasonal plus trend fit. Monthly variation in the seropositive rate was separately visualized using a seasonal cycle subseries plot which assumed yearly periodicity. The seasonal cycle subseries plot displays horizontal lines for the mean seropositive rate of each month, ie, all Januarys, all Februarys, etc., over the total period; and the end of vertical lines emanating from the horizontal line indicates the specific seropositive rate for that month in each year of the data.

To statistically compare average monthly seropositive rates, univariate logistic regression models were constructed for each region with odds ratio (OR) and 95% CI calculated for each month. The lowest average monthly rate was used as the reference month, which was February for each region except the CS region (where January was the reference). All data were presented in Excel[b] format and analyzed using R[c] and STATA[d] statistical software. Statistical significance was set at $P < .05$.

Results

Two thousand and twelve of 44,916 (4.48%; 95% CI: 4.29–4.67) sera submitted from 2000 to 2010 in the total (US) dataset were positive at titers ≥1 : 3,200. One hundred and thirty-nine (6.91%) of total positive cases were based on seroconversion. In the overall dataset, marked fluctuations were noted with peaks occurring in a pattern suggestive of periodicity (Fig 2). Variation by year also occurred. The annual seropositive rate was the highest in 2004 (5.81%; 95% CI: 5.03–6.68) whereas the lowest rate (2.21%; 95% CI: 1.59–2.97) was observed in 2001. The STL plot of trend, seasonality, and remainder components indicated seasonality to be the strong-

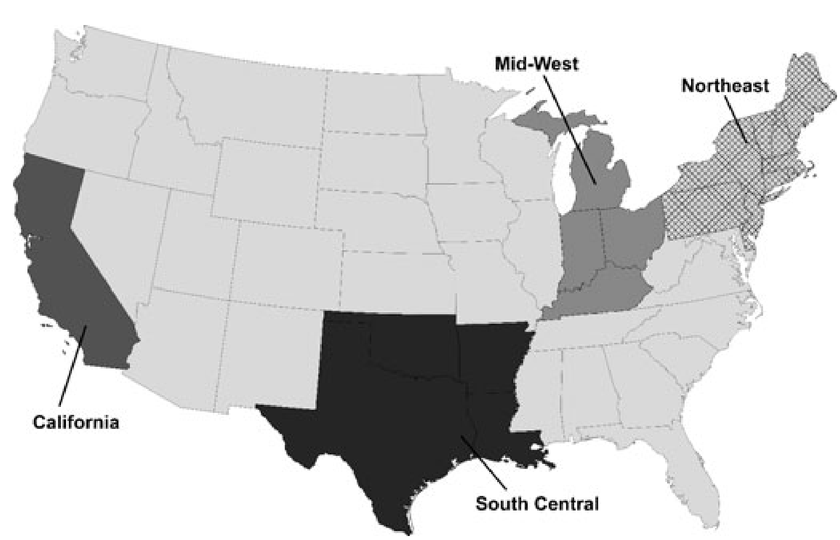

Fig 1. Four geographic regions used in assessment of temporal patterns of seropositivity to leptospirosis in dogs, 2000–2010.

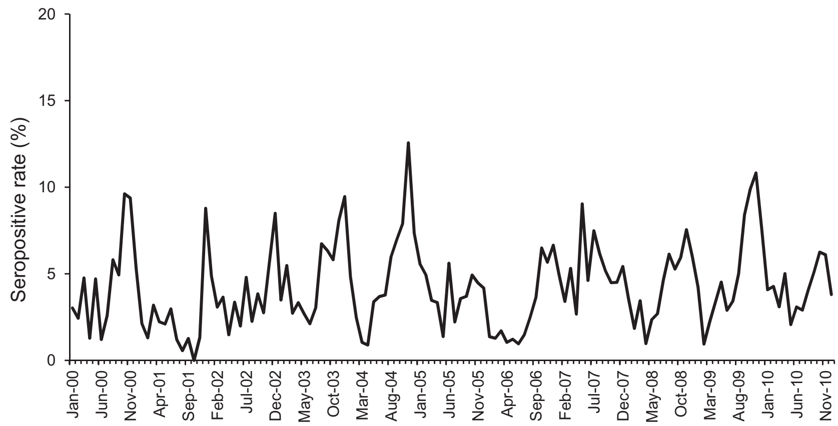

Fig 2. Seropositive rate (%) of microscopic agglutination tests using 1 : 3,200 titer cutoff for canine leptospirosis in the United States by month from January 2000 through December 2010.

