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Lyme disease (LD) has become the most prevalent vector-borne disease in the United States and the sixth Nationally Notifiable disease. Surveillance of Lyme disease from the 1992-2016 has shown a sustained documented expansion of LD moving south into the border of Virginia and North Carolina, west into West Virginia, Tennessee, northwest into North Dakota, and North into Canada. This expansion of LD seems to be associated with expansion of the disease vector *Ixodes scapularis*, with newly established populations in the southwestern Appalachian and Piedmont regions of Virginia. The goal of the study was to characterize the entomological risk of the spread of LD from VA into NC. To determine the distribution and infection prevalence of *I. scapularis* along a northeastern-to-southwestern gradient from VA to NC, tick-flagging and hunter-harvested deer tick collecting approaches were used with samples tested by the CDC for infection. Flagging was comprised of periodic sampling sessions from October 2015 to July 2017, conducted at Fairy Stone, Mayo River, Hanging Rock, Pilot Mountain, Yadkin Island Park, and Lake Norman State Parks. Hunted deer processing stations Hilltop Farms (Walnut Cove, NC) and Game Butchers (Troutman, NC), were used for collecting ticks from hunter-harvested deer covering counties for the northern, central and southern North Carolina Piedmont regions.

Ticks collected by flagging were suggestive of a north-to-south trend with no significant difference among the northernmost State Parks and a significant difference in

abundance between the northern and southernmost State Parks. The highest number of *I. scapularis* ticks (0.7 per 100m) was collected from the north-most Virginia's Fairy Stone and Hanging Rock State Parks, but no *I. scapularis* were collected from the southernmost Lake Norman location. Infection prevalence of ticks collected by flagging exhibited a general north-to-south declining trend. Though not statistically significant with highest infection rate approximately 25% at the north-most Fairy Stone State Park. For deer collected ticks, there was a significant north-to-south decrease in tick burden per deer, with the northern region located on the VA-NC border having the highest number of *I. scapularis* (6.0 per deer), followed by the central and the southern regions of NC. Infection prevalence of sampled ticks from deer are suggestive of a declining trend although not significant, with the northern region having the highest (17%), followed by the central region (11%), and no infection present in the southern region. *Ixodes scapularis* results collected from flagging, and hunter-harvested deer are highly suggestive of a north-to-south gradient in *I. scapularis* densities with Alexander and Iredell being the south-most *I. scapularis* positive counties. *Borrelia burgdorferi* infection results also suggest a north-to-south distribution, with *B. burgdorferi* appearing to have only made it as far south as the central counties of Yadkin and Forsyth. Entomological risk estimates for density of infected nymphs (DIN) and adults (DIA) of flagging and hunted deer also showed a north-to-south trend with Fairy Stone State Park having the highest (0.033) DIN and northern NC region having the highest (0.808) DIA. The results are consistent with first the spread of the vector followed by the pathogen.

Keywords: *Ixodes scapularis*, ticks, Lyme Disease, *Borrelia*, blacklegged tick

ASSESSMENT OF ENTOMOLOGICAL RISK FOR LYME BORRELIOSIS

ALONG A NORTH-TO-SOUTH GRADIENT

FROM SOUTHERN VIRGINIA

INTO NORTH CAROLINA

by

Jimmie Lee Teague III

A Thesis Submitted to
the Faculty of The Graduate School at
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Approved by

____
Committee Chair

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I dedicate this thesis to the Lord for whom all strength and knowledge to complete this work comes from. I also dedicate this to my wife Kaylie who has supported and loved me throughout this journey, and to our new daughter Autumn.

APPROVAL PAGE

This thesis written by Jimmie Lee Teague III has been approved by the following committee of the Faculty of the Graduate School at the University of North Carolina at Greensboro.

Committee Chair ______________________

Committee Members ______________________

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Date of Acceptance by Committee

____
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CHAPTER I

INTRODUCTION

Background

Infectious disease epidemiology

An increasing trend of infectious disease emergence and resurgence has been observed globally during the past 70 years[1]. Among these emerging infectious diseases (EID) zoonoses (a disease which has a vertebrate, non-human, animal source) and vector-borne diseases comprise approximately 83% of them[1; 2]. This newly recognized group of infectious diseases necessitates the application of an ecological approach providing new theory, methodology, and analytic tools to understand better their structure and function[3; 4]. Anthropogenic environmental changes (e.g., climate change, land-use change), drug or pesticide resistance, as well as international travel and commerce have been identified as critical drivers of disease emergence[5]. For a pathogen to successfully spread in a host population the ecological niches of the pathogen, host, and (sometimes) vector must overlap within a permissive environment[6]. Hence, changes to any of the components of this epidemiologic triad (Fig. 1.0): pathogen, host, vector, or the environmental could potentially turn an endemic disease into an epidemic or even a pandemic[7].

Lyme disease

The first documented case of Lyme Disease (LD) dates back to a 5300-year-old human that was recovered from a frozen glacier in the Italian Alps[8; 9]. Though it is clear that *Borrelia burgdorferi* was pre-existing, it was not discovered in the US until the 1970's in Lyme Connecticut during an investigation into an unexpected number of juvenile rheumatoid arthritis cases. The outbreak was found to be caused by a *Borrelia* bacterium, later named after the medical entomologist Dr. Willy Burgdorfer[10; 11]. This late manifestation of Lyme was identified as a multisystem disease transmitted by the tick vector *Ixodes dammini* (later reclassified as *Ixodes scapularis*)[12]. Further investigation into LD discovered that the physical manifestation of the signs and symptoms of LD had been medically documented in the US and Europe before the Connecticut outbreak, but these episodes were poorly understood and were often misdiagnosed[13]. Further DNA testing for *B. burgdorferi* in museum tick and mice specimens from the late 19th and 20th century confirmed the presence of the bacterium before the widely-publicized Connecticut incident[14; 15].

The pathogen

Lyme Disease is caused by a spirochete bacteria, comprised of inner and outer membranes, with a distinct morphology consisting of a spiral, wavelike body, and flagella [16; 17]. The *Borrelia* genus comprises at least 18 genospecies, of which *B. burgdorfei* sensu lato (*B. burgdorferi, B. garinii, B. afzelii*) are responsible for most human LD cases worldwide [18]. In the US, however, it is only *B. burgdorferi* sensu strictu that is known to be the etiological agent responsible for LD[16]. *Borrelia burgdorferi's* life

cycle consists of an arthropod vector and mammalian, reptilian, or avian host[18]. The gene expression of a variety of lipoproteins produced by *B. burgdorferi,* such as OspC and OspA, enables the bacteria to establish infection in a range of hosts (lizards, birds, humans, small and large mammals)[19-21]. Lyme disease is known as an inflammatory infection that targets areas rich in collagen, like the musculoskeletal, cardiac, and nervous systems[8]. The most notable clinical manifestation of LD is the Erythema multiforme (EM) rash commonly called the "bullseye rash" which occurs at the initial infection site[8]. *Borrelia burgdorferi's* ability to evade a host immune response is attributed to the pathogens ability to coat antigenic components and outer surface proteins with plasmin from the host[8]. This subversion tactic allows the bacteria time for replication and phenotypic changes, delaying the manifestation of symptoms and making a diagnosis of LD difficult[8; 21]. The difficulty in LD diagnosis also leads to longer periods of infection and gross underrepresentation of the actual number of reported cases per year[22].

The vector - Ixodes scapularis

Ticks belong to the class Arachnida, subclass Acari, order Parasitiformes. The tick *I. scapularis,* commonly known as the blacklegged tick, is a member of the Ixodidae (hard-bodied) family. The developmental stages of *I. scapularis* consist of egg, larvae, nymph, and adult (Fig. 1.1)[23]. Blood meals are needed for molting into each developmental stage and for reproduction. *I. scapularis* exhibits a complex three-host life cycle that, typically, spans over a two-year period (Fig. 1.1)[24]. The life cycle of *Ixodes* spp. under field conditions, is regulated by two main dormancy mechanisms[25]. Taken together these dormancy mechanisms reduces the exposure of *Ixodes* spp. to unfavorable

environmental conditions (dehydration, freezing), while synchronizing host-seeking and development processes with the seasons[25]. These dormancy mechanisms are classified as either a type of developmental diapause or a behavioral diapause[25]. Developmental diapause relates to delays or halting of the development process of engorged ticks (larvae, nymphs), oviposition, and development of eggs through hormonal controls in response to adverse environmental conditions[25; 26]. Behavioral diapause (overwintering) involves the suppression of host-seeking (questing), and attachment activities of unfed ticks, during periods of hazardous environmental conditions[25; 26]. The life cycle of *I. scapularis* begins in the late fall and early winter with the eggs being laid by engorged female ticks in ground leaf litter, for protection against environmental conditions during development[27]. Upon hatching in mid-spring (Fig. 1.1), larvae will then begin host-seeking (host 1: small mammals, lizards, birds) by questing near leaf litter and remaining active during the summer and fall months (year 1). This stage is typically when the pathogen is acquired. After blood-feeding the larval tick will drop off and enter developmental diapause, and then molt into nymphs during late fall or early spring of the subsequent year. The nymphs emerge in early spring (year 2) and begin host-seeking (host 2: small to medium-sized mammals) remaining active through the summer months into the early fall (Fig.1.1). *Ixodes scapularis* is a potential carrier or co-carrier of 7 different human pathogens: *Borrelia burgdorferi* (Lyme Disease), *Borrelia mayonii* (Lyme Disease), *Borrelia miyamotoi* (Tick-borne Relapsing Fever), *Anaplasma phagocytophilum* (Human Granulocytic Anaplasmosis), *Babesia microti* (Babesiosis), *Ehrlichia muris*-like (Ehrlichiosis), and Powassan virus (Lineage 2 POW)[28; 29]. It is during the nymphs period

of activity that the majority of *B. burgdorferi* transmission to humans occurs and why nymphal density is considered the key epidemiological risk factor for LD[30]. Following blood-feeding, nymphs will drop off and molt into adults (Fig. 1.1) during fall of the second year. The adults (year 2) remain active through late-fall/early winter months questing for their third and final host typically large-sized mammals. Blood-engorged females will lay approximately 2000 eggs and then die[27]. This type of cycle results in a tick spending a majority of its lifespan off-host, either seeking blood meals, molting to the next developmental stage or diapausing[31]. During the ticks attachment phase, a questing tick attaches to the host and will begin actively seeking a favorable location for feeding[27]. The tick will then probe the selected area of the host using its mouthparts before insertion[27]. *Ixodes scapularis* mouthparts consist of palps and a barbed structure referred to as the hypostome[23]. The attachment of the tick begins with the insertion of the hypostome into the dermis of the host[32]. The tick will then secrete a cement-like substance that ensures firm attachment and protection of the mouthparts against a hosts immune response[32]. The salivary secretion of *I. scapularis* during feeding prevents clotting, induces dilation of capillaries, and possesses immunosuppressing characteristics[23; 33]. During the slow and fast feeding phase of ingestion, the tick will concentrate the blood meal by removing the excess water, allowing for increased blood intake during engorgement[23; 27]. The amount of time required for the tick vector to successfully feed on is 3 to 5 days for the larvae and nymph stages and 5 to 10 days for the adult stage[21; 23]. This slow feeding process has been associated with the ticks' need to produce cuticle during feeding in accommodation for the increased blood volume[23].

