Spatial and Temporal Emergence Pattern of Lyme Disease in Virginia

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Abstract. The emergence of infectious diseases over the past several decades has highlighted the need to better understand epidemics and prepare for the spread of diseases into new areas. As these diseases expand their geographic range, cases are recorded at different geographic locations over time, making the analysis and prediction of this expansion complicated. In this study, we analyze spatial patterns of the disease using a statistical smoothing analysis based on areal (census tract level) count data of Lyme disease cases in Virginia from 1998 to 2011. We also use space and space–time scan statistics to reveal the presence of clusters in the spatial and spatiotemporal distribution of Lyme disease. Our results confirm and quantify the continued emergence of Lyme disease to the south and west in states along the eastern coast of the United States. The results also highlight areas where education and surveillance needs are highest.

INTRODUCTION

Lyme disease has become the most commonly reported vector-borne disease in the United States.¹ The disease is caused by the bacterium *Borrelia burgdorferi* sensu stricto, which is transmitted to humans by *Ixodid* species ticks.² Patients suffering from Lyme disease can have acute symptoms, such as skin rash, fever, headache, and fatigue during the early stage of infection.³ Patients not treated during the early stage of infection may develop severe, chronic multi-systemic symptoms, such as arthritis in major joints, shooting pains, numbness or tingling in the hands or feet, and memory problems.³ A 1998 study estimated that treatment costs of Lyme disease in the United States were about \$2.5 billion over a 5-year period.⁴ Thus, Lyme disease has become a significant public health burden in the United States.

Although Lyme disease was initially endemic to New England and a few other northeastern states, over the last several decades, it has expanded its range southward, westward, and northward from its initial endemic range. Since the first identification of the illness in 1975 in the town of Lyme,⁵ Connecticut, cases of the disease have been reported in the northeastern United States, the upper Midwest, and California.^{6–8} From 1992 to 2006, 248,000 Lyme disease cases were reported, and several states, including Virginia, showed an expansion of the endemic range of Lyme disease.⁹ Specifically, within Virginia, the number of cases has more than tripled in the 5-year period from 2007 to 2011 compared with the previous 5-year period (Figure 1). According to the Virginia Department of Health (VDH), this increase is not solely because of reporting or diagnostic changes; rather, it is the result of increased cases, because it has spread southward into Virginia from neighboring states that are endemic for Lyme disease.¹⁰

Spatial smoothing and cluster analyses are often used to enhance visualization of disease spatial patterns and spread.^{8,11} Identification of disease incidence clusters provides public health officials with the ability to assess where scarce resources

should be applied. In this broader context, the goal of this paper is to quantify spatial and temporal emergence patterns of human cases of Lyme disease in Virginia between 1998 and 2011 to assess where and when human case clustering occurs.

MATERIALS AND METHODS

Study area. The study examined the spread of Lyme disease incidence within the state of Virginia (latitude: 36°32' N to 39°28' N; longitude: 75°15' W to 83°41' W), which is located in the South Atlantic region of the United States (Figure 2). Total population of the state was around 8 million according to the 2010 census. Lyme disease emergence was first detected in the Delmarva Peninsula of Virginia as isolated cases. Subsequently, it became prevalent in the densely populated northern Virginia region; then, during the past two decades, the disease spread southward and westward into the less developed and less densely populated central and southern regions (Figure 2). Although there are limitations in treating Virginia as a closed system, the focus of this paper addresses the emergence of Lyme disease from Virginia's northern counties toward the south rather than on the initial introduction of Lyme disease into the state. Rapid suburban/periurban development and the southward expansion of the disease into Virginia (with many cases occurring in areas with a high level of development) makes the state a valuable model for addressing important scientific questions regarding the role of environmental conditions and anthropogenic habitat change on Lyme disease emergence at multiple spatial scales.

