LYME DISEASE EMERGENCE IN VIRGINIA:
AN EXAMINATION OF THE DEMOGRAPHIC AND ENVIRONMENTAL
VARIABLES CORRELATED TO THE SPATIAL PATTERN OF DISEASE INCIDENCE

SARA ELIZABETH DYMOND

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Korine N. Kolivras, Committee Chair
Stephen P. Prisley
James B. Campbell

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Liz Dymond

ABSTRACT

Since its initial identification in 1975, Lyme disease has become a public health concern in the U.S. Increased concern is sparked by the rapid rate at which the disease is emerging into new areas. One area of disease emergence is the state of Virginia which has been experiencing exponentially increasing rates of the disease. This research studies Virginia's landscape-level habitats to explore demographic and environmental variables related to the spread of Lyme disease.

The land cover data came from the National Land Cover Database (2006), demographic data came from the U.S. census (2010), and Lyme disease case data came from the Virginia Department of Health (2006-2010). Key variables examined in this statewide study include the percentages of landscape types measured inside each census tract, measures of forest fragmentation, and measures of land cover interspersion inside state census tracts

Analysis was carried out using a spatial Poisson regression model. Of the original 15 variables, 10 were significantly correlated to Lyme disease. The six that were positively correlated with disease incidence include percent herbaceous land, percent water, two edge contrast measurements of herbaceous-forest land, median age, and average income. The four that were negatively correlated were percent developed, population density, and two edge contrast measurements of developed-herbaceous land.

Overall results indicate that specific environmental and demographic variables are associated with increased disease incidence as Lyme disease emerges in Virginia. Results from this study could help create a predictive statewide map for Lyme disease incidence and aid in disease awareness and resource allocation.

ACKNOWLEDGEMENTS

ATTRIBUTION

Dr. Korine Kolivras is my academic advisor and committee chair. Her experience in Medical Geography gave her insight into constructing the research design, interpreting the results, and suggesting applications for public health outcomes. She is the principal investigator on the NSF research team and led most of the research coordination and design. She also aided in the analysis and writing process.

Dr. Yili Hong is a research team member from the Statistics department. In collaboration with Dr. Jie Lie, he performed the statistical analysis and generated several of the tables and figures. He also aided in research design and interpretation of the results.

Dr. Jie Li is a research team member from the Statistics department. In collaboration with Dr. Yili Hong, she performed the statistical analysis and generated several of the tables and figures. She also aided in research design and interpretation of the results.

Dr. Stephen Prisley is my committee member and a member of the research team. His background in Forestry as well as his experience with Geographic Information Systems allowed him to give insight into the research design. He also aided in data generation and checked data accuracy.

Dr. James Campbell is my committee member and a member of the research team. His background in land use and land cover change provided insight into how changes in environmental variables may impact Lyme disease emergence. He provided valuable input into research design.

Dr. David Gaines collaborated with the research team and produced the Lyme disease case information from the Virginia Department of Health. He also assisted in overall research design with expert advice on entomology and biology.

Dr. Randel Dymond, with his expertise in Civil Engineering and Geographic Information Systems, assisted in technical aspects of the research project related to data generation and provided expert advice.

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CHAPTER 1: INTRODUCTION & STATEMENT OF PURPOSE

1.1 THE INCREASING SPREAD OF LYME DISEASE

Lyme disease is an ancient affliction with a long history of occurrence in humans. Evidence of DNA from Borrelia burgdorferi, the bacterium which causes Lyme disease, was discovered by archaeologists in a 5,300 year old ice man in 1991 in the Ötztal Alps (Hall 2011). It is the oldest known documentation of Lyme disease in the world (Hall 2011). However, the first documented modern cases of Lyme disease identified in North America in 1975 in the town of Lyme, Connecticut (Steere 1978). Within a few years of initial discovery, 512 patients had been diagnosed with Lyme disease along three foci: the northeastern coast, Wisconsin, and California/Oregon (Steere 1979). With each passing year, Lyme disease incidence has continued to increase at exponential rates.
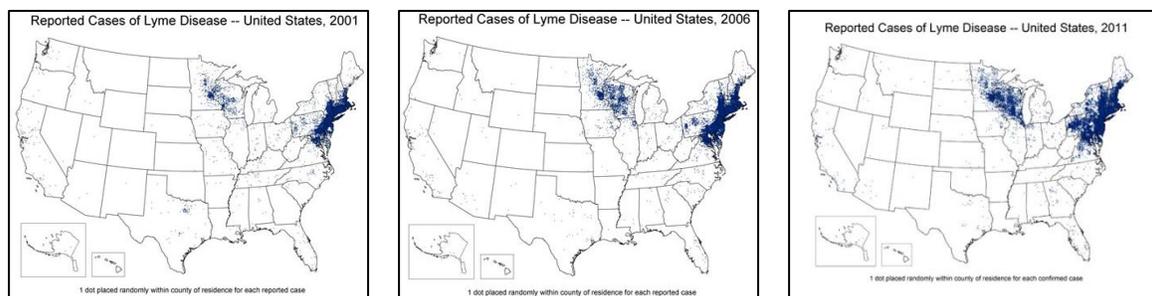
Figure 1.1: Spread of Lyme Disease in the U.S.: 2001, 2006, 2011
Source: (Centers for Disease Control and Prevention 2011)

According to the CDC's Morbidity and Mortality Weekly Report, an annual count increase of 101% was recorded between the years of 1992 to 2006 (Bacon 2008). According to this same report, the number of cases of Lyme disease totaled 248,074 in 2006, making it the most commonly reported vectorborne disease in the United States. However, increases in incidence and prevalence are not the only changes observed. Along the eastern coast, Lyme

disease continues to spread both north and south, as seen in Figure 1.1. Though U.S. incidence of Lyme disease is increasing slowly, Virginia is one of the regions experiencing a dramatic expansion of Lyme disease occurrence with numbers increasing from 259 cases in 2002 to 1,023 in 2011 (Table 1.1, Figure 1.2). According to the Virginia Department of Health, this increase is due to both a genuine increase in Lyme disease occurrence and an increase in case follow-ups and public awareness, not strictly one or the other (Virginia Department of Health 2011).

Table 1.1: Virginia Lyme Disease Cases
Source: (Virginia Department of Health 2011)

Lyme Disease in Virginia	
Year	# of Cases
2002	259
2003	202
2004	216
2005	274
2006	357
2007	959
2008	933
2009	908
2010	1,245
2011	1,023
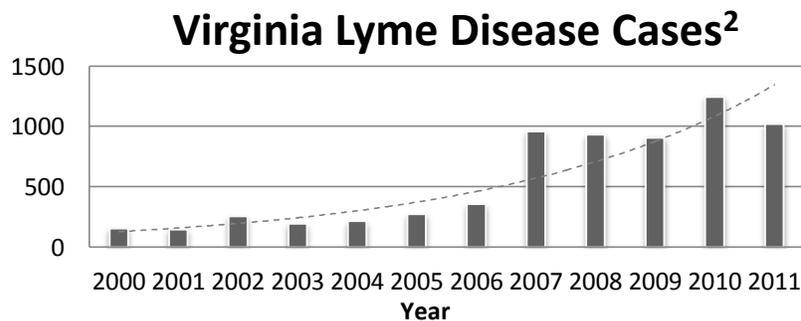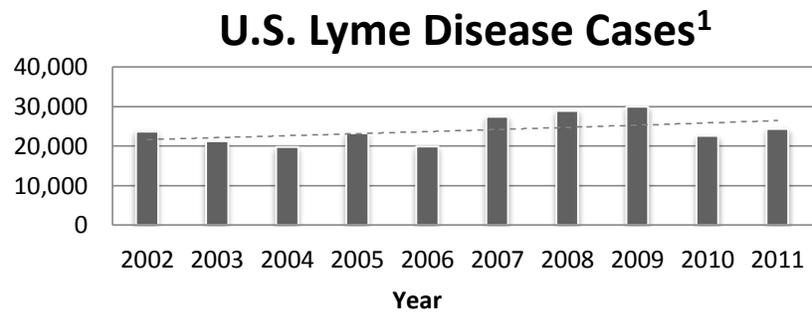
Figure 1.2: Comparison of U.S. Lyme Disease Cases
& Virginia Lyme Disease Cases
Sources: (Centers for Disease Control and Prevention 2012)[1],
(Virginia Department of Health 2011)[2]

1.2 LYME DISEASE IMPACT

The primary way to reduce the impact of an infectious disease is to prevent disease occurrence through preemptive measures. Currently, the most effective preventions available for Lyme disease include landscape modifications, host management, and education of the public to

wash after time spent outdoors to thoroughly check for ticks (O'Connell 2010, Connally 2009). Although these preventative measures are protective when practiced consistently, a vaccine is often a preferred method of prevention. A Lyme disease preventative vaccine was developed and approved by the Food and Drug Administration (FDA) in 1998 (Centers for Disease Control and Prevention 1999). The rOspA vaccine, LYMErix™ created by SmithKline Beecham Biologicals in Belgium, was a three dose vaccine indicated for use in people aged 15-70 years old (Centers for Disease Control and Prevention 1999). However, in 2002 the vaccine was discontinued due to many issues, the primary one cited by the manufacturer being low public demand (Poland 2011). Although it is possible that more effective prevention measures may become available for Lyme disease, the burden of disease continues to grow as more and more people are affected.

Once the bacterium enters the body, it can begin to cause musculoskeletal and neurological side effects, such as arthritis, fatigue, headaches, and a skin rash called erythema migrans in the early stage of the disease. The late stages of the disease can result in heart and nervous system damage (Wormser 2006). The disease can be physically debilitating and can cause serious damage to quality of life. Lyme disease is currently considered an extremely rare cause of death in the United States. In fact, a study looking at death certificates in the U.S. between the years 1999-2003 found that, upon review of patient symptoms, only one record was found to be consistent with clinical manifestation of Lyme disease (Kugeler 2011). Thus, while seriously debilitating to quality of life, Lyme disease is not currently known to be the sole cause of fatality and is a contributing factor to fatality only in extremely rare circumstances.

Though the symptoms of Lyme disease can be very serious, treatment is available. However, the effectiveness and cost of treatment varies with the length of time that passes between disease contraction and diagnosis. Early-stage Lyme disease is highly treatable with antibiotics such as oral doxycycline or tetracycline for 10 to 30 days if it is caught within a few weeks or months of infection (Strickland 1994). However, once the disease becomes disseminated within the body, intravenous antibiotics may be recommended to rid the body of disease due to the belief that the bacterium can persist in human tissue, especially within the central nervous system (Wormser 2006). These antibiotics, including intravenous ceftriaxone or penicillin, tend to have greater side effects and higher costs (Wormser 2006, Strickland 1994). The difference in treatment effectiveness and costliness as a result of time taken to diagnose the disease is a major issue.

In fact, treatment cost, or the financial burden of Lyme disease, increases as the length of time between infection and diagnosis increases. This is a result of a decreased effectiveness of treatment options after the early stage of infection (Wormser 2006). A study estimating the economic burden of Lyme disease assessed that, in the year 2000, Lyme disease patients at the clinically-defined early stage incurred a mean cost of $464 for direct medical treatment (Zhang 2006). However, patients at the clinically-defined late stage had a mean cost of $1,380 (Zhang 2006). Thus, the average cost of treatment for a late stage patient almost $1,000 more than that of an early stage patient. The difference in treatment costs between early stage and late stage Lyme highlights the importance of early diagnosis and treatment.

Though this disease is concerning at an individual level due to the decrease in quality of life along with a hefty financial burden for treatment, collectively Lyme disease creates a

startling economic burden. Maes et al. (1998) estimated that the overall burden of Lyme disease treatment in the U.S. was $2.5 billion over a span of 5 years to treat 4.73 cases per 100,000 population (Maes 1998). A different study, published in 2006 but looking at data from 2002, estimated that the U.S. burden was $203 million annually (Zhang 2006). This figure was based on the number of cases reported to the CDC though researchers believed that this figure could be much higher in the real world, due to the belief that Lyme disease is a much underreported disease (Meek 1996). Clearly, Lyme disease is a significant cost burden to the United States and one which could increase over the future years if incidence continues to grow.