Transmission cycle

Borrelia burgdorferi's expression of OspC enables the pathogen to invade the tick's salivary glands, thereby capitalizing on the host suppressed immune system during tick feeding[34]. This increases the bacteria's transmission efficiency from vector to host. The pathogen is maintained in the enzootic cycle by reservoir hosts[35]. A reservoir host is a susceptible host that allows the pathogen to survive and multiply during times of vector inactivity and permits transmission to another susceptible host and maintenance of the pathogen in the system during the non-transmission periods[36; 37]. Transmission of *B. burgdorferi* can also occur via a non-systemic tick-to-tick pathway referred to as co-feeding transmission[38]. This non-systemic form of transmission of *B. burgdorferi* along this tick-to-tick pathway is facilitated by the fact that ticks exhibit a high degree of aggregated distribution on and off hosts[39; 40]. Co-feeding transmission occurs instantaneously through the ingestion of the pathogen, via the saliva from an infected tick feeding alongside an uninfected tick[38; 39]. Transmission of *B. burgdorferi* through this non-systemic pathway allows for a higher rate of infection in immature ticks because it is not dependent on the level of infection or susceptibility of the host to the pathogen[39].

Hosts

Ixodes scapularis is considered a host generalist, feeding on more species in North America than any other tick[23; 34]. During larval and nymphal states, *I. scapularis* typically feeds on at least 52 different mammal species, 60 species of birds, and 8 reptile species [23]. Conversely, adults primarily feed on medium to large mammals with an inclination to white-tailed deer (*Odocoileus virginianus*)[23]. Tick larvae limit themselves

to the lower strata of leaf litter to minimize desiccation[27]. This behavior puts larvae in closer proximity to smaller mammals, like the white-footed mouse (*Peromyscus leucopus*), lizards, and ground feeding birds such as the American robin (*Turdus migratorius*). The nymphal stage can physically tolerate questing higher out of the lower strata than the larval stage and is why it is found on both smaller medium and sometimes larger hosts[41]. Adult ticks quest even higher up the vegetation becoming increasingly restricted to larger hosts as smaller mammals become out of range and no longer accessible[24]. Given that these are the main blood-host of adults, deer population densities are inextricably linked to tick densities[34]. The pathogen *B. burgdorferi* is also a host generalist, capable of surpassing the host's immune response for many types of mammals, birds, and reptiles[34]. Upon exposure to *B. burgdorferi*, a host is classified as either competent, less competent, or non-competent based on certain criteria (Table 1.0)[27]. These criteria cover the host's susceptibility to the infection when bitten, the ability of the pathogen to amplify and persist in the host, and the efficiency of the host at transmitting the infection back to feeding vectors[42]. Competent hosts that demonstrate those characteristics play a role in the propagation of the pathogen in the system[27]. Non-competent hosts like the white-tailed deer do not propagate the pathogen back to subsequent tick generations but are critical in the maintenance and amplification of vector populations[34]. Sometimes the pathogen is transmitted to an incidental host (dead end), like humans for LD that do not contribute to the maintenance of the pathogen[43]. The dilution effect hypothesis suggests that a relationship exists between host diversity and the transmission rate of a pathogen[11]. That for any given habitat the frequency of

competent hosts in relation to non-competent hosts in the landscape has an effect on human LD disease risk[44]. For example fragmented forested patches consisting of low-diversity host communities, with a relatively high frequency of competent hosts, such as the white-footed mouse will have a higher infection risk, while large forested patches with highly diverse biotic community will have a lower frequency of competent than non-competent hosts and therefore will have a lower infection risk[42]. The risk of infection to humans is directly related to the decrease in the host diversity caused by anthropogenic forest fragmention[45]

Expansion

Increased incidence

Biological and nonbiological factors, such as but not limited to increasing abundance and range expansion of wildlife, human encroachment, and creation of habitats that attract ticks and wildlife hosts, are associated with the increased incidence of LD[46]. Through these ecological changes, environmental conditions became more favorable for the tick and pathogens life cycle[39]. The face of the landscape began to change, with deforested areas and farms being gradually reverted back to wooded areas for parks and suburbs[13]. Habitat generalists like the white-footed mouse, as well as edge-liking species like the white-tailed deer, thrive in this newly fragmented forest mosaic landscape[45]. As a result, white-tailed deer populations have rebounded from overhunting, and white-footed mouse populations multiplied, creating an ideal niche setting for *B. burgdorferi* pathogen propogation[45]. Therefore, producing increased number of fragmented areas in the landscape accompanied with reduced biodiversity, which results

in high infection risk. These same type of small, high-risk, forests are those that are commonly being used for human outdoors recreation creating a “perfect storm” in terms of elevated LD exposure risk[27].

Geographical spread

After the initial detection of LD in the 1970’s, the disease was shown to be concentrated in the northeastern US mainly New England [47]. By the early 2000’s the number LD cases had increased, becoming concentrated along the East Coast of northern New England and northern Virginia, with the remainder of the cases in Minnesota and Wisconsin[22; 48]. For southeastern states in the early 2000’s such as North Carolina, Tennessee, and South Carolina the number of LD cases reported on average was low, being only 92, 23, and 19 cases per year, respectively[22]. Since 2016 there are 30,000 reported cases of LD annually in the US alone, making it the highest vector-borne disease, and the sixth Nationally Notifiable disease in the country[22]. There has been a sustained documented expansion of LD moving south into the border of Virginia and North Carolina, west into West Virginia, Tennessee, northwest into North Dakota, and North into Canada[22; 48-50]. This southwestern expansion of LD seems to be associated with the expansion of the northern *I. scapularis* tick populations into areas of known low tick densities, as suggested from studies conducted by Herrin and Brinkerhoff[51; 52]. Recent spatiotemporal cluster analysis conducted by Lantos at the Duke Global Health Institute (Fig. 1.2) showed that the expansion of LD in VA was occurring more rapidly southwest along the Appalachians to the border of NC compared to other areas of VA[48]. Based on the results of the study Lantos did predicate that with the current trajectory of

LD, NC should anticipate growth in the number of LD cases, particularly in the Piedmont Mountain region[48].

"Who's first" – hypotheses regarding Lyme Disease expansion

With recent studies establishing the relationship between the spread of LD to expansion of the vector, the question remaining is whether the vector brings the pathogen with it or are there some other ecological mechanisms taking place? A study on the spread of LD and *I scapularis* to lower Michigan led to the development of three possible ecological ("tick-first," "dual-invasion," "spirochete-first") scenarios on the emergence of LD[46]. In the "tick-first" scenario *I. scapularis* ticks are able to establish uninfected populations in new areas via dispersal by white-tailed deer, a known incompetent host, and the bacteria, *B. burgdorferi* enters the system at a later time, as a result of a slower secondary invasions by the migration of small mammals and birds[46]. Under the second scenario, known as the "dual-invasion" scenario, competent mammalian or avian host distribute *I. scapularis* and *B. burgdorferi* concurrently establishing new populations of both the vector and the pathogen into new areas[46]. Under the third scenario, known as the "spirochete-first" scenario, established populations of the bacteria are already present in particular areas and are maintained enzootically by cryptic vectors that are wildlife host specialist (e.g., *Ixodes dentatus*)[46; 53]. Thus, *B. burgdorferi's* transmission cycle which is usually undetected in the area has no direct impact on human LD risk. As populations of *I. scapularis* spread into these established areas, amplification of pathogen transmission within the enzootic system to these newly introduced bridge vectors occur, creating bridging opportunities to humans and increasing the impact on LD risk to humans [46; 53].

The results of the Lower Michigan study found support for all three scenarios across the four different study sites, showing the complexity involved in the expansion of LD in the US[46]. Within the context of the recent apparent expansion of LD into NC, these three hypotheses will be used as a conceptual framework.

Study Goal

The epidemiological and entomological information from Virginia presented above indicates that LD has spread southwestward along the eastern Appalachian Piedmont foothills, with a current wavefront occurring along the VA-NC border. While the number of human reported LD cases in NC has increased during the last 7 years, no entomological information is available regarding the distribution and abundance of *I. scapularis* in NC[54]. As part of an active entomological surveillance effort supported by the North Carolina Department of Health and Human Services (DHHS), our general goal in this study is to characterize the entomological risk of LD spread from VA into NC. Such information can help identifying potential routes of LD invasion from VA to NC and inform subsequent public health policies such as medical and educational interventions[55].

Study Questions and Hypotheses

Question one

Does Lyme Disease spread from Virginia into North Carolina?

Hypothesis: I hypothesize that the increased number of Lyme Disease cases in North Carolina is driven by population expansion of the vector I. scapularis and the

pathogen *B. burgdorferi* from southwestern Virginia into northwestern North Carolina along the Appalachian eastern foothills.

Prediction: I predict to find a northeast-to-southwest gradient in tick abundance and *B. burgdorferi* infection prevalence.

Question two

What are the mechanisms driving this expansion?

Tick-first hypothesis: Uninfected *I. scapularis* ticks establish populations into new areas followed later by migration of the pathogen *B. burgdorferi*.

Prediction: Uninfected *I. scapularis* ticks detected at the southern edge of tick distribution, followed by detection of infected *I. scapularis* ticks in the northern region of distribution.

Dual invasion hypothesis: *I. scapularis* and *B. burgdorferi* concurrently establish populations into new areas.

Prediction: Infected I. scapularis ticks detected at the southern edge of tick distribution.

Specific Aim I

Determine the distribution, and relative abundance of, *I. scapularis*, using tick flagging and collections from hunter-harvested white-tailed deer.

Approach

Using tick flagging at NC and southern Virginia state parks and tick collection from hunted deer at deer check stations, I sampled ticks along a northeast-to-southwest transect from southern VA into NC along the Appalachian eastern foothills region.

Prediction

Using both collection methods, *I. scapularis* densities are expected to decrease in a north-to-south direction along the Appalachian foothills region.

Specific Aim II

Determine geographical *B. burgdorferi* infection patterns in *I. scapularis* ticks collected by tick flagging or from hunter-harvested white-tailed deer.

Approach

Ticks collected at Aim 1, were sent to the Centers for Disease Control and Prevention (CDC) for testing for *B. burgdorferi.*

Prediction

Given that tick infection rate should be positively correlated with the vector-to-host density ratio and that tick density is expected to decline along a north-to-south gradient (Aim 1), tick infection prevalence was predicted to exhibit a north-to-south decline.

CHAPTER II

METHODS

Strategy

Tick flagging

Assessment of tick distribution in southern VA and NC state parks along a north-to-south gradient using tick flagging: Sampling locations were chosen along the suspected path for the spread of LD into North Carolina from Virginia. The selected sites were Fairy Stone State Park, Mayo River State Park, Hanging Rock State Park, Pilot Mountain State Park and Lake Norman State Park (Fig. 2.0). Yadkin Island State Park was added to the selected sampling sites during year 2 of the flagging season to adjust for the distance gap between Hanging Rock and Lake Norman State Parks. Tick abundance is measured as mean tick number per 100 m flagging transect.

