EFFECTIVENESS OF PRESCRIBED BURN TREATMENT ON FORESTED LAND AS A METHOD TO REDUCE LYME DISEASE HUMAN-CONTRACTIONS IN THE STATE OF VIRGINIA

by
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ABSTRACT

Virginia, and the greater United States, are currently experiencing an expansion in the range and increase in cases reported of Lyme disease, caused by the bacteria *Borrelia burgdorferi*. A parasitic tick, *Ixodes* sp., is the primary vector of the disease causing bacteria in the Eastern United States. The vector tick has been increasing its range to the north, west, and south, leading many land management professionals to inquire into the effectiveness of stopping the spread of Lyme disease by reducing *Ixodes* sp. range expansion. Understanding how the effects of natural resource management on the expanding range and subsequent expanding number of Lyme disease cases is paramount in determining best practices for mitigating the Lyme disease human risk. Prescribed burn treatment of viable forested habitat has more recently been touted as viable and potentially effective natural resource management method to reduce tick populations. The effectiveness of prescribed burn treatment to actually reduce rates of human Lyme disease case reporting in Virginia, however, is not fully understood.

This study examined the spatial patterns of Center for Disease Control Lyme disease case reports in Virginia and whether the current usage of prescribed burn treatment reduces the reported contraction of Lyme disease in human populations. A spatial autocorrelation analysis concluded Lyme disease estimated incidence rates per 100,000 persons by county exhibited clustered spatial patterns. These clusters reflected areas of high population density and suitable forested habitat for *Ixodes* sp. and host species. A regression analysis examining the effects of the number of acres applied with prescribed burn treatment on the reduction of estimated incidence rates is yet unclear. Further analysis is of paramount importance in the decisions made by public and private landowners in how to implement land management practices that reduce the risk of Lyme disease.

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1.Introduction

As interactions between natural resource management and disease epidemiology have become more evident in the past few decades with the onset of new scientific avenues of inquiry, such as landscape ecology, it is critical that we understand the impact and effectiveness that land management practices have on the spread, emergence, or reduction of diseases (Turner, 2005, Brearley et al., 2013, Wheeler & Waller, 2008) From the observed causal relationships of human-modified landscapes on the prevalence of wildlife disease to the effect of heterogeneous landscape on rabies transmission in raccoon populations, the importance of considering spatial relationships when mitigating disease and determining natural resource management policies has become paramount (Brearley et al., 2013, Wheeler & Waller, 2008). Prescribed burn treatment is a more recently utilized forest management tool including in the mitigation of disease transmission (Allan, 2009, Arthur et al., 2012, Brose et al., 2001, Gleim et al., 2014, Gleim et al., 2019, Hodo et al., 2019, Tripp, 2017).

Recent studies have begun to take a more in depth look at the complex ecological phenomenon driving the progress of Lyme disease across the United States (Kugler et al., 2015, Wilson, 2005, Allan, 2009, Gleim et al., 2014, Gleim et al., 2019, Tripp, 2017). These studies have shown an interaction with the density of *Ixodes* sp. ticks, infamous for being carriers the Borrelia burgdorferi bacterium that causes Lyme disease, and the rate of human-contracted cases of Lyme disease (Kugler et al., 2015, Wilson, 2005). This is of paramount interest to land management officials and stakeholders as counties in the Northeastern and Mid-Atlantic United States have identified a >320% increase in Lyme disease contractions in recent years (Kugler et al., 2015). Prescribed burn treatment of forested areas known to hold high density populations of *Ixodes* sp. have begun to be examined within the past decade as a viable natural resource management policy for the reduction of Lyme disease contractions in local human populations (Gleim et al., 2014, Gleim et al., 2019, Hodo et al., 2019, Tripp, 2017). These studies showed that long-term prescribed burning was shown to significantly reduce tick abundance as well as reduce the density of infected ticks by 35 times in burned sites, drastically reducing the risk of human contraction of Lyme disease in burned sites (Gleim et al., 2014, Gleim et al., 2019, Hodo et al., 2019, Tripp, 2017). However, the effects of the size of prescribed burn treatment and the relative importance of other environmental characteristics, such as forest composition, on the subsequent reduction on human contractions of Lyme disease is unclear (Hodo et al., 2019, Tripp, 2017). Virginia, which lies in the transitional zone between the Northeast and Southeast regions of the United States, has a history of prescribed burn treatment usage in forest management policies, and is in the top 15 states that report Lyme disease cases in the nation (CDC, 2015). However, the relationship between prescribed burn treatment and the subsequent reduction of Lyme disease case reporting in Virginia has not been studied to date.

The overall goal of this paper was to perform a pilot study examining the current state of Lyme disease contractions in Virginia and the potential effects existing prescribed-burn treatment regimens may have on the pathogen prevalence in local human populations. This paper will investigate whether the federal Lyme disease case reporting surveillance data has spatial patterns and if the usage of prescribed burn treatment reduces the reported contraction of Lyme disease in local human populations within the state of Virginia. Through accounting for acres of prescribed burn treatment, population density, and probable and confirmed CDC Lyme disease case reports by county, I evaluated the current state of Lyme disease dispersal in Virginia and the potential role prescribed burn treatment has on its risk to humans. I hypothesize that there will be observable spatial patterns of Lyme disease case reporting indicating clustering of counties with either low or high reporting due to the range distribution of *Ixodes* sp., the carrier tick species. I also hypothesize that there will be a negative correlation between acres of prescribed-burn and number of Lyme disease cases per county, as prescribed burning will likely cause a significant decrease in the abundance of infected ticks in an area, ultimately leading to a decreased risk to human health. To test these hypotheses, I completed these two specific objectives:

1. Determine if the current state of Lyme disease in Virginia exhibits spatial patterns and if those patterns are dispersed or clustered in nature.

    H_0: The current state of Lyme disease in Virginia is randomly distributed indicating no spatial patterns.

    H_1: The current state of Lyme disease in Virginia does exhibit spatial patterns in a clustered nature.

    H_2: The current state of Lyme disease in Virginia does exhibit spatial patterns in a dispersed nature.

2. Determine if there is an observable correlation between acres of prescribed burn treatment and number of confirmed and probable Lyme disease case reports per county in Virginia.

    H_0: There is no statistically significant correlation between acres of prescribed burn treatment and number of confirmed and probable Lyme disease case reports per county in Virginia.

    H_1: There is a statistically significant positive correlation between acres of prescribed burn treatment and number of confirmed and probable Lyme disease case reports per county in Virginia.

H_2: There is a statistically significant negative correlation between acres of prescribed burn treatment and number of confirmed and probable Lyme disease case reports per county in Virginia.

