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LYME DISEASE ECOLOGY IN THE EASTERN UNITED STATES: AN OVERVIEW

by

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Under the direction of Dr. Elizabeth Gleim
Senior Thesis in Biology

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Abstract

Lyme disease is the most common vector-borne disease in the United States of America (USA). In North America, Lyme disease is caused by *Borrelia burgdorferi* sensu stricto (s.s.) and vectored by the blacklegged tick, *Ixodes scapularis*. Historically, Lyme disease has been primarily restricted to the northeastern USA, despite *I. scapularis* occurring throughout the entire eastern region. There are genetically unique northern and southern populations of *I. scapularis*. The differences between these two populations have been hypothesized to be driving the difference in Lyme disease case incidence in the northeastern and southeastern United States. For example, *I. scapularis* nymphs feed before larvae in northern populations, but feed at the same time (or the larvae feed just before the nymphs) in southern populations. This impacts Lyme disease incidence because if an infected nymph feeds on a host before larvae, the larvae are more likely to be infected during their first feed. Accordingly, this would result in an increased pathogen prevalence in northern populations of *I. scapularis* and thus, increased numbers of Lyme disease cases. Also, northern ticks demonstrate different host seeking behavior than southern ticks. Specifically, northern populations host seek above the leaf litter, compared to southern ticks that host seek below leaf litter. This leads to more Lyme disease cases in the North because ticks that host seek above leaf litter are more likely to attach to humans. There is strong evidence indicating that northern *I. scapularis* are migrating northwards and southwards which is resulting in the emergence of Lyme disease in new regions. This review will examine the ecology of Lyme disease and its associated vector in the United States, including its more recent expansion into Canada and the southeastern USA.

Lyme Disease General Background

Lyme disease was first described in 1977 following an investigation conducted by the Centers for Disease Control and Prevention (CDC) in Lyme, Connecticut (the place for which Lyme disease later received its name) after multiple children in the area exhibited signs of arthritis (Bacon et al. 2008, Steere et al. 1977). *Borrelia burgdorferi* sensu stricto (s.s.). was later identified as the pathogen that causes Lyme disease, and it was the first *Borrelia* member of the *Borrelia burgdorferi* sensu lato (s.l) species complex (Bacon et al. 2008, Burgdorfer et al. 1982, Benach et al. 1983, Steere et al.1983, Rudenko et al. 2011). It was not until 1981, that the tick species, *Ixodes scapularis* (Acari: Ixodidae: formerly known as *Ixodes dammini*) was confirmed to be the vector of those first reported cases (Burgdorfer et al. 1982, Oliver et al. 1993, Smart & Caccamise 1988).

Today, with an estimated 476,000 cases occurring annually, Lyme disease is the most common vector-borne disease in North America (CDC 2024c). The majority of the cases of Lyme disease occur in the northeastern USA and upper Midwest (CDC 2024d), even though *I. scapularis* ticks occur throughout the entire eastern USA (more on this later). The most common symptoms of Lyme disease include rashes, joint and muscle aches, fevers and chills, headaches, stiff neck, and erythema migrans (which is frequently referred to as a “bulls-eye rash”). If Lyme disease is not treated promptly, more severe symptoms can develop including facial palsy, arthritis with severe joint pain and swelling, irregular heartbeat, inflammation of brain and spinal cord, and nerve pain. When treated promptly, antibiotics are an effective treatment for Lyme disease, and will have no long-term impacts on the patient. However, in some cases, particularly when diagnosis and/or treatment is delayed, ongoing symptoms can occur after treatment which is referred to as post-treatment Lyme disease syndrome (PTLDS) (Middelveen et al. 2018). Symptoms of PTLDS include speech problems, decreased short-term memory, decreased concentration, pain and swelling in major joints, aching muscles, and restless sleep (Giorgi 2018).

It's not entirely clear what causes PTLDS (CDC 2024b), but there are three main theories, 1) *B. burgdorferi* s.s. clusters together such that antibiotics are not able to fully access and kill all of the bacteria (Lantos 2015, Gurarie 2023). These remaining bacteria can later reactivate and cause inflammation, 2) Lyme disease may trigger an underlying autoimmune disorder, meaning patients will show symptoms of illness without active *B. burgdorferi* s.s. infection (Gutierrez-Hoffman et al. 2020, Gurarie 2023), or 3) the cell debris of inactivated bacteria post-antibiotic treatment triggers an immune response in some patients (Bockenstedt et al. 2012, Rebman et al. 2017, Gurarie 2023).

The medical community is still working to determine treatments for PTLDS. Historically, doctors frequently prescribed antibiotics to treat PTLDS long-term and today, some doctors still utilize this approach (Wong et al. 2021, Maksimyan et al. 2021, Chung et al. 2023). However, multiple studies have shown that prolonged use of antibiotics is not effective in resolving symptoms associated with PTLDS (CDC 2024b). Furthermore, long-term use of antibiotics is especially concerning given the fact that it can disrupt the gut microbiota of the patient and/or lead to antibiotic resistance which can ultimately lead to potentially fatal complications like sepsis and colitis (Baggs et al. 2018, CDC 2024b).