Hunter-harvested deer

Tick collection from hunter-harvested deer: Designated hunted deer check and deer processing stations located in north-western NC Piedmont and south-western NC Piedmont regions, were used to collect *I. scapularis* samples from deer. The ticks were collected from the ears, underbelly, and genital area of the deer for consistency and comparison. The deer are identified by age, sex, and county hunted in.

Characterization of entomological risk

The epidemiological risk of LD to humans is defined as the product of two parameters: entomological risk (calculated as a product of vector abundance and infection prevalence) and human exposure[56]. Given that infected *I. scapularis* nymphs are the key source of human exposure to the pathogen[8], the entomological risk for LD is defined as the density of host-seeking *B. burgdorferi* infected nymphal ticks[56]. The risk of exposure is highest during the emergence of nymphal ticks (Fig. 1.1), which are hard to detect (due to their small size) and coincides with the human population's most active periods outdoors in the spring and summer months[8]. A similar but smaller peak of increased incidence of infection is observed in the fall and early winter, coinciding with the emergence of infected adults who might remain, intermittently active until early spring[21]. The vector abundance parameter for determining the entomological risk level is classified into three categories, based on CDC risk map criteria[56]

- Established populations: ≥ 6 ticks or multiple life stages collected per area;
- Reported occurrence: < 6 ticks and only one life stage collected per area;
- Absence of ticks or missing data from collection.

This classification method was used to characterize the tick establishment status of my sampling sites and counties across the Virginia/NC border. Entomological risk for LD is also evaluated by the infection prevalence among the nymphal or adult ticks. In this study, I characterized both parameters in my study sites, which enabled me to evaluate whether LD entomological risk varies along a north-to-south transect between Virginia and NC.

Collection Methods

Tick flagging sites

Locations were chosen because they form a northeast to southwest transect along the suspected region for LD invasion into North Carolina from Virginia (Fig 2.0). State parks range from southwestern Virginia (Fairy Stone State Park) through northwestern NC (Mayo River State Park, Hanging Rock State Park, Pilot Mountain State Park, Yadkin Island State Park) to southwestern North Carolina (Lake Norman State Park). The parks are relatively similar regarding their habitat composition comprising deciduous forest, grass field habitats, soil, and vegetation suitable for *I. scapularis* habitats[57]. Preliminary dragging of Pilot Mountain State Park and reports by local park rangers of no known tick burden resulted in a decision to eliminate Pilot Mountain State Park from further sampling.

Tick collection by flagging

Flagging or drag sampling is frequently used in collecting ticks from all life stages[58]. In flag sampling, the flag is constructed of a small wooden dowel and cloth and is carried alongside the investigator[59]. Whereas in drag sampling the drag is constructed from a long wooden dowel and large cloth with a rope or chain attached to each end of the wooden base so that it can be dragged behind the investigator[60]. Questing ticks are seeking a blood meal and will rest on vegetation to detect vibration, heat, shadow, odor, and CO_2 of a potential host passing by [23]. Flagging has been considered a more efficient method of sampling in comparison to dragging[59]. This is because flagging allows the investigator to sample areas of dense undergrowth, allowing the flag to make better

contact with the leaf litter surface [61]. For this reason, flagging has been one of the most widely-used methods in investigating tick abundance [59].

The flagging apparatus is composed of a 1.22 m long wooden pole to which a white flannel sheet approximately 1 m^2 is attached (Fig. 2.1). The Flannel material mimics the consistency of animal's fur while the white color makes it easier to detect larval and nymphal ticks attached to it. The flag is swept across the top of brush, leaf litter, rocks and low vegetation. The brushing motion of the flag is to mimic the passing by of a potential host and triggers tick attachment. In each site, sampling is stratified by habitats such that common habitats are sampled adequately. Flagging was conducted along 100 m walking transects, stopping every 20 m to check both sides of the flag for ticks. The sampling effort for flagging is comprised of sampling sessions conducted periodically for each state park with the largest locations Fairy Stone, Hanging Rock, and Lake Norman having 15 transects which total 1500 m per session, and the smaller Mayo River State Park locations having 10 transects totaling 1000 m per sampling session. The ticks collected were placed in vials containing 95% ethanol. These vials were then be placed in the -20°C freezer upon returning to the lab for later identification and pathogen testing. The flagging sessions covered the entire life cycle of *I. scapularis*, with sessions completed from October 2015 through July 2017

Hunter-harvested deer sites

Sites used for deer herd health checks and hunted-deer check stations were determined by the North Carolina Wildlife Resource Commission (NCWRC) and the North Carolina Division of State Parks. Hunted-deer processing stations Hilltop Farms

(Walnut Cove, NC) and Game Butchers (Troutman, NC) (Blue "D" markers, Fig. 2.0) are in Stokes and Iredell counties, respectively. Deer herd health check sites were conducted on Lake Norman (Red 5 marker, Fig. 2.0) and South Mountain (Light Blue "H" marker, Fig. 2.0) State Park land.

Hunting season collection

Tick sampling from hunted deer was conducted over three consecutive hunting seasons in cooperation with NCWRC. Sampling in year 1 was performed in northwestern North Carolina at the opening of the central rifle season November 14, 2015 - January 1, 2016. In year 2 sampling was continued in northwestern and southern locations at the opening of rifle season November 12, 2016 – January 2, 2017. In year 3 sampling was continued in the northwestern location only at the opening of rifle and black powder season November 6, 2017 – January 2, 2018. The ticks were sampled from hunted deer brought in for weighing and cleaning at deer processing centers. Hunted deer were brought to Hilltop for processing from Surry, Stokes, Rockingham, Yadkin, Guilford, and Forsyth counties. Hunted deer were brought to Game Butchers for processing from Iredell, Davie, Rowan, and Catawba counties. Since hunters typically designate deer hunt-location by county, this is also the spatial resolution of this data. Such sampling strategy allowed for comparison of tick burden per deer and comparison of infection rates between counties. The deer brought in were first identified by age, sex, weight, and county location by a representative of NCWRC. We then examined deer for ticks, with the focus on ears, stomach, and genital regions. Recovered ticks were removed using tweezers by grabbing the tick at the attachment site and pulling the tick up and out. The

ticks collected were placed in vials containing 95% ethanol and stored at -20˚C for later identification and PCR testing. Since *B. burgdorferi* detectability is substantially reduced in adult blood-fed ticks[34], only non-blood fed tick samples collected from deer were sent to the CDC to test for *B. burgdorferi* infection[11; 34].

Herd health collection

Annual herd health check sampling was carried out on February 21st and 28th 2017 in southern North Carolina in coordination with North Carolina State Parks and NCWRC. It provides the means to determine the Abomasal Parasite Count (APC) of the deer population[62]. This APC count is used to measure the overall health of the deer population in the State Parks[62]. The Wildlife Commission then uses the APC in determining deer population control measures for the area. This deer herd health assessment is conducted once every five years for various North Carolina State Parks. The two sites sampled in 2017 covered Lake Norman State Park located in Troutman, NC (Red 5 marker in Iredell County, Fig. 2.0), and South Mountain State Park located in Connelly Springs, NC (Light Blue “H” marker in Burke County Fig. 2.0). The ticks collected were placed in vials containing 95% ethanol and stored at -20˚C for later identification and PCR testing.

General Methods

Tick identification

Individual ticks collected from flagging and hunted-harvested deer were morphologically identified to the species level using published keys[63; 64].

Tick infection testing

Confirmed non-blood fed nymph and adult tick samples were sent to the CDC for testing in collaboration with the Communicable Disease Branch of the NC Division of Public Health and the CDC's Division of Vector-Borne Disease. The extraction of DNA from individual ticks was completed by the CDC using a modified version of the protocol for DNA extraction from field-collected ticks[29; 65]. In the extraction of individual *I. scapularis* DNA, the tick was first homogenized using 545 mg 2.0 mm yttria-stabilized zirconium oxide beads in an Qiagen 470 µl lysis mix comprised of buffer ATL, 20 µl proteinase K, and 0.5% DX anti-foaming reagent[29]. The sample was then disrupted for 2 min using a Biospec Mini-Beadbeater-96 before incubating for approximately 10-12 min at 56° C[29]. Once incubating was complete the sample was centrifuged for 30 s at 1000 x g, and 200 µl was processed using the Qiagen (QIAcube HT) automated nucleic acid isolation system as well as the Qiagen (Cador Pathogen 96 kit)[29]. The sample was then combined with Qiagen VXL mixture and binding buffer ACB, to 650 µl and subjected to 3 min vacuum at 35 kPa[29]. Once vacuuming was complete, the column was washed using 600 µl of an AW1 buffer and vacuumed for 2 min at 35 kPa[29]. At which time the DNA was finally eluted by the addition of 100 µl AVE buffer to the column, incubated for 2 min, then vacuumed for 6 min at 55 kPa[29]. Each extract was then screened for *Borrelia*, *Anaplasma phagoctytophilum*, and *Babesia microti* using a pair of multiplex real-time PCR assays[66]. Modifications include the use of a pan-*Borrelia* 16S target in place of the *B. burgdorferi* "gB31" target[67]. Samples that tested positive for *Borrelia* underwent additional testing to detect and distinguish *B. miyamotoi*, *B. burgdorferi s.s.*, and *B.*

mayonii using a duplex real-time PCR assay targeting the oppA2 gene in *B. burgdorferi s.s.* and *B. mayonii*[68].

Permits and Collaborations

In cooperation with Virginia and North Carolina Division of State Parks, all permits were obtained for sampling and removal of ticks collected during flagging conducted on state-owned land. Ticks collected from deer herd health checks and hunted deer processing facilities were obtained in collaboration with NCWRC's yearly deer surveillance program.

Statistical Analysis

Tick flagging

Statistical analysis was performed using RStudio statistical software (version 1.1453)[69]. Tick sampling data were analyzed with a generalized linear model (GLM) using a negative binomial distribution. The number of ticks per 100m flagging transect was used as the response variable and tested against the following predictor variables: location, year, season, altitude, and habitat. A likelihood ratio test was used to determine the best fit model, by dropping one predictor variable at a time and checking for goodness-of-fit[70]. A robust variance estimator (robust standard error) was used to adjust for the overdispersion effects of tick count data[71]. This analysis was conducted for each species of collected tick[72].

Hunter-harvested deer

Hunter-harvested deer tick sampling data were analyzed with a generalized linear model (GLM) using a negative binomial distribution. To evaluate the north-to-south tick

spread hypothesis, the counties represented by the deer's hunter reported location were grouped as either North, Central, and South (Fig. 2.2) as well as West, Central, East. Number of ticks collected from hunter-harvested deer was used as the response variable and tested against the following predictor variables: deer's county of location were grouped north-to-south, west-to-east, year, deer age, and deer sex[72].