2. Literature Review

2.1 Lyme Disease Epidemiology

Lyme borreliosis, commonly called Lyme disease, is caused by the bacterium spirochete *Borrelia burgdorferi* (Gould, 2008). This spirochete has been found in fleas, mosquitoes, and other biting flies, but is only vectored by ticks (Beisiada et al., 2010). The major vector group are the *Ixodes* sp. complex, most often *Ixodes scapularis* in the Eastern United States (Gould, 2008, Beisiada et al, 2010). Regionality of tick species and genotype coincide with different regional strains of Lyme disease (Wormser et al., 2006). Lyme disease can be severely debilitating to victims, particularly if not treated with antibiotics within the twenty-four hour post-bite infection window (Biesieada et al., 2010, Wormser et al., 2006). Early symptoms of infection include skin lesions and sporadic muscle or joint pain (Biesieada et al., 2010). The bacteria spreads throughout the body after infection, attacking multiple organ systems including the muscles, nervous system, heart, and bone with different symptom variation occasionally occurring with different strains of Lyme disease (Wormser et al., 2006). As the infection progresses, symptoms can include heart disease, internal organ lesions, and severe neurological pain and abnormalities (Biesiada et al., 2010).

2.2 Tick Life History and Ecology

Ixodes scapularis is an ectoparasitic arachnid with three distinct life stages, larvae, nymphs, and adults, over the course of a two-year life cycle (Gould, 2008). Trans-ovarial transmission of Lyme disease from the mother to egg is extremely rare yielding larvae uninfected after their midsummer hatch (Gould, 2008, Guerra et al. 2002). Before transitioning into the nymph life stage, approximately eighty percent of tick larvae die, with mortality rates reducing while remaining high during subsequent life stages (Wilson, 1998). Larvae become infected once they have attached to an infected host, such as small mammals and birds, emerging as nymphs the following spring (Gould, 2008). With near-microscopic size, higher abundance, and early summer timing of questing when human activity in forested areas is high, nymphs are the most likely life-stage of disease vector for humans (Ostfeld et al., 2006). Ticks remain in the nymph stage for half a year before emerging as adults in the fall season resuming questing activity for large and medium-sized mammal hosts (Gould, 2008).

Hosts range in size from small rodents to large ungulates, with a single animal potentially acting as host to multiple ticks at a single time, leading to a potential transfer of Lyme disease at exponential rates (Gould, 2008, Otsfeld et al., 2006). This activity of questing is the search of a vertebrate host for attachment and a blood meal (Duffy & Campbell, 1994, Gould, 2008). Adults quest on forest floor vegetation and in leaf litter from fall to spring with an ambient air temperature tolerance of 4° degrees C (41° F) (Duffy & Campbell, 1994, Schulze, Jordan, & Hung, 2001). The rest of the tick's life is spent hidden in leaf litter molting, overwintering, and mating (Otsfeld et al., 2006). Larval and nymph ticks have minimized water storage capacity and permeable cuticles making them sensitive to changes in relative humidity leading to potential desiccation (Vail & Smith, 2002). Changes in habitat such as reductions in leaf litter, duff, or understory cover, that increase light penetration and reduced humidity, can lead to a substantial reduction in tick abundance (Ginsberg & Stafford, 2005).

2.3 Preferred Tick Habitat

Environmental characteristics that determine an area's potential habitat suitability for Ixodes sp. include humidity, seasonal temperature, soil conditions, and vegetative cover and composition (Diuk-Wasser et al., 2006, Guerra et al., 2002). Areas of high humidity and variable seasonal temperature have been shown to have a strong *Ixodes* sp. population presences on a national scale, such as in Long Island and coastal New England (Diuk-Wasser et al., 2006). A perfect medium of soil conditions are needed to optimize tick habitat suitability as well. Soils that are too dry can lead to tick desiccation and death, while soils of high moisture lead to negative effects on overwintering capabilities in leaf litter and upper horizons of soils, ruling out much wetland habitat (Guerra et al., 2002). Coarse soils, such as sandy barrens, are more likely to harbor high tick density populations than thicker clays where water drainage is minimal (Guerra et al., 2002).

Ixodes sp. prefer early and secondary successional forested habitat where the vegetative cover in forests is an environmental characteristic that strongly effects microhabitat suitability and distributions of ticks (Schulze & Jordan, 2005, Tripp, 2017). Differences on minute spatial scales, across otherwise homogeneous areas, have been shown to have a strong influence on tick habitat suitability (Schulze & Jordan, 2005, Tripp, 2017). Such variations include leaf litter and shrub layer compositions which can alter the pH and offer greater shading, altering microclimates (Shulze & Jordan, 2005, Tripp, 2017). Higher densities of shrub layers stabilize temperature and increase humidity by limiting air flow between forest strata, as well as reducing predation on ticks and their host species (Tripp, 2017).

The culmination of these environmental characteristic lead to general patterns of tick preference for deciduous rather than coniferous forest habitat, particularly in forests dominated by maple species over oak forests (Guerra et al., 2002, Tripp, 2017). Studies of tick populations in New Jersey found that tick populations were more prevalent with habitat fragmentation, hardwood tree dominance, higher shrub density, and higher leaf litter depth (Schulze & Jordan, 2005). It was concluded that the comparative pine forest areas of the study exhibited undesirable comparatively high temperatures and low humidity, limiting tick survival (Schulze & Jordan, 2005).

2.4 Lyme Disease Dispersal

As neither host nor vector are known to pass the bacterium on to their offspring, Lyme disease is reliant on the continual transmission between host and vectors to reproduce and expand in range (Humphrey, Caporale, & Brisson 2010). Lyme disease has spread from its endemic range in New England to much of the northeastern United States, upper Midwest, and California (Murphree Bacon, Kugeler, & Mead, 2008). First being identified in 1975 in Connecticut, there were 248,000 cases reported in several states between 1992 to 2006, a substantial increase in the disease's range and incidence rate (Kugeler et al., 2015 Murphree Bacon, Kugeler, & Mead, 2008). Our understanding of the spatial distribution and progression of Lyme disease in human populations is entirely dependent on surveillance data collected from case reporting by physicians and health officials to state and federal government officials (CDC, 2017, Hendricks & Mark-Carew, 2017). A multi-state spatial analysis study encompassing Kentucky, Maryland, Ohio, Pennsylvania, Virginia, and West Virginia revealed that case reporting to state health departments by counties between the years of 2010 to 2014 showed high amounts of clustering, indicating the presence of spatial relationships in county level case reporting and the need for further examination of how Lyme disease reporting effects the accurate estimation of Lyme disease progression (Hendricks & Mark-Carew, 2017).