Borrelia burgdorferi sensu lato & sensu stricto

Since its first description in 1977, locally acquired cases of Lyme disease have since been documented throughout much of North America, Europe, and parts of Asia and more recently appears to be emerging in Central America, South America, and Asia (Ozdenerol 2015, Lucca et al. 2024). We now know that globally, Lyme disease is caused by several different members within the species complex, *B. burgdorferi* s.l. (a diverse and complex group of closely related bacterial spirochete species around the world), and that *B. burgdorferi* s.l. is vectored by ticks from the *Ixodes* genus (Hao et al 2011, Rudenko et al. 2011). Historically, *B. burgdorferi* s.l. only included eight *Borrelia* species (Wang et al. 1999), although not all species within the complex are pathogenic. However, we now know that there are 20 accepted species in *B. burgdorferi* s.l. (Wolcott et al. 2021). Specifically, Europe and Asia have the following *B. burgdorferi* s.l. species: *Borrelia bissetitiae*, *Borrelia spielmanii*, *Borrelia lusitaniae*, *Borrelia garinii*, *B. burgdorferi* s.s., *Borrelia valasiana*, *Borrelia bavariensis*, *Borrelia afzelli*, *Borrelia turdi*, *Borrelia yangtzensis*, *Borrelia japonica*, and *Borrelia tanukii* (Wolcott et al. 2021). North, Central, and South America have the following *B. burgdorferi s.l.* species: *Borrelia mayonii*, *Borrelia andersonii*, *Borrelia kurtenbachii*, *Borrelia califroniensis*, *Borrelia bissettiae*, *B. burgdorferi* s.s., *Borrelia carolinensis*, *Borrelia americana*, and *Borrelia chilensis* (Wolcott et al. 2021).

While there are a total of 20 species of *Borrelia* in the sensu lato complex, only four of these species have been documented to cause illness (Rudenko et al 2011, Ivanova et al 2014). Specifically, *B. burgdorferi* s.s., *B. afzellii*, *B. garinii*, and *B. spielmanii* are pathogenic and cause Lyme disease in humans (Baranton & De Martino

2009). *B. burgdorferi* s.s. is found in North America and Europe (Castillo-Ramírez et al. 2016), *B. afzellii* occurs in Europe and Asia (Demicco et al. 2018), *B. garinii* occurs in Europe and North America (Smith Jr. et al. 2006), and *B. spielmanii* is found in Europe (Herzberger et al. 2007). While there are many species in the sensu lato complex, only *B. burgdorferi* s.s. causes cases of Lyme disease in the United States (Baraton & De Martino 2009). In particular, because Lyme disease was first discovered in the United States, *B. burgdorferi* s.s. is arguably the most well-studied cause of Lyme disease compared to the other three pathogenic species in the sensu lato complex.

Strain-typing of *B. burgdorferi* s.s.

While Lyme disease has been a focus of many studies since its first discovery, it was not until the mid-1990s that the scientific community began working to understand how different strains of *B. burgdorferi* s.s. can cause different symptoms in patients. A restriction fragment length polymorphism (RFLP) analysis which targeted the 16S-23S rRNA internal transcribed spacer (ITS) region of *B. burgdorferi* s.s. was developed by Liveris et al. (1996). Through this work, blood and skin cultures from patients with Lyme disease in New York were analyzed and three different strains of *B. burgdorferi* s.s. were identified: type 1, type 2, and type 3 (Liveris et al. 1996). These different strains of the pathogen are also associated with different levels of disease severity. Strain type 1 is associated with more severe symptoms, while strain type 3 is associated with less severe symptoms (Wormser et al. 1999). However, type 3, while generally resulting in mild symptoms, is known to have more variability in symptoms in patients and in some instances, the inability to cause disease at all (Wang et al. 2002). Interestingly, it’s possible for some patients to be infected with more than one strain type. This implies that a patient can be infected from multiple tick bites and/or one tick with multiple strains of *B. burgdorferi* s.s. and this can impact the way in which Lyme disease presents in these patients (Liveris et al. 1996, Liveris et al. 1999).

General Overview of *Ixodes scapularis* and its Life Cycle

The main vector of *B. burgdorferi* s.s. on the East coast of the USA is the blacklegged tick, *I. scapularis* (Re III et al. 2004). *Ixodes scapularis* has a range encompassing the entire eastern United States and parts of southern Canada (CDC 2022), reaching as far west in the USA as Texas, Oklahoma, Kansas, Nebraska, North Dakota, and South Dakota (CDC 2022, Eisen & Eisen 2023). There are two different clades of *I. scapularis* ticks, the American clade, and the southern clade. The southern clade of *I. scapularis* only occurs in the southeastern United States and has high genetic diversity (Xu et al. 2020). The American clade occurs throughout the entire range of *I. scapularis* on the East coast of the United States (Xu et al. 2020). The American clade is believed to be the result of a small subset of ticks from the southeastern USA that repopulated the Northeast after the most recent glaciation event (Van Zee et al. 2013). Therefore, founder effects have led to the American clade having low genetic diversity but notably are the only clade that occurs in the northeastern USA (Humphrey et al. 2010).

Ixodes scapularis is a three-host tick and usually takes 2-3 years to complete their life cycle (CDC 2021b, Vandyk et al. 1996). Specifically, *I. scapularis* adults will lay their eggs in the spring, and by the summer, the eggs hatch into larvae (CDC 2021b). The larvae then have their first blood meal on a vertebrate host which is typically a smaller host like birds and rodents. In the southeastern USA, larvae can also feed on lizards (Main et al. 1982, Bosler et al. 1984, Anderson et al. 1987a, Lane et al. 1991).