Infection prevalence

Tick infection status is a binary outcome (infected =1 and noninfected = 0) and was tested using a logistic regression model with a binomial distribution, testing the effects of the independent variables such as county, direction, year, and parks has on tick infection status[72].

CHAPTER III

RESULTS

Distribution and Relative Abundance of *I. scapularis*

Tick flagging

A total of 132 *I. scapularis* ticks, comprising of 99 larvae, 24 nymphal, and 9 adult stages were collected from the 6 State Park sites (Table 2.0). The distribution of *I. scapularis* per 100 m is given in (Fig. 3.0) with the error bars representing the calculated normal standard error for each park (Fairy Stone 0.400, Mayo Waterfall 0.250, Mayo Park 0.335, Hanging Rock 0.551 Yadkin Island 0.167, Lake Norman 0.00). The highest number of *I. scapularis* ticks per 100m was collected from north-most Virginia's Fairy Stone State Park and Hanging Rock State Park and slightly less in Mayo Waterfall Park and Mayo River Park (Fig. 3.0). All these locations, however, are relatively northern state parks. In contrast, *I. scapularis* ticks were completely absent from the southmost Lake Norman State Park (Fig. 3.0). The density of *Ixodes scapularis* was best predicted by a negative binomial model (Table 2.1). A likelihood ratio test was performed to determine the best-fit model and showed that the predictor variables altitude and habitat were not a good fit for the model and removed (Table 2.1 A). The reduced best-fit model used in evaluation of tick distribution collected by flagging included State Park locations (6 levels), season (3 levels), and year (2 levels). As predicted, tick density decreased as the distance from Virginia decreased (Table 2.1). The other predictor variables, season and

year also had significant effects on tick abundance. Tick abundance for year 2 showed a significantly higher distribution compared to year 1 collection. There were significantly more ticks in the fall season compared to the spring.

Hunter-harvested deer

A total of 130 deer (Table 2.2) were sampled over a three-year period, yielding 549 adult ticks (Male and Female) over the represented counties. The north-to-south distribution of *Ixodes* per deer is shown in (Fig. 3.2) with the given error bars representing the calculated normal standard error for each region (North 0.738, Central 0.810, South 0.193). There is a clear trend of north-to-south decrease in tick burden per deer (Fig. 3.2) with the northern region located on the VA-NC border having the highest tick burden, followed by the central and the southern regions of NC. This result seems to be consistent with the flagging data (Fig. 3.0) which also showed a higher density of *Ixodes* for the northern regions. However, in contrast with flagging results, I did find ticks on hunted deer (Table 2.2) for the southern counties (total of 10 ticks collected from 26 deer) of Davie, Alexander, Iredell, and Rowan Counties.

The density of *I. scapularis* was best predicted using a negative binomial model (Table 2.3). A likelihood ratio test was performed to determine the best-fit model and showed that the predictor variables deer age and West-to-East were not a good fit for the model and removed (Table 2.3 A). The reduced best-fit model used in evaluation of tick distribution collected from hunted deer included latitude (North-to-South, 3 levels), year (3 levels), and deer sex (2 levels). Ticks distribution showed marginal significant ($P < 0.1$) difference between the central and northern regions (Table 2.3). The other predictors

year and deer sex also showed to be significant in the distribution of I. scapularis (Table 2.3). There showed to be a clear increase in tick burden per deer in years 2 and 3 compared to year 1 with year 2 having the highest density of ticks collected.

Infection Patterns

Tick flagging

In total, 33 ticks, consisting of 12 (1 male, 1 female, 10 nymphs) from Fairy Stone, 8 (5 male, 3 nymphs) from Hanging Rock, 5 from Mayo River (all nymphs), 7 from Mayo River Waterfall (1 male, 1 female, 5 nymphs) and 1 nymph from Yadkin Island State Parks were screened for tick-borne pathogens. The infection prevalence of *Borrelia* collected by flagging is given in (Fig. 3.2) with the given error bars representing the calculated binomial standard error for each park (Fairy Stone 0.13, Mayo Waterfall 0.13, Mayo Park 0.00, Hanging Rock 0.12). Infection prevalence of flagging submitted samples exhibited a general north-to-south declining trend with highest prevalence at the north-most VA Fairy Stone State Park (25%) and (14-13%) at the NC northern state parks (Fig. 3.2). Given that the 1 tick submitted to the CDC for testing from Yadkin Island State Park came back inconclusive, and no ticks were found at the southern Lake Norman site (Iredell County), no information on tick infection level is available from those sites for tick flagging samples. With the very small sample sizes submitted for each park the binomial model best for the distribution of *B. burgdorferi* was the model that had no predictor variables.

Hunter-harvested deer

A total of 262 adult ticks collected from deer were screened for presence of tick-borne pathogens. The north-to-south infection prevalence of *Borrelia* from hunted deer is shown in (Fig. 3.3) with the given error bars representing the calculated binomial standard errors for each region (North 0.02, Central 0.05, South 0.0). Infection prevalence in northern counties was highest at 17% followed by 11% for the central counties (Fig. 3.3), but no infection was detected in southern counties (but mind the small sample size in the latter, n=5). Infections prevalence at the county level showed that the north-most Rockingham (22%) and Stokes (19%) counties had the highest level of infection, followed by Surry (15%), Yadkin (17%), and Forsyth (13%) counties. The binomial model selection for prevalence of *B. burgdorferi* from hunter-harvested deer was a simplified model using only 1 predictor variable latitude (North-to-South, 3 levels). There was no significant difference in prevalence between the northern and central NC regions and a significant difference ($P < 0.05$) between the southern and northern as well as the southern and central regions.

Combined infection prevalence

As predicted, infection rates appear to follow clear north to south decreasing trend when tick samples from tick flagging and hunter-harvested deer are examined together. With approximately a 25% infection prevalence in northern Virginia, 17% infection prevalence for NC counties that border VA, 11% infection prevalence for the central NC counties and 0.0% southern NC counties. The prevalence of *B. burgdorferi* was predicted by using a binomial model with 2 predictor variables (Table 2.5), direction (north-to-

south 4 categories), and tick's life stage (2 categories). The difference in infection prevalence was marginally significant ($P < 0.1$) between VA and the northern and central counties of NC but significantly less at the south-most counties (Table 2.5). Showing that as distance increased from VA the prevalence of the pathogen also decreased. The difference in infection prevalence between the adult and nymphal tick stage was also marginally significant with the prevalence of infected adults being higher.

Screening for other pathogens

Testing of collected *I. scapularis* tick samples for other pathogens, showed 2 ticks collected from northern Rockingham County being positive (5%) for *Borrelia miyamotoi* responsible for Tick-Relapsing Fever (TBRF) as well as 8 ticks testing positive (20%) for *Aanaplasma phagocytophilum* responsible for Anaplasmosis. Of those ticks testing positive for infection in Rockingham County, 1 (2%) sample exhibited coinfection for *B. burgdorferi* with *Aanaplasma phagocytophilum* as well as a 1(2%) for coinfection of *Borrelia miyamotoi* and *Aanaplasma phagocytophilum*. Surry County also had 1(4%) tick test positive for *Borrelia miyamotoi*. *Aanaplasma phagocytophilum* was also detected in 1 tick from Forsyth (13%) and 2 from Stokes (4%) counties with no positives for coinfections.

Tick establishment classification and estimated entomological risk

There was a 60% increase in entomological classification status change for sampled counties in North Carolina compared to the prior 2015 classification (Table 2.4). With a majority of northern and central counties sampled reaching the established classification, and southern sampled counties becoming classified as a newly reported

occurrence. The estimated entomological risk of the density of infected nymphs (DIN) for flagging collected ticks (Table 2.0) showed a north-to-south decreasing trend in infection risk with the highest risk at the northernmost Fairy Stone State Park (0.033). The density of infected adults (DIA) for flagging collected ticks (Table 2.0) showed no apparent trend in entomological risk. Estimated entomological risk of DIA for deer collected ticks (Table 2.2) showed no apparent trend at the county level. When the counties were grouped by region north-to-south, the average calculated DIA showed a clear north-to-south trend. With the northern NC region DIA (0.808) being highest followed by the central NC region DIA (0.522), and no reported DIA risk for the southern region.

Abundance Patterns of Other Tick Species

Amblyomma americanum

The highest number of *A. americanum* ticks per 100m was collected from the north-most Mayo River and Mayo Waterfall State Parks (Fig.4.7) and much less in Fairy Stone and Hanging Rock State Parks. This is consistent with data on *A. americanum* tick densities per deer being very similar for the north and central NC regions (Fig. 4.8). In contrast, *A. americanum* ticks were completely absent from the southern Yadkin Island and Lake Norman State Parks (Fig. 4.7) and in the southern region of deer collected samples (Fig. 4.8)

Dermacentor albipictus

Two species of Dermacentor ticks were collected form flagging and hunter-harvested collections. In total, only 3 *Dermacentor variabilis* adults were collected from

tick flagging over a two-year period. For tick collections from hunter-harvest deer (Fig. 4.9), *Dermacentor albipictus* was the more abundant species and had the highest density for the central region of NC.

CHAPTER IV

DISCUSSION

Lyme Disease emergence in the Northeastern and Western parts of the United States has increased at an alarming rate. and has been linked to the migration of the vector *I. scapularis* [73]. Though this trend has been taking place in the northern US for some time, it is a fairly recent phenomenon in the lower Southern US. With most analysis on the distribution and expansion of LD focusing on number of human LD cases, there is very little systematic surveillance being done on *I. scapularis*[74]. This leaves little to no entomological data on the distribution of *I. scapularis* and infection prevalence to accurately determine the entomological risk of LD for NC[74]. By conducting tick surveillance from tick flagging and hunter-harvested deer in investigating LD emergence in NC, I first evaluated the evidence that distribution of *I. scapularis* and *B. burgdorferi* infection prevalence into NC was from VA. Second, I evaluated two alternative mechanisms "tick-first" and "dual invasion" that could be underlying the spread LD into NC. Then I evaluated infection prevalence of *B. burgdorferi* to determine the entomological risk of LD in NC.

Evidence for Lyme borreliosis from VA into NC

Ixodes tick populations were either absent or concentrated in the coastal plains region of NC, with no detectable levels of *Borrelia* prior to the recently increased emergence of LD[52; 75]. In NC as with many other states the emergence of LD has risen

sharply in concurrence with the rising white-tailed deer populations[46]. It is possible that the mountain ecology such as climate, landscape characteristics, and wildlife populations that makes up the Appalachian and Piedmont regions running through VA and NC are sustaining the expansion into NC of both tick and pathogen populations[45; 48]. The data from ticks collected thus far from flagging and hunted-harvested deer show there to be a significant north-to-south gradient from VA into NC in *I. scapularis* abundance with Alexander and Iredell counties being on the southernmost edge of NC tick distribution. This result is suggestive of deposited tick populations in southern regions of VA rapidly reached population density levels that supported further migration south into NC. This trend is consistent with the prediction in (AIM I) that *I. scapularis* densities are expected to decrease in a north-to-south direction along the Appalachian foothills region.