Lyme disease data. Statewide surveillance for Lyme disease in Virginia started in 1989 when it became a reportable disease.¹² This study examined all Lyme disease-positive laboratory reports received by the VDH for patients from commercial testing laboratories as well as Lyme disease case reports provided to the VDH by physicians as part of Virginia's Lyme disease case surveillance. Lyme case classification at the VDH was based on the National Surveillance Case Definition for Lyme disease.¹³ The National Surveillance Case Definition for Lyme disease changed in 2008. Before 2008, a case could be confirmed by the presence of an erythema migrans (EM) rash alone or the manifestation of one late-stage symptom with laboratory confirmation.¹⁴ However, after 2008, cases could be confirmed by known exposure or the appearance of an EM

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FIGURE 1. Counts of human cases of Lyme disease in Virginia from 1998 to 2011.

rash only when it occurred in an already endemic county. In a non-endemic county, a positive laboratory test was required.¹⁵ We believe that the more rigorous definition after 2008 could possibly reduce the number of false positives. To account for the change in the case definition, we screened the case data and identified 44 cases in 32 census tracts that had cases reported before 2008 but none after 2008 according to the new definition. We considered these 44 cases as possible misdiagnoses and removed them from our data analysis, resulting in a total of 6,714 cases for our analysis.

Lyme disease cases that were counted after 1998 were mapped by patient address for geographic analysis at the VDH using Centrus Software.¹⁶ We obtained case data with coordinate information from the VDH. We then aggregated the cases to census tracts for analysis according to the latitude and longitude of each case. Census tracts were selected as the unit of analysis for the following reasons: (1) the availability of population data as well as other demographic information for each census tract, facilitating standardization of the raw count data; (2) census tracts boundaries are based, in most cases, on permanent features, such as roads, rivers, or railroads, which constitute potential barriers to the movement of mice or deer, compared with arbitrary political boundaries, such as zip code boundaries; and (3) a previous study found that road-bounded polygons of varying sizes,¹⁷ which are similar to census tracts, produced stronger models in a study of Lyme disease and forest-edge habitats than a study design using grid cells of standard sizes.

The time frame considered in this paper is from 1998 to 2011, excepting 1999 and 2002. Years 1999 and 2002 were removed from the analysis, because we did not have case data at the census tract level for those 2 years. Population information for 2010 for each census tract was downloaded from the US Census Bureau (<http://www.census.gov/>). For all other years, we used intercensal estimates at the county level (the 2000 census tract boundaries differ from those of 2010; hence, the 2000 census tract populations cannot be used directly). The yearly population in each census tract (i.e., the population for census tract i in year j) is determined by its county's intercensal-estimated population in year j prorated by a factor. The factor is computed as the ratio of the tract's population over its county's population based on the 2010 census data.

Spatial smoothing. A disease rate map is useful for public health practitioners to determine high-risk areas. The mapping

FIGURE 2. Lyme disease raw rates at the census tract level in 2003, 2006, 2007, and 2010. Light blue color represents a rate below 0.3 per 10,000 population in the census tract. The upper left plot shows labels for the neighboring state/region. The color class intervals were determined by Jenks optimization method. DP = Delmarva Peninsula in Virginia; KY = Kentucky; MD = Maryland; WV = West Virginia.

of raw disease rates, however, may obscure spatial patterns in disease risk, especially when raw rates are computed from regions that exhibit large variability in population.¹¹ In our study, the maximum population for a census tract was 22,060, whereas the minimum was only 1 based on Virginia's 2010 census data. The maps based on raw rates, as shown in Figure 2, suffered from high variability in rate estimation at different tracts. Thus, it was necessary to obtain smoothed rates to account for spatial variability of raw estimated rates. Also, it was easier to examine spatial patterns in smoothed disease rate maps.

To address instability of disease rates in census tracts with low populations, we used a locally weighted average approach. The approach used a kernel function to smooth the rate for each census tract by averaging values associated with neighboring regions.¹¹ Let $N(s_i, t)$ be the aggregated case number for census tract i during year t , $i = 1, \dots, n$, and $t = 1, \dots, T$. Here, n is the number of census tracts in Virginia (per 2010 definition), and T is the number of years when data at the census tract level are available. Let E_{it} be the population for census tract i at year t and d_{ij} be the distance between the centroid of census tracts i and j . The raw disease rate for census tract i during year t , $\lambda(s_i, t)$, is computed as $N(s_i, t)/E_{it}$. The locally weighted estimates of the disease rate $\hat{\lambda}(s_i, t)$ for

census tract i during year t were obtained using the following formula (equation 1):

$$\hat{\lambda}(s_i, t) = \frac{\sum_{j=1}^n K_b(s_i, s_j) N(s_j, t)}{\sum_{j=1}^n K_b(s_i, s_j) E_{jt}},$$

where $K_b(s_i, s_j)$ is a bivariate kernel function with bandwidth parameter b . The kernel function assigns more weight to observations that are closer to location s . Although there are several choices for the kernel function (e.g., bivariate Gaussian and bivariate quadratic function), we found that the bivariate Gaussian function worked well through cross-validation (e.g., pp. 48–53 in ref. 18).