Borrelia burgdorferi s.s. is not transovarially transmitted (e.g. from mother to offspring), which means that the larvae that hatch cannot be infected with *B. burgdorferi* s.s. Therefore, *B. burgdorferi* s.s. is maintained in the wild via a sylvatic cycle (i.e., a cycle between animal hosts and the vector; Donohoe et al. 2015). Specifically, when an *I. scapularis* attaches to a host, the ticks release proteins in their saliva that inhibit pain, inflammation, and blood clotting in the host (Stewart & Bloom 2020). Should the tick feed on a host that is infected with a pathogen like *B. burgdorferi* s.s., the pathogen is ingested and then circulates throughout the tick (Stewart & Bloom 2020). The bacteria will ultimately establish residency in the midgut of the tick (Stewart & Bloom 2020). It is hypothesized that the midgut is the best place for pathogens to thrive because it lacks digestive enzymes and therefore has a neutral pH that will not kill pathogens (Stewart & Bloom 2020, Hajdusek et al. 2013). Once a tick is infected with *B. burgdorferi* s.s., it remains infected for life. Therefore, infected *I. scapularis* will infect any subsequent uninfected hosts it feeds on, thus perpetuating the pathogen’s cycle.

Once the larvae have completed feeding, they drop off of the host and remain in the leaf litter over the winter. Then in the spring, the larvae molt and emerge as nymphs (CDC 2021b). The nymph will then feed on another small vertebrate host, drop off into the leaf litter, molt, and emerge as an adult in the fall and winter seasons (CDC 2021b). Adults will find one more vertebrate host to feed on in order to have enough energy to reproduce and then females lay eggs in the leaf litter to complete the life cycle (CDC 2021b). Adult *I. scapularis* usually prefer larger hosts, like white-tailed deer (*Odocoileus* *virginianus*) and other mesomammals (Wilson et al. 1985, Bosler et al. 1984, Anderson et al. 1987b). The female and male *I. scapularis* ticks most frequently mate on the host (Kocan et al. 2015). Male and female *I. scapularis* ticks can sometimes mate off the host, but this is less likely (Kocan et al. 2015).

Pathogens of *Ixodes scapularis*

While *B. burgdorferi* s.s. is the most well-known pathogen associated with *I. scapularis,* it is not the only pathogen that *I. scapularis* can vector. For example, *I. scapularis*, can also vector *Anaplasma phagocytophilum* (anaplasmosis), *Babesia microti* (babesiosis), and Powassan virus (Aliota et al. 2014, Nelder et al. 2016, Xu et al. 2016). These three pathogens all share fairly non-descript symptoms including fevers, chills, nausea, headaches, loss of appetite and muscle and body aches (CDC 2019, CDC 2023b, CDC 2023d). Anaplasmosis can also cause vomiting and diarrhea (CDC 2019) and Powassan virus can frequently lead to more severe disease including encephalitis and meningitis (CDC 2023d).

These other diseases associated with *I. scapularis* have lower incidence rates as compared to Lyme disease. For example, the most recently published data indicates that there were 47 cases of Powassan virus in 2022, 1,827 cases of babeosis in 2020, and 6,729 cases in 2021 of anaplasmosis (Schwartz et al. 2021, Kugeler et al. 2021, CDC 2024b, CDC 2023c, CDC 2023a, CDC 2024a). While these cases are much lower than those that we see with Lyme disease, it’s strongly suspected that these other diseases are underreported for a number of reasons. For example, all of these diseases are associated with flu-like symptoms (fevers, chills, sweats, headache, body aches, nausea, fatigue, or loss of appetites), and frequently do not result in severe illness except for in people with pre-existing health conditions (CDC 2023b). Because of this, people with mild cases are unlikely to seek medical attention. Furthermore, there's generally less awareness of these diseases within the medical community which results in them rarely being tested for and diagnosed (CDC 2023b). Thus, it's strongly suspected that there's a higher incidence of these other tick-borne diseases than is currently being reported.

Interestingly, a single *I. scapularis* can be co-infected and transmit multiple pathogens (Aliota et al. 2014, Xu et al. 2016), which can impact the clinical signs that are seen in the infected host (Moutallier et al. 2016, Duik-Wasser & EPJ 2016, Swanson et al. 2006). For example, Swanson et al. (2006) found that along the East Coast of the United States, and Europe, 0-28% of *Ixodes* spp. were co-infected with *B. burgdorferi* s.s. and one other pathogen (either *A. phagocytophilum*, *B. microti*, or *B. divergens*). Other studies have also found *I. scapularis* co-infected with *B. burgdorferi* s.s. and other pathogens at lower rates (1-1.9%; Schauber et al.1998, Holman et al. 2004).

Ixodes scapularis hosts

Hosts are essential to both the maintenance of the *B. burgdorferi* s.s. transmission cycle and the *I. scapularis* life cycle. Generally speaking*, I. scapularis* are generalist feeders. Generalist feeders means that these ticks can feed on a variety of hosts, which can range from mice to humans (Tietjen et al. 2020). However, hosts for *I. scapularis* ticks are different depending on their life stage. Furthermore, there are genetically distinctive northern and southern populations of *I. scapularis* in the USA which are known to exhibit different host seeking behaviors. This different host seeking behavior, in conjunction with different host species distributions across the USA, has led to some notable differences in the hosts used in the northern versus southern USA.