The increase in Lyme disease is correlated with an increase in the range of *Ixodes* sp. throughout the continental United States (Ogden et al., 2006). Models have shown that density of tick species is extremely important when determining a region's risk of human contraction of Lyme disease, as the density of tick populations increase, so does the likelihood of infected ticks within the populations by, potential increase in the risk of human contraction (Diuk-Wasser et al., 2006). Counties that reported established tick populations rose 44.7% between 1996 and 2015 (Eisen et al., 2016, Dennis et al., 1998). This increase in range is propelled by multiple factors, including the increase in preferred successional habitat, climate change, and movements of host species such as migrating birds (Kugeler et al., 2015, Ogden et al., 2006). Fragmentation by anthropogenic activity that reduces mature forested habitat and increases early and secondary successional habitat, preferred by *Ixodes* sp., has increases as human development increases, subsequently increasing the range of ticks and Lyme disease (Kugeler et al., 2015, Ogden et al., 2006). In the northern part of the tick's range, increase ambient temperatures associated with climate change has been modeled to contribute to the northern expansion of tick populations (Ogden et al., 2006).

It has been shown that host species, such as migrating birds and small mammals play a large role in the distribution and enlargement in range of the Ixodes sp. (Van Buskirk & Ostfeld, 1995, Madhav et al., 2004). Different host species for Ixodes sp. have varying levels of competency in acting as a reservoir for the Lyme disease causing bacteria, Borrelia burgdorferi, as well as the capability to carry and pass on the disease when acting as a blood meal for ticks (Humphrey, Caporale, & Brisson, 2010). Mammals that have shown the highest competency in acting as reservoirs for Lyme disease in the Northeast are the white-footed mouse and the white-tailed deer (Ostfeld et al., 2006). The seasonal migration of such species, as well as long ranging species such as the American robin, have contributed greatly to tick and Lyme disease dispersal (Guerra et al., 2002, Tripp, 2017).

2.5 Lyme Disease Management Techniques

Mitigation efforts to control the spread of Lyme disease focus either on the reduction of human/ tick interactions or through the disruption in the *Ixodes* sp. population density (Ginsberg, 1994, Gould 2008). Traditionally, mitigation measures include emphasizing personal protection techniques and pesticide applications (Ginsberg, 1994). However, these methods have fallen out of favor either from lack of efficacy or disapproval from the general public towards the increased usage of chemical management methods (Gould, 2008). Studies have shown that only 40-50% of adults take precautions (personal wear of deterrent spray, minimizing exposed skin, and full-body checks after exposure to high-risk areas, etc.) even when they are aware of the risk of Lyme disease exposure (Poland, 2001). In the past few decades, mitigations that focus on the reduction of Lyme disease through the reduction of *Ixodes* sp. population density or through the disruption of the interaction between the host species and potentially infected tick populations has gained more popularity (Gleim, 2014, Gleim 2017). .

2.6 History of Fire Suppression

Federal forest policy was dominated by fire suppression, beginning with the establishment of national forest in 1891, and was supported by studies that stated the danger of uncontrollable and destructive wildfires was too great were used to justify these policies throughout the early 1900's (Stephens & Ruth, 2005). However, beginning in the 1940's, studies documenting increased fuel loads and fire risk caused by decades of fire suppression helped push through the 1963 Leopold Report officially connecting fire suppression policy with adverse environmental effects such as the lack of hardwood tree recruitment greater instability in species composition (Stephens & Ruth, 2005). In recent decades, scientific consensus and government policies favor prescribed burn treatments as an effective ecosystem management strategy to maintain the health of ecosystems throughout the United States (Greenberg, Otis, & Waldrop, 2006).

2.7 Effects of Prescribed Burn Treatment on Ecosystems

Prescribed burn treatment is essential to hardwood species recruitment in the Appalachian forests and current studies are being done to determine the best methods of increasing prescribed burn protocols (Brose et al., 2001). Before the fire suppression policy of the late 1800's, fire was a regularly occurring disturbance that kept non-oak species at manageable numbers in oak deciduous forests. Fire-suppression protocols allow for an increase in non-oak species, changing forest dynamics in a multitude of ways (Shumway et al. 2001). The primary objective of prescribed burning is to alter the characteristics of existing vegetation while maintaining quality or quantity of desirable characteristics (Wilkinson, 1979). While varying between ecosystems, prescribed burning also effects all aspects of an ecosystem including but not limited to changes in plant species composition, soils, wildlife, nutrient pools, integrity and function of the forest floor, water quality and yield, air quality, and long-term forest productivity (Tiedemann, Klemmedson, & Bull, 2000). Areas restored with prescribed burn treatment often exhibit high tree mortality, increased snags, decreased understory density, increased light penetration, and reduced leaf litter and duff layers (Greenberg, Otis, & Waldrop, 2006). There may also be shifts in forest compositions, such as the shift from maple to oak dominance, resultant in the change of microclimates under the canopy and leaf litter (Arthur et al., 2012).

2.8 Effects of Prescribed Burn Treatment on Ticks

The change in forest composition caused by prescribed burn treatment has the potential to decrease in habitat viability of Ixodes sp., who depend on low light penetration and high humidity by reducing leaf litter depth and clearing shrub layers that provide shading (Ginsberg & Stafford, 2005). In the states of Georgia, Florida, Pennsylvania, and South Carolina, forested areas of long-term prescribed burning have been shown to have significantly reduced tick counts than their un-burned counterparts by approximately 78% (Gleim et al., 2014, Gleim et al., 2019, Hodo et al., 2019, Tripp, 2017). Large area burns reduce tick and host repopulation while repeated and consistent prescribed burn treatment may deplete tick source populations for increased long-term results (Gleim et al., 2014). Most recently, a study examining the interaction between prescribed burn regimes and disease vector tick species in Georgia, Florida, and South Carolina found strong evidence that, while prescribed burns did not reduce the pathogen prevalence in tick populations, these burns resulted in the reduction of overall tick population and a 98% lower encounter rates with infected ticks than unburned forested sites (Gleim et al., 2019).