est component in the overall data during the study period (Fig 3). The STL plot of the trend component showed only minor fluctuations during the 11 years without an overall increasing or decreasing pattern. The seasonal component indicated a single peak at the end of each year, consistent with increased positive cases in the fall. The remainder component appeared to have random variation although large positive residuals were noted in October 2000, December 2001, November 2004, and May 2007. The seasonal cycle subseries plot showed that mean monthly seropositive rates were greatest in November, followed by December and October (Fig 4). Overall, rates were greatest in the fall (September–December) although a smaller peak in spring (May) is also noted. The cycle subseries plot exhibited interannual variation by month as noted by the vertical bars, eg, a large increase above average occurred in May of 1 year (2007) compared to other years.

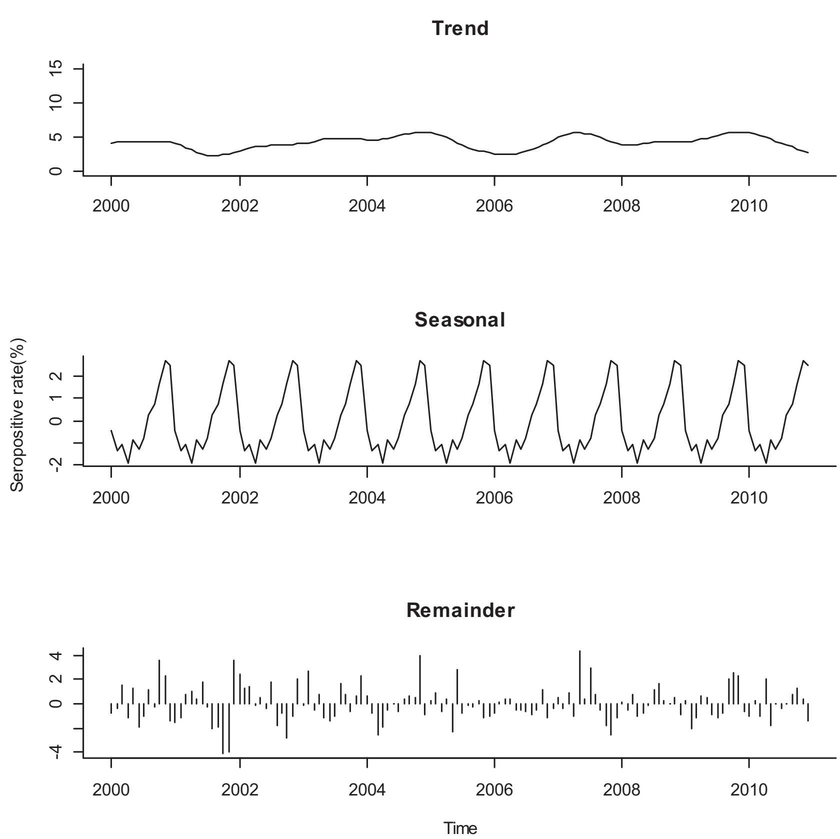

Fig 3. Seasonal-trend decomposition of the monthly seropositive rate (%) for canine leptospirosis in the United States, 2000–2010, displayed in its 3 components of trend, seasonal, and the remainder.