What has become clear by looking at the impact of Lyme disease on the U.S. is that Lyme disease can seriously affect the quality of life, both physically and financially, of individuals and subsequently of the greater U.S. However by increasing preventative and awareness measures, the overall cost burden of Lyme disease could be lowered significantly. The length of time between diagnosis and treatment could be significantly reduced in areas across Virginia through greater public and physician awareness of disease risk. One of the issues of Lyme disease emergence is that the disease can go unnoticed for long periods of time and may not be tested for systematically because case emergence is sporadic rather than consistent in some areas across the state. That is why it is critical that people are aware of the disease and what types of environments it is most closely associated with. Understanding which areas are seeing a greater influx of the disease and which types environments may pose the greatest risk, physicians and patients may be able to more quickly identify Lyme disease, thus ameliorating some of the more serious outcomes of the disease. This thesis research aims to address this issue by better understanding how Lyme disease is emerging across the state and what landscape characteristics are associated with that emergence.

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1.3 PROBLEM THESIS

      This research project will assert that, in light of the research performed and discussed in the literature review in Chapter 2, a serious gap exists in the knowledge about Lyme disease spread in a newly endemic area such as Virginia and what factors contribute to the emergence of Lyme disease in new areas. Most research examining links between Lyme disease emergence and environmental characteristics has been conducted in endemic areas. This research seeks to examine those potential links in a newly endemic area where Lyme disease is currently emerging. Lyme disease incidence continues to grow exponentially in Virginia and more research must be done to determine where the disease will appear in the future and what factors contribute to its continued emergence. This research project has one specific objective which is: to determine which ecological and demographic factors are related to the emergence of Lyme disease in Virginia.

      This project is part of a larger NSF sponsored project developed to explore through a combination of epidemiological, geographical, and statistical methodologies how specific demographic and environmental variables such as forest fragmentation, land cover change, and human characteristics are linked to the emergence of Lyme disease in Virginia. The end goal of this research is to aid in disease risk prediction by creating a statewide, census tract specific, disease risk map and, in turn, to aid in the allocation of resources for the prevention and awareness of Lyme disease. The research presented here contributes to that goal by elucidating the underlying factors involved in Lyme disease emergence in Virginia. The health and monetary burdens faced by the state and the increase in case numbers highlight the dire need for education, awareness, and early detection programs. A better understanding of Lyme disease emergence

will help the government know where best to allocate precious resources for education and awareness programs.

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CHAPTER 2: LITERATURE REVIEW

2.1 INTRODUCTION

This chapter reviews literature on the Lyme disease ecological cycle and the demographic and environmental variables that affect the spatial distribution of disease occurrence. Section 2.2 begins the discussion of the primary academic discipline this research falls under, medical geography, and takes a closer look at the frameworks within medical geography that are applied to this research, disease ecology and landscape epidemiology. Section 2.3 describes how disease emergence is an inherently geographic phenomenon. Section 2.4 outlines a broad overview of Lyme disease ecology including the history of the disease in the U.S., and the vector, host, and disease reservoir animals that play different roles in the transmission and maintenance of Lyme disease in the environment. Section 2.5 emphasizes the role climate variables play in determining the spatial distribution of Lyme disease. The final section, 2.6, discusses the impact of humans and human environments on the ecological niche of Lyme disease and how these variables may act as predictors of the spatial spread of disease and disease risk.

2.2 THEORETICAL FRAMEWORK

This research is situated within the field of medical geography, which employs geographical concepts and techniques to study issues related to disease and health (Rawlings 1996). Medical geography integrates the biological, environmental, and social sciences as well as qualitative and quantitative methodologies such as cartography, statistics, participatory fieldwork, and interviews in order to examine health issues (Meade 2010). Historical figures in the field of medical geography include Hippocrates (400 B.C.) who examined the impact of cultural-environmental interactions on health as well as John Snow (1854) who mapped the

Broad Street cholera epidemic in London and stopped the outbreak by discovering contamination in a communal water source (Meade 2010).

By understanding the nature of the disease, its habitat, and how it spreads, researchers can begin to prevent further human exposure through environmental and behavioral modifications (Lambin 2010). This project therefore draws from disease ecology and landscape epidemiology, frameworks within medical geography, to understand the complex ecological cycle that the Borrelia burgdorferi bacterium must cycle through in order to cause Lyme disease in humans.

Disease ecology is the study of the relationships between populations and their changing environments to determine how the interactions between the two can influence disease emergence, incidence, and spatial distribution (Meade 2010). The triangle of human ecology looks at the dynamic equilibrium between population, behavior, and physical and biological habitats (Meade 2010). Together, these three elements affect the state of health. A change in any of these three categories impacts the other two and creates a disturbance in the equilibrium. An example of human disease ecology study in Lyme disease may be a change in the pattern of decision making when building homes. City planners may choose to create more medium density residential zones instead of either high or low density zones. That decision would most likely affect where developers choose to build, in this case pushing development forward in medium density zones such as suburbs. Developers would then usually clear a farm field or a forest in order to build a suburban development, often leaving forested areas in proximity to those residential zones. If this process were to continue on a broader scale, it could create hot zones for Lyme disease occurrence by changing the average habitat in proximity to human

habitation, allowing more frequent interaction between ticks and infectious disease reservoirs, and also humans and newly infected ticks in those suburban developments. Though hypothetical, this decision making chain could be a contributing factor to the influx of Lyme disease into the east coast.

An additional hypothetical framework combines with disease ecology to better explain disease emergence. Landscape epidemiology, or pathoecology, a term created by Reinhard in 1974, according to Anne Grauer is a "retrospective analysis of environmental data" which explains the natural and social influences on the development of disease conditions (Pg. 176 Grauer 2012). This area of study looks at several elements contributing to disease including the biotic, abiotic, and social variables that play into creating the ideal geographically-situated niche for a disease. In simple terms, it seeks to explain why a disease occurs in a specific location. The field is based on work done by Pavlovskii (1966), who introduced the concept of a "nidus". A nidus explains the outbreak of disease in a limited social or geographic space. A nidus can be an individual's environment or a large community and the surrounding area. It is the analysis of ecological factors, such as vectors, reservoirs, humans, and their interactions which contribute to the formation of an ideal niche for disease occurrence. Lyme disease can be studied through landscape epidemiology, examining why Lyme occurs where it does. In the past Lyme disease had a more limited nidus or zone of infection but with landscape changes and changes in host and vector habitats, that nidus is expanding. This expansion of the nidus has caused Lyme to emerge into non-endemic areas. This study will ask what factors, biotic, abiotic and social, could be encouraging the expansion of the Lyme disease nidus into previously unaffected areas in the state of Virginia.

This type of niche disease study using a landscape epidemiological approach and based on analysis of both social and ecological data can be conducted at multiple spatial scales. On an individual level, this theory looks at the direct geographical and social interactions of a person or animal. For example, a person may have a bucket of drinking water inside their home which contributed to mosquito larvae formation and that individual's subsequent infection with malaria. On a community level, landscape epidemiology permits the study of the complex patterns of interactions between hosts, vectors, humans and all other ecological aspects. The social habit of keeping standing drinking water in and around living spaces in a community could be contributing to an overall outbreak of malaria at a specific geographic location.

Understanding social and ecological variables contributing to an outbreak can aid in prevention through landscape modification, in this case example, lowering the rate of malaria within a community by removing standing water. Landscape modification "can [either] create, or be used to prevent, the establishment of disease cycles" (Page 111 Meade 2010). Previous studies of other zoonotic disease such as Bovine Spongiform Encephalopathy (BSE) (Hope 2012) or Chagas disease (Abad-Franch 2009), successfully demonstrated landscape or environmental modification approaches to disease prevention and paved the way for this investigation into Lyme disease.

2.3 THE GEOGRAPHIC NATURE OF EMERGING INFECTIOUS DISEASES

Emerging infectious diseases are an inherently geographic phenomenon. Emerging diseases are defined as those that have just entered into a population or those that have existed but have an increasing incidence rate or an expanding geographic range (Morse 1995). Emerging diseases in humans are almost always precipitated by a disturbance in the current equilibrium or a change in an ecological, environmental, or demographic factor (Morse 1995).

This factor then causes a change in the geographic relationship of humans to a new microbe or a host organism, bringing them into closer contact with one another (Morse 1995).

There are many well-known historical examples of emergent infectious diseases. In each example the disease organism can be traced back to its source nidus, geographically, where it was either amplified or originated. Population expansion pushed exploratory ships and diseased sailors from Europe into the Americas, leading to widespread infiltration of smallpox and other communicable diseases into areas that had never seen them before (Daszak, Cunningham and Hyatt 2000). Construction of the Aswan River dam led to a dramatic amplification of schistosomiasis, a parasitic disease whose lifecycle depends on a water-loving snail, in the areas surrounding the dam (Mayer 2000). Perhaps the emergent disease example that is most well-known to public health workers and epidemiologists is that of John Snow and the cholera epidemic of 1854 (Smith 2002). Cholera emerged into London as a result of a change in population and increasing pressure on the sewage system that led to dumping of contaminated water into the River Thames. John Snow mapped the outbreak by afflicted residents' home addresses to find that a Broad Street water pumping station formed the epicenter of the emergence.

There are many more contemporary examples of emerging diseases in the U.S. including HIV/AIDS, hantavirus, and Lyme disease (Mayer 2000). Inside each disease emergence is a unique story of a culmination of environmental factors leading to contact of a human with a disease agent. Lyme disease emergence is due to the increasing pressure of human inhabitants on surrounding environments and, possibly, the reemergence of deer populations. It is thought that suburban development along the northeastern coast has fostered greater contact between

humans through the residential environment and the environment of tick hosts, such as deer and small rodents. This contact, or intersection of habitation spheres, is primarily geographical in nature and is a phenomenon that can be mapped and studied to prevent unchecked spread of the disease organism.

2.4 LYME DISEASE ECOLOGICAL CYCLE

2.4.1 Overview & History of Lyme Disease Ecology

Lyme disease is considered a zoonotic disease because it is caused by a pathogen transmitted between animals; humans are incidental, or dead-end hosts. In the case of Lyme disease, the vector, a tick, infects hosts during a blood exchange or a "blood meal". These vectors are originally infected by feeding off of other animals, known as disease reservoirs, which harbor the pathogen. Disease reservoirs can either be highly competent, meaning they infect a high percentage of the ticks that feed off of them, or be incompetent. A low-competency reservoir (LCR) means that the animal will infect very few of the ticks that feed off of it even though it may harbor the disease. (Ostfeld 2011)

The bacterium B. burgdorferi is unique because it can be passed from ticks into many different host organisms. In fact, there are over 125 species in North America that can serve as a host for the bacterium (Keirans 1996). Of these, the white-footed mouse (Peromyscus leucopus) is known as the most competent reservoir (Levine 1985, Donahue 1987). When a tick feeds on a white-footed mouse it usually becomes infected with the B. burgdorferi bacterium and it can remain infectious for several months, during which time previously uninfected ticks may feed, thus continuing the bacterium transmission cycle between generations. Other host organisms play an important role in the maintenance and movement of tick populations without serving as a competent disease reservoir. The primary host animal in this role is the white-tailed deer

(Stafford 2007, Spielman 1979). Together these organisms make up the chain of transmission and disease maintenance on the eastern coast of the United States. However, the question remains: where did Lyme disease come from?

In 1972, a strange outbreak of arthritis in children began the investigation into Lyme disease in the United States (Steere 1978). Though it appeared that Lyme disease was just emerging in the U.S., it is actually an ancient affliction that has long existed in the United States and around the world. However, the ecological changes in the eastern U.S. during the time of European settlement caused great upheaval in the disease ecosystem. It is thought that when the Northeast was deforested and the wildlife population was harvested for food and profit during the 18th and 19th centuries that Lyme disease became nearly eradicated. Almost all the deer populations thinned or died out, with some pockets remaining on island refuges such as Long Island (Barbour 1993; Persing 1990; Cronon 1983).