2.9 Study Area

The Eastern United States is known for deciduous, dry to mesic forests, and alfisol-type soils overlying sedimentary rock preferred by ticks (Guerra et al., 2002). Virginia, on the border of the Northeast and Southeast regions, with ample hardwood deciduous forests and high Lyme disease contractions, has been overlooked by previous studies investigating this phenomenon, despite Virginia's habitat suitability (Gleim et al., 2014, Gleim et al., 2019, Tripp, 2017, Hodo et al., 2019). Much of the state is characterized by heavily forested hardwood deciduous habitat, most of which is under State of Federal protection in the western portion of the state and privatized in the eastern creating a mosaic in land management practices that are preferred, largely determined by historical precedence or individual preference (Wilson, 2005). The climate of the region is humid subtropical, with definite seasonality throughout the year. Average temperatures range between 61°F (16.1°C) and 89°F (31.7°C) and average precipitation falls between 38.29 inches to 40.74 inches annually (Virginia is for Lovers, 2020). The primary land use is agricultural with pockets of urbanization, often along the riverine systems that feed into the Chesapeake Bay Watershed (Wilson, 2005). Virginia's Shenandoah Valley to the west is characterized by two national forests that are historically managed by prescribed burns regimes as well as having neighboring counties within the region that exhibit high reporting of Lyme disease, which makes it an ideal region to examine the relationship between prescribed burn regime and reported human contraction of Lyme disease within the context of the state as a whole (Brose, P. et al., 2001, Kugeler, K.J., et al., 2015). Cases of Lyme disease in Virginia are most frequently observed in the northern and eastern counties along the US Interstate Highways I-18, I-95, and I-64 corridors (Li et al., 2014). This is thought to be connected with population growth and an increase in habitat fragmentation leading to greater amounts of suitable *Ixodes* sp. and host species early and secondary successional habitat (Li et al., 2014).

3. Methods

3.1 Data acquisition and management

Lyme disease surveillance data for all counties in Virginia was obtained from the Centers of Disease Control publicly available servers (https://www.cdc.gov/lyme/resources/LD-Case-Counts-by-County-00-18.csv, March 2020). Complete case totals were obtained for the years 2010 to 2017 and were limited to confirmed and probable case counts, the national reporting standard (CDC, 2017). Surveillance data was specially obtained for the years 2010 to 2017 to utilize the stricter diagnostic criteria of Lyme disease case definitions established in 2008 and 2011 (Figure 1, Li et al., 2014). Lyme disease case classifications standards in Virginia are based on the current Council of State and Territorial Epidemiologists (CSTE) Case Definition for Lyme disease and reflect a stricter criterion for case determination with increased consistency of laboratory and medical evidence among reported cases (CDC, 2017). The decision diagram shown in Figure 1 summarizes the Lyme disease case classification criteria (WV Bureau of Public Health, 2016).

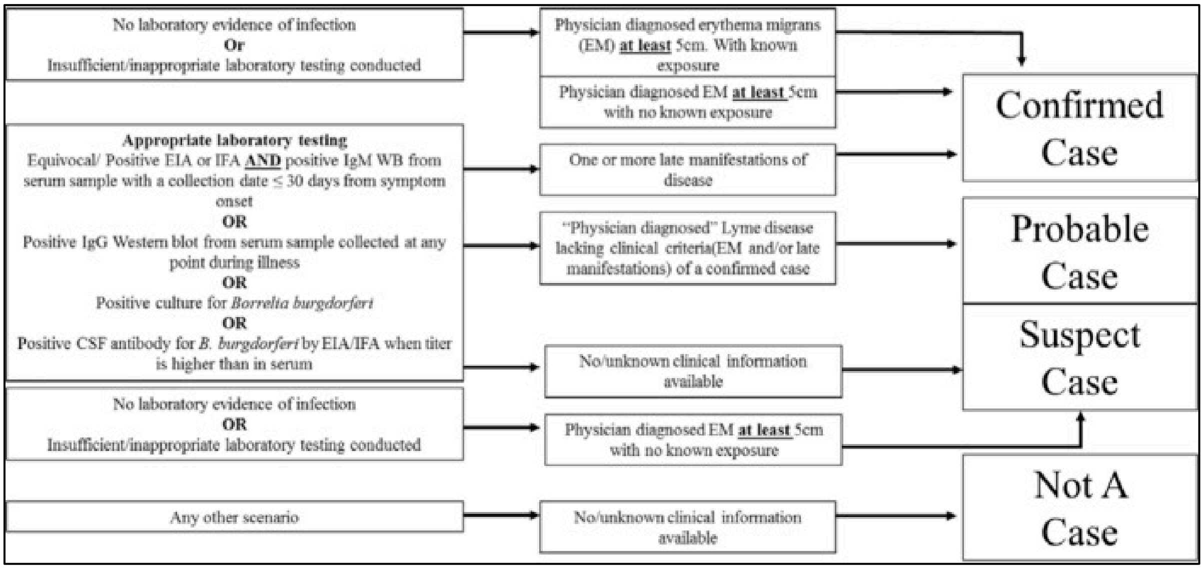

Figure 1. Visual representation of case classification from the CSTE Lyme disease case definition. Diagram was obtained from the 2016 West Virginia Bureau of Public Health Lyme disease Case Ascertainment Toolkit.

Surveillance data were sorted in Microsoft Excel 2016 by reporting year, county and city/township. Complete annual totals were paired with U.S. census county level population estimates by year and county (Bureau USC, 2018). Excel spreadsheets containing the county level case information and population census data were joined within ArcGIS Pro 2.5.1 to a United States Counties and Localities shapefile obtained from the U.S. Department of

Agriculture National Resources Conservation Service Geospatial Gateway (USDA: NRCS) (ESRI, Redlands CA, USDA: NRCS: Geospatial Gateway, 2019).

Acreage of land burned in forest fires was obtained from the Monitoring Trends in Burn Severity (MTBS) server dating from 1986 -2017 in the form of a geospatially reference shapefile (Eidenshink et al., 2007). The MTBS is an interagency program designed to consistently map the burn severity and extent of large fires across all lands of the United States from 1984 to present by analyzing Landsat satellite data at a standardized 20 meter resolution (Eidenshink et al., 2007). This data was then filtered down to only prescribed burn instances, limiting the range from 2010 to 2017.

3.2. Descriptive analysis

Sum total acreage of land treated with prescribed burn was determined for each county. Estimated incidence rate by county was calculated by dividing the mean number of Lyme disease cases across all years by the respective county's 2010 census estimates then multiplied by 100,000 persons (CDC, 2012). Those counties that had a history of lack of reporting were removed to limit the influence of false negatives and outliers, reducing the total number of counties to 128 of 133 (Glavanakov et al., 2001). This estimated incidence by locality was then visualized to display apparent variation in the spatial distribution in Lyme disease reporting.