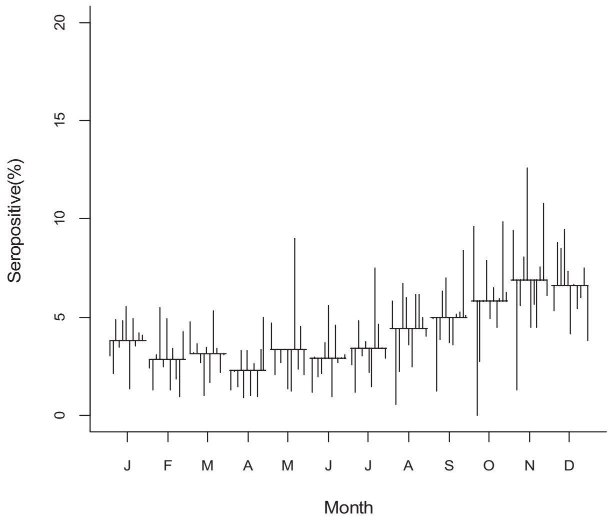

Fig 4. Seasonal cycle subseries plot of the monthly seropositive rate (%) for canine leptospirosis in the United States, 2000–2010. Horizontal lines display the overall mean seropositive rates for each month in the 11-year period. Each vertical line above or below the horizontal bar revealed the difference from the overall monthly average in each year of the data.

STL Decomposition by Region

In the MW region, 169 (4.25%; 95% CI: 3.64–4.92) sera were considered positive in 3,980 submitted samples. The MW samples were 8.9% of the total 44,916 submissions. The STL trend component presented a fluctuating pattern without an apparent overall increase or decrease (Fig 5A). The STL seasonal component had a broad peak in the latter third of the year. The remainder component showed only mild variation in residuals in the 2nd half of the time period except for a large residual in May 2007.

In the NE region, 616 (4.20%; 95% CI: 3.88–4.54) of 14,657 canine sera were positive at a titer ≥1 : 3,200. Contributions from this region represented 32.6% of the total dataset. The STL decomposition showed that the trend component had minimal fluctuation and a slight overall increase during the study period (Fig 5B). The seasonal component was unimodal with a single monthly peak occurring near the end of each calendar year. The remainder component displayed varying residuals during the time series.

For the CS region, there were 234 (3.52%; 95% CI: 3.09–3.99) positive submissions among 6,655 total sera. Sera from this region were 14.8% of the total MAT tests. The STL trend component was relatively flat with minimal fluctuations (Fig 5C). The seasonal component, however, had a primary peak early in the calendar year. Large positive variations in residuals were principally noted in 2000 and 2001.

In the SC region, 311 (7.34%; 95% CI: 6.58–8.17) sera were positive of 4,235 sera submitted for testing. This region accounted for 9.4% of all submissions. The STL trend component for this region displayed the largest fluctuations in the study period (Fig 5D). The seasonal component displayed more peaks per year compared to other regions. The primary peak was at the end of the year, but secondary and tertiary peaks were noted earlier in the year. In the remainder component, large residuals were primarily noted before 2004.

Monthly Evaluation by Region

In the MW region, the seasonal cycle subseries plot showed that on average the seropositive rate in December was greatest, followed closely by October (Fig 6A). Monthly rates in February were less than half of December rates, but gradual increases in seropositive rates occurred during the year. There was variation between years, and high rates (compared to normal for the month) were noted in February, May, July, and September in different years. In regression analysis of month for the MW region, odds of a positive MAT titer were significantly increased in May (OR: 4.53; 95% CI: 1.29–15.93), July (OR: 4.72; 95% CI: 1.35–16.51), August (OR: 3.92; 95% CI: 1.10–14.00), September (OR: 4.51; 95% CI: 1.30–15.66), October (OR: 6.31; 95% CI: 1.86–21.34), November (OR: 6.31; 95% CI: 1.86–21.43), and December (OR: 6.35; 95% CI: 1.86–21.70) compared to February.

The seasonal cycle subseries plot for the NE region showed that the average seropositive rate was greatest in November (Fig 6B). Second and 3rd highest rates were observed in December and October, respectively. Variation in the mean seropositivity between months was greater in this region than in other regions analyzed. Mild variations were noted between years. In the NE region, there were significantly increased odds of a positive test in August (OR: 2.03; 95% CI: 1.21–3.41), September (OR: 2.64; 95% CI: 1.59–4.38), October (OR: 3.26; 95% CI: 2.00–5.30), November (OR: 4.80; 95% CI: 2.96–7.77), and December (OR: 3.64; 95% CI: 2.23–5.96) compared to February.