Specifically, small mammals (such as white-footed mice, *Peromyscus leucopus*) are the main host for larvae and nymphs in the North. While small mammals also serve as hosts to larvae and nymphs in the Southeast, lizards are also important hosts for these life stages in the southeastern USA (LoGiudice et al. 2003, Apperson et al. 1993, Kollars Jr. et al. 1999, Durden et al. 2002, Spielman 1985). However, lizards are not typically utilized as hosts in the northeastern USA in part due to lizards being less common in the northeastern USA (Pester 2022).

White-footed mice are highly competent reservoirs (which means they have the ability to acquire and transmit the pathogen) for *B. burgdorferi* s.s. (Barbour 2017) while lizards are not (Clark et al. 2005). Furthermore, some lizards such as *Sceloporus occidentalis* (Western fence lizard) and *Elgaria multicarinata* (Southern alligator lizard) are able to clear the *B. burgdorferi* s.s. infection from *I. scapularis* during feeding (Lane & Quistad 1998, Swanson & Norris 2007). This is due to a protein in the lizards' blood that is a borreliacidal antibody that kills the bacteria (Lane & Quistad 1998). Thus, it has been hypothesized that this notable difference in hosts utilized by *I. scapularis* larvae and nymphs is one key factor impacting Lyme disease incidence in the North versus the South (Lane & Quistad 1998, Barbour 2017, Clark et al. 2005, Apperson et al. 1993).

Multiple types of birds can also serve as hosts to *I. scapularis* larvae and nymphs. Importantly, some songbird species, including American Robins (*Turdus migratorius*), Song Sparrows (*Melospiza melodia*), and Northern Cardinals (*Cardinalis cardinalis*) act as competent reservoirs for *B. burgdorferi* s.s. (Scott et al. 2015, Ritcher et al. 2000, Stafford III et al. 1995, Ginsberg et al. 2005). Furthermore, birds can also play a key role in long distance dispersal of these ticks (Scott et al. 2016, Cumbie et al. 2021, Brinkerhoff et al. 2019, Mitra et al. 2014). Indeed, songbirds can move ticks into new regions when they are migrating (Scott et al. 2016) and have even been implicated in establishing new populations of *I. scapularis* in the upper Midwest near the Great Lakes (CDC 2022). Migratory birds may also have played a role in Lyme disease expanding into Canada, and the southern USA (Ogden et al. 2008, Scott et al. 2019, Cumbie et al. 2021, Scott et al. 2020).

Ixodes scapularis adults tend to feed on larger sized animals like white-tailed deer throughout their entire range (Schotthoefer & Frost 2015). While white-tailed deer are not competent hosts for *Borrelia burgdorferi* s.s., they are important to the *I. scapularis* life cycle, as they serve as one of the primary hosts for adult ticks (Huang et al. 2019, Piesman & Spielman 1979, Kilpatrick et al. 2014). Furthermore, male and female *I. scapularis* ticks also mate while on white-tailed deer (Piesman & Spielman 1979, Kilpatrick et al. 2014). Thus, white-tailed deer sometimes play an indirect role in Lyme disease incidence since their population sizes have in some instances been found to influence *I. scapularis* population size (Huang et al. 2019, Kilpatrick et al. 2014, Magnarelli et al. 1993). For example, one study found that an increase in deer that are hunted in a year is linked to an increase in Lyme disease in the area (Robertson et al. 2019). This is because an increase in the number of deer hunted means that there was an increase in the abundance of deer that year which led to a higher density of ticks (Robertson et al. 2019, Kilpatrick & LaBonte 2003, Anderson et al. 1987b, Wilson et al. 1988, Wilson et al. 1990, Stafford 2001, Kilpatrick et al. 2014).

White-footed mice are the most competent reservoir for *B. burgdorferi* s.s. and several other species including squirrels, voles, and chipmunks have also exhibited a surprisingly high diversity of *B. burgdorferi* s.s. genotypes, arguably implying that they may be highly competent reservoirs for *B. burgdorferi* s.s. as well (Mechai et al. 2016). A link between biodiversity and the prevalence of *B. burgdorferi* s.s. has been observed and this has led to a concept called the dilution effect (Ostfeld & Keesing 2000). The basic concept of the dilution effect is that because different hosts have different reservoir competencies, when there is an increase in biodiversity, this decreases *B. burgdorferi* s.s. prevalence in a system where multi-host ticks live (Ostfeld & Keesing 2000, Schmidt & Ostfeld 2001, Van Buskirk & Ostfeld 1995, LoGiudice et al. 2003, Ostfeld & LoGiudice 2003, Allan et al. 2003, LoGuidice et al. 2008, Keesing et al. 2006). This is because, the more host species that you have in one region, the more species who have low reservoir competency will be present, thus “diluting” the number of highly competent reservoirs, like the white-footed mouse. This dilution of competent reservoirs will reduce the prevalence of *B. burgdorferi* s.s. in *I. scapularis*.

