Title

Regional and Temporal variations of Canine Leptospirosis across the United States, 2000-2010

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Short title

Regional and temporal variations of canine leptospirosis

Keywords

Leptospira; dogs; MAT, seropositive; STL; seasonal cycle subseries plot

List of abbreviations used in the manuscript

MAT microscopic agglutination test, CI confidence interval, STL seasonal-trend decomposition procedure based on loess, OR odds ratio, NE North-east, MW Mid-west, SC South-central, CS California-southern west coast

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Abstract

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: The main objective of this study was to evaluate and compare seasonal patterns in seropositivity for canine leptospirosis for four US regions [north-east (NE), mid-west (MW), south-central (SC), and California-southern west coast (CS)].

Animals: 44,916 canine serum samples submitted for microscopic agglutination tests (MAT) from 2000 through 2010.

Methods: Positive cases were defined as MAT titers ≥ 1: 1,600 for at least one of 7 tested serovars. Four geographical regions were defined, and MAT results were included in regional analyses based on hospital zipcode. A seasonal-trend decomposition method for time series was utilized for the analysis. Monthly variation in the seropositive rate was evaluated using a seasonal cycle subseries plot and logistic regression.

Results: Of the total submissions in the 11-year period, 3,503 (7.80%) were considered seropositive. Significantly higher seropositive rates occurred in November and December in the MW and NE regions, and in December only in the SC region. High/higher monthly seropositive rates also 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 leptospirosis differed by geographical region. Although risk of infection in dogs may occur year-round, knowledge of seasonal trends may assist veterinarians in formulating differential diagnoses and evaluation of exposure risk.

Introduction

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 historically being the major serovars contributing to canine infection.[5,6] Dogs usually become infected when in contact with urine or water containing *Leptospira* bacteria.[6,7] Diagnosis is often made via serology, and the microscopic agglutination test (MAT) has been 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 may be erroneous due to cross-reactions between serogroups.

Increased incidence of leptospirosis in humans has been associated internationally with weather producing high rainfall and flooding because 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 domestic animal leptospirosis.[11-13] In California, one study found the number of canine cases seen at a university referral hospital was correlated (71%) to the annual rainfall amount at a nearby metropolitan area.[11] In another study it was suggested that rainfall in the previous three months had a 41% correlation with leptospirosis in dogs in Indiana between 1983 and 1998.[12]

More commonly, canine leptospirosis in the US has been associated with season of year, with increased diagnoses noted in fall.[6,14,15] In New York City, more canine cases were reported between October and December.[16] In Washington State, seropositive rates in dogs were higher in late summer and fall.[17] This time of year however does not always correlate with highest rainfall in many regions of the US, nor do all US regions note highest incidence in this season.[a]

To our knowledge, no studies have been conducted to evaluate possible seasonal variations of canine leptospirosis at various locations across the United States in the same period. The main objective of this study was to evaluate the seasonal patterns of canine leptospirosis in four US regions [north-east (NE), mid-west (MW), south-central (SC) and California-southern west coast (CS)] using MAT results from a single laboratory 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, patient ID, and patient name were included in the dataset.

In order to reduce the number of seropositive cases associated with post-vaccination titers, positive results were defined as MAT titers ≥ 1:1,600 for at least one of the tested serovars. Approximately, 9% of the tested dogs were brought in more than once to the clinics. Only one MAT result was used for each dog. If discernible (what does that mean…how can the visit be discerned), only the first hospital visit per dog was used in analysis unless a subsequent MAT showed seroconversion after the initial submission. The monthly seropositive rate (%) with 95% confidence interval (CI) was calculated by dividing the number of positive results by the total number of submitted canine samples for that month. The total dataset was first analyzed to investigate the overall trend and seasonality of leptospirosis in the United States.

Four regions (state abbreviations shown) were then defined [north-east (NE): ME, NH, VT, MA, RI, CT, NJ, NY, PA and DE; mid-west (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. The NE, MW and SC regions were determined using zip codes starting with 0, 4, and 7, respectively, while the CS region was defined using zip codes between 90001 and 96100. 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: trend, seasonality 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. 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 average seropositive rate of each month over the total period, and each vertical line above and below the horizontal line indicates the specific seropositive rate for that month in each year of the data.