In CS region, the highest rate on the seasonal cycle subseries plot was in March (Fig 6C). The March rate was closely followed by rates observed in February and adjacent months. Greatest annual variations were noted in higher rates in May, August, October, and November in 2000. In regression analysis, odds of a positive test in any month were not significantly different compared to January as the reference month, except for reduced odds in September (OR: 0.39; 95% CI: 0.16–0.93).

In the SC region, the highest observed average seropositive rate on seasonal cycle subseries plot was in December with a lesser rate in January (Fig 6D). Secondary peaks were observed in July and May. Marked annual variation was noted, but large increases above average were noted in April 2003 and September 2003. For the SC region, the months at significantly increased risk compared to February were January (OR: 2.34; 95% CI: 1.17–4.66) and December (OR: 1.74; 95% CI: 1.04–2.87).

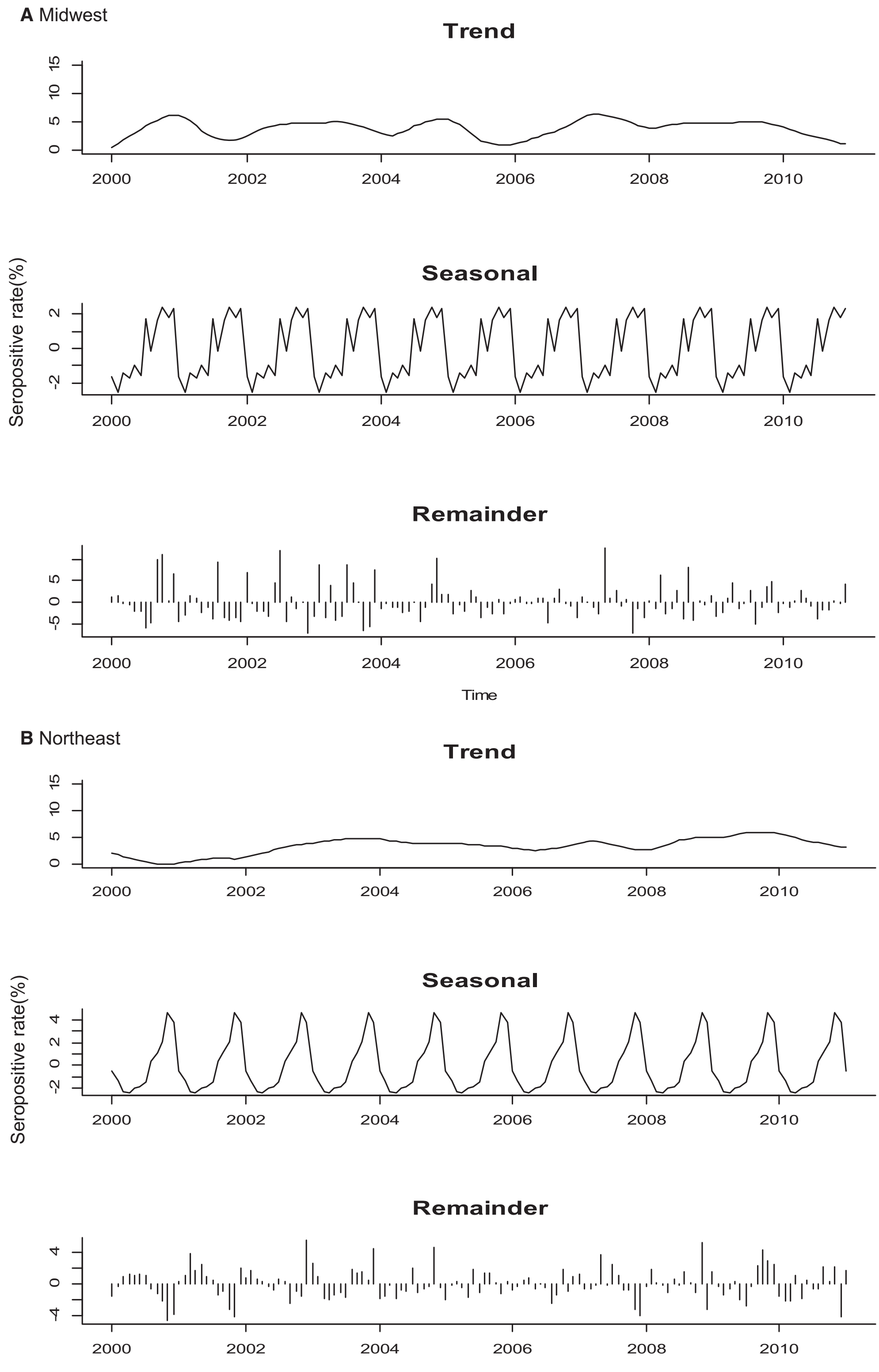

Fig 5. Seasonal-trend decomposition plots of the seropositive rate (%) on a monthly basis for canine leptospirosis in 4 US regions.