In addition to white-tailed deer there are several other species that are considered to have low reservoir competency (and therefore are considered dilution hosts) including the Virginia opossum (*Didelphis virginiana*), which nymphs may feed on during their blood meal (LoGiudice et al. 2003). In addition to Virginia opossums having low reservoir competency, they are also good at grooming off and killing ticks by consuming them (Elzinga 2020). Thus, it’s been speculated that the Virginia opossum may play a role in decreasing or controlling Lyme disease (Kirchner 2021, Elzinga 2020, Hennessy & Hild 2021). Other low reservoir competency hosts include raccoons

(*Procyon lotor*), southern flying squirrels (*Glaucomys volans*), eastern gray squirrels (*Sciurus carolinensis*), North American red squirrels (*Tamiasciurus hudsonicus*), Veeries (*Catharus fuscescens*), Gray Catbirds (*Dumetella carolinensis*), Wood Thrushes (*Hylocichla mustelina*), and American Robins (*Turdus migratorius*; Hersh et al. 2014, Brisson & Dykhuizen 2004, Brisson et al. 2008, LoGiudice et al. 2003).

In addition to hosts that have high and low reservoir competency, some hosts have moderate competency. Moderately competent hosts for *B. burgdorferi* s.s. include masked shrews (*Sorex cinereus*) and eastern chipmunks (*Tamias striatus*; Hersh et al. 2014, Brisson & Dykhuizen 2004, Brisson et al. 2008, LoGiudice et al. 2003). While these hosts are somewhat considered dilution hosts as compared to white-footed mice and other highly competent species, they do still play a role in the sylvatic cycle of *B. burgdorferi* s.s.

Differences Between Northern and Southern *I. scapularis* Populations

As previously noted, there are two distinct populations of *I. scapularis*, a northern and southern population. Southern populations are below Pennsylvania, Ohio, Indiana, and Illinois (Sakamoto et al. 2014). This cut off was because of a glacial event killing off Northern ticks, and then southern ticks expanding back up into the North (Sakamoto et al. 2014). One distinct difference between northern and southern populations is their host seeking behavior (Arsnoe et al. 2019). Specifically, the larvae and nymphs of northern populations host seek above leaf litter, while the larvae and nymphs of southern populations host seek below the leaf litter. For example, one study that tested nymphs in the South vs the North showed nymphs in southern populations quest at lower heights and exhibit hiding behavior (Tietjen et al. 2020) in which the tick tucks itself in the clay or under leaf litter and remains motionless (Tietjen et al. 2020).This is contrasted with northern ticks that spent more time questing, e.g. host seeking at the top of low-lying vegetation (Tietjen et al. 2020). These differences in host seeking behaviors between *I. scapularis* larvae and nymphs has been suggested to explain, in-part, the variation of Lyme disease risk in the northern versus southern USA (Arsnoe et al. 2019, Arsnoe et al. 2015). Specifically, since northern *I. scapularis* nymphs seek above host litter they are more likely to attach to and infect humans, compared to southern nymphs that quest below the leaf litter where they are less likely to encounter and crawl onto humans.

It's thought that these differences in host seeking behavior are likely due to differences in climate between the northern and southern USA. Specifically, southern populations of *I. scapularis* tend to have greater mortality risk due to increased desiccation stress in the South associated with its warmer climates (Ginsberg et al. 2017). Accordingly, southern *I. scapularis* larvae and nymphs exhibit behaviors like laying under leaf litter, which helps retain moisture and therefore reduces their mortality risk (Ginsberg et al. 2017).

It has also been hypothesized that these differences in host seeking behavior also make northern ticks more likely to get on a competent host like white-footed mice which are more likely to travel on top of the leaf litter rather than through it. Alternatively, southern ticks staying down in the leaf litter are less likely to get on white-footed mice and humans and more likely to get on non-competent reservoirs like lizards which may spend more time down in the leaf litter (Morris et al. 2022). These differences in the wildlife hosts that northern and southern ticks use are thought to be another reason why

Lyme disease is so common in the Northeast and yet rarely occurs in the Southeast (Ginsberg et al. 2017, Arsnoe et al. 2015, Ginsberg et al. 2021).

While all *I. scapularis* ticks have a similar lifecycle, the phenology (e.g. when the different life stages are active throughout the year) of northern and southern populations of *I. scapularis* also differ in some important ways. For example, adult *I. scapularis ticks* in northern populations reach peak activity in autumn and early spring and are not active during winter (due to extreme cold temperatures), while adult *I. scapularis* in southern populations are active throughout the winter (Ogden et al. 2018). Furthermore, nymphs feed in the spring and early summer, before larvae emerge in the northeastern USA (Fish 1993, Gatewood et al. 2009, Ogden et al. 2018). However, in the southeastern USA we see *I. scapularis* nymphs and larvae either feed during the same time or larvae feed just prior to the nymphs (Clark et al. 1998, Ogden et al. 2018).

These phenological differences are an important factor when it comes to establishing or breaking the sylvatic cycle for the maintenance of *B. burgdorferi* s.s. in the wild. Specifically, when the nymphs feed before the larvae, which is what occurs in the North, this allows the nymphs to infect wildlife hosts, which will then infect the larvae once they emerge and begin feeding on those same wildlife hosts (Fish 1993). However, southern larvae feed prior to or at the same time as nymphs, making it less likely for larvae to feed on infected hosts (Clark et al. 1998, Ogden et al. 2018). Therefore, the order in which larvae and nymphs feed, relative to one another, is hypothesized to be critical to maintaining *B. burgdorferi* s.s. in *I. scapularis* populations from year to year and is thought to be yet another reason that northern populations of *I. scapularis* have such a high prevalence of *B. burgdorferi* s.s. compared to southern populations. This, in turn, translates to high Lyme disease case incidence in the Northeast (Gatewood et al. 2009).