In order to statistically compare average monthly seropositive rates, univariate logistic regression models were constructed for each region with odds ratio 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 $p<0.05$.

Results

A total of 3,503 (7.80%; 95% CI: 7.55-8.05) sera were positive at any titer ≥ 1:1,600 among 44,916 submitted samples (one sample per dog) from 2000 to 2010. Marked fluctuations between months were noted with peaks occurring in a pattern suggestive of periodicity (Fig. 1). Variation by year also occurred. The annual seropositive rate was the highest in 2009 (10.15%; 95% CI: 9.42-10.92) while the lowest rate (4.31%; 95% CI: 3.44-5.32) was observed in 2001. The STL plot of trend, seasonality and remainder components indicated a strong seasonal component in the overall data during the study period (Fig. 2). The STL plot of the trend component showed mild fluctuations during the eleven years without an overall increasing or decreasing trend. 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 a large positive residual is noted in May 2007. The seasonal cycle subseries plot showed that on average monthly seropositive rates were greatest in November, followed by December and October (Fig. 3). Overall, rates were greatest in the fall (September-December) although a smaller peak in spring (May) is also noted. The cycle subseries plot exhibited inter-annual variation by month as noted by the vertical bars, e.g. a large increase above average occurred in May of one year (2007) compared to other years.

STL decomposition by region

In the Midwest (MW) region, 353 (8.87%; 95% CI: 8.00-9.80) 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. 4A). The STL seasonal component was unimodal, but the peak was accompanied by a ‘shoulder’ of high rates also preceding this peak. The remainder component showed mild variation in residuals, except for one large residual which occurred in 2000.

In the north-east (NE) region, 1,042 (7.11 %; 95% CI: 6.70-7.54) of 14,657 canine sera were positive for a diagnosis of leptospirosis. 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. 4B). The seasonal component was unimodal with a single monthly peak occurring near the end of each calendar year. The remainder component displayed relatively small residuals each month of the time series.

For the California-southern west coast (CS) region, there were 410 (6.16%; 95% CI: 5.59-6.77) 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. 4C). The seasonal component however had a primary peak early in the calendar year with a smaller secondary peak occurring approximately 9 months later. Large variations in residuals were principally noted in 2000.

In the south-central (SC) region, 500 (11.81 %; 95% CI: 10.85-12.82) 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. 4D). 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 noted in early 2002 and 2003.

Monthly evaluation by region

In the Mid-west (MW) region, the seasonal cycle subseries plot showed that on average the seropositive rate in December was greatest, followed closely by November (Fig. 5A). Monthly rates in February were approximately half of December rates but gradual increases in seropositive rates were noted during the year. Variation did occur between years, and unusually high rates (compared to normal for the month) were noted in February, March, May, June, July and September in different years. In regression analysis of month for the MW region, odds of a positive MAT titer were significantly increased only in November (OR: 2.27; 95% CI: 1.27-4.06) and December (OR: 2.17; 95% CI: 1.20-3.91) compared to February.

The seasonal cycle subseries plot for the north-east (NE) region showed that the average seropositive rate was greatest in November (Fig. 5B). Second and third highest rates were observed in December and October, respectively. Variation 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: 1.55; 95% CI: 1.06-2.27), September (OR: 2.26; 95% CI: 1.57-3.26), October (OR: 2.32; 95% CI: 1.62-3.31), November (OR: 3.54; 95% CI: 2.49-5.02) and December (OR: 2.31; 95% CI: 1.60-3.32) compared to February.

In California-southern west coast (CS) region, the highest rate on the seasonal cycle subseries plot was in February (Fig. 5C). The February rate was closely followed by rates observed in

March, April, and November. Greatest annual variations were noted in higher rates in May, August, October and November in 2000. In regression analysis only February (OR: 2.06; 95% CI: 1.27-3.36) and March (OR: 2.17; 95% CI: 1.35-3.49) were significantly increased in odds of a positive test compared to January as the referent month.

In the south-central (SC) region, the highest observed average seropositive rate on seasonal cycle subseries plot was in December (Fig. 5D). The secondary peak was in May with lesser rates seen in January, April, July, and November. Marked annual variation was noted, but large increases above average were noted in April 2003 and May 2002. For the SC region, the only month at significantly increased risk compared to February was December (OR: 1.74; 95% CI: 1.04-2.87).