Borrelia burgdorferi infection results also demonstrated a clear north-to-south distribution, with *B. burgdorferi* appearing to have only made it as far south into the central region of NC, with counties such as Yadkin and Forsyth being on the edge of the pathogens distribution. With NC infection prevalence illustrating a declining trend within a marginal degree of significance coupled with the southernmost sampled regions having no detectable levels of infection, supports the prediction in AIM II that the pathogen would also exhibit a north-to-south gradient.

Changes in the *I. scapularis* entomological classification status of NC sampled counties showed that most counties in the northern and central NC regions were now being classified as having established *I. scapularis* populations and half of the counties in the southern region that previously had no reported occurrence, are now being classified

as having reported *I. scapularis* populations. This trend in entomological classification status change also supports the prediction of a north-to-south gradient of *I. scapularis* from VA into NC. North Carolina LD entomological risk showed a clear north-to-south trend in DIN for flagging collected tick samples, with VA Fairy Stone State Park having the highest DIN (0.033) followed by NC Mayo Waterfall and Hanging Rock State Park locations. This north-to-south trend was further support by the DIA of deer collected ticks with the northern counties bordering VA having the highest DIA (0.97) followed by the central region (0.35) and no recorded entomological risk for the southern region of NC.

Lyme borreliosis Invasion Mechanism

The three evasion mechanisms "tick-first," "dual invasion," and "pathogen first" is a product of either the vector arriving first followed by the pathogen, the vector and pathogen arriving together, or the pathogen preexisting and the vector arrives later. These three scenarios used to examine LD expansion into NC can also be seen in other vector-borne disease systems as well. For instance, Dengue and Zika viruses in South America is a representation of a "vector-first" invasion scenario. The vector *Aedes aegypti* was introduced first to South America from Africa creating the appropriate setting for pathogen reception which occurred much later via introduction of the pathogen by an infected traveler from source regions[76; 77]. Known as one of the most deadliest pandemics in history the plague is, unfortunately, a great example of the "dual invasion" mechanism in action[78]. The plague is caused by a bacteria that is maintained by an zoonotic life cycle involving rodents and a flea vector[79]. China was found to be the origin of the plague's, where the pathogen, host, and vector were spread simultaneously through the

use of trade routes along the silk road into new regions[78]. Eastern Equine Encephalitis (EEE) is a representation of a “pathogen fist” invasion scenario and was first discovered in 1831 in horses in the US, but the first documented outbreak occurred in 1933[80]. The pathogen was maintained in the system by the *Culiseta melanure* mosquito, which does not typically bite humans, followed by the invasion of bridge vectors that occurred later. With the data collected from this study depicting a clear declining trend in tick abundance in a north-to-south direction, a reported tick presence having no detectable infection prevalence for the southernmost edge is suggestive of the “tick-first” hypothesis could be the driving force of LD expansion into NC. However, it is important to consider that with such a small sample size tested for infection in the southern region, and small mammal trapping not conducted in the current study, to rule out “dual invasion” or “spirochete-first” as possible mechanism for LD expansion into NC would be premature.

Public Health Implications

This study was aimed specifically to evaluate the invasion of *I. scapularis* and *B. burgdorferi* from VA-to-NC. The implications of this study allowed us to track in real time the dynamics of LD expansion into NC and provide better ecological insight into the LD system. That will lead to developing LD models for NC that can be used to predict human risk assessments at the county and State Park levels. This information can be used by public health officials in NC, for reevaluation of current LD treatment practices for areas with high infection prevalence (≥ 20%) and update medical practitioners on protocols for recommended prophylactic antibiotic treatment intervention. More importantly, the information can be used to raise public awareness of LD risk at the state

and local government levels in NC. For that reason, it would be beneficial to continue this study and increase the amount of systematic surveillance conducted in the NC region.

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APPENDIX A

TABLES

Table 1.0. South Eastern US Vertebrate Species Competency Status in Lyme borreliosis Propagation.

Competent reservoir hosts	Mammals -	*Blarina brevicauda* (Northern short-tailed shrew), *Lepus spp.* (hares), *Microtus pennsylvanicus* (Meadow vole), *Peromyscus leucopus* (White-footed mouse), *Sciurus griseus* (Western gray squirrel), *Sorex spp.* (Shrew), *Tamias spp*. (Chipmonk)
	Birds -	*Turdus migratorius* (American robin), *Fratercula arctica* (Puffin)
	Lizards -	*Eumeces inexpectatus* (Southeastern skink)
Less competent reservoir hosts	Mammals -	*Canis latrans* (Coyote), *Didelphis virgianus* (Virginia opossum), *Procyon lotor* (Raccoon), Sciurus carolinensis (Eastern gray squirrel)
	Birds -	*Cardinalis cardinalis* (Northern cardinal), *Melospiza melodia* (Song sparrow)
	Lizards -	*Anolis carolinensis* (Carolina anole), *Sceloporus undulatus* (Eastern fence lizard)
Incompetent hosts	Mammals -	*Dama dama* (Fallow deer), *Odocoileus hemionus* (Mule deer), *Odocoileus virginianus* (White-tailed deer)
	Birds -	*Dumatella carolinensis* (Gray catbird), *Pipilo erythrophthalmus* (Eastern towhee), *Toxostoma rufum (Brown thrasher)*
	Lizards -	*Elgaria multicarinata* (Southern alligator lizard), *Sceloporus occidentalis* (Western fence lizard)

Table 2.0. Summary of Tick Abundance and Estimated Entomological Risk by State Parks for Ticks Collected by Flagging. The table depicts the total number each life stage of *I. scapularis* ticks collected from flagging, the calculated infection rates, and density of infected nymphs and adults. Density of nymphal and adult ticks was calculated from collected number of nymphs and adults divided by sampling effort. Density of infected nymphs and adults (DIN, DIA) was calculated by nymphal and adult density times infection rate.

State Park	Sampling Effort **	Larvae	Nymph	Adult	Nymphal Density	Adult Density	Number Tested	*B. burgdorferi*	Infection Rate	DIN	DIA
Fairy Stone	75	39	10	2	0.13	0.03	12	3	0.25	0.0333	0.0067
Mayo Waterfall	40	17	5	2	0.13	0.05	7	1	0.14	0.0179	0.0071
Mayo Park	30	8	5	0	0.17	0.00	5	0	0.00	0.0000	0.0000
Hanging Rock	60	35	3	5	0.05	0.08	8	1	0.13	0.0063	0.0104
Yadkin Island	6	0	1	0	0.17	0.00	1	NA	NA	NA	NA
Pilot Mountain	12	0	0	0	0.00	0.00	0	0	0.00	0.0000	0.0000
Lake Norman	45	0	0	0	0.00	0.00	0	0	0.00	0.0000	0.0000

** Total number of 100 m transects sampled for each park.

Table 2.1. Negative Binomial Regression Model of Factors Affecting Abundance of *Ixodes scapularis* Based on Tick Flagging. A) Deviance table testing factors for their affect on tick abundance. Likely Ratio Test (LRT) of each predictor variable with its associated p-value. B) Best-fit negative binomial regression model. Baseline (intercept) comprised Fairy Stone for State Park, year 1, and spring for the effect of season.

A

Full Model:

Ixodes ~ State Park + Altitude + Season + Year + Habitat

	Df	Deviance	LRT	Pr(>Chi)
< None >		70.93		
StatePark	5	88.49	17.56	0.004 **
Altitude	1	72.56	1.63	0.201
Season	2	84.39	13.46	0.001 **
Year	1	80.88	9.95	0.002 **
Habitat	1	71.34	0.41	0.522

signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

B

Reduced Model:

Ixodes ~ State Park + Season + Year

Parameter	Estimate	St. Error	z value	Pr(>|z|)
Intercept	-3.53	0.94	-3.77	0.000 ***
Mayo Waterfall	-0.02	0.67	-0.03	0.762
Mayo River	-0.17	0.89	-0.19	0.853
Hanging Rock	0.89	0.79	1.11	0.265
Yadkin Island	-4.17	1.33	-3.15	0.002 **
Lake Norman	-35.16	0.46	-76.91	2.20E-16 ***
Summer	0.95	0.86	1.10	0.271
Fall	2.96	0.96	3.09	0.002 **
Year 2	2.94	0.67	4.39	1.13E-05 ***

ΔAIC 2.21

Table 2.2. Summary of Tick Abundance and Estimated Entomological Risk by County for Ticks Collected from Hunted Deer. The table depicts the total number of adult *I. scapularis* ticks collected by county, the calculated infection rates, and density of infected adults. Density of infected adults (DIA) was calculated by adult density times infection rate.

County	Sampling Effort **	Adult	Tick Burden	Number Sent	*B. burgdorferi*	*Infection Rate*	*DIA*
Surry	11	53	4.8	31	*6*	*0.194*	*0.933*
Stokes	56	300	5.4	140	*21*	*0.150*	*0.804*
Rockingham	19	129	6.8	59	*13*	*0.220*	*1.496*
Caswell	1	3	3.0	0	*0*	*0.000*	*0.000*
Yadkin	2	15	7.5	6	1	*0.167*	*1.250*
Forsyth	12	36	3.0	19	2	*0.105*	*0.316*
Guilford	3	3	1.0	2	0	*0.000*	*0.000*
Davie	1	0	0.0	0	0	*0.000*	*0.000*
Burke	7	0	0.0	0	0	*0.000*	*0.000*
Alexander	1	2	2.0	1	0	*0.000*	*0.000*
Iredell	15	8	0.5	4	0	*0.000*	*0.000*
Rowan	2	0	0.0	0	0	*0.000*	*0.000*

** Total number of deer sampled per county.

Table 2.3. Negative Binomial Regression Model of Factors Affecting Abundance of *Ixodes scapularis* Based on Tick Collected from Hunted Deer. A) Deviance table testing factors for their affect on tick abundance. Likely Ratio Test (LRT) of each predictor variable with its associated p-value. B) Best-fit negative binomial regression model. Baseline (intercept) comprised northmost counties region, year 1, and female.

A

Full Model:

Ixodes ~ Latitude + Year + Sex + AGE(Factor) + Longitude

	Df	Deviance	LRT	Pr (>Chi)
< None >		135.74		
Latitude	2	160.32	24.58	4.59E-06 ***
Year	2	142.06	6.33	0.042 *
Sex	1	141.03	5.29	0.021 *
AGF	2	136.35	0.61	0.737
Longitude	2	136.11	0.38	0.828

signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

B

Reduced Best-fit Model:

Ixodes ~ Latitude + Year + Sex

Parameter	Estimate	St. Error	z value	Pr(>|z|)
Intercept	0.651	0.350	1.859	0.063 .
Central	-0.641	0.255	-2.510	0.012 *
South	-2.505	0.494	-5.076	3.85E-07 ***
Year 2	0.823	0.280	2.937	0.003 **
Year 3	0.557	0.243	2.290	0.022 *
Male	0.657	0.300	2.185	0.029 *
ΔAIC 6.98				

Table 2.4. Entomological Classification of Tick Establishment Status of Sampled Counties in North Carolina.