3.3. Cluster analysis

In order to examine the potential current spatial relationships of Lyme disease incidence rates in Virginia a spatial autocorrelation analysis was chosen. The autocorrelation of incidence rates as a function of distance between county centroids was determined using Moran's statistic *I* as a spatial autocorrelation coefficient (Cliff & Ord, 1981, Sokal & Oden, 1978). This was done in ArcGIS Pro 2.5.1 utilizing the Spatial Autocorrelation (Global Moran's I) tool. This measures the spatial autocorrelation based on Lyme disease incidence rates location and values simultaneously, calculating the I index value and both a p-value and z-score to evaluate the significance of the index (Getis & Ord, 2010; Glavanakov et al., 2001). The Moran's I statistic for spatial autocorrelation is given as:

$$I = \frac{n}{S_0} \frac{\sum_{i=1}^{n} \sum_{j=1}^{n} w_{i,j} z_i z_j}{\sum_{i=1}^{n} z_i^2}$$

where z_i is the deviation of the estimated incidence rates i from its mean ($x_i - X$), $w_{i,j}$ is the spatial weight between feature i and j, n is equal to the total number of counties, and S_0 is the aggregate of all the spatial weights:

$$S_0 = \sum_{i=1}^{n} \sum_{j=1}^{n} w_{i,j}$$

The z_I-score for the statistic is computes as:

$$z_I = \frac{I - \mathrm{E}[I]}{\sqrt{\mathrm{V}[I]}}$$

where:

$$\begin{aligned} \mathrm{E}[I] &= -1/(n-1) \\ \mathrm{V}[I] &= \mathrm{E}[I^2] - \mathrm{E}[I]^2 \end{aligned}$$

Specifically, the mean and variance for the estimated Lyme disease incidence rate is calculated, then for each county the mean is subtracted, creating a deviation from the mean (Getis & Ord, 2010). Deviation values for all neighboring counties are then multiplied together to create a cross-product, which is summed in the numerator of the Moran's I statistic, Index, and normalized by the variance so that Index value always falls between -1.0 and +1.0 (Getis & Ord, 2010). If counties with high estimated incidence rates tend to be near other counties with high estimated incidence rates the statistic will be positive, but if the opposite is true, and counties with high estimated incidence rates tend to repel other high rate counties then the Index will be negative (Getis & Ord, 2010). If there are equal positive and negative cross-products, then the index will be zero, meaning that the estimated incidence rate of Lyme disease per counties is randomly dispersed and counties' estimated incidence rate are not correlated with their neighbor's rates (Getis & Ord, 2010).

Contiguity edges only, which characterize polygons that share a common border as neighbors, were chosen as the neighbor criterion due to the greater likelihood that neighboring counties have increased spatial interaction and therefore only neighboring counties that share a boundary influence computations for the target county (Hendricks & Mark-Carew, 2017, Szonyi et al., 2015).

3.4. Regression analysis

A regression analysis was done in Excel 2016, comparing the total acreage of prescribed burn treatment and the estimated incidence rate of Lyme disease per 100,000 persons by county throughout the entire state. Results were displayed in a scatterplot, demonstrating any potential causal effect prescribed burn treatments may have incidences rates in the state. A second targeted regression analysis was completed comparing only those 19 counties that had a history of prescribed burn treatment, in an effort to reduce false negatives. These results were also displayed in a scatterplot, to visualize any relationships between the variables.

4. Results

4.1 Descriptive analysis

Overall from 2010 to 2017, 9828 confirmed and probable Lyme disease cases were reported in Virginia (Table 1). The estimated incidence for the state during this time was 15.35 per 100,000 persons (Table 1, Figure 2). Floyd county had the highest estimated incidence of Lyme disease (269.98 per 100,000 persons). Prescribed burn treatment was utilized in 19 of 134 counties, with a total of 70848.07 acres across the state (Table 1, Figure 3). Madison county had the largest number of treated acres (13933.88 acres).

Table 1. Average Human Lyme disease case counts within Virginia Counties, 2010 to 2017.

County or Township	Population (2010 U.S. Census)	Average Annual Reported Lyme disease Cases (confirmed and probable)	Estimated Incidence Rate x 100,000 persons	Total Acres Prescribed Burn Treatment
Arlington	207627	17.38	8.37	0.00
Clarke	14034	12.5	89.07	0.00
Culpeper	46689	9.88	21.15	0.00
Fairfax County	1081726	196	18.12	0.00
Fauquier	65203	24.5	37.57	0.00
Frederick	78305	46.63	59.54	0.00
Highland	2321	0.13	5.39	102.74
Loudoun	312311	205.38	65.76	0.00
Madison	13308	5.13	38.51	13933.88
Page	24042	9.75	40.55	2944.92
Prince William	402002	45.75	11.38	0.00
Rappahannock	7373	5.38	72.9	10938.08
Rockingham	76314	25.63	33.58	8338.67
Shenandoah	41993	17	40.48	0.00

County or Township	Population (2010 U.S. Census)	Average Annual Reported Lyme disease Cases (confirmed and probable)	Estimated Incidence Rate x 100,000 persons	Total Acres Prescribed Burn Treatment
Stafford	128961	12.13	9.4	0.00
Warren	37575	26	69.19	0.00
Alexandria	139966	10.5	7.5	0.00
Fairfax City	22565	0.38	1.66	0.00
Falls Church	12332	0.5	4.05	0.00
Harrisonburg	48914	3.75	7.67	0.00
Manassas	37821	2.5	6.61	0.00
Manassas Park	14273	1.13	7.88	0.00
Winchester	26203	9.63	36.73	0.00
Bland	6824	0.5	7.33	1275.81
Buchanan	24098	0.25	1.04	0.00
Carroll	30042	25.75	85.71	0.00
Dickenson	15903	0.13	0.79	0.00
Giles	17286	9.75	56.4	1287.16
Grayson	15533	8	51.5	541.29
Lee	25587	-	-	-
Pulaski	34872	34.25	98.22	0.00
Russell	28897	0	0	0.00
Scott	23177	0.38	1.62	1708.89
Smyth	32208	1.25	3.88	839.19
Tazewell	45078	0.25	0.55	604.90
Washington	54876	0.75	1.37	0.00
Wise	41452	-	-	-
Wythe	29235	14.5	49.6	2194.04
Bristol	17835	-	-	-
Galax	7042	7.25	102.95	0.00
Norton	3958	-	-	-
Accomack	33164	5.25	15.83	0.00
Albemarle	98970	35.38	35.74	2637.63
Alleghany	16250	0.38	2.31	0.00
Amelia	12690	1	7.88	0.00
Amherst	32353	6.25	19.32	0.00
Appomattox	14973	1.38	9.18	0.00
Augusta	73750	30.75	41.69	12399.65
Bath	4731	0.13	2.64	6844.28
Bedford County	74898	10	13.35	532.66
Botetourt	33148	1.63	4.9	2442.92