Discussion

This study used a single dataset of MAT results from practitioner-submitted canine sera to determine that seasonality of *Leptospira* seropositivity in dogs differed by region in the United States from 2000 to 2010. Various risk factors for leptospirosis, such as amount of rainfall, contact with contaminated water

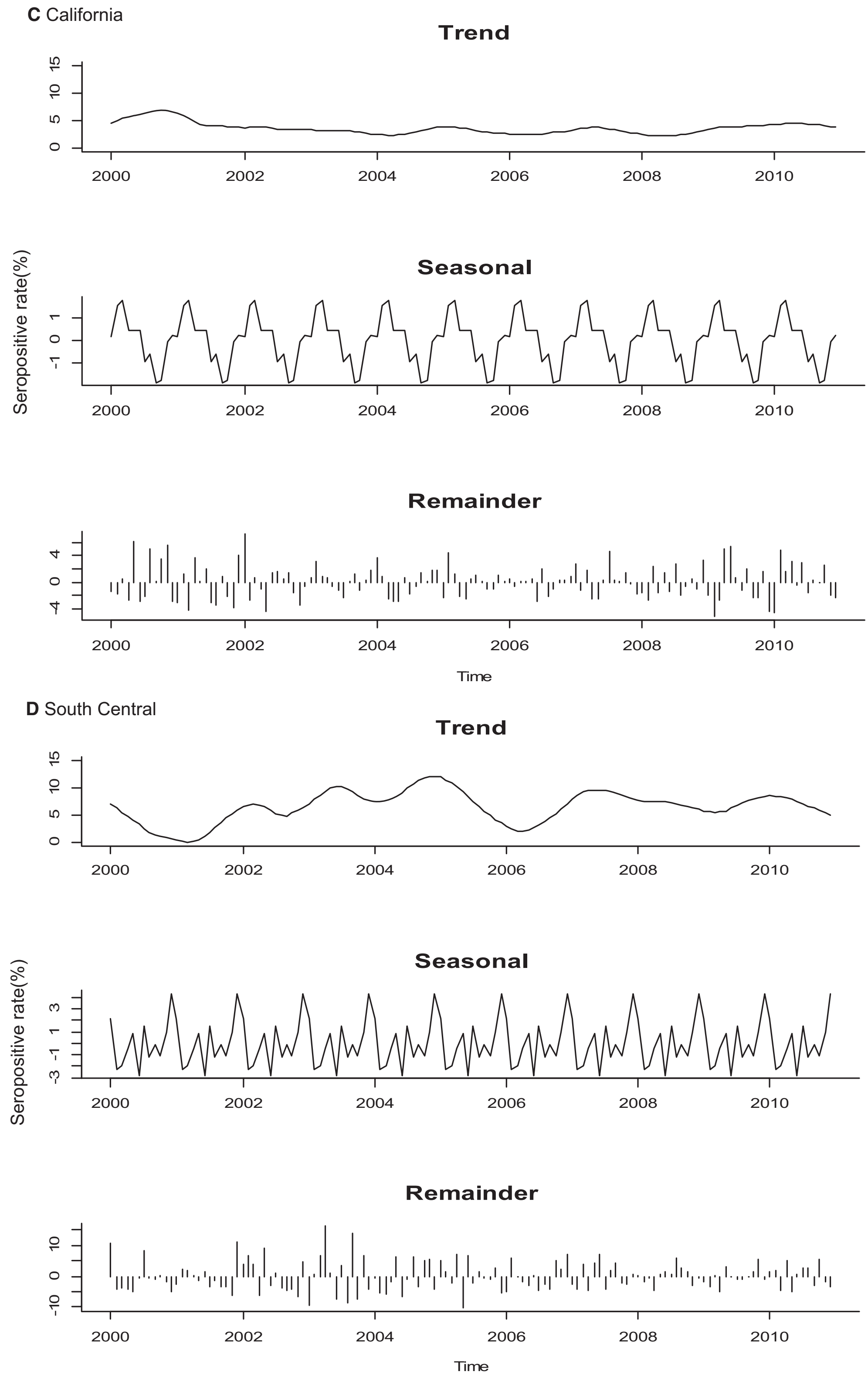

Fig 5. (Continued).