The cross-validation was completed using the leave-one-out method. In particular, we left one census tract out and used the rest of the data and equation 1 to calculate the smoothed rate for the omitted tract. We then calculated the square of the difference between the smoothed rate (obtained by using the leave-one-out data) and the actual observed rate. This was done repeatedly for each tract to obtain the sum of the squared differences. The kernel function that we chose was the function that minimized the sum of squares of differences. The bandwidth parameter determines how many neighboring

FIGURE 3. Lyme disease smoothed rates at the census tract level in selected years. Light blue color represents a rate below 0.3 per 10,000 population in the census tract. The color class intervals were determined by Jenks optimization method.

regions should be included in the locally weighted averaging. The choice of the bandwidth parameter was determined for each year using cross-validation. The implementation of the spatial smoothing was done by using R.¹⁹

Spatial and temporal clustering. It is important to know if the cases vary randomly over space/time or if there are spatial/temporal clusters to better understand the emergence pattern of a disease. The spatial scan statistics can be used to locate disease clusters.^{20,21} Using a circular window with a continuously adjusted radius, this spatial statistical technique can detect probable size-varied geographic clusters as the center of the window moves over the study area. This technique has been used to detect potential geographic clusters of various human and equine diseases.^{22–25} In this study, we applied the spatial clustering method to investigate the spatial pattern of the Virginia human Lyme disease case data in each year of the study period.

To incorporate the time dimension into the analysis, a space–time scan statistic was used, where the scan window becomes a cylinder with an additional time dimension.^{26–28} With a varying time period for the cylinder, the space–time scan statistic can identify significant space–time clusters, such as those found in an equine West Nile epidemic study.²⁴ We also applied the space–time scan statistic to the Virginia Lyme case data, which included the entire study period, implemented both prospectively and retrospectively. The

prospective space–time scan statistic moves from the starting point of the entire time period to the end to detect possible clusters, whereas the retrospective space–time scan statistic moves in the reverse direction. Both the spatial and space–time scan statistics are implemented in the publicly available software SaTScan (www.satscan.org).

RESULTS

There were 6,714 cases of Lyme disease identified in the study period (1998–2011). The cases identified in the last 5 years of the study period (i.e., from 2007 to 2011) account for 74% of the total cases. There are 1,907 census tracts, with a mean (SD) tract area equal to 58.14 km² (117.86 km²), a mean (SD) tract population equal to 4,191 (1,811), and a mean (SD) tract population density equal to 1,204/km² (1,851/km²). For conciseness, Figure 3 shows the smoothed rate for only selected years. As is evident from Figure 3, years 2003 and 2006 are within the time period in which the case number is low but steadily increasing. After year 2006, the disease counts were at a relatively high level and reached a peak in 2010. Compared with the raw rate maps shown in Figure 2, the smoothed maps show more continuity in the disease rate, and hence, they are more valuable in identifying trends in emergence. At the beginning of our investigation period, Lyme disease cases were mainly found in the northern

FIGURE 4. Lyme disease clusters in selected years.

TABLE 1
Lyme disease primary cluster summary by year

Year	Annualized rate (per 10,000 population)	Primary cluster location	No. of census tracts included in the primary cluster	Population of the primary cluster (%)	Area of the primary cluster (%)	RR of the primary cluster	Expected cases of the primary cluster	Observed cases of the primary cluster
1998	10	Eastern shore	4	0.26	0.87	38.41	1	6
2000	20	Northern VA	55	2.10	1.82	30.49	3	57
2001	21	Northern VA	270	14.03	4.18	14.26	21	107
2003	25	Northern VA	78	3.78	2.77	20.98	7	84
2004	28	Northern VA	59	2.88	1.77	17.59	6	72
2005	35	Northern VA	124	6.86	3.22	15.92	18	143
2006	46	Northern VA	332	17.67	3.81	11.75	62	252
2007	123	Northern VA	279	15.67	7.91	10.11	148	618
2008	119	Northern VA	253	14.43	7.05	9.74	135	580
2009	110	Northern VA	328	18.63	8.97	6.57	162	521
2010	154	Northern VA	426	24.86	16.67	6.38	306	836
2011	123	Northern VA	295	17.47	20.12	6.96	172	585

VA = Virginia.