As previously noted, there are multiple strains of *B. burgdorferi* s.s. that are associated with more or less severe Lyme disease symptoms in humans. Strain type 1, which is associated with more severe cases of Lyme disease, has been found to be selected for in *I. scapularis* populations that exhibit these non-synchronous nymph and larvae peaks which is observed in northern populations (Gatewood et al. 2009). Interestingly, these non-synchronous peaks have been observed in Southwest Virginia where Lyme disease has more recently become endemic (Lantos et al. 2015). Therefore, it's hypothesized that strain type 1 could be more prevalent in southwestern Virginia and potentially contributing to the higher Lyme disease incidence in this region as people are more likely to seek medical attention and get diagnosed if they have more severe symptoms.

The Expansion of Northern Populations of *Ixodes scapularis*

Northern *I. scapularis* populations are expanding both northwards into Canada and southwards into the southeastern USA (Brinkerhoff et al. 2014, Morris et al. 2022, Ogden et al. 2008, Scott et al. 2019, Cumbie et al. 2021, Scott et al. 2020, Ogden et al. 2006). These range expansions have led to Lyme disease emerging in these locations in which it has not historically occurred (Ogden et al. 2008). In addition to Lyme disease, other diseases associated with *I. scapularis* have emerged or begun increasing in these locations as well (Bouchard et al. 2019).

Numerous studies have examined the northern expansion of *I. scapularis* into Canada. Ogden et al. (2008) captured 39,095 birds and tested the ticks found on them to predict how there will be a northern expansion of Lyme disease in eastern Canada, and Ontario. Scott et al. (2019) tested ticks on songbirds that were flying into Canada from the Neotropics, Mexico, and the United States, and their findings supported the idea that migratory songbirds' long distance northern migrations was spreading ticks that are infected with *B. burgdorferi* s.s. into Canada. Furthermore, Scott et al. (2020) collected *I. scapularis* ticks in Quebec from birds and tested them for pathogens and found that it would only take six infected *I. scapularis* to establish a Lyme disease endemic area. Finally, multiple studies have predicted the expansion of Lyme disease into more northern regions of eastern Canada and also into West Canada based on models using to predicted temperature changes that will be caused by climate change. (Simon et al. 2014, Cheng et al. 2017, Ogden et al. 2005, Leighton et al. 2012).

Historically, Lyme disease has been restricted to the northeastern USA, not extending beyond Maryland. Interestingly, one study that tested museum specimens of ticks and mice showed that *B. burgdorferi* s.s. was actually present in parts of New England as far back as the late 19th century (Steere et al. 2004). However, the southern expansion of Lyme disease was first observed in 2007 when there was a substantial increase of cases in Virginia (Lantos et al. 2015). In 2006 there were about 400 cases of Lyme disease in Virginia, but in 2007, cases spiked to about 1,000 (Lantos et al. 2015). These cases have continued to grow since then with 1,350 cases of Lyme disease occurring in Virginia by 2016 (Virginia Department of Health 2016). Data indicates that this increase was not due to a change in the case definition, but rather appears to be a genuine increase in Lyme disease incidence (Lantos et al. 2015).

Following the marked increased in Lyme disease cases in Virginia, studies were launched to better understand why this increase in cases was occurring (Brinkerhoff et al. 2014, Lantos et al. 2015). Initial geospatial analysis of cases indicated a distinct spatial trend in which the increase in Lyme disease cases was concentrating along the Appalachian Mountains in western Virginia, with a distinct hotspot in southwestern Virginia (Lantos et al. 2015). Meanwhile, there were still few cases of Lyme disease in Southeast and Central Virginia (Lantos et al. 2015). Specifically, one study found that Southwest Virginia had 25-99.9 cases per 100,000 people, while counties in Southeast Virginia ranged from 0-49.9 cases per 100,000 people (Brinkerhoff et al. 2014).

To further examine this, Brinkerhoff et al. (2014) were the first to perform a field-based study to explore the drivers of this southern expansion of Lyme disease and furthermore, to examine why Lyme disease cases were concentrating in western Virginia. To do this, 204 *I. scapularis* nymphs were collected from 4 sites across Virginia (east to west) and tested them for *B. burgdorferi* s.s. (Brinkerhoff et al. 2014). Through that work, they found that the site tested in the Appalachian Mountains in western Virginia had the highest prevalence of *B. burgdorferi* s.s. at 20%. However, sites in Central and Southeast Virginia only had a pathogen prevalence of 0% to 0.6% (Brinkerhoff et al. 2014). This was notable because *Ixodes scapularis* are known to have a much higher prevalence of *B. burgdorferi* s.s. in the northeastern USA, where Lyme disease is endemic.

Brinkerhoff et al. (2014) also performed a phylogenetic analysis on the ticks they collected across the state and found that they were all from the American clade (Brinkerhoff et al. 2014), which occurs throughout the eastern USA but is the only clade to occur in the Northeast. However, previous studies that were conducted in the early 2000's in Virginia had found a mix of the American and Southern clade (Qiu et al. 2002). Collectively, this data, while not definitive, seemed to support the hypothesis that northern populations of *I. scapularis* were expanding southwards into Virginia and therefore driving the increase in Lyme disease cases in the state.