or urine, type and frequency of outdoor activity by reservoir hosts and by dogs, have been suggested in previous studies,[12,14,19,20] but times or "seasons" of greatest risk might differ for these factors and their interactions. Summary measures of large datasets can be influenced by selected subsets of the data, and

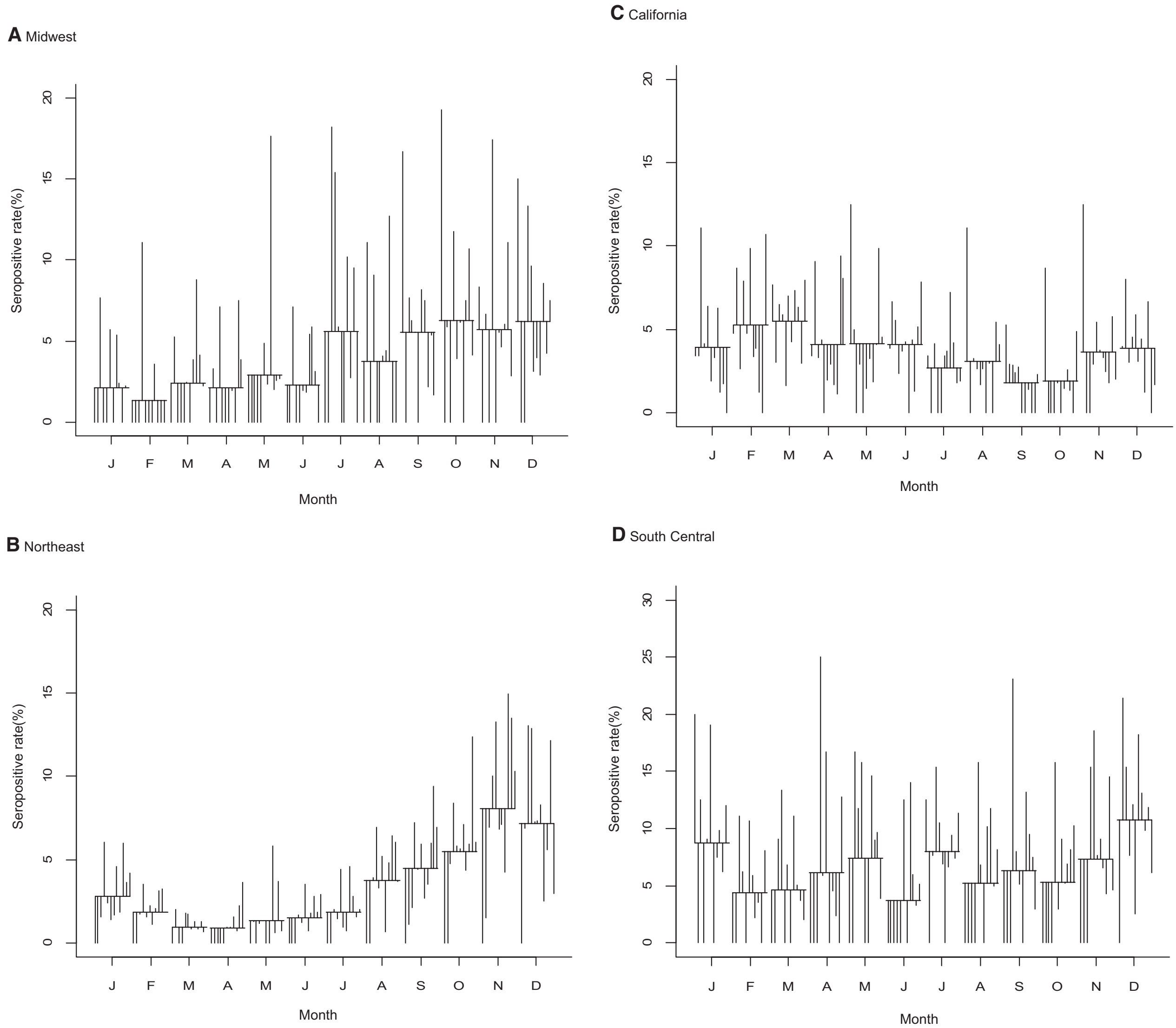

Fig 6. Seasonal cycle subseries plots of the seropositive rate (%) on a monthly basis for canine leptospirosis in 4 US regions.

epidemiological studies could fail to identify differences within distinctive subsets of the data. Variations could exist also within regions analyzed or within other geographic subsets, areas, or both that are not evaluated in the study.

Interpretation of these temporal findings should also consider that they are based on MAT results indicating the presence of anti-*Leptospira* antibodies. After exposure to *Leptospira*, it usually takes a minimum of 7–10 days for a dog to produce detectable serum antibodies.[2,8] The identification of a positive test in a particular month could therefore indicate exposure in the previous month(s). False-negative results can occur early in infection. For these reasons, inclusion of seroconverting cases was accomplished by searching for dogs brought in for additional testing after a negative 1st test. False positives for exposure to the *Leptospira* organism are uncommon with the MAT, but false positives can occur at the serogroup/serovar level because of cross-reactions between serovars or serogroups.[2] This study did not attempt to distinguish between serogroups/serovars. The cutoff titer of $\geq$1 : 3,200 was selected to minimize misclassification because of recent vaccination without elimination of true cases. A nonverifiable assumption was also made that the request for a MAT was predicated on clinical signs potentially associated with leptospirosis in dogs, rather than a request for titer testing after vaccination. The above considerations clearly limit the interpretability of raw percentages alone, but in relative comparisons within a large dataset would not be of consequence. If the data were in fact based on postvaccinal titers, then one would not expect seasonal variation—or possibly an extended level of titers during the summer that then decline would be seen if more vaccines are given in summer.

Analysis of the total dataset, evaluating submissions from all geographic areas together, indicated a strong seasonal increase in the fall. This is consistent with previous studies using multiregional contributions of clinical cases from veterinary hospitals in the United States and Canada.[14,16,20–22] The number of laboratory submissions were not homogeneous across geographic regions in this dataset, and there were some similarities and differences in seasonality in the 4 US regions investigated. The MW and NE regions are contiguous and of similar latitude, and the a priori assumption was that there would be minimal differences in these 2 regions. The SC and CS regions, however, are spatially distinct (from MW/NE and each other) and influenced by different weather patterns.