Discussion

This study used a single dataset of MAT results from practitioner-submitted dog sera to determine that seasonality of canine leptospirosis differed by region in the United States. Various risk factors for leptospirosis, such as amount of rainfall, contact with contaminated water/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 may differ for these factors and their interactions. Summary measures of large datasets may be influenced by selected subsets of the data, and epidemiological studies may fail to identify differences within distinctive subsets of the data. Variations, i.e. heterogeneity, may exist also within regions analyzed or within other geographical subsets/areas not evaluated.

Interpretation of these temporal findings should also consider that they are based on MAT results indicating serologic conversion. Following exposure to *Leptospira*, it generally takes 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 may therefore indicate exposure in the previous month(s). False-negatives occur early in infection. Therefore inclusion of seroconverting cases was accomplished by searching for dogs brought in for additional testing after a negative first test; 7.22% (253/3,503) of total positive cases were based on seroconversion. False-positives for exposure to the *Leptospira* organism are uncommon with the MAT, but false-positives can occur at serogroup/serovar level due to cross-reactions.[2] This study did not attempt to distinguish between serogroups/serovars. Antibody production also occurs after exposure and subclinical disease, persisting at low titers, as well as after vaccination. The cutoff titer of ≥1:1,600 was selected to attempt to minimize misclassification without inappropriate elimination of true cases. A non-verifiable assumption was also made that the request for a MAT was predicated on clinical signs potentially associated with canine leptospirosis. The above considerations clearly limit the interpretability of raw percentages alone, but may be considered non-differential in assessing relative comparisons within a large dataset.

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 multi-regional contributions from veterinary hospitals in the United States and Canada. [14,16,20-22] It was noted however that the numbers of laboratory submissions were not homogeneous across geographical regions in this dataset, and some similarities and differences in seasonality were noted in the four 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 two 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 November and 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 were reported 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 may 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 may influence patterns. Historical monthly precipitation (rain or snow) data by state indicates October and November are the wettest months in parts of the NE region, e.g. Massachusetts, but in the MW region, e.g. 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 could therefore support a relationship between rainfall and leptospirosis, showing a delay in positive tests due to disease incubation and seroconversion. 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, since 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 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 may have more opportunities to come into contact with organisms shed by wild animals in fall and early winter due to increased movement of urban wildlife (such as raccoons and skunks) in a dispersion period of wildlife family units seeking shelter for the winter period.[27,28] Risk patterns associated with spring may also include increased movement of raccoons, primarily males, as a result of mating periods.[29] Further investigation into the possible role of different wildlife species or environmental conditions contributing to the infection is needed in each region.

STL 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 as in seasonal cycle subseries plots also allows identification of time points, e.g. specific years, that deviate from the mean of the data. Other methods used to analyze epidemiological data collected over time include generalized linear models or time series methods focusing on evaluating change-point of parameters rather than decomposing and describing its elements. A recent paper that reviewed methods used to assess seasonality in epidemiological studies of human infectious disease did not include methods such as STL.[30] STL methods have not been commonly employed in veterinary research, perhaps due to lack of time series data conducive to its use.

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 in this study was made by univariate logistic regression in this study. The calculation of odds ratios is influenced by selection of the referent 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 referent group, but this was not the same month in all regions.

Temporal and spatial parameters of laboratory 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 canine leptospirosis are challenging, but detectable patterns can help direct future investigations.

In this study using serological test results for canine leptospirosis, different geographical regions were found to have somewhat 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 may 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; Development Core Team, 2011

[d] STATA version 11.2, STATA Corp., College Station, TX, USA

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Figure legends

Figure 1. Seropositive rate (%) of microscopic agglutination tests for canine leptospirosis in the United States by month from January 2000 through December 2010.

Figure 2. Seasonal-trend decomposition of the monthly seropositive rate (%) for canine leptospirosis in the United States, 2000-2010, displayed in its three components of trend, seasonal, and the remainder.

Figure 3. Seasonal cycle subseries plot of the monthly seropositive rate (%) for canine leptospirosis in the United States, 2000-2010. Horizontal lines display the overall average seropositive rates for each month in the 11 year period, and each vertical line above or below the horizontal bar revealed the difference from the overall monthly average in each year of the data.

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

Figure 5. Seasonal cycle subseries plots of the seropositive rate (%) on a monthly basis for canine leptospirosis in four US regions.