This disappearance of deer could have decimated the tick population across the coast. In fact, historical records point to that theory. In 1748-1750, a Swedish naturalist named Pehr Kalm wrote about how abundant and bothersome ticks were during his travels across the United States (Stafford 2007). A century later, entomologist Asa Fitch found that ticks were nearly extinct along the same route Kalm had traveled years earlier (Stafford 2007). This extreme reduction in tick populations could have reduced the number of disease reservoirs over time to near extinction, except in isolated areas such as islands where hunting and habitation was rare and from which Lyme disease would likely later re-emerge (Stafford 2007). Since the early 1900's, forested areas have been reestablished in the Northeast in increasing numbers, often interspersed with housing and small farms. Deer rehabilitation has also been a major project in many states

along the coast, including Virginia, bringing populations back from virtual extinction at the beginning of the 20th century to overpopulation in many parts of the state today (Virginia Department of Game and Inland Fisheries 2007). As the forests returned, so did the deer, the ticks, and the bacterium that infects the ticks. It is possible that now the bacterium is slowly re-emerging back into the original territory it once affected in even closer contact with humans due to the interspersion of forest and habited land.

2.4.2 The Vector Species: Ticks

Ticks are a type of arthropod, closely related to mites and spiders. Although there are over 80 species of ticks living in the United States, only two of them are important in the transmission of Lyme disease. Both Ixodes scapularis and Ixodes pacificus are species of ticks that pass Lyme disease on to humans. I. scapularis inhabits the East coast and Midwest while I. pacificus is seen on the West coast. B. burgdorferi can be passed from a feeding tick to a human host in the course of a blood meal after the tick has been attached for 36 to 48 hours (Centers for Disease Control and Prevention 2007). The infecting ticks, both Ixodes scapularis and Ixodes pacificus, have a complex four -stage life cycle consisting of egg, larval, nymphal, and adult stages. Each summer, tick eggs hatch and release larvae which attach to small birds or mammals, including the white-footed mouse (Peromyscus leucopus), for their first blood meal. At this point, ticks may become infected with the bacteria through an animal disease reservoir and will remain infected for the remainder of their lives. The following summer, the larvae will have grown into nymphs and will seek another blood meal in order to develop into the adult stage. The tick stage that transmits Lyme disease to humans most frequently is a nymph because its small size, potentially less than 2mm in length, inhibits human detection. As adults, ticks will seek blood once again during the summer months in order to lay eggs and complete their life

cycle. During the spring and summer months, Lyme disease infections are highest because of this cycle of host seeking. In total, most ticks will only take three blood meals during their life cycle - as larvae, as nymphs, and as adults. Though ticks take a limited amount of blood meals, during a limited time frame of warm months, they still manage to infect a large number of people each year. It is important to know what other factors drive human infection rates up, including concentrations of disease reservoir hosts. (Centers for Disease Control and Prevention 2007)

2.4.3 The Primary Disease Reservoir: White-footed Mice

When ticks were identified as the vector of Lyme disease, the next question asked by disease ecologists was: what animal is the disease reservoir? In other words, how did the tick acquire Borrelia burgdorferi? The idea is that by reducing the population of the disease reservoir animal, the disease could be controlled. This question was addressed by a group of researchers led by Bosler in 1983 (Bosler 1983). The scientists examined blood from white-footed mice and deer and found that both samples carried B. burgdorferi spirochetes. The ticks collected off the animals indicated that more ticks from the white-footed mice were infected than those taken from the deer. Another study, in 1987, looked simply at whether or not the white-footed mouse, Peromycus leucopus, was a competent reservoir (Donahue 1987). This study not only confirmed that white-footed mice can serve as disease reservoirs, but also that only one infected nymph taking a blood meal from a mouse was necessary to infect that mouse, leading to many subsequent tick infections from that mouse. The study confirmed that tick progeny are not born with B. burgdorferi so the bacterium must be acquired after birth. It was determined that mice are more efficient disease reservoirs than hamsters or rabbits. Thus far, research had only confirmed that white-footed mice are the most efficient reservoir, with tick infection rates of 75-95% after ticks feed on an infected mouse. (Ostfeld 2011).

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Scientists then began to look at whether greater abundance of mice led to greater tick abundance as well as greater tick infection rates. Ostfeld, a primary researcher in this subject area, continued his studies on white-footed mouse competency over a period of 13 years (Ostfeld 2006). The hypothesis of the overall study was that in years where mouse abundance was high, tick infection rates would be high as well. A second hypothesis tested the theory that mice were the ideal host for ticks, predicting that when mice abundance was high, tick populations would be high as well. The results of the study showed that while mice were good predictors of both tick population densities as well as tick infections rates, they only explained 58% of the variation in the statistics. Chipmunk abundance was equally good at predicting tick population densities as mouse abundance. Furthermore, a related study looked at a comparison of mice, chipmunk, shrew, and low-reservoir-competence (LRC) hosts (Brisson 2008). The results of the study illustrated that, while chipmunks and mice are significantly good at infecting ticks, combined they only made up 16-25% of the hosts that ticks preferentially fed on in the study. There were clearly more variables that needed to be considered aside from mouse abundance when looking at both tick abundance and infection rates.

These studies led to the idea of a more complex ecosystem which included the interaction of ticks with multiple disease reservoirs having varying competency levels for tick infection. In the Brisson et al. (2008) study mentioned earlier, shrews actually hosted 30% of the ticks and yielded a higher percentage of infected ticks than did either mice or chipmunks. However, while these three species combined did infect 80-90% of the ticks that contracted B. burgdorferi, they ended up hosting less than half of the total tick population in the study. The results of this study indicated that outcompeting efficient disease reservoirs with low-competency reservoirs (LCR) could result in a decreased number of infected ticks, reducing overall Lyme disease transmission.

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In sum, these statistics discredited the idea that white-footed mice are the most influential reservoirs of Lyme disease just because they are the most competent at infecting ticks. Future studies on disease reservoirs began to focus more on the more complex idea of host competition and species richness in reducing Lyme disease.

2.4.4 Complexity of Host Biodiversity & Reservoir Decay

In Brisson et al. (2008), discussed previously, the ideas of multiple hosts and biodiversity were introduced. In the earlier discussion about the limitation of deer in predicting tick habitats, the study by Rand et al. (2004) concerning deer removal yielded another interesting result. The research showed that removal of deer actually increased tick infection rates for Borrelia burgdorferi, possibly due to an increase of feeding on other hosts more likely to transmit disease, such as mice. This idea of host competition is very important to the ultimate goal of Lyme disease research: to lower the human infection rates. Vectors that have many hosts are less influenced by the abundance of a few hosts in particular, but, with a greater number of hosts present, the dispersion of blood meals will be evenly spread across many hosts (Ostfeld 2011). This "spread" across many hosts can lead to reduced feeding on highly competent reservoirs, such as mice and chipmunks, and, therefore, a decreased number of infected ticks transmitting Lyme disease to humans.

The concept of host biodiversity has been illustrated in many different types of studies, including a study led by Ostfeld (2000), which looked at how species richness and evenness can affect the prevalence of Lyme disease. This study used a computer simulation model to illustrate how the relative abundance of diverse species, including ground-dwelling birds, small mammals, and lizards, can influence infection prevalence in nymphal ticks. Both ground-dwelling birds

and small mammals showed a "rescue effect" phenomenon. When species richness declined, Lyme disease increased and vice versa. Therefore, the overall conclusion was that increased species richness decreases the risk of Lyme disease.

Another study, led by Keesing (2009) focused on how ticks are influenced by specific hosts rather than broader factors such as climate or habitat. This study used wild captured animals and proceeded by placing 100 larval ticks on each animal to record how many ticks survived feeding for a blood meal, which usually requires attachment for a substantial period of time. Ticks that fed off of white-footed mice were more likely to survive and be full. Those ticks that fed off opossums, in contrast, had much lower survival rates due to the animal's grooming practices. As a result, the density of infected ticks increased when ticks fed less on animals with grooming habits. This result supports the idea that species diversity affects disease prevalence and that by increasing the biodiversity of hosts on which ticks can feed, overall tick infection rates are lowered. This idea becomes important when looking at specific ecologies associated with Lyme disease occurrence and how species diversity could potentially contribute to Lyme disease reduction or increase. Though the question of where high concentrations of infected ticks may occur is partially answered, another question remains. How is the disease spreading so rapidly into new areas and what is carrying infected ticks into these new territories?

2.4.5 The Primary Host Animal: Deer

The first culprit that many people will think of when addressing Lyme disease habitats is deer. The stigma associated with deer developed, in part, from the informal title of "deer tick" previously given to Ixodes scapularis, one of the ticks that transmit Lyme disease. While deer do not serve as a reservoir for B. burgdorferi and thus cannot transmit the bacterium to ticks, they

have been cited as the "definitive host"(pg. 150 Spielman 1979) and the "primary source of nourishment for gravid [pregnant] female I. scapularis" (pg. 783 Rand 2004). The initial discovery of this fact was made while deer hunting on Nantucket Island in 1976-1977 (Spielman 1979). All stages of tick life were present on the deer in large amounts, including larvae numbers of over 467 per deer, signaling an important role of deer in tick ecology as a sort of habitat unto itself on which ticks can travel, live, feed, and mate. Following this line of logic, much research was done regarding deer in the control of tick populations.

One of the primary studies was performed by Wilson et al. (1984) on Great Island, Cape Cod, Massachusetts. Following a large scale operation of deer removal, killing ~70% of the 30 deer present on the island, the result of the study was unexpected. By examining the numbers of larval and nymphal ticks on white-footed mice (Peromycus leucopus), pre- and post-deer removal, the scientists came to the conclusion that eliminating deer from the island made little impact on tick abundance numbers. However, a contrasting study performed on Monhegan Island, Maine, showed a drastic reduction of juvenile and adult ticks on the main disease reservoir present on the island, rats (Rattus norvegicus), two years after completely removing the deer population on the island (Rand 2004). In addition, the state of Connecticut witnessed correlating drops in both nymphal tick infections and human Lyme disease cases when the population of deer declined (CT Department of Environmental Protection 2007, Stafford 2003).

One reason scientists speculate that the studies can be contradictory is the result of new knowledge about the variety of hosts ticks can survive on. Tick species that transmit Lyme disease do not specifically depend on deer populations. In fact, according to Keirans (1996), I. scapularis can depend on 125 other types of North American vertebrates for blood meals.

However, it is clear that deer are a very important host in tick reproduction cycles, especially in some areas. Controlling deer populations may be part of the key to controlling the spread of Lyme disease. There are many ways to do this including hunting permits, bow hunting permits for semi-urban areas, incentivized doe hunting, and even deer birth control. However, most of these deer control methods are expensive and, without constant maintenance, can easily relapse back to the original deer overabundance issue due to the fact that deer populations can easily double in as little as a year. In areas less isolated than an island or more densely populated, deer population control may be unfeasible. However, deer habitats and their intersection with human populations may be a key indicator in where we can expect to see higher incidence of Lyme disease.

2.5 IMPACT OF CLIMATE ON DETERMINING SPATIAL DISTRIBUTION OF LYME DISEASE

2.5.1 Climate Mapping and Spatially Predicting High Risk Lyme Disease Area

The ability to predict vector expansion into new geographic areas allows scientists to predict where disease might appear in the future. Being able to predict spread is a powerful tool which allows for education, preparedness, and disease control. Since Lyme disease is transmitted through ticks and the ticks are infected by feeding off of disease reservoirs carrying the bacteria, the habitats of ticks and the disease reservoirs can determine where Lyme disease may occur. In the beginning, Lyme disease had three original foci of occurrence where these factors combined: the northeastern coastline, Wisconsin, and California, but it has since expanded. The total potential range for expansion could be determined by examining to the total possible area that Ixodes scapularis and Ixodes pacificus could potentially inhabit. Ticks are a hardy species as they can survive on animals at temperatures above 40°C and in temperatures

below -10˚C in lab settings (Ostfeld 2011). Scientists began to define and predict tick habitat expansion through climate and temperature variables.

One of the first attempted habitat suitability maps of the United States was produced in 1998 using NOAA satellite data, temperature minimums/maximums, and the normalized difference vegetation index (NDVI), which represents the greenness of different vegetation types (Estrada-Pena 1998, Ostfeld 2011). This habitability map was compared to known information about the current distribution of ticks to create a predictive map of future tick habitability using statistical analysis. While that predictive map did give a good starting idea for tick ranges, it had some inaccuracy due to the incomplete knowledge about the current tick distributions (Dennis 1998, Ostfeld 2011). Another map, created later by Brownstein et al. (2003), included more climate variables including the average, minimum, and maximum air temperatures as well as ground temperatures.