County Location	Deer	*Ixodes* / Deer	Flagging Transects	*Ixodes* / 100 m	Entomological Classification	
					Prior 2015 Classification	New 2017 Classification
Surry	11	4.8	12	0.0	Reported Occurrence	Established Populations
Stokes	56	5.4	60	0.7	Established Populations	Established Populations
Rockingham	19	6.8	70	0.5	Absence of Ticks	Established Populations
Caswell	1	3.0	-	-	Absence of Ticks	Reported Occurrence
Yadkin	2	7.5	6	0.2	Absence of Ticks	Established Populations
Forsyth	12	3.0	-	-	Reported Occurrence	Established Populations
Guilford	3	1.0	-	-	Reported Occurrence	Reported Occurrence
Burke	7	0.0	-	-	Absence of Ticks	Absence of Ticks
Alexander	1	2.0	-	-	Absence of Ticks	Reported Occurrence
Iredell	15	0.5	45	0.0	Absence of Ticks	Reported Occurrence
Rowan	2	0.0	-	-	Established Populations	Absence of Ticks

Table 2.5. Logistic Regression Model of the Effect of North-to-South Direction and Tick Stage Affecting *Borrelia burgdorferi* Infection Status of Collected *Ixodes scapularis* Ticks. The data is comprised of both flagging and hunted deer collected ticks. Baseline (intercept) comprised of Virginia region and adult stage.

Reduced Model:
B. burgdorferi ~ Latitude + Life Stage + Year

Parameter	Estimate	St. Error	z value	Pr(>|z|)
Intercept	-0.246	0.779	-0.317	0.752
Border	-1.315	0.775	-1.697	0.09 .
Central	-1.833	0.991	-1.850	0.064 .
South	-16.320	0.898	-18.175	2.00E-16 ***
Nymph	-1.065	0.548	-1.943	0.052 .

APPENDIX B

FIGURES

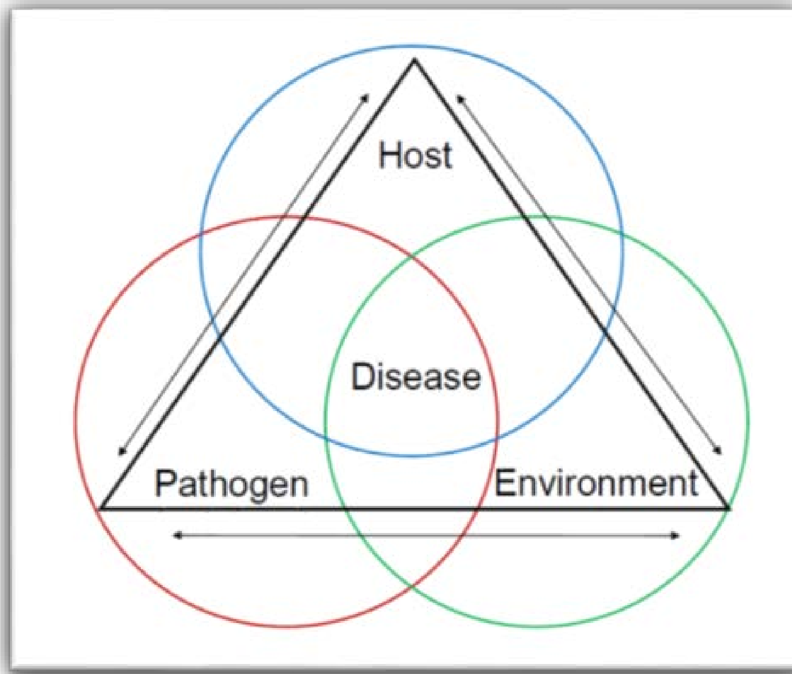

Figure 1.0. The Epidemiological Triad. The figure depicts the individual niche (color coordinated circles) for the pathogen, host, and environment as well as the interactions (arrows of the triangle) between each niche. It is at the nexus (overlap) of these niches that disease occurrence can take place in the environment.

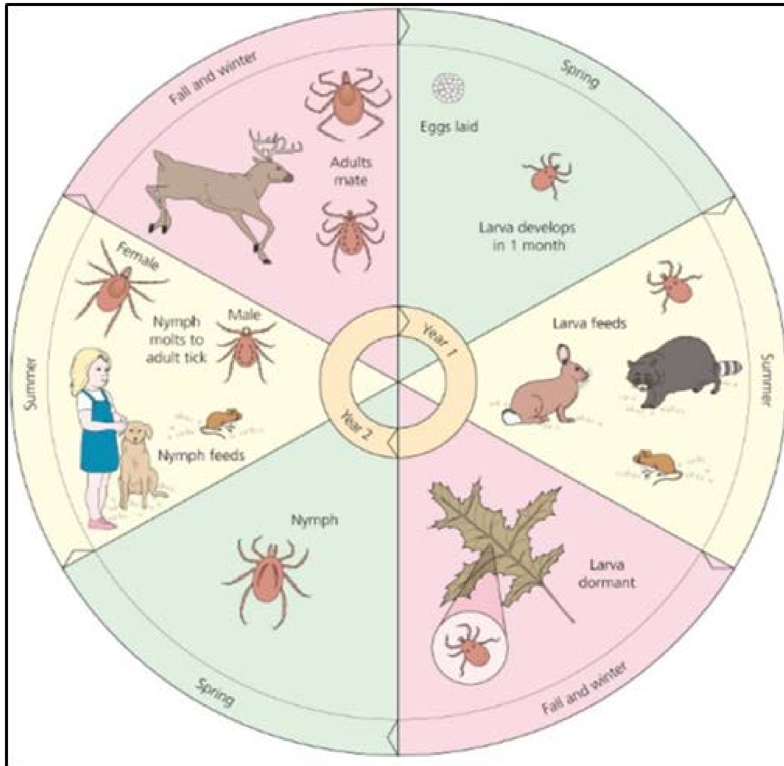

Figure 1.1. Two Year Life Cycle. The two-year life cycle of *I. scapularis* is divided into seasons beginning with spring of year 1 (larvae emergence) through fall of year 2 (adults lay eggs). The typical host fed on for each tick life stage is displayed in the season of activity[1;25].

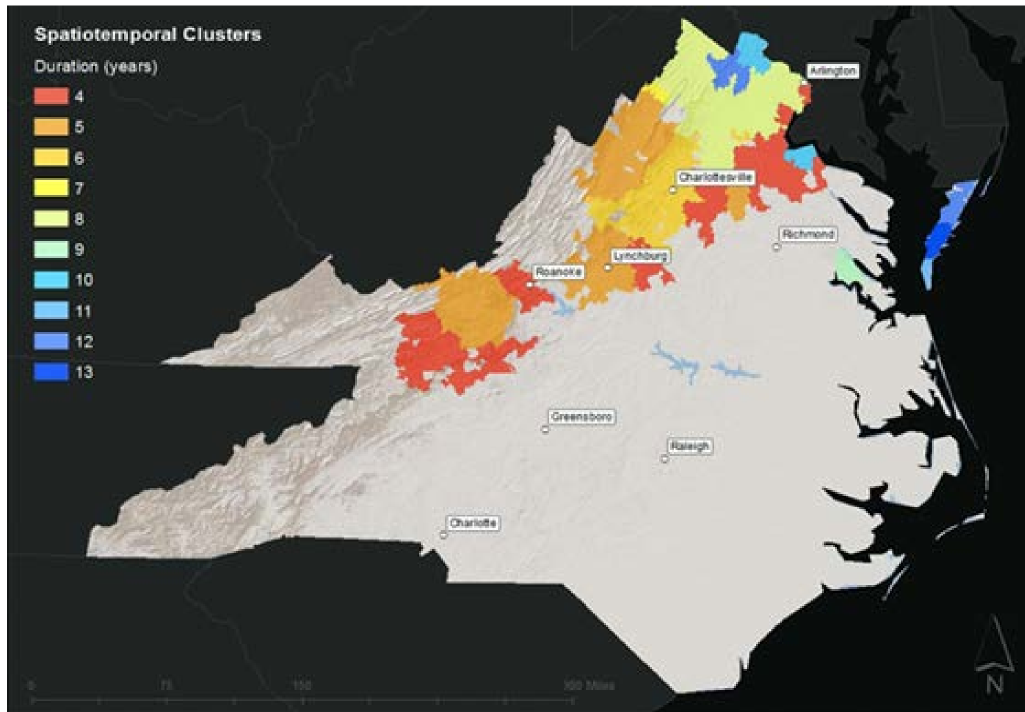

Figure 1.2. Results of a Spatiotemporal Cluster Analysis of Lyme Disease Cases from Virginia Conducted by Lantos et al. (2015). Results of this analysis indicates a southwesterly expansion of LD cases along the Appalachian Mountains.

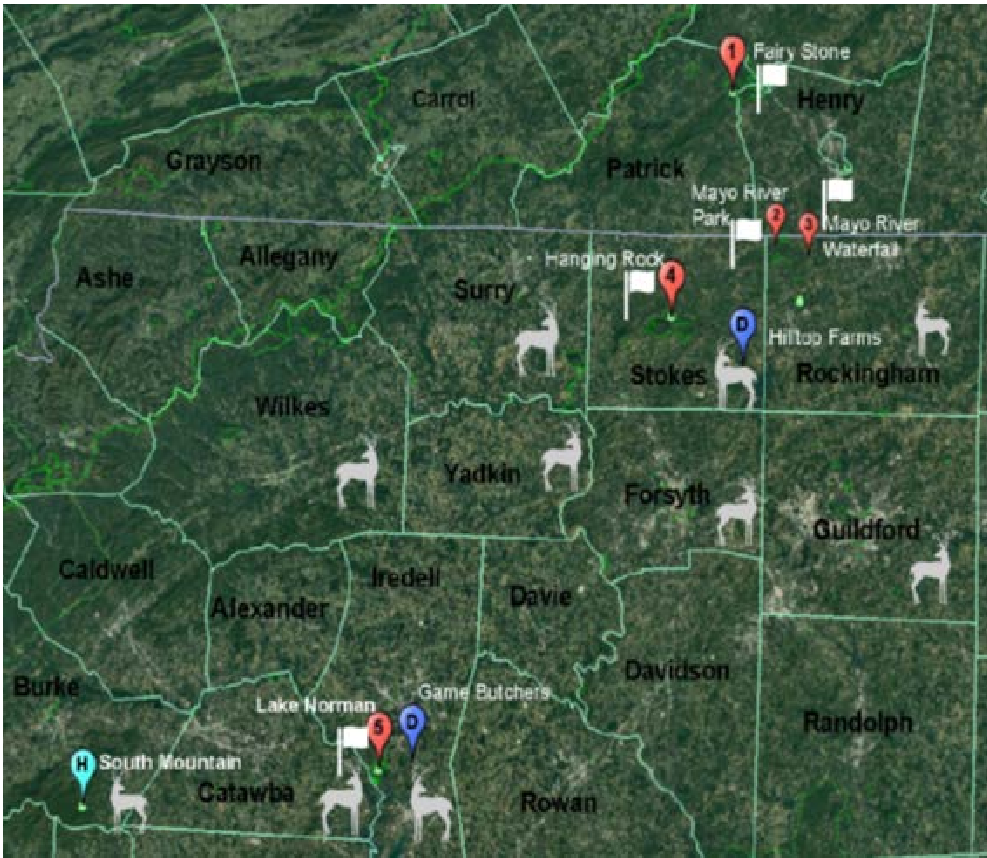

Figure 2.0. Flagging and Hunted-harvested Deer Study Sites. State park sites selected for surveillance are marked on the map in descending order North-to-South (1-5 Red Markers). Deer herd health checks (Light Blue "H" Marker and Lake Norman 5 Marker) were conducted at Lake Norman, and South Mountain state parks. Hunter-harvested deer (Blue "D" Markers) facilities were located at Hilltop Farms and Game Butcher's locations. Deer symbols indicate counties represented from which deer were harvested from.