Virginia area. Then, the main focus of disease occurrence area shifted southward to less developed and less heavily populated areas. By the end of the investigation period, the disease had spread to the southern boundary of the state. The eastern shore of Virginia (on the Delmarva Peninsula) has a moderate disease rate in all time periods. Note that the population in that area is low, and therefore, increases in rates might be elevated artificially.

Setting the maximum spatial cluster size to be less than or equal to 50% of the state's population, we identify statistically significant spatial clusters for each year (P value < 0.05). Figure 4 shows geographical locations of primary and secondary clusters for selected years. The primary cluster is the cluster that is least likely to have occurred by chance, which was identified by maximum likelihood estimation. Secondary clusters are those ranked after the primary cluster by their likelihood ratio test statistics. Table 1 gives detailed information for the primary clusters for each year.

For year 2003, the primary cluster is in the northern Virginia area in the semirural counties west of Washington, DC. The cluster includes 78 census tracts and only 3.78% of the state's population. For year 2006, the primary cluster is in the same region as in year 2003, but the cluster expands to include more populated tracts. This cluster includes 332 census tracts, 3.81% of the state's area, and 17.67% of the state's population, with 252 observed cases versus 62 expected cases and a relative risk (RR) of 11.75. The RR indicates that the risk of becoming a Lyme disease case is 11.75 times higher for individuals residing inside the primary cluster than individuals living outside the cluster. The disease has been expanding toward the southwestern part of the state since 2006. For year 2010, the primary cluster includes 426 census tracts, 16.67% of the state's area, and 24.86% of the state's population, with 836 observed cases versus 306 expected cases and an RR of 6.38. The RR indicates that the risk of Lyme disease for those who reside inside the

primary cluster is 6.38 times higher than for those who reside outside the cluster. Note that, from 2006 to 2008, the percentage of the population in the primary cluster did not increase. This effect is because of the fact that the primary cluster shifts from a more populated area to a less populated area from 2006 to 2008 (Figure 4). The area of the primary cluster mostly increases during the study period (Table 1), expanding into the less populated western part of the state.

Different from the spatial clustering, the space-time scan statistic can take the time dimension into account. Figure 5 shows that the primary prospective cluster, which is located in the northern Virginia area, covers the period from 2008 to 2011 (Table 2). This space-time cluster covers 426 census tracts with an average of 24.69% of the state's population. The cluster has 2,748 observed cases versus 673 expected cases and an RR of 6.71, indicating that the risk of Lyme disease for those residing in this region during 2008 to 2011 is 6.71 times higher than for those residing outside this space-time cylinder when we study the entire period prospectively. Note that the space-time cluster can provide an RR for a period of time within a certain spatial region, whereas a spatial cluster cannot provide this information in the time dimension. Similarly, Figure 6 shows that the primary retrospective cluster covers the period from 2010 to 2007 (Table 3). The cluster is also located in the northern Virginia area, with 268 census tracts and 15.34% of the state's population. The cluster has 2,336 observed cases versus 415 expected cases and an RR of 8.6, indicating that the risk of Lyme disease for those residing in this region from 2001 to 2007 is 8.6 times higher than for those residing outside this space-time cylinder when we study the entire period retrospectively.

DISCUSSION

Most disease pattern studies involve only a 4- or 5-year period.^{29,30} Investigating a time period of more than a decade

TABLE 2
Lyme disease space-time cluster analysis (prospective) summary

Cluster location	Cluster indicator	Time frame of the cluster	No. of census tracts included in the cluster	Average % of the population of the cluster	Area of the cluster (%)	RR of the cluster	Expected cases of the cluster	Observed cases of the cluster
Northern VA	Primary	1/1/2008 to 12/31/2011	426	24.69	16.67	6.71	673	2,748
Southwestern VA	Secondary	1/1/2008 to 12/31/2011	21	1.35	3.07	4.34	37	157
Eastern shore	Secondary	1/1/2008 to 12/31/2011	12	0.58	2.55	4.35	16	68
Central VA	Secondary	1/1/2010 to 12/31/2011	97	5.18	15.43	1.7	71	120

VA = Virginia.