As climate change became more quantifiable, scientists began to incorporate the new data on greenhouse gas emissions and the resulting global temperature changes into their disease prediction maps. In 2005, Brownstein's original map work was altered to take into consideration the probable scenarios of climate change and how that might affect tick habitat suitability (Brownstein 2005b). The study utilized data on land cover in conjunction with climate change data from the Intergovernmental Panel on Climate Change (IPCC). In Canada, Ogden et al. continued research on suitable Lyme disease climates, producing risk maps focusing more specifically on the Canadian perspective (Ogden 2006). They also produced 3 risk maps for 2020, 2050, and 2080 by incorporating the predicted climate change scenarios generated by the Canadian Coupled Global Climate Model 2 (Ogden 2008). The climate maps generated using

temperature and climate scenarios were at a very broad scale over the entire continent of North America and did not look at state or county specific tick distribution densities. They also did not indicate the probable infection rates for B. burgdorferi in the ticks that inhabit different areas. Scientists attempted to identify these risks by looking at specific factors which could immediately point to either high tick abundance or high tick infection rates.

2.5.2 Elevation, Precipitation & Acorn Data as Predictors of Lyme Disease Risk

Though climate maps are good for estimating overall tick habitation, scientists hoped to be able to hone in on high risk areas for Lyme disease by looking at one or two climate-related variables at a time. One study looked specifically at elevation, predicting that Ixodes scapularis could only survive in low-elevation sites in New England because winter temperatures at higher elevations further inland would be prohibitive (McEnroe 1977). This original hypothesis turned out to be incorrect as they discovered that ticks did survive at higher elevations in New England. However, further research did prove that elevation changes definitely do impact tick habitats. A study done by Jouda et al. (2004) clearly illustrated that elevation has an impact on tick habitats and Borrelia burgdorferi infection rates. To do this research, the scientists took measurements at three different elevations (620m, 740m, and 900m). There tended to be a higher proportion of adult ticks to nymph ticks, a lower density of ticks, and lower infection rates in ticks at increasing elevations (Jouda 2004).

In Jones and Kitron's (2000) study, the focus was on rainfall and climate variables in Illinois. They concluded, after an 8 year study, that white-footed mice and larval tick abundance decreased after extreme droughts. However, a contrasting study, also done over an 8 year period from 1998-2005, was performed by Schulze et al. (2009) looking solely at precipitation and

temperature to measure tick abundance. This study found no correlation between precipitation or temperature variations on the amount of nymphal tick abundance. Altogether, rainfall was not a consistently predictive variable for determining high density tick habitats.

More research was performed in New York State over a period of 13 years (Ostfeld 2006, Ostfeld 2001). While the original study (2001) saw little to no influence of precipitation and no influence of deer abundance, it did find a positive correlation between acorn levels in the fall and both mouse and larval tick infection levels the next summer. Ostfeld et al. (2006) concluded again that precipitation and deer had no influence while acorns from two years previous and mouse/chipmunk populations from one year previous did have significance in tick infection rates. With the two studies in agreement, acorn production and mouse abundance seemed like a promising correlation with both tick and mouse infection rates

However, the results that Ostfeld et al. (2006) found in their studies were ultimately inconclusive when compared to other studies. Schauber et al. (2005) compared weather, mouse, and acorn variables in seven states and one specific county, Duchess County, NY. This study compared Lyme disease incidence to weather, mouse, and acorn data. While the results did trend differently for each state, there was no variable that proved to be predictive overall. The researchers hypothesized that this could be a result of different factors being more or less influential in specific areas. For example, a certain type of ecology needs to be present for acorn production to be a predictive variable.

As researchers began to look at particular habitats for tick and Lyme disease emergence, the limitations of climate and environmental models became evident. The number of variables that need to be considered in the tick's complex host habitats and their hardy survivability make

appropriate climate conditions very hard to predict. Research projects have been performed to assess almost every climate aspect constraining tick habitats - including the effect of climate, climate change, and precipitation. While climate and precipitation proved to have inconclusive impacts on tick abundance and infection rates, acorn abundance and the effect it had on mouse abundance showed promise. However, as research has continued, these variables have been shown to be important only in small, specific regions. When looking at creating predictions for a statewide infection risk, none of the variables discussed show promise for this research project as a consistently useful study variable.

2.6 IMPACT OF HUMANS & DEVELOPMENT ON LYME DISEASE ECOLOGICAL NICHE
2.6.1 Impact of Residential Landscape Characteristics on Lyme Disease Risk

Another way in which researchers began to trace Lyme disease risk was by looking at areas where there is an increased likelihood of tick and human interaction. As most people affected by Lyme disease cannot pinpoint any specific areas for tick encounters other than their residences, successful studies have been done focusing on the residences as the potential areas of contraction (Glass 1995). Researchers began to look at these narrowly focused portions of land to determine which specific features encourage tick populations. This area is known as the residential landscape.

Dister et al. (1997) performed a remote sensing characterization of 337 residential properties in Westchester, New York. Properties were evaluated using Landsat TM to determine several variables influencing the specific habitat of each lawn including: brightness values, wetness values, greenness values, USGS elevation data, and infrared photos. Tick densities were determined through random sampling to create categories of no, low, and high risk properties. When compared with the other variables, high risk properties were identified as being primarily

greener and wetter, meaning more broadleaf and woody areas than those with large lawns. Several other studies using different techniques discovered similar findings. Frank et al. (1998) studied types of lawn features looking at lawn, ornamental features, ecotone features, woods, and stone walls on residential properties to determine tick presence. The results agreed with the earlier study that lawns correlated with lower tick densities and woods correlated with higher tick densities.

In addition to wooded landscapes, it was also a general trend that people tended to contract Lyme disease in non-urbanized settings. Cromley et al. (1998) performed a study that looked at the types of residential settings, based on population density. The study area was 325 square miles and centered around 12 towns, including Lyme, CT, the initial epicenter of the disease outbreak. The results indicated that people living in medium residential density, or in towns/villages, were less likely to be infected than those living at lower densities. Together, the results of these three studies indicated an overall risk association with non-urban, partially forested properties. This characterization led researchers to look at the risk factors associated with suburbanized properties and farmlands with large amounts of fragmented forests.

2.6.2 Impact of Forest Fragmentation on Incidence of Lyme Disease

Forest fragmentation is a process that occurs when large, unbroken forests are sectioned into small pieces by roads, agriculture, urbanization, or other types of development. Fragmented land cover is significant, as discussed above, because it often occurs in suburbs where there are small patches of forests located close to homes or farm properties, leading to greater levels of Lyme disease risk. However, forest fragmentation is significant in terms of biodiversity, a useful variable in predicting tick infection rates. A study conducted by Yahner (1992) looked at clear-

cutting cycles over three periods. In particular, the study focused on small rodent diversity and abundance, particularly Peromyscus leucopus, the white-footed mouse and the most competent reservoir for B. burgdorferi. Overall, results showed that, as forest fragmentation increased, as a result of clearcuts, some rodents increased in abundance including, most notably, the white-footed mouse. Clearcutting and forest fragmentation led to an overall decrease in biodiversity and an increase in high competency reservoirs, i.e. white-footed mice, for the Lyme disease bacterium.

In a related study, Allan et al. (2003) looked at forest fragments and nymphal tick infection rates to determine potential dependence on smaller or larger fragment sizes. In this study, small forest fragments were those that were less than 2 hectares (ha) in area and large forest fragments were between 2 and 8 ha in area. The hypothesis was based upon the idea that mouse reservoir densities increase and biodiversity of hosts decrease with smaller patch size (Yahner 1992). This study examined 14 forest fragments in Dutchess County, NY, concluding that ticks collected in smaller forest fragments (<2ha) carry a higher density of infected nymphal ticks than those collected in larger fragment sizes (>2ha). From these two research projects, new studies were designed to tie reported human Lyme disease incidence with forest fragmentation and forest patch size.

Brownstein et al. (2005a) designed a study based on 30 sites around Lyme, CT to determine overall relationships between forest fragmentation levels and documented human Lyme disease incidence rates. Fragmentation levels were incorporated into a Poisson regression model with average disease incidence rates of each town. The results did indicate a positive correlation between increasing fragmentation levels and higher tick infection prevalence.

However, increased forest fragmentation was negatively correlated with human disease incidence rates- indicating that another variable was influencing the suburban environment.

A related study (Jackson 2006) looked at land cover data for the entire US state of Maryland and 12 assorted counties. The landscapes were divided according to major federal roads to imitate potential deer boundaries. Incidence rates and location of infections from the last 5 years were collected and incorporated. Fragmentation levels of each bounded polygon were determined using FRAGSTATS software. Results of this study indicated that the most predictive land cover variable for Lyme disease infections was the proportion of forest-herbaceous/scrub edge and the percentage of forested areas within the polygon. Income was also positively related. This result indicated that ecotone areas, or areas of transition between forest and herbaceous/scrub habitats, could be primary indicators of Lyme disease incidence. The researchers found that the number of small forest fragments (<2 ha) present in each polygon was also positively correlated to disease incidence. Together, Yahner (1992), Allan et al. (2003), and Jackson et al. (2006) conclude that higher levels of fragmented forest could lead to higher tick densities and higher incidence rates of both tick and human infections with Borrelia burgdorferi.

These studies are especially important because they indicate consistent trends using environmental variables for predicting tick habitats, tick infection rates, and Lyme disease risk areas. In addition, Jackson et al. (2006) generated a statewide risk pattern that included risks for each individual polygon identified. The land cover data (NLCD) used are easily accessible to each state and do not require intensive data collection. This concept of utilizing residential landscape characteristics such as forest fragmentation rates as prediction variables of Lyme disease incidence appears to answer the research questions posed during the literature review.

Forest fragmentation may be the ecological process most influential in the prediction of Lyme disease spread and could aid in the successful construction of a statewide risk map for Virginia.

2.7 CONCLUSION

Over the years since Lyme disease has emerged, more and more questions about the disease have been answered. Though control of disease spread and prevention of human infection is still ongoing, this literature review provides a base for knowing where and why Lyme disease may occur at increasing rates. Therefore, this research specifically examines this gap in knowledge for the state of Virginia, which forms the southern edge of an expanding zone of disease emergence. In particular, this research fills the gap by examining if the processes of forest fragmentation and land cover change are important predictors of Lyme disease incidence and emergence in Virginia. The results could help by confirming ideas validated by previous studies. In addition, human variables such as age, gender, and income can be examined in Virginia to see whether they are correlated with Lyme disease. In addition, the research would contribute new knowledge about Lyme disease patterns in emerging areas, rather than those already endemic with the disease. Many of the research studies, including both Brownstein et al. (2005a) and Jackson et al. (2006), examined Lyme disease in endemic areas. There is a possibility that areas experiencing a new emergence of the disease will have dissimilar results to those inundated by disease incidence. Through research and data analysis, the gaps in knowledge about how and where Lyme disease is spread in newly emerging areas can be filled.

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CHAPTER 3: LYME DISEASE EMERGENCE IN VIRGINIA: AN EXAMINATION OF THE DEMOGRAPHIC AND ENVIRONMENTAL VARIABLES CORRELATED TO THE SPATIAL PATTERN OF DISEASE INCIDENCE

Sara Elizabeth Dymond[1][**] *(sdymond@vt.edu)*, Korine N. Kolivras[1] *(korine @vt.edu)*, Yili Hong[2] *(yilihong@vt.edu)*, Jie Li[2] *(jieli@vt.edu)*, Stephen P. Prisley[3] *(prisley@vt.edu)*, James B. Campbell[1] *(jayhawk@vt.edu)*, David N. Gaines[4] *(david.gaines@vdh.virginia.gov)*, Randel L. Dymond[5] *(dymond@vt.edu)*

1. Department of Geography at Virginia Tech, 115 Major Williams Hall, Blacksburg, VA 24061-0439

2. Department of Statistics at Virginia Tech, 406-A Hutcheson Hall, Blacksburg, VA 24061-0439

3. Department of Forest Resources and Environmental Conservation at Virginia Tech, 313 Cheatham Hall, Blacksburg, VA, 24061-0439

4. Virginia Department of Health, Epidemiology, 109 Governor St., Richmond, VA, 23219

5. Department of Civil and Environmental Engineering at Virginia Tech, 200 Patton Hall, Blacksburg, VA, 24061-0439

**Corresponding Author:
Sara Elizabeth Dymond
College of Natural Resources, Virginia Polytechnic Institute and State University
115 Major Williams Hall (0115)
Blacksburg, VA 24061
Tel.+ 540 231 6886
Email: sdymond@vt.edu

ABSTRACT

Background

Since its emergence in 1975, Lyme disease has become the most significant vectorborne illness in the United States. Virginia has experienced Lyme disease expansion, with steadily increasing rates over the past decade and disease spread from the northern part of the state to the southwestern end. This study examined correlations between demographic and environmental variables and the spatial pattern of Lyme disease emergence in Virginia. Specifically, this study investigated whether areas with greater concentrations of certain land cover types, more interspersion of different landscape types, or areas with a greater abundance of fragmented forests were at higher risk for Lyme disease incidence. The ultimate goal of this project was to identify which demographic and environmental factors correlated with higher Lyme disease incidence across the state as the disease emerged and to propose what strategies state health departments can implement to reduce disease incidence in the future.