County or Township	Population (2010 U.S. Census)	Average Annual Reported Lyme disease Cases (confirmed and probable)	Estimated Incidence Rate x 100,000 persons	Total Acres Prescribed Burn Treatment
Brunswick	17434	0.38	2.15	0.00
Buckingham	17146	1	5.83	0.00
Campbell	54842	3.63	6.61	0.00
Caroline	28545	3.25	11.39	0.00
Charles City	7256	0.5	6.89	0.00
Charlotte	12586	0.38	2.98	0.00
Chesterfield	316236	16.13	5.1	0.00
Craig	5190	-	-	-
Cumberland	10052	0.5	4.97	0.00
Dinwiddie	28001	1.38	4.91	0.00
Essex	11151	0.38	3.36	0.00
Floyd	15279	41.25	269.98	0.00
Fluvanna	25691	5.5	21.41	0.00
Franklin County	56159	1.5	2.67	0.00
Gloucester	36858	1.38	3.73	0.00
Goochland	21717	1.38	6.33	0.00
Greene	18403	7.88	42.79	606.46
Greensville	12243	0.13	1.02	0.00
Halifax	36241	0.13	0.34	0.00
Hanover	99863	4.13	4.13	0.00
Henrico	306935	10.63	3.46	0.00
Henry	54151	2	3.69	0.00
Isle of Wight	35270	0.88	2.48	0.00
James City	67009	2.38	3.54	0.00
King and Queen	6945	0.38	5.4	0.00
King George	23584	5.75	24.38	0.00
King William	15935	0.63	3.92	0.00
Lancaster	11391	0.63	5.49	0.00
Louisa	33153	6.38	19.23	0.00
Lunenburg	12914	0.5	3.87	0.00
Mathews	8978	0.63	6.96	0.00
Mecklenburg	32727	0.38	1.15	0.00
Middlesex	10959	1.13	10.27	0.00
Montgomery	94392	57.13	60.52	0.00
Nelson	15020	5.38	35.79	0.00
New Kent	18429	0.5	2.71	0.00
Northampton	12389	1.88	15.13	0.00

County or Township	Population (2010 U.S. Census)	Average Annual Reported Lyme disease Cases (confirmed and probable)	Estimated Incidence Rate x 100,000 persons	Total Acres Prescribed Burn Treatment
Northumberland	12330	1.13	9.12	0.00
Nottoway	15853	1.25	7.88	0.00
Orange	33481	5.13	15.31	0.00
Patrick	18490	1.63	8.79	0.00
Pittsylvania	63506	2.5	3.94	0.00
Powhatan	28046	1	3.57	0.00
Prince Edward	23368	2	8.56	0.00
Prince George	35725	1	2.8	0.00
Richmond County	9254	0.13	1.35	0.00
Roanoke County	92376	10.75	11.64	0.00
Rockbridge	22307	5.88	26.34	0.00
Southampton	18570	0.25	1.35	0.00
Spotsylvania	122397	12.5	10.21	0.00
Surry	7058	0.38	5.31	0.00
Sussex	12087	0.63	5.17	674.91
Westmoreland	17454	0.63	3.58	0.00
York	65464	2	3.06	0.00
Buena Vista	6650	0.63	9.4	0.00
Charlottesville	43475	10.63	24.44	0.00
Chesapeake	222209	4.5	2.03	0.00
Colonial Heights	17411	1.13	6.46	0.00
Covington	5961	0.38	6.29	0.00
Danville	43055	1.63	3.77	0.00
Emporia	5927	-	-	-
Franklin City	8582	0.38	4.37	0.00
Fredericksburg	24286	3.13	12.87	0.00
Hampton	137436	1.75	1.27	0.00
Hopewell	22591	0.5	2.21	0.00
Lexington	7042	0.75	10.65	0.00
Lynchburg	75568	22	29.11	0.00
Martinsville	13821	0.13	0.9	0.00
Newport News	180719	2.38	1.31	0.00
Norfolk	242803	2.63	1.08	0.00
Petersburg	32420	0.38	1.16	0.00
Poquoson	12150	0.38	3.09	0.00
Portsmouth	95535	0.25	0.26	0.00
Radford	16408	11.13	67.8	0.00

County or Township	Population (2010 U.S. Census)	Average Annual Reported Lyme disease Cases (confirmed and probable)	Estimated Incidence Rate x 100,000 persons	Total Acres Prescribed Burn Treatment
Richmond City	204214	5.63	2.75	0.00
Roanoke City	97032	11.13	11.47	0.00
Salem	24802	1.88	7.56	0.00
Staunton	23746	10.13	42.64	0.00
Suffolk	84585	1.25	1.48	0.00
Virginia Beach	437994	8.63	1.97	0.00
Waynesboro	21006	4.13	19.64	0.00
Williamsburg	14068	1.88	13.33	0.00

*Counties with no reported Lyme disease cases are represented with "-".

County- level Lyme disease incidence per 100,000 between 2010 to 2017 for all Virginia counties is displayed in Figure 2. Counties with higher estimated incidence rates are displayed in dark brown with correspondingly lighter browns as estimated incidence rates lessen. The majority of counties (59.4%) have fewer than 5 estimated cases of Lyme disease per 100,000 persons.

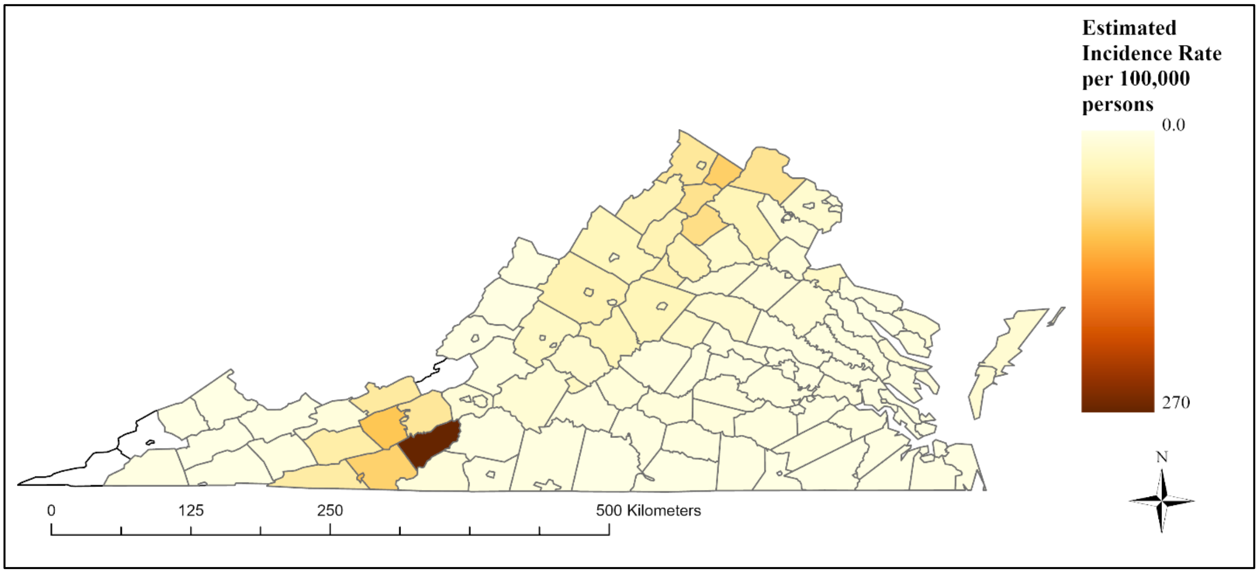

Figure 2. Quantile choropleth map displaying estimated Lyme disease incidence rates by county per 100,000 persons for the state of Virginia from 2010 to 2017.