TABLE 3
Lyme disease space–time cluster analysis (retrospective) summary

Cluster location	Cluster indicator	Time frame of the cluster	No. of census tracts included in the cluster	Average % of the population of the cluster	Area of the cluster (%)	RR of the cluster	Expected cases of the cluster	Observed cases of the cluster
Northern VA	Primary	1/1/2007 to 12/31/2010	268	15.34	7.85	8.6	415	2,336
Central VA	Secondary	1/1/2010 to 12/31/2011	173	10.09	18.7	3.39	139	446
Southwestern VA	Secondary	1/1/2008 to 12/31/2011	21	1.35	3.07	4.34	37	157
Eastern shore	Secondary	1/1/2003 to 12/31/2005	11	0.60	2.11	5.42	12	62

VA = Virginia.

allows us to detect not only the spatial pattern but also, the temporal pattern of disease emergence. Understanding spatial and temporal emergence patterns pinpoints priority areas for the education of physicians, so that they are aware that their patients could be affected by the disease, and the general public, who can use preventative measures to avoid contracting the illness. Additionally, an understanding of diffusion patterns can aid in the creation of predictive models for future spread.

Our study finds that the spatial distribution of Lyme disease in Virginia is not random but clustered, which is consistent with a previous study of Lyme disease's emergence in New York.³¹ Clusters are also observed in the occurrence of other tick-borne diseases, such as human granulocytic anaplasmosis.³⁰ Clusters are important to public health practitioners, because they indicate the areas that were most highly affected by a disease in the past. For example, physicians in the primary cluster area should be educated to recognize that patients in this area are under a high risk of Lyme disease infection. More importantly, newly emerging clusters should be given special attention, because they usually indicate the direction and velocity that a disease is spreading. Specifically, Lyme disease in Virginia is clearly spreading toward the southwestern part of the state, which is indicated by the expansion of the primary cluster in this direction. From the direction and speed of Lyme disease's expansion shown by the analysis, it is likely that this primary cluster will further expand to the southwestern part of Virginia in the next few years. Actions from public health practitioners and the general public are needed to minimize additional cases in this area. Lyme disease diagnostic tests should be performed, and appropriate therapy should be initiated in patients with symptoms.

Lyme disease cases in Virginia mostly occurred in the northern and eastern counties, and they were generally along the US Interstate Highways I-81, I-95, and I-64 corridors. Those regions have experienced pronounced population growth and accompanying urban/suburban development in recent decades. Suburbanization has brought residential land use into landscapes that were formerly characterized by agricultural land

mixed with small woodlots and other forested land. The resulting disturbance of this pattern has created a fragmented mix of land cover types that form habitat favoring white-tailed deer (*Odocoileus virginianus*) and white-footed mice (*Peromyscus leucopus*). The white-footed mouse is the most competent host and reservoir for *B. burgdorferi* in the eastern United States, whereas there are a number of reservoir hosts that can transmit *Borrelia* species to ticks in the western United States. In our study area, white-tailed deer provide a blood meal for egg production by female ticks and serve as an important dispersal mechanism for ticks across the local landscape.³² It is known that changes in land cover in the northeastern United States (i.e., suburbanization and reforestation of former agricultural land) played a role in the emergence of Lyme disease in the 1970s.³³ People who moved into those areas were likely to encounter land inhabited by rodents and white-tailed deer. Also, a number of studies^{34–37} have shown that the emergence or re-emergence of vector-borne diseases often is strongly associated with landscape features of the suburban residential environment.

Much of Virginia has land tracts abundant in forested and herbaceous land cover, which provide an ideal habitat for animals, such as white-tailed deer and white-footed mice. Because these animals play important roles in tick reproduction and Lyme disease transmission, it is not unexpected that Lyme disease would eventually become more prevalent in Virginia. One of our next undertakings in our Lyme disease research in Virginia is to use environmental data and population data to build a model relating Lyme disease occurrence and environmental and demographic variables.¹² This analysis will help us to identify the factors that affect Lyme disease infection risk, explain patterns of Lyme disease spread noted in this study, and provide better guidance to public health practitioners.