Methods

This study utilized environmental data from the National Land Cover Database, Lyme disease case data from the Virginia Department of Health, and population data gathered from the U.S. census. A spatial Poisson regression model was used to determine whether or not land cover, landscape features, and population characteristics were significantly correlated with Lyme disease incidence in Virginia.

Results

Analysis indicated that herbaceous land cover is positively correlated with Lyme disease incidence. Those areas with more interspersion between herbaceous and forested land were also positively correlated with incidence. In addition, income and age were both positively correlated with disease incidence. Highly developed areas, interspersion of herbaceous and developed land, and areas with high population densities were negatively correlated to disease incidence. Abundance of forest fragments less than 2ha in area were not significantly correlated.

Conclusions

Our research shows that several environmental and demographic variables are correlated with Lyme disease incidence in an area with recent disease emergence, which supports the results of research conducted in endemic areas. These variables could be predictive of where Lyme disease may emerge in the future and could help state health departments better direct prevention campaigns as Lyme disease continues to emerge across the state and country.

Keywords

Lyme disease, GIS, Medical Geography, Virginia, Spatial Poisson Regression

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3.1 INTRODUCTION

      Lyme disease, caused by the bacterium *Borrelia burgdorferi* and transmitted through the bite of an *Ixodes scapularis* or *Ixodes pacificus* tick, was first described by physicians in the early 1970s due to an outbreak of arthritis in young children near Lyme, Connecticut [1]. Since then Lyme disease has continued to widen its geographic spread and its prevalence across the U.S. Lyme disease is now the most significant vectorborne disease in the U.S. with the highest reported number of confirmed cases at 29,959 in 2012 [2]. It has three major geographic focal points in the U.S. including the Northeast coast, the Midwest around Wisconsin and Minnesota, and along the West coast [3]. Virginia, the study area for this research, has seen a clear expansion of Lyme disease with a dramatic increase from 2000 to 2011 illustrated in Figure 3.1. Though U.S. rates appear to be slowly increasing over time, rates within Virginia are dramatically increasing. According to a report by the Virginia Department of Health, this increase is due to several factors, one of which is an increase in case reporting practices and public awareness [4]. We seek to identify other potential factors relating to underlying characteristics in the human and physical environments.

      One way to examine the increasing incidence of Lyme disease and predict where Lyme disease might occur in the future is to determine where the environments of amplifying organisms and humans intersect. White-tailed deer, primary hosts for adult ticks, thrive in many landscapes but most specifically in what are known as "edge" environments, intermediate zones between forest fragments and open, vegetated spaces [5]. This ecotone, or transitional area between two biomes, allows deer to feed and forage in the open, vegetated space but also allows deer to hide and retreat when necessary into forested areas. White-footed mice, primary disease reservoirs for *B. burgdorferi*, thrive in most environments but become opportunistic and out-

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compete other small mammals in disrupted areas of small, fragmented forests, which are created when a larger forest is cut into smaller pieces as a result of development or agricultural expansion [6]. Studies suggest that most human Lyme disease cases in the northeastern US are contracted through exposure to ticks around the peridomestic or home environment [7-9]. This combination of landscapes including mixed forested and open areas with small forest fragments near human residences typically exists in suburban or low developed residential areas. It has been suggested in previous research that low-density residential areas do tend to be correlated with higher rates of Lyme disease incidence [10].

This research sought to quantify relationships of different land cover types, with a focus on the area devoted to different land cover categories, the degree of forest fragmentation, and the intersection of different land cover types, to the rate of disease incidence in order to observe trends across the state of Virginia. Lyme disease is suspected to operate at a landscape level, suggesting that zoonotic processes supporting disease incidence occur at a larger than individual or residential home scale. Though many studies have looked at the land cover mixtures or ecotones at the individual residential environment level [7-9], few have tried to capture the broad-scale land cover pattern, reflecting how land use decisions can affect broad scale disease incidence over a relatively large area. This study is informed by broad-scale land-cover studies done by Jackson [10] and Brownstein [11] that sought to determine the land-cover patterns associated with higher disease incidence. However, those studies were performed in Maryland and Connecticut, respectively, after Lyme disease had already become endemic. The research presented here examined the landscape level factors associated with the emergence of Lyme disease because little is known about the role of environmental factors in facilitating the spread into new areas across the southeastern U.S.; this study attempts to fill that gap. A better

understanding of the factors that are influencing the spread of disease in Virginia could lead to better prediction of where the disease will occur in the future, increasing awareness and decreasing the physical and monetary costs of Lyme disease by reducing diagnosis time.

The research objective of this project was to examine ecological and demographic factors that may have influenced the emergence of Lyme disease in Virginia. Specifically, based on previous research, we hypothesized that the greater interspersion of forest and herbaceous land cover and greater counts of small fragmented forests (<2 ha) would correlate with greater Lyme disease emergence and incidence.

3.2 BACKGROUND

Once a person becomes infected with *B. burgdorferi* following a tick bite, a range of symptoms may appear. Early stage Lyme disease is characterized by an *Erythema migrans*, a bull's-eye shaped rash, and flu-like symptoms including a low-grade fever, fatigue, and muscle aches. As the disease progresses into a later stage, symptoms can include arthritis, neurological symptoms, musculoskeletal, and cardiac issues [12]. Though the disease has been documented as a contributing cause of death, it is an extremely rare cause of death in medical records [13]. More often the disease can cause significant loss in quality of life unless promptly treated, and additionally, treatment costs can be high. The disease, though treatable with antibiotics, becomes difficult to eradicate once it is spread throughout the body. The treatment cost for late stage disease patients has been estimated to be, on average, $16,199 while the average cost of treatment for an early stage patient was only $1,310 [14]. The debilitating physical symptoms and financial burden of Lyme disease can be quite serious, especially if diagnosis and treatment are delayed. Delays in diagnosis could be common in areas that have not experienced many

Lyme disease cases in the past because the early stage symptoms can be ambiguous and Lyme can easily be mistaken for something else. However, this study intends to improve diagnosis time by increasing our understanding and awareness of the demographic and environmental factors associated with the continued emergence of Lyme disease.

Lyme disease is a zoonotic disease that is transmitted between animal reservoirs by ticks; humans are accidental, dead-end hosts for the bacterium *B. burgdorferi*. The *Ixodes scapularis* tick transmits the bacteria in the Northeast and Midwest United States while the *Ixodes pacificus* tick is the vector along the West coast [15]. A tick has three life stages after hatching (larva, nymph, and adult) and must take a blood meal before passing from one life stage into the next [16]. The bacterium is usually picked up by *Ixodes* ticks during a blood meal in the larval or the nymphal stage [16] . Although ticks have over one hundred potential hosts in the U.S., the principal hosts for young ticks along the East coast are small rodents and birds. Birds can be a major host for immature *I. scapularis* and could be important in the long-distance diffusion of infected ticks. The primary disease reservoir responsible for transmitting *B. burgdorferi* to the majority of young ticks is the white-footed mouse, *Peromyscus leucopus* [17]. White-tailed deer (*Odocoileus virginianus*) play a major role in the reproductive cycle of ticks as the preferred host for adult ticks and pregnant female ticks [16], and although deer do not pass the *B. burgdorferi* bacterium to ticks, they increase the geographic range of ticks by transporting them across distances. Thus, the relative population abundance and preferred habitat environments of ticks, white-footed mice, and white-tailed deer heavily influence where Lyme disease will occur.

One way in which researchers identify areas of high Lyme disease risk is by determining habitat areas where the Lyme vectors, hosts, disease reservoirs and humans are most likely to

interact. Much research has examined the residential landscape as the point of contact between the tick vectors and hosts. Several studies [e.g., Dister et al. [8] and Frank et al. [18]] have examined environmental data at different residential sites to determine which variables may encourage higher tick densities. The density of ticks present on a property could affect the likelihood of human-tick interaction and thus, Lyme disease incidence. Both studies concluded that a lawn was negatively correlated to tick density while wooded land was positively correlated to tick density. This finding indicated that higher proportions of forest in residential areas may lead to greater Lyme disease risk. In addition to wooded landscapes, population density was another factor taken into account. Cromley et al. [9] looked at residential settings and found that people living in high and medium density zones were less likely to contract Lyme disease than those living in low density developments. Thus it appeared that low density residential zones with at least partially forested lots are at greater risk for Lyme incidence.

Researchers have also questioned whether or not the size of forest fragments around homes may impact disease risk. The significance of forest fragments was brought to light through initial research by Richard Yahner [6] who looked at the relative abundance of white-footed mice in clearcut environments. He found that the relative abundance of white-footed mice as compared to other small mammals increased as the size of forest fragments decreased. Yahner's research led Allan et al. [19] to theorize that increased densities of white-footed mice, the most competent reservoirs for Lyme disease, would lead to higher nymphal tick infection rates. As hypothesized, the final results of Allan's research illustrated that smaller forest fragments (<2 ha) had a higher density of *B. burgdorferi* infected nymphal ticks than larger forest fragments (> 2ha). Brownstein et al. [11] yielded similar results, finding that small forest fragments were positively correlated to nymphal tick infection rates. However, in Brownstein et

al.'s study, small forest fragments were negatively correlated with human disease incidence rates, indicating that some other variable could be influencing the rate of human disease incidence.

A related study by Jackson et al. [10] examined trends of forest fragmentation as well as ecotone or land cover type transition zones, linking these patterns to Lyme disease incidence within a 12 county area in Maryland. They found that human Lyme disease infections were positively correlated with an index of forested-herbaceous land edge contrast, percentage forest cover, and income. The study also found a positive correlation between a greater number of small forest fragments (< 2ha) and higher incidence of Lyme disease. All these study results combined seem to indicate a general trend in the predictive value of environmental variables at the local, peridomestic level, as well as a broad scale state level. The variables investigated in these research projects formed the basis of this research in the state of Virginia. The key difference in this research project is that this research was done to identify factors that correlate with emergent Lyme disease rather than endemic Lyme disease.

It is recognized that many factors other than land cover pattern are relevant to the study of *B. burgdorferi* infection rates. These factors include acorn abundance [20, 21] and climate variations including rainfall and temperature as well as humidity [22]. However, land-cover variables were the primary factors investigated in this study due to the broad scale of analysis and the success of previous studies which indicated that land cover variables were significantly correlated with Lyme disease incidence across both small and large study areas.

3.3 STUDY AREA

Virginia was chosen as the study area because Lyme disease has emerged and increased across the state, particularly over the past decade. Shortly after statewide surveillance for Lyme disease began in 1982, isolated Lyme disease cases were discovered in the northern part of the state, around the Washington, D.C. suburbs and on the Delmarva Peninsula. Since that time the disease has spread southward into suburban and urban centers connected by major highway routes such as I-81, I-64, and I-95. It is now dispersing outwards from those concentrated zones, as shown in Figure 3.2. While Virginia is not an entity unto itself and represents a small part of a larger disease expansion, state lines provide useful boundaries for the division of the study area, and the quintupling of cases between 2004 and 2011 along with landscape characteristics make it a useful model in which to study Lyme disease's emergence.

Virginia is 39,490 square miles in area and is home to more than 8 million people [23]. The majority of the population is concentrated in what is known as the "urban crescent" which includes a somewhat contiguous urbanized area connecting northern Virginia around Washington, D.C., Fredericksburg, Richmond, and Hampton Roads (Figure 3.3) [24]. The urban crescent, which tends to have higher incomes, is projected to see the greatest population change from 2010 to 2035, accounting for 76% of the population change forecasted for those years within Virginia [24]. Outside of these urban centers, the state is much less populated with lower incomes. These demographic trends and areas of population growth could be important to the study of Lyme disease, especially considering landscape risk factors. Population growth tends to yield suburbs and low density home development surrounding urbanized population centers, and this type of development could be a factor in the amplification of Lyme disease in the state.