To follow-up on these findings, Morris et al. (2022) compared the phenology and abundance of *I. scapularis* in southwestern and southeastern Virginia, which was the first comparative study of its kind in the state. This study showed that nymphal activity occurred before larval activity in southwestern Virginia (as is seen in northern populations; Fish 1993, Gatewood et al. 2009, Ogden et al. 2018), and in southeastern Virginia, nymphs and larvae were active at the same time, which is more like southern populations (Roger 1953, Clark et al. 1998, Ogden et al. 2018). Furthermore, Morris et al. (2022) found that more *I. scapularis* larvae and nymphs were collected in southwestern Virginia than in southeastern Virginia. Because all life stages of *I. scapularis* had been collected and a comparative study design had been utilized, they were able to determine that the differences in number of ticks collected were not due to differences in abundance, but rather, differences in host seeking behaviors. Specifically, the Southwest locations collected more nymphs and larvae but had collected the same number of adults as compared to the southeastern locations (Morris et al. 2022). This difference in host seeking behavior could help support the theory of expansion of northern *I. scapularis* into western Virginia since seeking on or above leaf litter is more associated with northern populations of *I. scapularis* (Arsnoe et al. 2015, Arsnoe et al. 2019).

Subsequent studies doing phylogenetic analyses of *I. scapularis* in Virginia have provided additional data indicating that *I. scapularis* from northern populations have expanded southwards (Van Zee et al. 2015, Xu et al. 2020, Jackson 2020). For example, Van Zee et al. (2015), conducted genetic analyses of *I. scapularis* from throughout its range and found that there is unidirectional gene flow from the North to the South (Van Zee et al. 2015). This implies that northern populations of *I. scapularis* are moving down into southern populations but that southern populations are not migrating northwards.

Furthermore, Xu et al. (2020) found that in Virginia the ticks belonging to the haplotypes of the southern populations had a lower prevalence of *B. burgdorferi* s.s. than ticks from Virginia that had haplotypes of northern populations. Finally, Jackson (2020) tested *I. scapularis* collected during the field work conducted by Morris et al. (2022) in southwestern Virginia and found that all *I. scapularis* collected there were from the American clade. Furthermore, Jackson (2020) found 16 different haplotypes within southwestern Virginia. Of the 16 haplotypes, seven had been previously reported in Virginia (Brinkerhoff et al. 2014). However, 9 of the haplotypes identified were unique (Jackson 2020). This diversity of haplotypes may indicate that these populations in Southwest Virginia are distinct from elsewhere in the state which would further support the hypothesis that *I. scapularis* are expanding from the North (Brinkerhoff et al. 2014, Jackson et al. 2020)

Jackson (2020) also tested *I. scapularis* nymphs collected from southwestern Virginia for *B. burgdorferi* s.s., but only found a 3.8% prevalence. *B. burgdorferi* s.s. prevalence in nymphs in the Northeast ranges from 21% - 38% (Foster et al. 2023, Lehane et al. 2021), however, the pathogen prevalence Jackson (2020) found was closer to 0-3% prevalence in the southeastern USA (Foster et al. 2023, Little et al. 2019, Lehane et al. 2021). A subsequent study conducted in southwestern Virginia found a 15.3% prevalence in nymphs (Whitlow et al. 2022). The lower prevalence found by Jackson (2020) may have been due to the fact that 1) ticks were collected in 2018 through 2019 [earlier than Whitlow et al. (2022)], and 2) were further south than the western site utilized from Brinkerhoff et al. (2014). Therefore, the nymphs tested by Jackson (2020) may have been at the frontline of the southern expansion in Virginia at that time, when *B. burgdorferi* s.s. would have just been establishing itself in the wildlife and tick populations in the region. The fact that several years passed between when Jackson (2020) had collected ticks and Whitlow et al. (2022) collected ticks likely indicates that *B. burgdorferi* s.s. is now better established in Southwest Virginia.

This higher pathogen prevalence in western Virginia has also been reported in adult *I. scapularis*. For example, *I. scapularis* adults collected in Southwest Virginia have been found to have a 36.3% *B. burgdorferi* s.s. prevalence (Aguirre & Gleim, unpublished data). Two studies also done in Blacksburg (a city in Southwest Virginia) found that *I. scapularis* adults had a pathogen prevalence of 32.9% (Herrin et al. 2014), and 37.6% (Whitlow et al. 2022). Collectively, the pathogen prevalence in adult ticks from southwestern Virginia is like the prevalence of *B. burgdorferi* s.s. in northern populations of *I. scapularis*. Specifically, *I. scapularis* adults in the Northeast have been found to have *B. burgdorferi* s.s. prevalence ranging from 33% - 58% (Foster et al. 2023, Little et al. 2019, Lehane et al. 2021).

Lyme disease has now become endemic in western Virginia and has continued to expand further southwards along the Appalachian Mountains into North Carolina and Tennessee. For example, the highest Lyme disease incidence rates in North Carolina now occur in the northwest region of the state along the Appalachian Mountains (Quillin 2020). Furthermore, in Tennessee, case numbers have been steadily increasing since 2014, with 39 cases occurring in 2023 (Tennessee Department of Health 2024). The highest case numbers in Tennessee occur on the northeastern side of the state in the Appalachian Mountains (Dellinger 2024).