County- level acres of prescribed burn treatment as well as the location and shape of prescribed burn treatments are displayed in Figure 3. Counties with a high total of prescribed burn treatment are displayed in dark green with the majority of counties (85.7%) containing 100 or less acres of prescribed burn treatment. The location and shape of all prescribed burn treatments in Virginia between 2010 to 2017 are displayed as orange polygons.

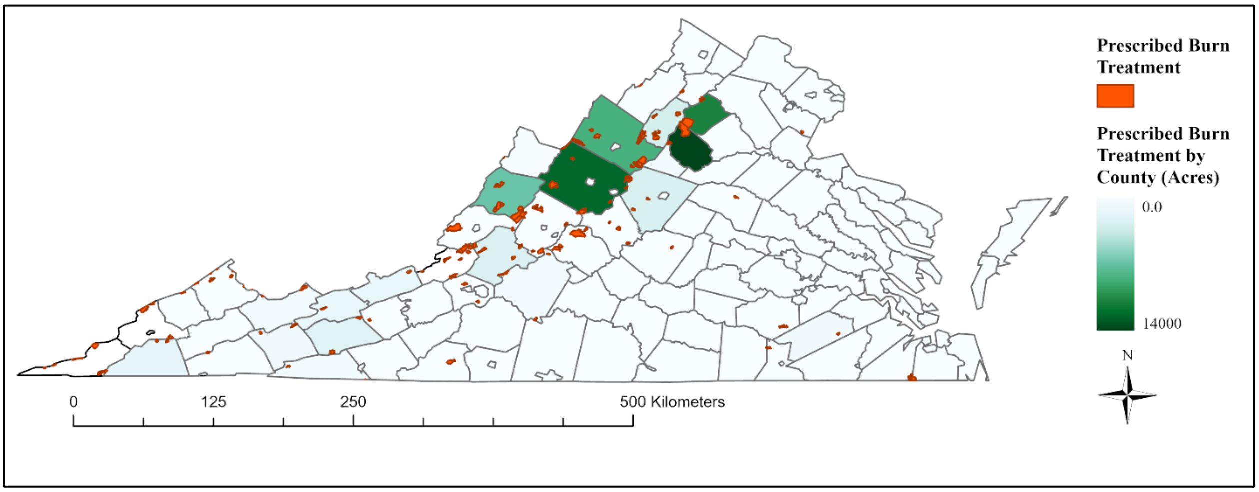

Figure 3. Quantile choropleth map displaying total sum of acres with prescribed burn treatment with spatial location and shape of prescribed burn treatments.

4.2 Cluster analysis

Moran's I estimates of spatial autocorrelation for incidence rates during the time frame of 2010 to 2017 were significant (P= 0.00) with a value of 0.44. The critical value, z-score, was 7.69 indicating that there is a less than 1% likelihood that the observed clustered pattern is a resultant of random chance. Looking at Figure 2, areas where clusters of counties with high estimated incidence rates can be observed along the mountainous region to the west of the state, as well as in the densely populated northern region surrounding Washington, D.C. Clusters of low Lyme disease incidence rate can be observed in much of the eastern region as well as in the south-western tip of the state.

4.4. Regression analysis

As shown in Figure 4, the regression analysis resulted in a weak positive relationship between acres of prescribed burn treatment and estimated Lyme disease incidence rate by county. The regression analysis examining the entire state had a R^2- value of 0.02, while the targeted regression analysis (Figure 5) of only those counties with a history of prescribed burn treatment showed a slightly stronger relationship (R^2= 0.16).

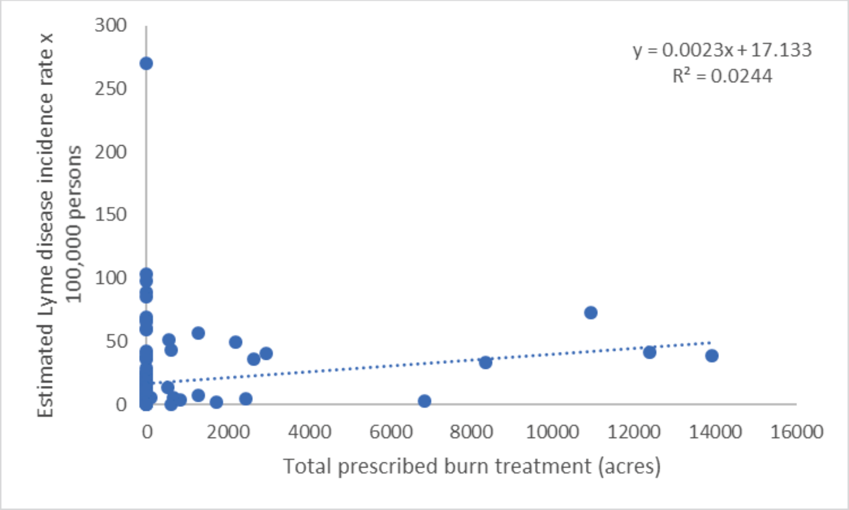

Figure 4. Graphical representation of the relationship between sum total acres of prescribed burn treatment and estimated Lyme disease incidence rate per 100,000 persons within Virginia, 2010-2017.