Our analyses have several limitations. First, the spatial analyses were based on locations where the cases were reported (i.e., home addresses), which may not necessarily identify the place where the infection was acquired. Second,

FIGURE 5. Lyme disease prospective clusters.

FIGURE 6. Lyme disease retrospective clusters.

in a minority of cases (less than 5% of our case data in any year), we encountered cases identified only by a post office box number instead of a street address. (We geocoded these cases at the centroid of the available zip code.) In the very few cases where we had no street address, no box number, and no zip code but were given the name of a small town or independent city within which the case resided, we used the centroid for that town or city. Note that, in our analysis, however, we aggregated case data to the census tract level; thus, the geocoding error was of less concern, and any introduced error was minimal.

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REFERENCES

1. Mead P, 2009. *Lyme Disease*. Available at: <http://wwwnc.cdc.gov/travel/yellowbook/2010/chapter-5/lyme-disease.aspx>. Accessed October 6, 2009.
2. Steere AC, Hardin JA, Malawista SE, 1978. Lyme arthritis: a new clinical entity. *Hosp Pract* 13: 143–158.
3. *Lyme Disease*. Available at: http://www.cdc.gov/lyme/signs_symptoms. Accessed April 11, 2014.
4. Maes E, Lecomte P, Ray N, 1998. A cost-of-illness study of Lyme disease in the United States. *Clin Ther* 20: 993–1008.
5. Steere AC, 1982. Lyme disease. *MMWR Morb Mortal Wkly Rep* 31: 367–368.
6. Waller LA, Goodwin BJ, Wilson ML, Ostfeld RS, Marshall SL, Hayes EB, 2007. Spatio-temporal patterns in county-level incidence and reporting of Lyme disease in the northeastern United States, 1990–2000. *Environ Ecol Stat* 14: 83–100.
7. Kitron U, Kazmierczak JJ, 1997. Spatial analysis of the distribution of Lyme disease in Wisconsin. *Am J Epidemiol* 145: 558–566.
8. Eisen RJ, Lane RS, Fritz CL, Eisen L, 2006. Spatial patterns of Lyme disease risk in California based disease incidence data and modeling of vector-tick exposure. *Am J Trop Med Hyg* 75: 669–676.
9. Bacon RM, Kugeler KJ, Mead PS, 2008. Surveillance for Lyme disease – United States, 1992–2006. *MMWR Morb Mortal Wkly Rep* 57: 1–9.
10. Virginia Department of Health (VDH), 2008. *Reportable Disease Surveillance in Virginia (Annual Reports) 1996–2008*. Richmond, VA: VDH Office of Epidemiology.
11. Waller L, Gotway C, 2004. *Applied Spatial Statistics for Public Health Data*, 1st Ed. Hoboken, NJ: Wiley-Interscience.
12. Seukep SE, Kolivras KN, Hong Y, Li J, Parisley SP, Campbell JB, Gaines DN, Dymond RL. An examination of the demographic and environmental variables correlated with lyme disease emergence in Virginia. Submitted to *EcoHealth*.
13. Centers for Disease Control and Prevention, 2013. *National Notifiable Diseases Surveillance System for Lyme Disease*. Available at: <http://wwwn.cdc.gov/NNDSS/script/conditionssummary.aspx?CondID=100>. Accessed December 12, 2013.
14. *Lyme Disease, 1996 Case Definition*. Available at: <http://wwwn.cdc.gov/NNDSS/script/casedef.aspx?CondYrID=750&DatePub=1/1/1996%2012:00:00%20AM>. Accessed April 11, 2014.
15. *Lyme Disease, 2008 Case Definition*. Available at: <http://wwwn.cdc.gov/NNDSS/script/casedef.aspx?CondYrID=751&DatePub=1/1/2008%2012:00:00%20AM>. Accessed April 11, 2014.
16. 1996. *Centrus*. Stamford.