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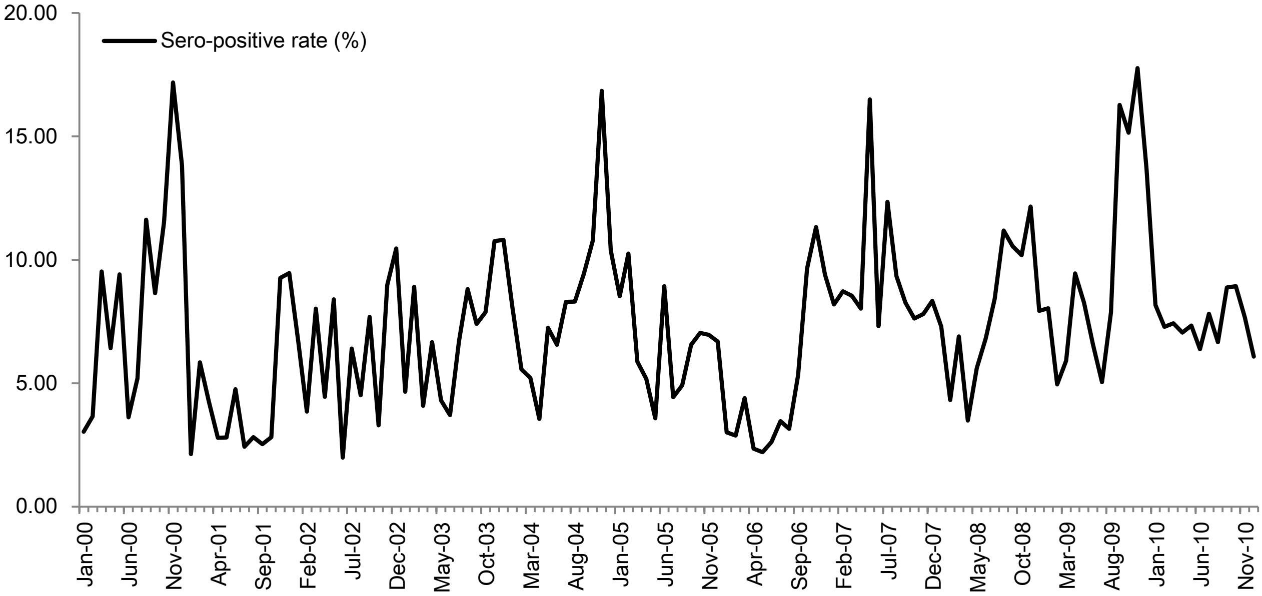