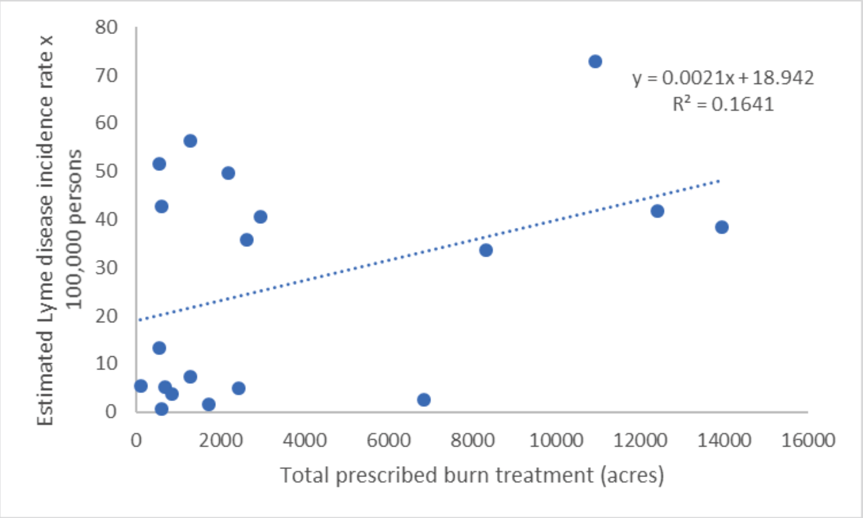

Figure 5. Graphical representation of the relationship between sum total acres of prescribed burn treatment and estimated Lyme disease incidence rate per 100,000 persons within Virginia counties targeting those counties with known history of prescribed burn treatments, 2010 – 2017.

5. Discussion

While spatial analyses have been used for some time in relevant literature, this is one of the few studies to apply spatial analysis to federally accumulated Lyme disease case reporting within Virginia during the time period of active prescribed burn treatment (Hendricks & Mark-Carew, 2017, Hu & Rao, 2009, Ozdenerol, 2015). Additionally, this is the first known study to compare estimated Lyme disease incidence rates by county per 100,000 persons to county-level acreage of prescribed burn treatment.

The strongly positive Moran's I Index value indicate that the spatial distribution of the high and low estimated incidence rates for Lyme disease by county per 100,000 persons is more spatially clustered than would be expected if underlying spatial processes were random. This concurs with other studies that have looked at spatial processes of state health department Lyme disease case reporting and found evidence of clustering in previous years, indicating that Lyme disease case reporting in humans continues to be spatially clustered, potentially due to human population density or *Ixodes* sp. population locations in forested locations (Hendricks & Mark-Carew, 2017, Ozdenerol, 2015, Li et al., 2014). Incidence rate visualization per 100,000 persons (Figure 2) revealed high estimated incidence rates in the western and northern portions of the state, coinciding with the Blue Ridge Mountain region and the Northern Virginia region surrounding Washington, D.C. There could be multiple factors determining the locations of these clusters including increased forested habitat in the Blue Ridge Mountain region preferable by ticks or the dense human populations of the Northern Virginia region increasing the likelihood of incidents (Guerra et al., 2002, Diuk-Wasser, 2006, Diuk-Wasser, et al., 2012). Locations of generally low estimated incidence rates can be observed in the sparsely populated southwestern corner of the state, as well as the throughout the middle and eastern portions of the state along the coast, where there are fewer reported tick populations (Figure 2; Guerra et al., 2002, Diuk-Wasser, 2006, Diuk-Wasser, et al., 2012). The presence of clustered spatial processes as well as the location of the clusters of high and low estimated incidence rates of Lyme disease human case reporting concur with previous research on comparable surveillance data from state health departments (Hendricks & Mark-Carew, 2017, and Li et al., 2014).

The number of counties with a history of prescribed burn treatment, as well as the spatial extent of those prescribed burn treatments were very low (Figure 3). When compared to the estimated incidence rate by county per 100,000 persons, a very weak positive trend was observed (Figures 4 and 5). This positive linear relationship hints at the potential spatial relationship between acreage of prescribed burn treatment and estimated incidence rate. However, this trend is not statistically strong enough to reject the null hypothesis indicating no causal relationship between prescribed burn treatment and estimated incidence rate in human contraction reporting of Lyme disease. This could be due to a lack of utilization and acceptance of prescribed burn treatment as a viable land management tool (Brose, et al., 2001, Arthur, et al., 2012).

The high clustering of estimated incidence rate by county per 100,000 persons for Lyme disease in Virginia and the weak positive relationship between that rate and the amount of acreage of prescribed burn treatment indicates the strong presence of spatial patterns within this ecological phenomenon. However, in the hopes of informing future natural resource decisions, further study should be done to illuminate these spatial patterns. The weak positive trend observed in the regression analysis would at first indicate that increased acreage of prescribed burn leads to higher estimated incidence of Lyme disease in Virginia Counties. This positive relationship could be due to statistical noise from a variety of factors including counties with high prescribed burn treatment acreage most likely have larger amounts of suitable habitat for *Ixodes* sp. or human behaviors such as participation in outdoor activities. The acreage of prescribed burns is comparably small as assessed with a county-wide assessment. For example, total acreage burned in Augusta County was 12,399.65 acres which only comprises 1.9% of the total area in the county.

It is important to note that these spatial analyses are particularly suited for identifying patterns of disease, but do not have the capability of determining the exact cause of such patterns, which would require much more in depth experimental design (Szonyi et al., 2015). The usage of surveillance data has other potential limitations associated that could result in under or over reporting (Diuk-Wasser et al., 2012). The variation in symptoms caused by Lyme disease among infected patients, lack of consensus on interpretations of Lyme disease case definitions, and inconsistent disease awareness among reporting officials such as physicians and public health professionals can all be possible sources of differential reporting bias (Hayes & Piesman, 2003, Murphree Bacon, Kugeler, & Mead, 2008, Bret et al., 2014). While this was minimized by limiting data acquisition to a time period when Lyme disease case definitions were the most stringent, there is no viable control over disease awareness and physician reporting (CDC 2016).

5.1. Further research

This study examined the current state of Lyme disease case reports in Virginia and if the current usage of prescribed burn treatment in forested areas significantly reduces the reported contraction of Lyme disease in human populations. Utilizing a spatial autocorrelation analysis, it was confirmed that Lyme disease estimated incidence rates per 100,000 persons by county exhibited clustered spatial patterns, but a regression analysis was unable to determine if the current amount of prescribed burn treatment in forest areas reduces the amount of reported cases of Lyme disease. Further research should be done on a finer resolution of Lyme disease reporting where specific forests with a history of prescribed burn treatment can be studied pre- and post- burn. This could be achieved by narrowing the scale of the study to specific forested habitats in Virginia with known *Ixodes* sp. populations and observing the change in incidence rates before and after the area is subjected to prescribed burn treatment. They should also include the potential confounding effect of landcover type, such as amount of suitable forest habitat for *Ixodes* sp. populations on the relationship between prescribed burn treatment and Lyme disease reporting. Further environmental effects that should be studied to contribute to understanding and mitigating the Lyme disease epidemic could be the influence of climate change on the effectiveness of prescribed burn treatment to reduce tick populations. This potential relationship could be examined by incorporating yearly temperature into the analysis, by comparing warmer and colder years for changes in spatial patterns of Lyme disease case reports or acres of prescribed burn treatment.

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