It is still not entirely clear why cases are emerging in the Appalachian Mountains and not elsewhere. However, it’s been hypothesized that the Appalachian Mountains are ideal habitat for *I. scapularis* and the main reservoir of *B. burgdorferi*, the white-footed mouse (Li et al. 2014). Specifically, it’s been found that higher elevation and increased vegetation density (both found in the Appalachian Mountains) are associated with an increase of Lyme disease cases in Virginia (Lantos et al. 2021). However, Morris et al. (2022) found no significant association with *I. scapularis* abundance and elevation. Thus, additional studies are needed to determine why Lyme disease cases are primarily confined to the Appalachian Mountains within the context of the southern expansion of Lyme disease. In particular, studies examining wildlife host movement and utilization within the Appalachian Mountains as well as weather and microclimatic conditions should be examined in the future.

Human Activity Impacts Lyme Disease Incidence

Human behavior and actions are known to impact *B. burgdorferi* s.s. prevalence and Lyme disease incidence by impacting *I. scapularis* populations and their hosts. One example would be the reforestation and land use change that is happening in the northeastern USA. The northeastern USA experienced extensive deforestation upon the arrival of European colonists. However, when colonists began expanding westward in the 1800's, some of the farms in the Northeast were abandoned, thus allowing them to become naturally reforested (Russo 2015). However, by the 1970s, people started moving back into the suburbs near forests (Brown et al. 2005, Russo 2015). Many epidemiological studies have since shown a positive correlation between Lyme disease case incidence and reforestation (Lastavica et al. 1989, Glass et al. 1995, Kitron & Kazmierczak 1997, Maupin et al. 1991). The proposed reason for this correlation is because reforestation leads to an increase in white tailed deer and other wildlife populations (Lastavica et al. 1989). Therefore, this increase in wildlife host abundance has been documented to lead to an increase in tick abundance (Barbour & Fish 1993, Piesman et al. 1979, Spielman et al. 1985). Since humans frequently live near or in these reforested areas, they are more likely to encounter ticks and thus contract *B. burgdorferi* s.s. (Russo 2015, Boyer et al. 2022). Thus, while reforestation is an important part of conservation efforts, the encroachment of humans on wildlife and their preferred habitats could have potential negative public health impacts by inadvertently increasing Lyme disease incidence, among other infectious diseases.

Another human activity that may impact Lyme disease incidence is through hunting. Hunting can have both negative and positive impacts on Lyme disease incidence. For example, hunting white-tailed deer can keep deer populations under control and thus reduce *I. scapularis* populations. In contrast, hunting of predators such as coyotes have led to increases in deer and rodent populations which are important host populations for *I. scapularis* (Kilpatrick et al. 2017). This increase in deer and rodent populations in certain regions leads to an increase in Lyme disease cases (Kilpatrick et al. 2017).

Interestingly, increases in Lyme disease cases has also been found to be correlated with the decline in red foxes in New England (which hunts small mammals such as the white-footed mice more so than coyotes), and the expansion of coyotes which directly compete with red foxes (Levi et al. 2012, Dantas-Torres 2015). The expansion of coyotes into the northeastern USA is attributed to the extirpation of gray wolves (*Canis lupus*) which historically were apex predators in the region prior to humans extirpating them in the early 1900's (Levi et al. 2012).

Finally, human-driven climate change has also impacted Lyme disease incidence in several ways. For example, as previously described, climate change has led to the expansion of Lyme disease into more northern regions (e.g., Canada) (Feria-Arroyo et al. 2014, Simon et al. 2014, Cheng et al. 2017). Ticks have also been found to have extended activity periods due to prolonged warm seasons and shorter winters (Bouchard et al. 2019, Süss et al. 2019). Climate change will not only impact Lyme disease cases in North America, but it's also predicted to impact cases in Asia and Europe (Revich et al. 2012, Voyiatzaki et al. 2022, Vladimirov et al. 2021). However, one potential positive impact that climate change may have on Lyme disease incidence is that high temperatures cause ticks to desiccate rapidly and may alter their host seeking behavior (keeping them down in the leaf litter where they are less likely to contact humans). In addition, it is thought that ticks in warmer climates may produce fewer eggs, thus reducing their abundance (Beament 1959, Fish 1993, Ginsberg et al. 2017).

Conclusions

Lyme disease is a public health threat in the United States due to the high number of cases that occur, and the potentially severe and/or chronic symptoms associated with the disease. Because of this, the need to understand the ecology of this complex vector-borne disease is critical to better mitigating disease risk to humans. Differences in the phenology, host seeking behavior, and wildlife host selection of northern and southern populations of *I. scapularis* seem to be the primary reasons that Lyme disease is so common in the northeastern USA, yet rarely occurs in the Southeast. Northern *I. scapularis* ticks are expanding both northwards into Canada and southwards into Virginia, North Carolina, and Tennessee, leading to increasing numbers of Lyme disease cases in these areas. While climate change is understood to be the driver of this northern expansion, it is still unclear what’s driving the southern expansion. Accordingly, additional studies to better understand what’s driving the southern migration of northern populations of *I. scapularis* are needed. Furthermore, efforts should be made to raise awareness of Lyme disease risk and prevention measures in these regions in which Lyme disease is now emerging.

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