3.4 DATA

This research was retrospective in nature, employing secondary data including Lyme disease cases, environmental data, and human demographic data. The latitudes and longitudes of residences for known Lyme disease cases for 2006 to 2010 (n=4377) were obtained from the Virginia Department of Health (VDH). We chose a five year time period for the study, also used by Jackson et al. [10], because the most recent land cover data available was from 2006, and the demographic data were collected in 2010.

The VDH receives the Lyme disease case data from healthcare providers with the home addresses of patients diagnosed with Lyme disease and converts these addresses into latitude/longitude to provide an added layer of protection and anonymity to the human case data. Cases are reported by physicians to the state Department of Health using the National Notifiable Diseases Surveillance Definitions. According to these definitions, from 1996 to 2008 cases could be confirmed by the presence of an *Erythema migrans* (EM) rash alone or through manifestation of one late-stage symptom with laboratory confirmation [25]. After 2008, cases could be confirmed by known exposure or the appearance of an EM rash only in an already endemic county or by a positive laboratory test [26].

The change in national reporting requirements would have most likely reduced the number of false positives reported after 2008 due to the emphasis on laboratory testing or diagnosis in already endemic counties. To take into account these reporting changes, we screened the case data. Those tracts that had cases reported before 2008 but none in the subsequent years were identified and the cases were considered to be probable misdiagnoses. These 44 cases were removed from the dataset due to the questionable nature of their occurrence, leaving 4,333 cases for the analysis.

Land cover data for Virginia for 2006 were acquired from the Multi-Resolution Land Cover Consortium [27]. National Land Cover Database (NLCD) data, with a spatial resolution of 30m, were aggregated into the major categories of water/wetlands, developed land, forest, and herbaceous/pasture land to simplify land cover distinctions (Laura Jackson, personal communication). The NLCD numbers assigned to each category are as follows: (1) Water/Wetlands: 0, 11, 12; (2) Developed: 22, 23, 24, 31; (3) Forest: 41, 42, 43, 90; (4): Herbaceous/Pasture: 21, 52, 71, 81, 82, 95. The water classification included all open bodies of water. Developed classes included low, medium, and high density developed zones as well as barren areas with no vegetation. Forest included deciduous, evergreen and mixed forest as well as woody wetlands. Herbaceous included both herbaceous and herbaceous grasslands as well as cultivated agricultural lands, developed open space (<20% impervious surface), and herbaceous wetlands.

In addition to land-cover factors, demographic data were included in the study to attempt to capture demographic variations across the study region. Average age, population density, and mean income for each census tract were obtained from the U.S. Census Bureau for 2010. The demographic data were used to characterize the underlying human population potentially at risk for Lyme disease and also to standardize case data within census tracts.

3.5 METHODS

The case data, land cover data, and human demographics data collected were aggregated into census tracts, which served as the units of analysis for this study. Census tracts are a more attractive way than zip codes or other arbitrary units of analysis to represent the proximal environment to Lyme disease cases because population data is available within tracts and because the tract borders are typically based upon features that to some degree may act as

potential barriers to deer and tick movement (rivers, roads, and other possible barriers). Jackson [10] successfully used polygons bounded by rivers or roads, comparable to census tract boundaries, to study forest edge or fragmented forest habitats in relation to Lyme incidence. Virginia's 1,907 census tracts, delineated in 2010, were used for this analysis.

Incidence was standardized by population for each Virginia census tract using yearly population estimates calculated through regression modeling of 2000 and 2010 census data. The number of cases present inside each tract was then divided by the total population estimate for the tract and multiplied by 1,000 people to calculate incidence per 1,000 people.

The four categories of NLCD data described in the data section were analyzed within census tract boundaries as well. Using ArcGIS software [28], the pixels of each land cover category were counted within each census tract. The number of raster pixels devoted to water, developed land, forest, and herbaceous land inside each tract was divided by the total number of pixels inside each tract to determine the percentage of the tract devoted to each land cover type.

The interspersion and mixture of different land cover types within the census tracts were determined using FRAGSTATS 4.1 [29]. This analysis allowed us to examine the hypothesis that certain types of land cover mixtures enhance the probability for disease host, deer, and human interaction. For example, the mix of herbaceous with more densely populated residential zones could encourage more deer foraging and infringement on individual's backyards. This hypothesis was tested using two variables calculated using the 2006 NLCD data in FRAGSTATS 4.1: Contrast-Weighted Edge Density (CWED) and Total Edge Contrast Index (TECI). To calculate CWED and TECI within each census tract, a weight matrix was formed to calculate intersections between herbaceous, developed land, and forested land. The three landscape intersections were weighted individually; for example forest-developed land

intersections were weighted, then developed-herbaceous, and then herbaceous-forest. TECI is a percentage computed by summing the edge lengths of the weighted landscape types divided by the total landscape edge length of all categories in the tract. It is essentially the percentage of the edge within a census tract that is made up of the two weighted intersecting land edges. Meanwhile, CWED is the sum of the weighted edge lengths divided by total landscape area (m^2), multiplied by 10,000 to convert to hectares. It is more of a measurement of the area of the edge, or the edge density, between the two intersecting land types within each census tract.

ArcGIS [28] was used to generate data on forest fragmentation within each tract from the 2006 NLCD data, specifically the number, frequency, size, and distribution of forest fragments within each census tract. The raster file of the forested layer was converted into a polygon shape file using the ArcGIS toolbox. We then isolated only those forested pieces that were less than or equal to 2 hectares (ha) in area. This forest size was demonstrated in previous research to have higher proportions of white-footed mice, greater risk of tick infection with *B. burgdorferi,* and thus enhanced risk for Lyme disease [10, 19, 30, 31]. This exercise generated three measures of forest fragmentation: the number of forested fragments less than 2ha inside each census tract, the percentage of the census tract covered by small fragmented forests (which is the total area of forested fragments divided by the tract area), and the total perimeter of forested fragments present inside each tract. These related measures help clarify the characteristics of forested land within each census tract. Those tracts with smaller, more numerous forests were thought to be associated with higher deer and mouse overlap, potentially leading to Lyme disease hot spots.

Several of the variables included in our model were highly correlated to each other, and therefore multicollinearity was present in the model. Multicollinearity could confound the results making them difficult to interpret. To resolve this issue, we used principal components

analysis (PCA) to transform the original correlated variables into a set of new linearly uncorrelated variables, or components. In order to do this, we first had to center (subtract the mean) and standardize (divide by the standard deviation) each variable, before running the PCA based on the standardized variables.

A statistical model using spatial Poisson regression analysis was built using the 12 environmental and land cover variables generated from the 2006 NLCD map, and the 3 demographic variables as the independent variables (Table 3.1). Cases (n=4333) from the five year time period from 2006 to 2010 were incorporated into the model as the dependent variable. Once the principal components analysis (PCA) was performed, the environmental and census variables were well-represented by the first eight components created using PCA, which explained 95% of the variability in the original variables. We proceeded with the spatial regression model using these 8 components. The parameters of the model were estimated using the Bayesian approach through Markov chain Monte Carlo (MCMC).

In the spatial Poisson regression analysis, counts y_i was modeled by a Poisson distribution, Poisson ($P_i\lambda_i$), with rate $P_i\lambda_i$, where P_i is the population of tract i and where λ_i is the population adjusted rate. Here, λ_i is modeled as

$$\log(\lambda_i) = \beta_0 + \beta_1 X_1 + \ldots + \beta_{15} X_{i,15} + \varepsilon_j$$

The variable ε_j is the spatially correlated random effect which accounts for the spatial correlation of case emergence. The statistical distribution of this random variable is specified using the conditional autoregressive model (CAR). In particular, it is described by the following equation:

$$\varepsilon_j | \varepsilon_{\_i} \sim N(\overline{\varepsilon}_{\_i}, \sigma^2/n_i)$$

The symbol $\varepsilon_{\_i}$ describes the adjacent neighbors to ε_j which were found by generating a spatial weights matrix of contiguity edges for the census tracts using the spatial statistics toolbox in

ArcGIS. The neighborhood determined for a tract using the spatial weights matrix were those tracts directly on the bordering edges of the tract, sharing a boundary line. After the regression model was run, the regression coefficients of each of the 8 PCA components were transformed into the regression coefficients of the original 15 variables using a reverse approach to the one used to create the linear components in the PCA analysis.

3.6 RESULTS AND DISCUSSION

This research study found significant correlations between land cover and demographic variables, and Lyme disease cases across the state. The results of the regression analysis showed that 10 of the original 15 variables had a statistically significant (α=0.05) relationship with Lyme disease incidence (Table 3.2 and Figure 3.4). The significant variables included percent water, percent developed, percent herbaceous, the TECI and CWED of forest-herbaceous edge, the TECI and CWED of herbaceous-developed edge, population density, age, and income. The variables which were not significantly correlated included percent forest, percent fragmented forest, fragment perimeter density, and the CWED and TECI of developed-forest edge.

The percent land cover inside each census tract was analyzed using four broad categories: developed, forest, water and herbaceous. Percent developed was a significant variable and had a negative correlation with Lyme disease incidence (Figure 3.4A). This result is not surprising as previous research suggested that areas with higher population densities, inside of cities, had a lower risk for Lyme disease incidence [9]. Heavily urbanized settings also typically have less potential habitat for vectors to interact with people. However, geographically Lyme disease does tend to concentrate around the edges of more populated areas, especially in the suburbs of D.C., indicating that more research may need to be done on this variable for it to be truly explanatory. Percent water was a positively correlated variable (Figure 3.4B). However, the positive

correlation to Lyme disease incidence could be misleading. The areas of the state that have the highest percentages of water occur in the northeast where the Chesapeake Bay separates the Delmarva Peninsula from the rest of the state. Census tracts in these areas extend out into the bay and thus have misleadingly high proportions of water. These areas in the state saw high case rates but it was most likely due to the proximity to the initial surge of Lyme into the Virginia from its more northern neighboring states rather than an actual correlation with the presence of water. Percent herbaceous was also positively correlated with Lyme disease incidence (Figure 3.4C). Herbaceous land, indicating a variety of land covers including residential lawns, parks, agricultural fields, etc., could provide good foraging ground for deer and mice, especially in the proximity of small forested patches which could provide cover for the foraging animals. Humans that live or work in closer proximity to these foraging animals and thus to the ticks that drop off of them could quite possibly be at greater risk for contracting Lyme disease. Percent forest was, surprisingly, not a significant variable. This is contrary to the results seen by Jackson et al. who found percent forest to be an important variable in Lyme disease incidence prediction [10]. However, in the case of Virginia this lack of a correlation could be due to the fact that portions of the state, especially those in more rural areas along the Blue Ridge Mountains, have large swathes of non-fragmented forests. It could be that forests themselves do not pose a risk but rather disturbed and fragmented forests near human habitation. The large swathes of undisturbed forests may have balanced out any correlation small fragmented forests may have had with Lyme disease incidence.

The indices that allowed us to examine the interspersion or intersections of two different land cover types were Contrast Weighted Edge Density (CWED) and Total Edge Contrast Index (TECI). The two indices are related so it is not surprising that the TECI and CWED for each

landscape intersection were the same in terms of significance. The TECI/CWED of developed-forest edge was not significant. This result is expected as developed-forest edge has not been shown to be significant in any of the previous studies. However, the other two edges that include herbaceous land, herbaceous-developed and herbaceous-forest, were significant variables. The interspersion of developed land with herbaceous land (Figures 3.3D and 3.3E) had a negative correlation with case rate while the interspersion of herbaceous and forested land (Figures 3.3F and 3.3G) had a positive relationship. In tracts where herbaceous land is adjacent to developed land, incidence is low, while tracts with herbaceous-forest boundaries had high incidence. The positive correlation of herbaceous-forest edge contrast was expected and it supports the results of Jackson et al. (2006). This type of environment with a mixture of herbaceous or open herbaceous land and forested land is favorable for deer, as discussed earlier, and it indicates that low density residential or suburban areas could be at a higher risk for Lyme disease. Herbaceous-developed land intersection was negatively correlated with Lyme disease incidence. Though little research has been done on this variable, it could be that developed edge with no forest cover does not provide the right environment for the interaction of ticks, deer, mice, and human hosts.