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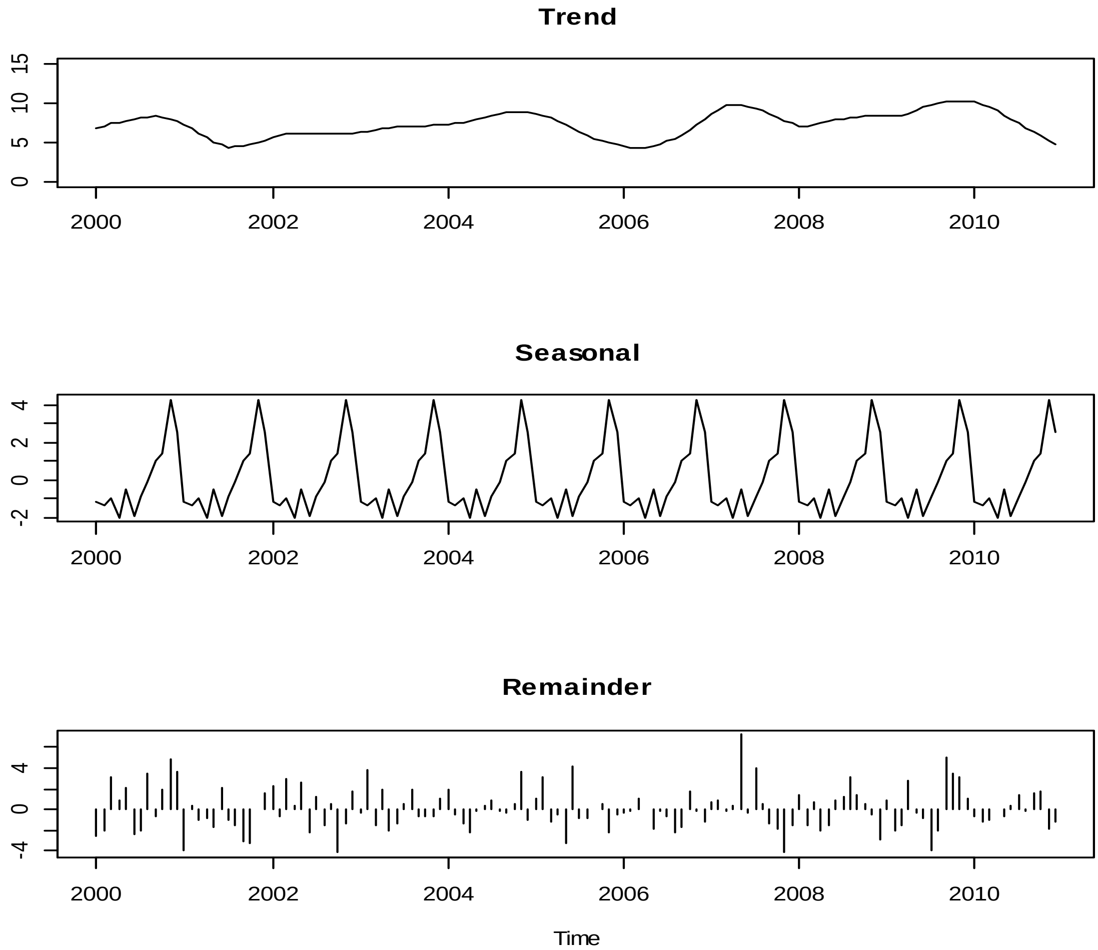

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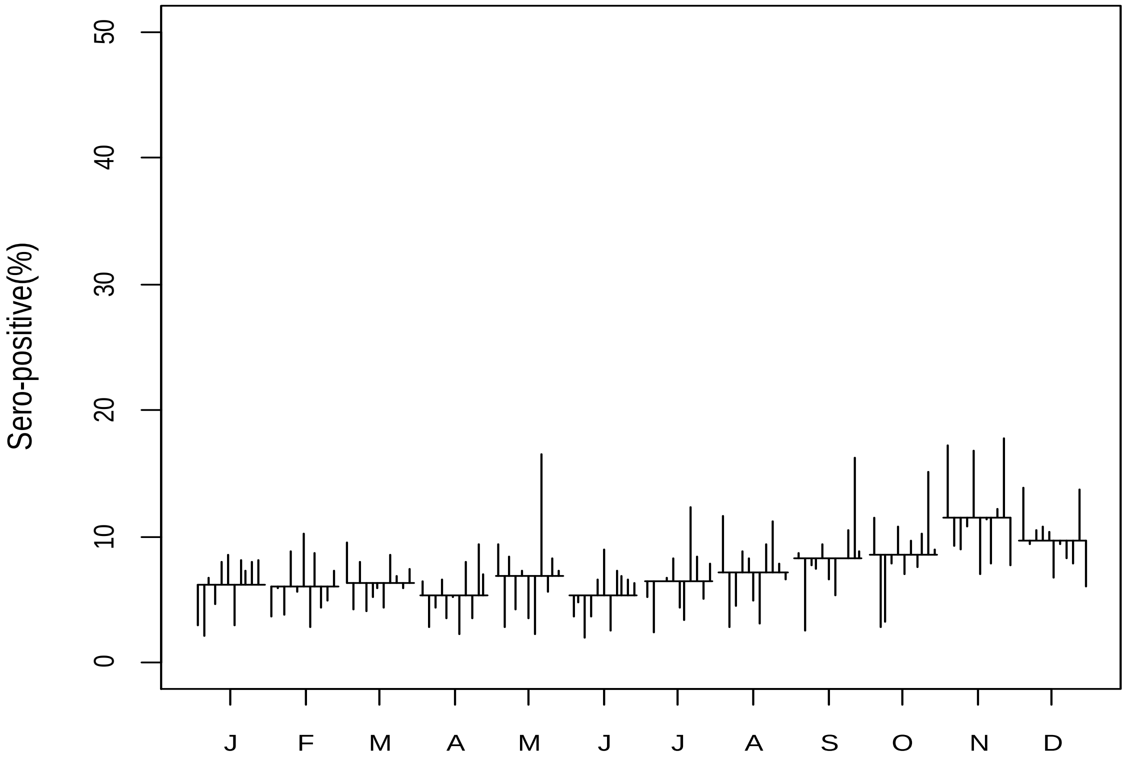