Contributing to the overall pattern, the highest seropositive rates in the MW and NE regions occurred in October through December. In both these regions of similar latitude, months in the spring, summer, and winter had average positive rates less than those in fall. In the NE region, significantly higher rates occur in August through December, consistent with published case series from this region.[23,24] Increased positive rates for fall, compared to summer months, were also noted in the CS and SC regions; but high/higher monthly rates also indicative of seasonality were observed earlier in the calendar year for both CS and SC regions (February and May, respectively). These late winter or early spring increases might be indicative of milder climates and increased precipitation without freezing.

Previous studies have attempted to discern an association with precipitation or rainfall,[11,12,25] although yearly weather variability can influence patterns. Historical monthly precipitation (rain or snow) data by state indicate October and November are the wettest months in parts of the NE region, eg, Massachusetts, but in the MW region, eg, Ohio, wettest months are May and June.[26] The time interval between these months and fall may explain the lag of 3 months found between precipitation and cases in a previous study.[12] May and June are also the wettest months in Texas (SC region),[26] which is somewhat reflected in the seasonal cycle subseries plot. In California, the months with the wettest weather are January and February.[26] The findings in this study therefore support a relationship between rainfall and leptospirosis, showing a delay in positive tests because of disease incubation and seroconversion. Our regional findings would also correlate with this temporal/seasonal pattern described in clinical cases in northern California.[27] Also, periods of extreme cold (winter in MW and NE) or extreme heat (summer in CS and SC) can be associated with reduced available surface water and reduced seropositivity rates, because leptospires can be killed by freezing or desiccation.[2] Further study is needed to truly assess the potential relationship between rainfall and positive MAT result in each region. Although the designation of regions in this study was not arbitrary, weather patterns are neither restricted to nor homogeneous across states, regions, or both. Future studies conducted with finer resolution of meteorological data could choose other variables for defining geographic areas.