The results considering forest fragmentation variables were quite surprising. Unlike the Jackson et al. study [10] and the study on forest fragmentation by Allan et al. [19] our results indicated that neither the percent of the tract covered by small forest fragments (<2 ha) nor the combined perimeter of the small forest fragments (<2 ha) within the tracts were significant. These variables were meant to indicate whether or not the presence small forest fragments, a habitat where white-footed mice out-compete other small rodents, were more likely to lead to human Lyme infection. Though unexpected, this finding supports the Brownstein [11] study

which found that although forest fragmentation does indicate increased rates of tick infection with *Borrelia burgdorferi,* it may not necessarily indicate higher rates of Lyme disease incidence in humans.

All three of the demographic variables included in the study were shown to be significantly related to Lyme disease incidence. Age was positively correlated with Lyme disease incidence, meaning that census tracts with an older population had higher incidence (Figure 3.4H). This result was expected due to the fact that Lyme disease tends to appear in specific age groups within the population. In general, incidence tends to be highest in children younger than 14 and adults older than 40 [16]. However, because the age range from 5-14 is much smaller than that of 40-70+, it is not surprising that the results were skewed to indicate that tracts with older age ranges were more positively correlated with Lyme disease incidence. It may be, in addition, that older individuals simply tend to prefer or are better able to afford residences in areas that are more associated with Lyme disease infection. Population density had a negative relationship with incidence (Figure 3.4I). Greater population density also tends to indicate more urban and developed environments with less habitat accessibility to ticks and to disease reservoirs. In addition, there is typically less human-environment interaction in very urban environments. However, as stated earlier with the percent developed variable, this variable could be misrepresentative. It is clearly not just very rural, unpopulated areas observing Lyme disease as seen in maps of case rates which illustrate Lyme disease cases concentrated just outside of heavily urbanized areas, in the suburbs or medium density developed areas. The last variable observed was average household income (Figure 3.4J). It was a significant variable with a positive correlation to Lyme disease incidence. Income was also found to be a significant indicator in the Jackson et al. [10] study. This result was surprising because income tends to be

highest in areas with moderate to high population densities around the edges of heavily urbanized areas, and population density was negatively correlated with incidence. However, counties in the Washington, D.C. suburbs in northern Virginia are among the wealthiest in the country, and Lyme disease incidence has been high in that region [32].

Limitations

There are several limitations to this study related to the NLCD data, the human case data, and the demographic variables. The land cover data used was at a 30m spatial resolution, limiting how fine or detailed our analysis could be, although the resolution was acceptable for our broad-scale analysis. In addition, the sub-categorization of the NLCD data into four larger categories made broad scale analysis much more efficient, but there is no indication as to what specific kind of herbaceous land is most associated with Lyme disease since herbaceous land includes several sub-categories of herbaceous cover and agricultural land. Given the importance of herbaceous cover indicated by our analyses, we suggest that future studies apply a greater focus on the specific type of herbaceous associated with an increased risk of Lyme disease. Whether it was a boundary of forest edge with pasture edge or forest edge with lawn edge, it is clear that herbaceous lands that border forested areas are at a higher risk. In addition to land cover spatial resolution, census tract sizes could be another factor with limitations in this study. The majority of the census tracts in the state are concentrated in the very urban, city centers. These are very small tracts with high development and high population concentrations. The remaining tracts throughout the state tend to be quite a bit larger, with much lower population concentrations due to the larger amount of area they encompass. The size of the tracts could

throw off the land cover variables because the two tract dichotomy may make it hard to capture intermediate zones or zones of land cover transition between very urban and very rural areas.

There are two main limitations associated with the Lyme disease case data: points that referred to a geocoded home addresses could be incorrect due to a geocoding error or infection could have occurred at an alternate location outside the home environment. Geocoding error is unavoidable, though it can be quantified using previous research by Cayo et al. [33]. According to Cayo's research, geocoded addresses are on average 30-40m off in urban areas and 50m off in rural areas. The greatest concern in our project was that cases would be incorrectly assigned to a census tract because of a geocoding error. To asses this, we determined how many cases were within 30m of another census tract. Only 5.6% of the total cases could have been incorrectly assigned and thus the effect of potential geocoding error is most likely quite low in our study. As to the alternate location for tick bite, it is very possible some people contracted Lyme disease outside of the residential environment. However, the home address remains a consistent reporting method as most people who contract Lyme are unaware of the location of tick attachment. In addition, the use of the home address has precedence as multiple studies of Lyme disease have successfully used residential location [7, 9]. An additional limitation in the data was due to the fact that cases are reported by the year diagnosed, not the year that the bacterium was actually contracted. In some instances, it can take much longer for a person to seek testing for Lyme disease, especially in areas where the disease has never been prevalent before [34]. Doctors may take longer to test for the disease and thus the time sequence of the disease events appearing across the state may have a slight lag in areas experiencing a new influx of the disease [34].

Future Research

In the future, this project could be expanded upon in several ways. Our next follow-up will use the results of this study as input data for a predictive map of disease emergence to encourage awareness and targeted surveillance in areas that may soon see higher rates of Lyme. This increase in awareness could shorten the length of time before treatment, lowering the physical and financial burden of the disease on individuals across the state. An additional follow-up will use higher resolution data in a small targeted study area to determine if certain landscape features or housing developments are associated with greater disease incidence. The data used in this project should be expanded upon to consider additional predictive variables for Lyme disease. Other environmental variables such as humidity and temperature as well as rainfall could be important indicators for Lyme disease emergence. Human variables such as patterns in behavioral practices including hobbies and occupation type could help elucidate why people in certain areas are contracting the disease at higher rates. In addition, since it is clear that herbaceous land is an important predictive variable, a follow-up looking at whether specific types of herbaceous, such as agricultural fields, open space in residential areas, or herbaceous pastures, are more highly correlated to Lyme disease incidence than others would be helpful.

3.7 CONCLUSION

Lyme disease in Virginia has become an increasing concern over the last several years as incidence within the state has continued to grow. Expansion of the disease has occurred spatially as well and the disease has disseminated from major population concentrations into other areas. The main objective of this project was to determine environmental and demographic factors correlated to increased Lyme disease incidence in a previously non-endemic area. Several of the

factors observed in this study were significantly related to Lyme disease incidence and thus could be facilitating the spread of the disease across the state.

We hypothesized that the percentage of land covered by certain land cover types and the interspersion of certain land cover types would have significant correlation to Lyme disease incidence. This hypothesis was not rejected due to sufficient evidence that higher percentages of herbaceous land and greater interspersion of herbaceous and forested land were both significantly positively correlated variables with increased Lyme disease incidence. Herbaceous land and developed land interspersion was negatively correlated with Lyme disease incidence. However, our second hypothesis, that higher amounts of small forest fragments inside census tracts would increase case rates in those census tracts was rejected. There was no statistical significance to support a relationship between higher amounts of forest fragmentation and Lyme disease incidence.

This research forms a starting point in Lyme disease emergence research in the state of Virginia and can be applied to other areas that are experiencing Lyme disease emergence. Furthermore, our findings support education efforts that may decrease diagnosis time through the increase in awareness, at both a state and individual levels. Although this research is only the beginning phase of predictive Lyme disease mapping, the Virginia Department of Health (VDH) can use the information presented to target areas of potential disease emergence, allocating more resources to educate and raise awareness. In addition, developers and planners may take this information into account when trying to protect communities from Lyme disease [10]. Low/medium density residential neighborhoods could be designed in such a way as to minimize herbaceous, as well as herbaceous-forest borders in the proximity of homes. Through

modifications such as these, city planners and developers may be able to aid in an overall reduction of Lyme disease risk over time.

Competing Interests

The authors do not have financial or non-financial competing interests by publishing this article

Authors' Contributions

SD generated most the geospatial data, performed most of the writing, and put together tables and figures. KK was the PI on the NSF grant and led most of the research coordination and design. She also aided in the analysis and writing process. YH and JL performed the statistical analysis and generated several of the tables and figures. They also formed part of the NSF proposal team. SP and JC checked data accuracy, aided in generation of the data and helped shape overall research objectives for project. They also formed part of the NSF proposal team. DG produced the Lyme disease case information from the Virginia Department of Health and assisted in overall research design with expert advice. RD assisted in technical aspects related to data generation and provided expert advice. All authors have read and approved the final manuscript.

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U.S. Lyme Disease Cases
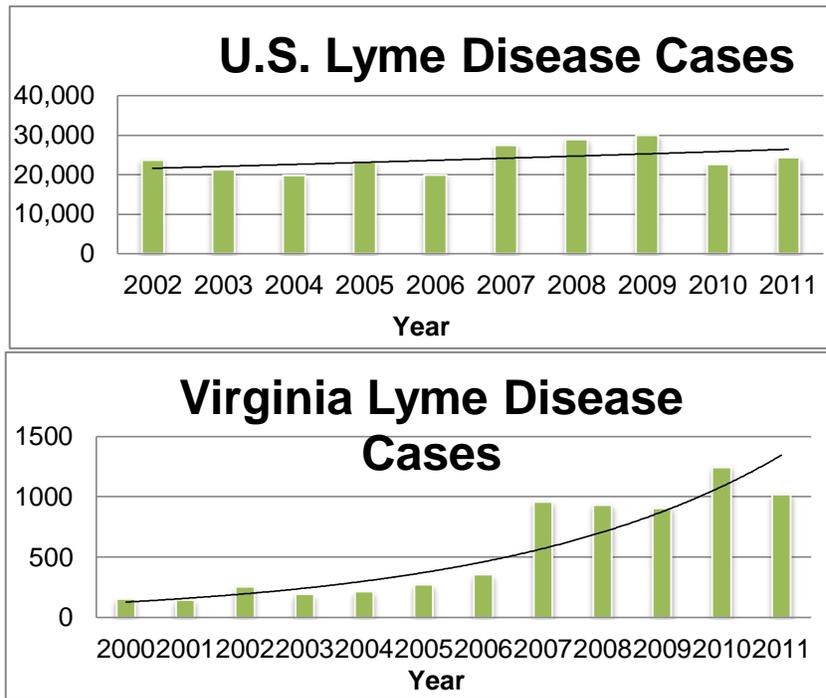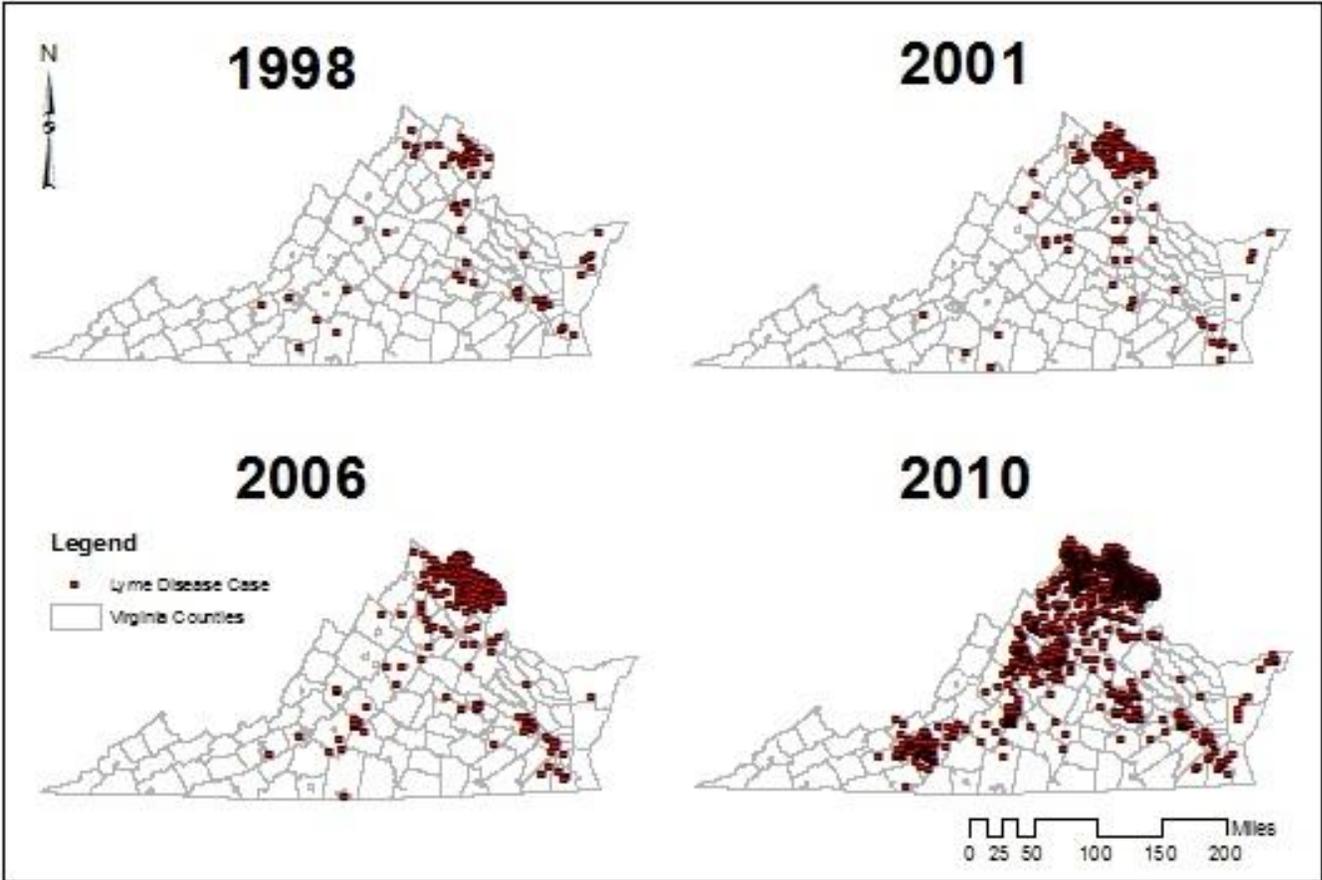