Figure 2.1. Tick Collection Flag. The flag was constructed using a .03 m by 1.22 m wooden dowel, with a 1 m^2 flannel sheet attached to the dowel using heavy gauge stables

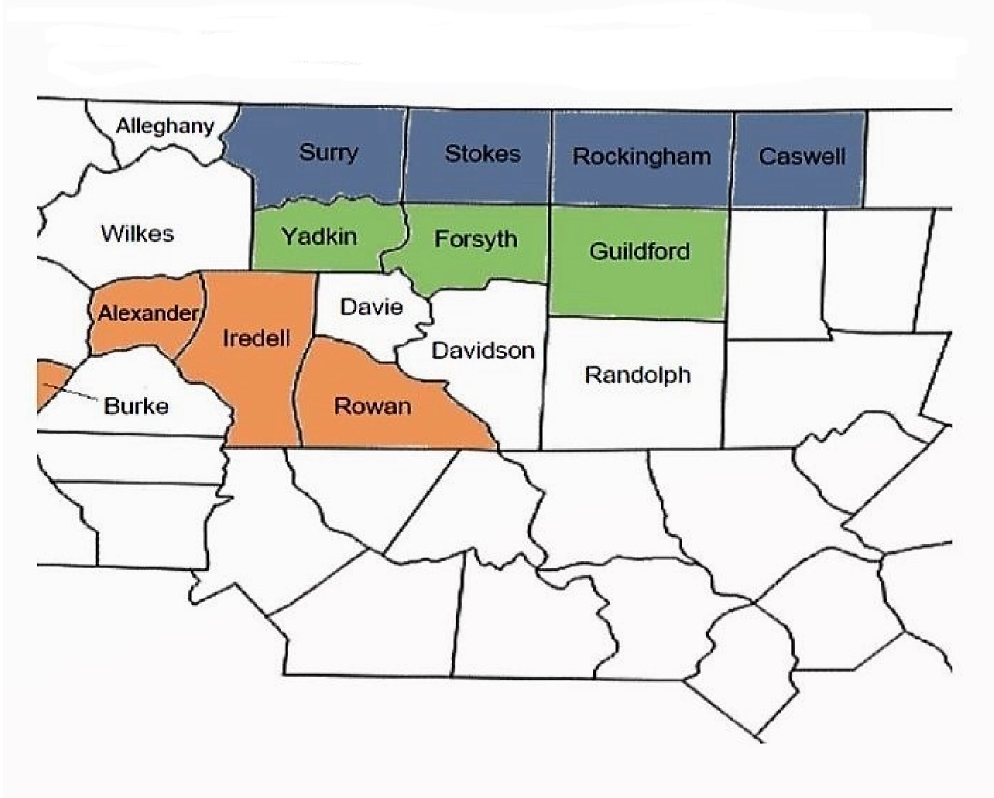

Figure 2.2. North to South Grouping of North Carolina Counties. The determined North-To-South grouping of counties in NC used for evaluating deer collected ticks. The counties considered northern are represented by blue, with counties considered more central represented by green, and counties considered to be southernly represented by light orange. Surrounding counties names are also given but had no collected deer samples.

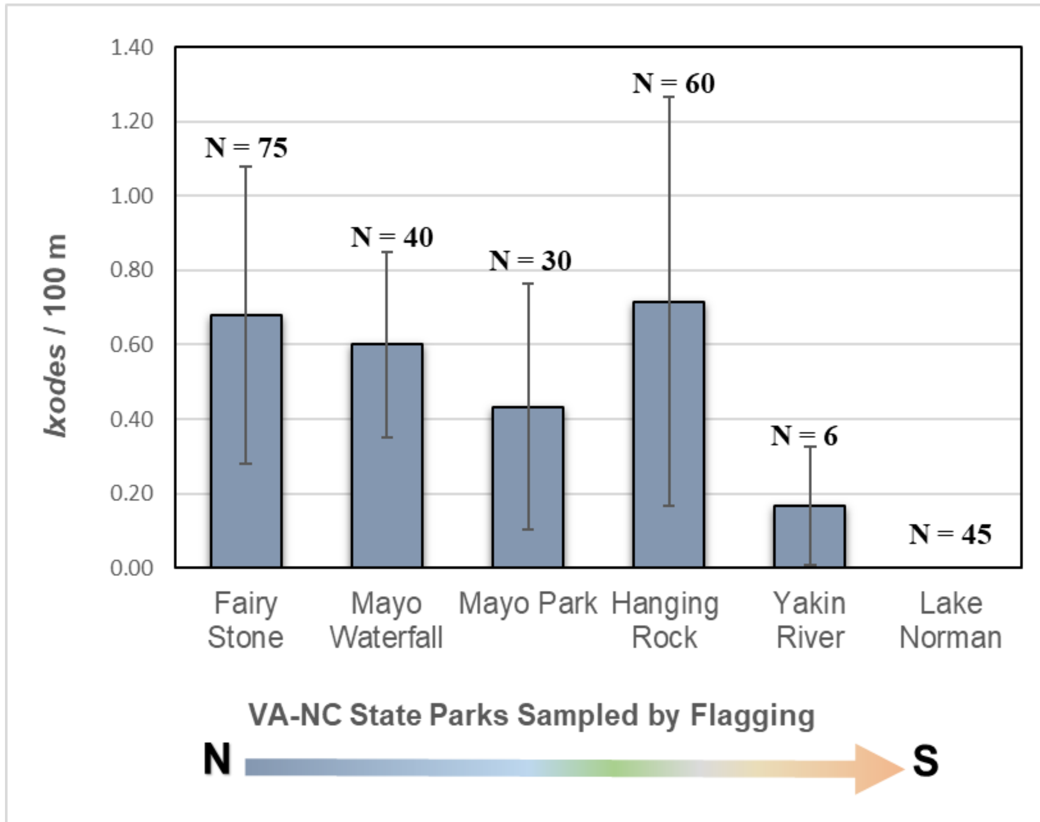

Figure 3.0. ***Ixodes scapularis*** **Relative Abundance for Ticks Collected by Flagging at State Parks.** State Parks sites are ordered along a north-to-south gradient. (N = Number of Transects, Error bars = SE).

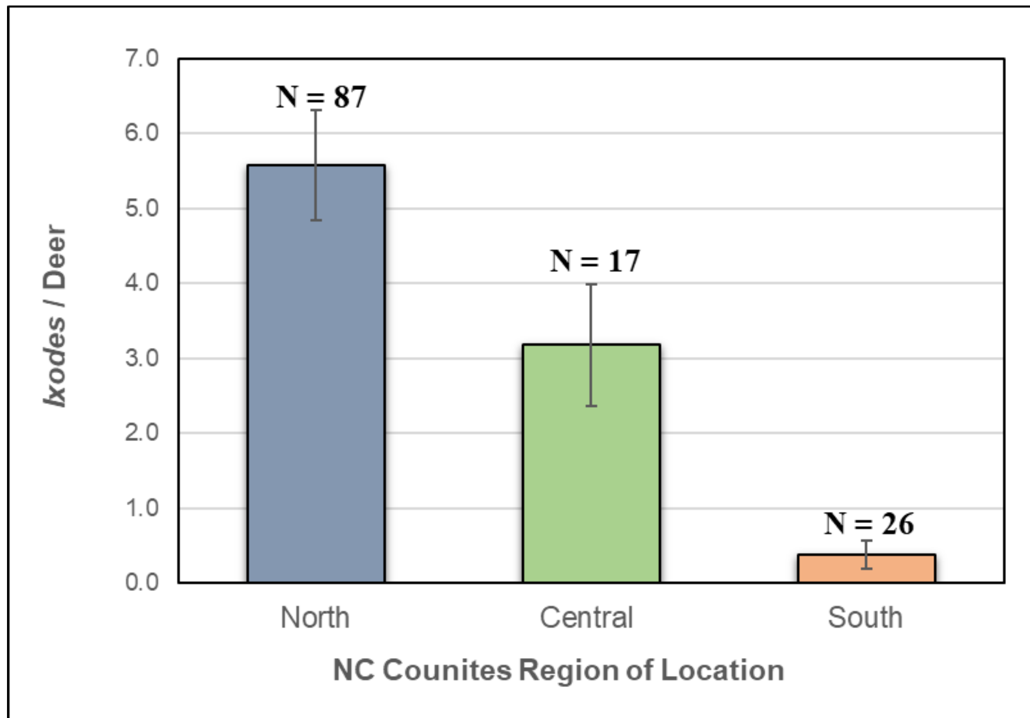

Figure 3.1. *Ixodes scapularis* per Deer in a North-to-South Distribution. North Carolina counties are ordered along a north-to-south gradient. (N = Number of Deer, Error bars = SE).

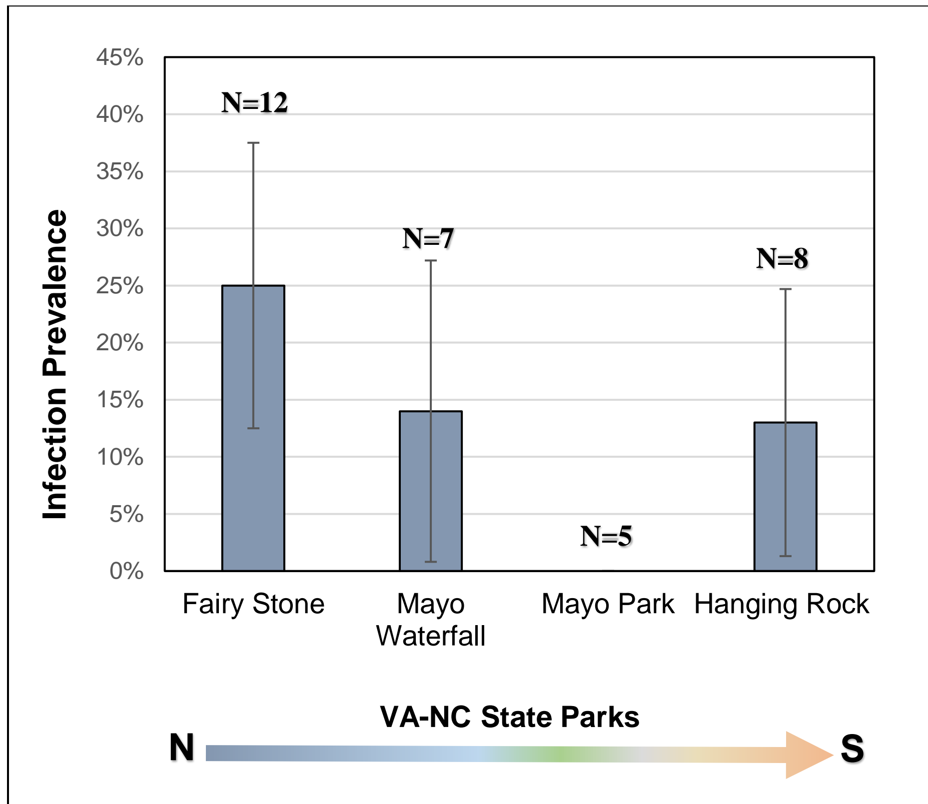

Figure 3.2. ***Borrelia burgdorferi*** **Infection Prevalence for Tick Flagging Samples Collected from State Parks Along a North-to-South Gradient.** Number of samples submitted to the CDC is represented above each park (Error bars = binomial SE). Yadkin Island and Lake Norman State Parks are not displayed, with the submitted sample for Yadkin Island inconclusive due to poor DNA and no samples submitted from Lake Norman.