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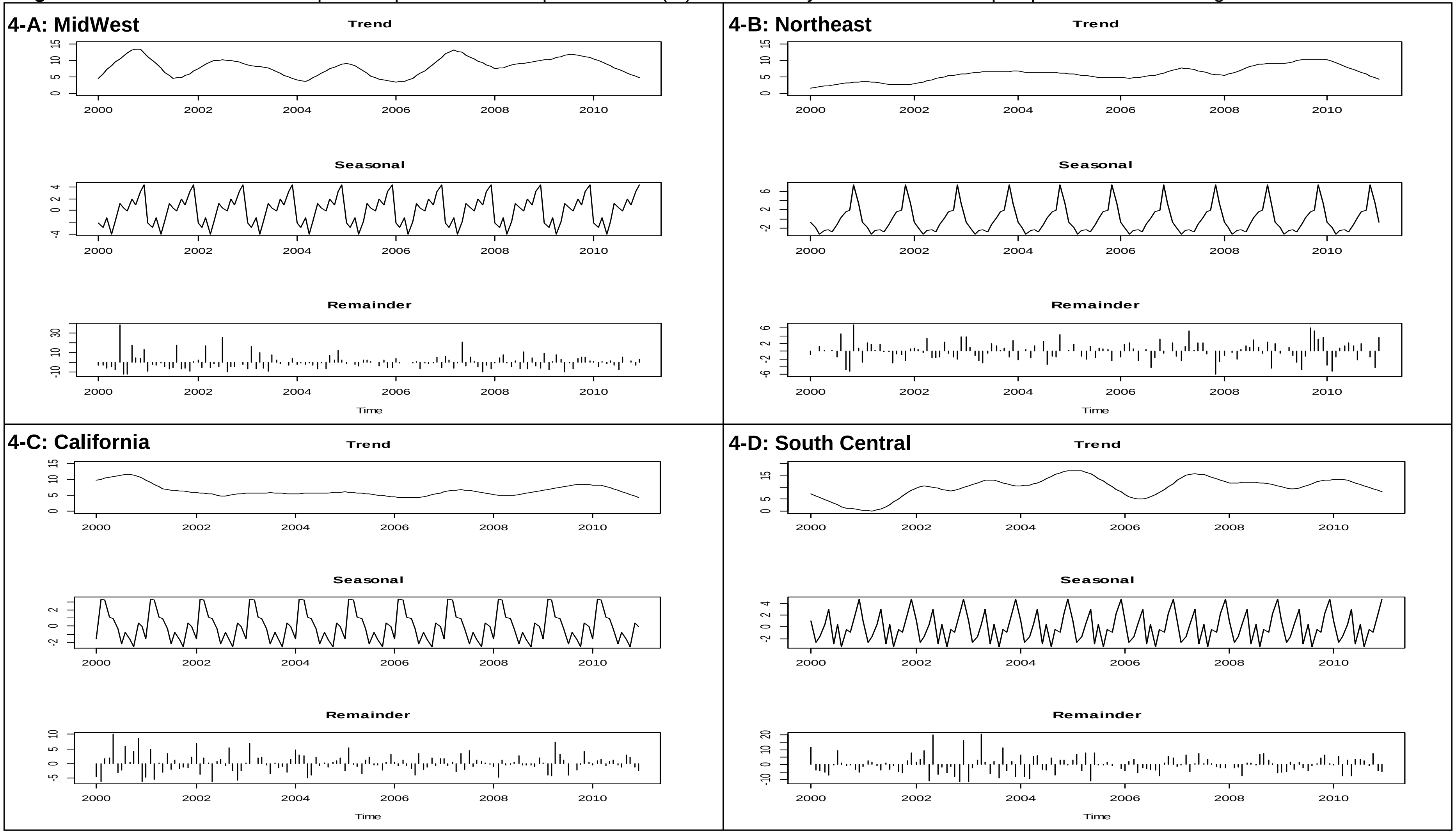

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