Although the amount of rainfall or flooding has been positively associated with increased cases of leptospirosis in some studies, urine from infected (usually reservoir) hosts is also required. Dogs could have more opportunities to come into contact with organisms shed by wild animals in fall and early winter because of increased movement of urban wildlife (such as raccoons and skunks) in a dispersion period of wildlife family units seeking shelter for the winter period.[28,29] Risk patterns associated with spring could also include increased movement of raccoons, primarily males, as a result of mating periods.[30] Further investigation into the possible role of different wildlife species or environmental conditions contributing to the transmission of disease is needed in each region.

Seasonal-trend decomposition procedure based on loess techniques provide an effective tool to visualize and explore time series events by dividing them into trend, seasonal, and remainders components that best fit the data.[18] Graphic presentation of the data such as seasonal cycle subseries plots also allows identification of time points, such as specific years that deviate from the mean of the data. Other methods used to analyze epidemiological data collected overtime include generalized linear models or time series methods focusing on evaluating change point of variables rather than decomposing and describing its elements.

Seasonal cycle subseries plots like other methods average individual seropositive rates for the same month in each year regardless of the number of submitted samples each month. Therefore, the average seropositive rate (horizontal line) can be influenced by large values, but these extremes can be identified in plotted vertical lines. These plots are visual representations of the data, not statistical comparisons. Assessment of statistically significant differences between monthly rates was made by univariate logistic regression in this study. The calculation of ORs is influenced by selection of the reference group/month, and confidence intervals may be more realistic indicators of differences than the point estimates. It was desirable to use the month with the lowest rates as the reference group, but this was not the same month in all regions.

This study's methodological approach obviously has limitations in its designation of geographic regions. Results related to spatial groupings can be very sensitive to changes in "region" particularly if changes cause the inclusion/exclusion of large subsets of data. Scientific inference also might be more focused on a level other than region or on specific climatic factors, such as precipitation. Erroneous conclusions or inferences about individual areas can be drawn from analysis of aggregated data.[31] This study was not designed a priori, however, as a study of climate or climatic zones, and the use of such data could still be limited by the temporal and spatial resolution of climate data and the uncertainty of the time and location of exposure to infective leptospires.

Temporal and spatial parameters of clinicopathologic data are also potentially affected by submission bias in those ordering the test, and it is not known to what degree biases differ by region. Investigating the epidemiology of any infectious disease is challenging, if clinical signs of the disease are not distinctive or easily recognized by clinicians, if clinicians do not have a reasonable suspicion of the disease, or if accurate economical tests are not available for a rapid diagnosis. Thus, many aspects of the epidemiology of leptospirosis in dogs are challenging, but detectable patterns can help direct future investigations.

In this study using serological test results as an assessment for leptospirosis in dogs, different geographic regions were found to have different seasonal patterns. Seropositive rates also showed differences in their variation by season for different US regions, and seropositivity could be found in sera submitted in any month of the year. Knowledge of seasonal trends can help veterinarians in formulating and ranking lists of differential diagnoses for their patients and can help in the evaluation of the risk of *Leptospira* exposure.

Footnotes

[a] Sykes JE, Bryan J, Armstrong PJ. Comparison of clinical findings associated with canine leptospirosis between two teaching hospitals. J Vet Intern Med 2007;21:624 (abstract)

[b] Microsoft Office Excel 2007

[c] R version 2.15.2; R Foundation for Statistical Computing, Vienna, Austria, 2011

[d] STATA version 11.2; STATA Corp, College Station, TX

Acknowledgments

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Conflict of Interest Declaration: Dr Moore is a consulting editor for experimental design and statistics with the *Journal of Veterinary Internal Medicine*.

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