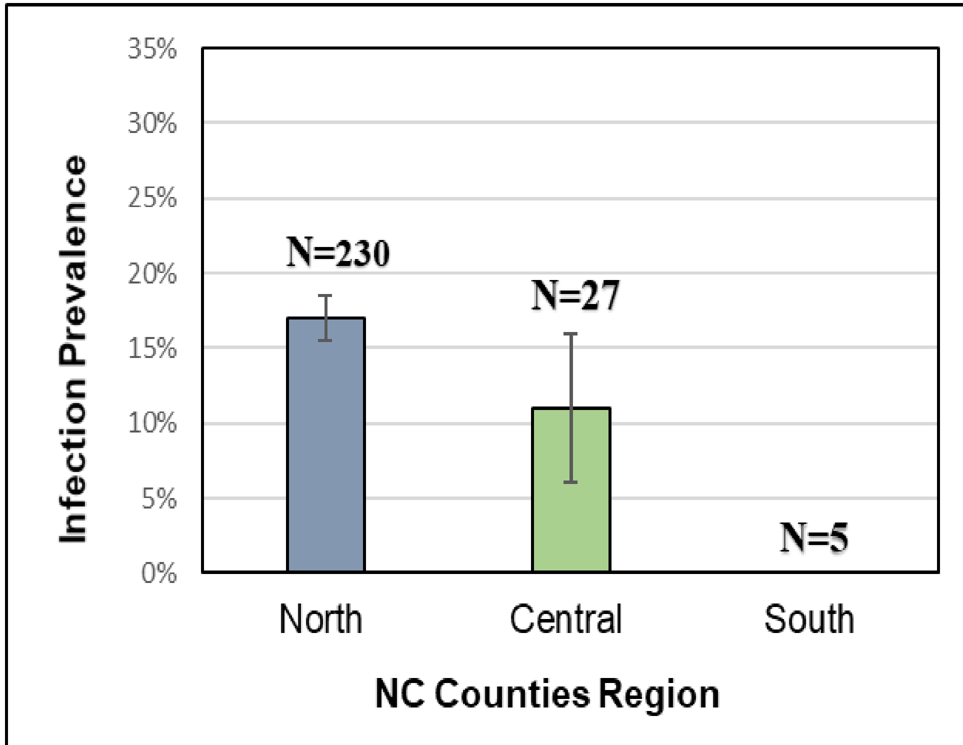

Figure 3.3. ***Borrelia burgdorferi*** **Infection Prevalence for Hunter-harvested Deer Tick Samples.** Number of samples submitted to the CDC is represented above each region (Error bars = binomial SE).

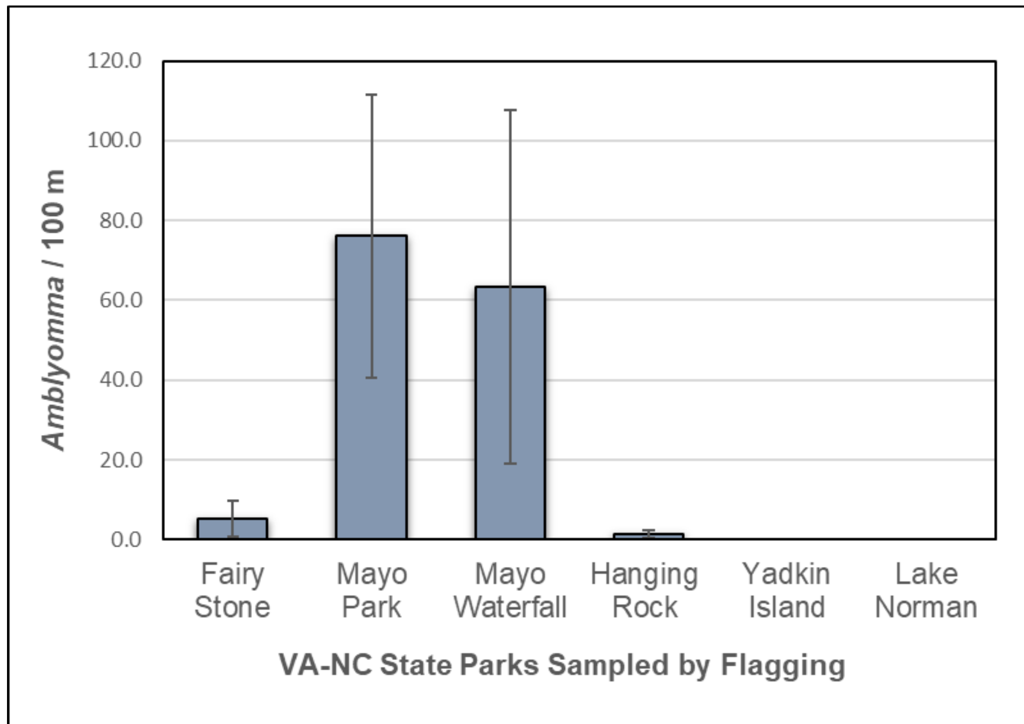

Figure 3.4. ***Amblyomma americanum*** **Relative Abundance for Ticks Flagging Collected Samples. S**tate Parks sites are ordered along a north-to-south gradient (Error bars = SE).

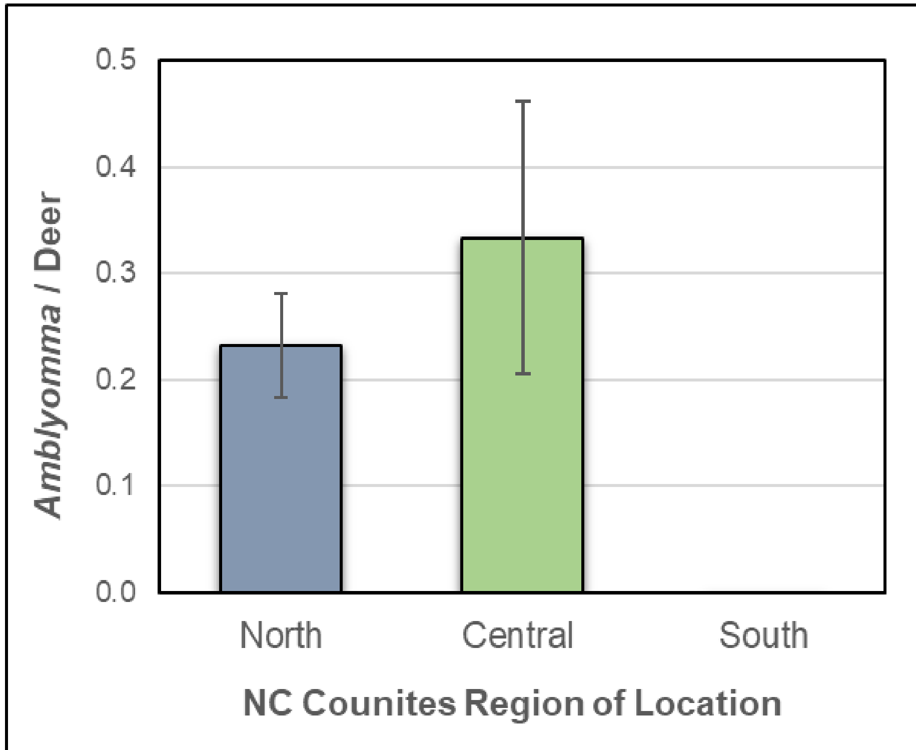

Figure 3.5. ***Amblyomma americanum*** **per Deer in a North-to-South Distribution.** North Carolina counties are ordered along a north-to-south gradient (Error bars = SE).

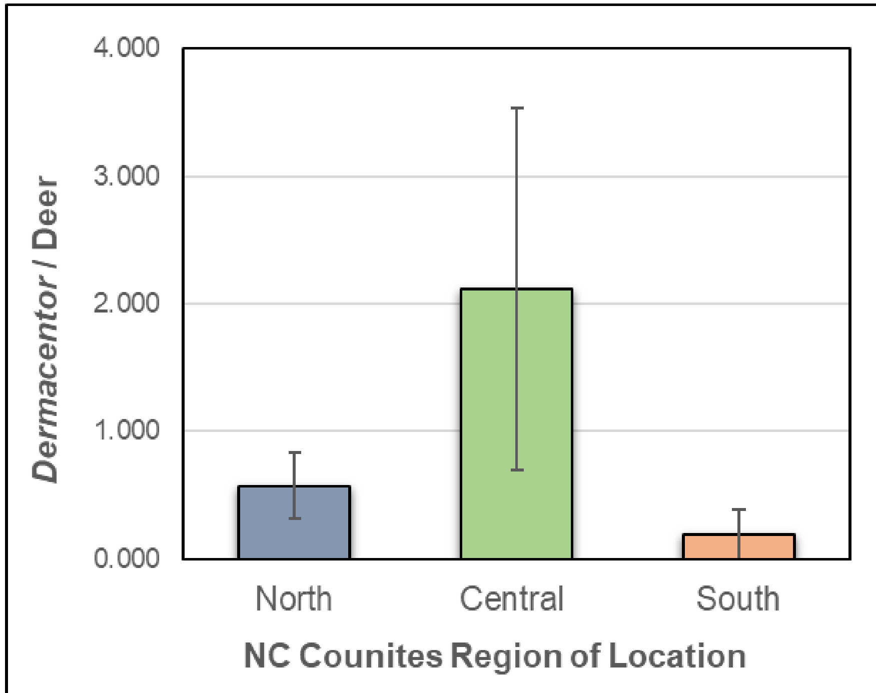

Figure 3.6. ***Dermacentor albipictus*** **per Deer in a North-to-South Distribution.** North Carolina counties are ordered along a north-to-south gradient (Error bars = SE).