17. Jackson LE, Levine JF, Hilborn ED, 2006. A comparison of analysis units for associating Lyme disease with forest-edge habitat. *Community Ecol* 7: 189–197.
18. Silverman B, 1986. *Density Estimation for Statistics and Data Analysis*. Boca Raton, FL: Chapman & Hall/CRC.
19. The R Project for Statistical Computing, 2013. *R*. Available at: <http://www.r-project.org/>. Accessed December 12, 2013.
20. Kulldorff M, 1997. A spatial scan statistic. *Comm Stat Theory Methods* 26: 1481–1496.
21. Kulldorff M, 1998. GIS and health. Gatrell A, Loytonen M, eds. *Statistical Methods for Spatial Epidemiology: Tests for Randomness*. London, England: Taylor & Francis, 49–62.
22. Hjalmars U, Kulldorff M, Gustafsson G, Nagarwalla N, 1996. Childhood leukaemia in Sweden: using GIS and a spatial scan-statistic for cluster detection. *Stat Med* 15: 707–715.
23. Viel JF, Arveux P, Baverel J, Cahn JY, 2000. Soft-tissue sarcoma and non-Hodgkin's lymphoma clusters around a municipal solid waste incinerator with high dioxin emission levels. *Am J Epidemiol* 152: 13–19.
24. Lian M, Warner RD, Alexander JL, Kenneth RD, 2007. Using geographic information systems and spatial and space-time scan statistics for a population-based risk analysis of the 2002 equine West Nile epidemic in six contiguous regions of Texas. *Int J Health Geogr* 6: 42.
25. Huang L, Tiwari RC, Zou Z, Kulldorff M, Feuer EJ, 2009. Weighted normal spatial scan statistic for heterogeneous population data. *J Am Stat Assoc* 104: 886–898.
26. Kulldorff M, Athas WF, Feuer EJ, Miller BA, Key CR, 1998. Evaluating cluster alarms: a space-time scan statistic and brain cancer in Los Alamos, New Mexico. *Am J Public Health* 88: 1377–1380.
27. Kulldorff M, 2001. Prospective time periodic geographical disease surveillance using a scan statistic. *J R Stat Soc Ser A Stat Soc* 164: 61–72.
28. Sonesson C, 2007. A CUSUM framework for detection of space-time disease clusters using scan statistics. *Stat Med* 26: 4770–4789.
29. Jackson LE, Hilborn ED, Thomas JC, 2006. Towards landscape design guidelines for reducing Lyme disease risk. *Int J Epidemiol* 35: 315–322.
30. Chaput EK, Meek JJ, Heimer R, 2002. Spatial analysis of human granulocytic ehrlichiosis near Lyme, Connecticut. *Emerg Infect Dis* 8: 943–948.
31. Glavanakov S, White D, Caraco T, Lapenis A, Robinson GR, Szymanski BK, Maniatty WA, 2001. Lyme disease in New York State: spatial pattern at a regional scale. *Am J Trop Med Hyg* 65: 538–545.
32. Mather TN, Wilson ML, Moore SI, Ribeiro JMC, Spielman A, 1989. Comparing the relative potential of rodents as reservoirs of the Lyme disease spirochete (*Borrelia burgdorferi*). *Am J Epidemiol* 130: 143–150.
33. Barbour AG, Fish D, 1993. The biological and social phenomenon of Lyme disease. *Science* 260: 1610–1616.
34. Boone JD, McGwire KC, Ottosen EW, DeBaca RS, Kuhn EA, Villard P, Brussard PF, St Jeor SC, 2000. Remote sensing and geographic information systems: charting Sin Nombre virus infections in deer mice. *Emerg Infect Dis* 6: 248–258.
35. Rogers DJ, Randolph SE, Snow RW, Hay SI, 2002. Satellite imagery in the study and forecast of malaria. *Nature* 415: 710–715.
36. Maupin GO, Fish D, Zultowsky J, Campos EG, Piesman J, 1991. Landscape ecology of Lyme disease in a residential area of Westchester County, New York. *Am J Epidemiol* 133: 1105–1113.
37. Li S, Hartemink N, Speybroeck N, Vanwambeke SO, 2012. Consequences of landscape fragmentation on lyme disease risk: a cellular automata approach. *PLoS ONE* 7: e39612.