Virginia Lyme Disease Cases

Figure 3.1: Comparison of U.S. Lyme Disease Cases & Virginia Lyme Disease Cases

Sources: [2, 35]

Figure 3.2 Map of Virginia Lyme Disease Geographic Case Emergence
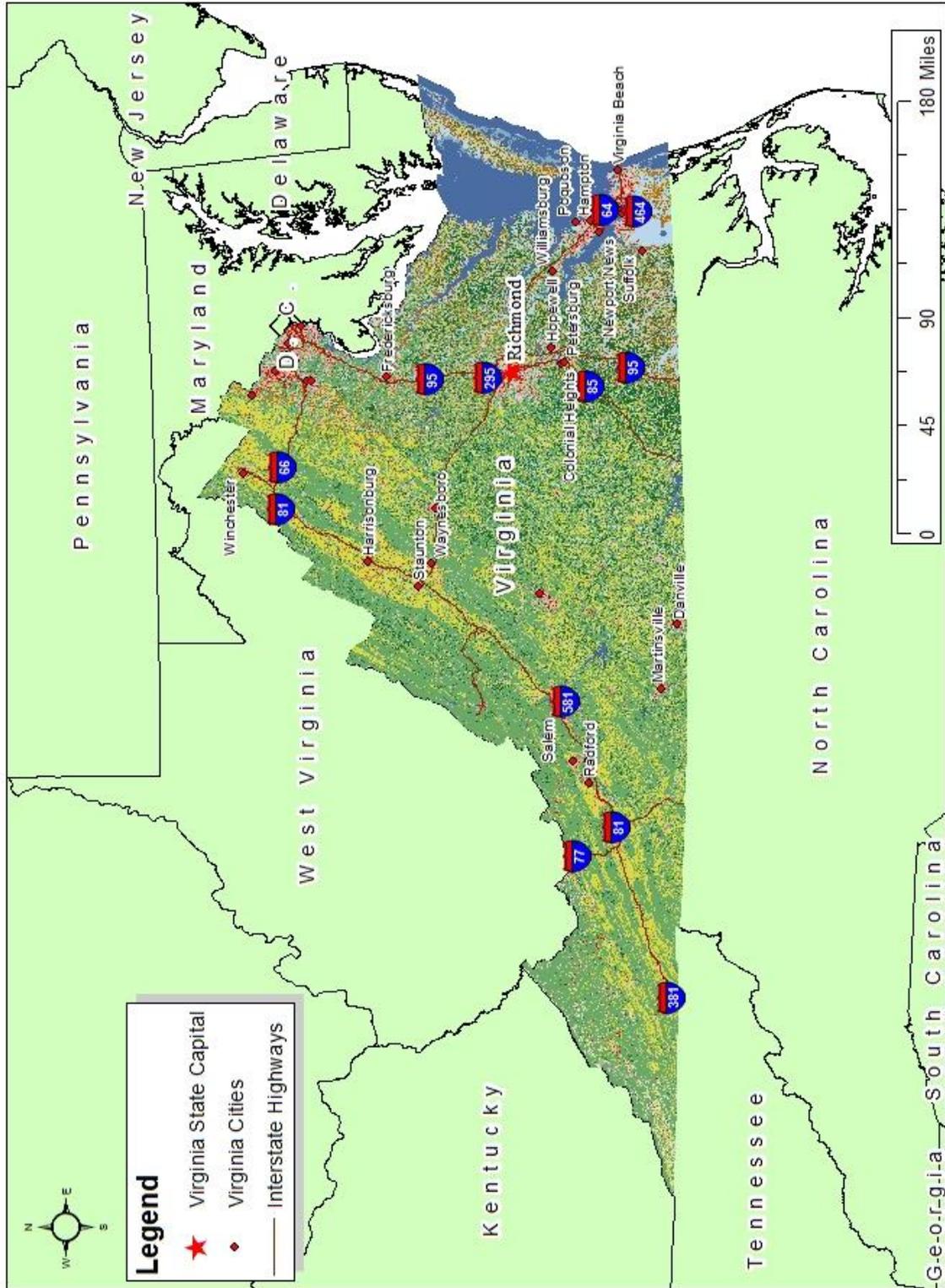Source: Virginia Department of Health
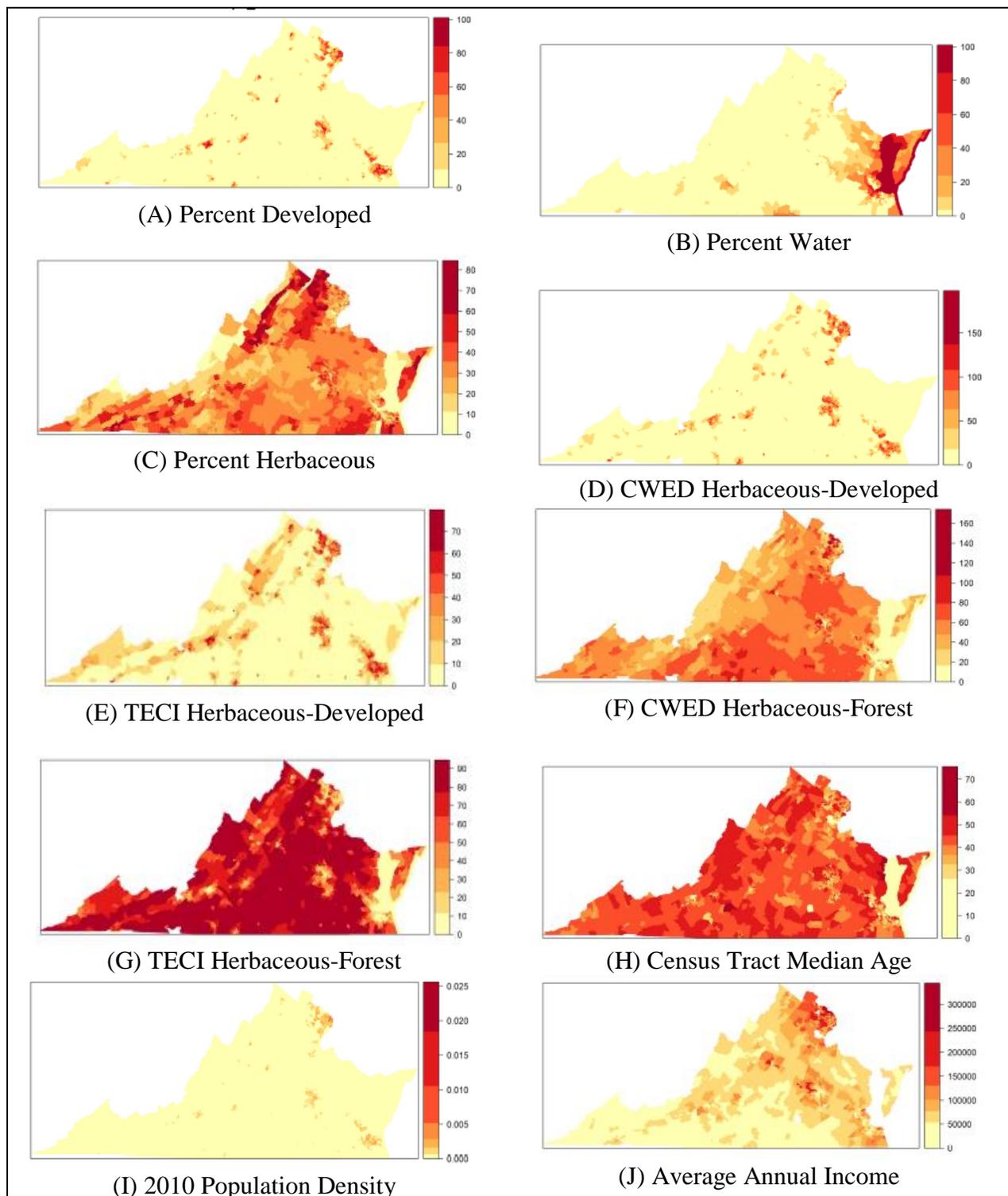
Figure 3.3 Reference Map of Virginia

Table 3.1 List of Variables Included in Spatial Poisson Regression Model

- Percentage of developed x_1, forest x_2, herbaceous x_3, water x_4 inside each tract
- Fragmented Forest area percentage x_5 (Sum of area of forested fragments (<2ha) inside each tract divided by the total tract area)
- Fragment perimeters density x_6 (Sum of forest fragment (<2ha) perimeters divided by the total tract area)
- CWED of developed-forest edge x_7 (Contrast Weighted Edge Density)
- TECI of developed-forest edge x_8 (Total Edge Contrast Index)
- CWED of forest-herbaceous edge x_9
- TECI of forest-herbaceous edge x_{10}
- CWED of herbaceous-developed edge x_{11}
- TECI of herbaceous-developed edge x_{12}
- Tract population density x_{13}, (The 2010 population per tract divided by the tract area)
- The median age at each tract x_{14}
- The mean income (inflation adjusted) at each tract x_{15}

Table 3.2 Estimated Regression Coefficients and Significance for Demographic and
Environmental Variables
(**bolded** and highlighted variables are statistically significant α=0.05)

Variable	Est.	SE	95% CI Lower	95% CI Upper
Pct Dvlpd	**-0.115**	**0.015**	**-0.144**	**-0.086**
Pct Forest	0.028	0.018	-0.007	0.064
Pct Herbaceous	**0.076**	**0.032**	**0.017**	**0.137**
Pct. Water	**0.137**	**0.040**	**0.053**	**0.211**
Pct Frag	-0.034	0.020	-0.073	0.006
Frag. Perim Den	-0.028	0.019	-0.066	0.010
CWED DF	0.014	0.020	-0.025	0.055
TECI DF	0.025	0.024	-0.21	0.071
CWED FS	**0.048**	**0.013**	**0.025**	**0.073**
TECI FS	**0.053**	**0.010**	**0.033**	**0.072**
CWED SD	**-0.048**	**0.016**	**-0.078**	**-0.015**
TECI SD	**-0.047**	**0.017**	**-0.079**	**-0.013**
Pop Den	**-0.086**	**0.037**	**-0.161**	**-0.017**
Median Age	**0.143**	**0.044**	**0.055**	**0.229**
Mean Income	**0.184**	**0.039**	**0.108**	**0.260**

(A) Percent Developed

(B) Percent Water

(C) Percent Herbaceous

(D) CWED Herbaceous-Developed

(E) TECI Herbaceous-Developed

(F) CWED Herbaceous-Forest

(G) TECI Herbaceous-Forest

(H) Census Tract Median Age

(I) 2010 Population Density

(J) Average Annual Income

Figure 3.4: Geographic Distribution of Significant Variables