BORRELIA BURGDORFERI PREVALENCE IN NYMPHAL *IXODES SCAPULARIS* ACROSS DELAWARE

by

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ABSTRACT

We performed a PCR protocol on 111 *Ixodes scapularis* nymphal ticks to identify *Borrelia burgdorferi* infections. The Delaware Department of Natural Resources and Environmental Control collected tick samples throughout New Castle, Kent, and Sussex County in the state of Delaware with the goal of calculating *B. burgdorferi* prevalence at each collection site. We found that the state of Delaware has an overall 27.03% prevalence of *B. burgdorferi* in *I. scapularis* ticks. *I. scapularis* is the main vector for *B. burgdorferi* in the northeastern United States and so sampling its population is crucial to gain a better understanding of the ecology of the bacterium. The PCR we used targeted the variable outer surface protein C locus. Future sequencing of this PCR amplicon will allow us to distinguish specific *B. burgdorferi* genotypes. Some *B. burgdorferi* genotypes appear more likely to infect humans than others. Identifying genotypes known to cause infection in humans may lead to us more accurately assesses the human risk of Lyme disease in Delaware.

Chapter 1

INTRODUCTION

Borrelia burgdorferi, the causative agent of Lyme Disease, is a gram negative obligate, internal parasitic bacterium transmitted between vertebrate hosts and hard-bodied ticks of the genus *Ixodes* (Obiegala et al. 2017). *Ixodes scapularis* vectors *B. burgdorferi* and thus plays a critical role in the bacterium's life cycle. *I. scapularis* larvae hatch and feed on the blood of their first host, typically a small mammal (e.g., white-footed mouse, *Peromyscus leucopus*) or a ground foraging bird, in the summer. After feeding, larvae molt into nymphs and overwinter on the forest floor. Nymphs become active the following spring, seeking and feeding on another host (including humans), before molting into adults. That fall, adult ticks will feed on their final host, reproduce, and then die (Ostfeld 2011). Brission and Dykhuizen (2004) and Ostfeld (2011) have suggested that *I. scapularis* most frequently becomes infected with *B. burgdorferi* as larvae while feeding on infected small mammals, making *I. scapularis* nymphs the first stage in which the tick can transmit the bacterium.

In humans, infection by *B. burgdorferi* causes Lyme disease, named after Lyme, Connecticut, the town where it was first described. Lyme disease is the most common vectored-transmitted disease in the United States (Snydam 2017). The symptoms of the disease may start with a slowly expanding skin lesion accompanied

by flu-like symptoms, and without treatment, can evolve into mono- or oligo-articular arthritis and/or neurologic abnormalities (Steere et al. 2004). Humans have a greater risk of coming into contact with *I. scapularis* nymphs infected with *B. burgdorferi* in fragmented habitats (e.g., parks, hiking trails in small forest patches, or small woodlots near homes) as reduction in forest patch size has been proposed to be positively correlated with Lyme disease risk (Ostfeld and Keesing 2000). *I. scapularis* is more likely to be infected with *B. burgdorferi* in fragmented habitats due to differences in the vertebrate communities of small, fragmented patches of forest relative to large expanses of forest. For example, fragmented forest patches may have fewer predators (e.g., red foxes, *Vulpes vulpes*) than bigger forest patches leading to larger populations of white-footed mice and other host species (e.g., eastern chipmunks, *Tamias striatus*) that are highly competent reservoirs of *B. burgdorferi* (Brisson et al. 2007; LoGuidice et al. 2008; Levi et al. 2012). White-footed mice also show a negative relationship between population density and habitat size, often leading to this species reaching higher population densities in small, fragmented habitat patches compared with large contiguous forests (Nupp and Swihart 1998).

Reservoir hosts are species infected by a parasite or pathogen that serve as a source of infection to another species, possibly through the use of a vector. Reservoirs that are highly competent have a high probability of passing the parasite/pathogen on to other hosts, often because they experience high infection intensities (i.e., high numbers of parasites infecting individual hosts). Furthermore, large forest patches can host a greater overall diversity of reservoir hosts, including low competence reservoir

hosts (i.e., reservoir hosts that are unlikely to lead to *B. burgdorferi* transmission), than smaller forest patches. This greater number of poor reservoir hosts in large forest patches can make *B. burgdorferi* become "diluted" within an ecosystem, leading to an overall decrease in *B. burgdorferi* transmission and fewer infected ticks (Schmidt and Ostfeld 2001).

B. burgdorferi is the causative agent of Lyme disease in humans, but humans seem to only become infected from *B. burgdorferi* that have particular *ospC* alleles that allow the pathogen to overcome the human immune system (Brission and Dykhuizen 2004; Dykhuizen et al. 2008). The *ospC* gene is the most variable gene in the *B. burgdorferi* genome and codes for a cell surface protein that is one of the first and most targeted antigens by the vertebrate host's immune system (Grimm et al. 2004; Wilske et al. 1993). Variability in *ospC* can be explained by strong selective pressure from the vertebrate immune system (Wang et al. 1999). Different host species have been found to be infected with unique combinations of *ospC* genotypes (Brission and Dykhuizen 2004). Genotypes are lineages of the bacterium characterized by their unique *ospC* allele. These genotypes appear to be non-recombining (Brisson et al. 2012). While only a subset of *ospC* genotypes infect humans, estimates of the prevalence of *B. burgdorferi* in *I. scapularis* ticks rarely differentiate between *ospC* genotypes. Thus, prevalence estimates of *B. burgdorferi* may overestimate infection risk to humans. By gaining a better understanding of the distribution of *ospC* genotypes, more targeted measures can be taken to decrease the risk of Lyme disease in humans.

The Delaware Department of Natural Resources and Environmental Control (DNREC) collected nymphal *I. scapularis* ticks across the state of Delaware and provided them to us for our study on ospC allele diversity. We screened these samples for *B. burgdorferi* infection using a PCR designed to amplify the *ospC* gene. PCR results (i.e., whether *B. burgdorferi* DNA was amplified or not as determined through gel electrophoresis) allowed us to calculate overall prevalence across the state. PCR amplicons from positive samples have been sent to the University of Delaware's Genotyping and Sequencing Center at the Delaware Biotechnology Institute for library building and short-read sequencing on an Illumina MiSeq machine. Once we have *ospC* sequences back, we will determine the prevalence of specific *ospC* genotypes. This information will allow us to more accurately estimate Lyme disease risk to humans in Delaware and serve to indirectly indicate the presence of other reservoir species (through the presence of known *ospC* alleles) in the locations the ticks were samples from. In this study, we calculated *B. burgdorferi* prevalence in *I. scapularis* nymphs across the state of Delaware and we plan to determine *ospC* genotype prevalence shortly.

Chapter 2

STUDY AREA

The state of Delaware is bordered by the Atlantic Ocean and Maryland, as well as the states of Pennsylvania and New Jersey, and hosts a variety of habitat types. Flat, sandy lands with some coastal wetlands make up most of Delaware's habitat; however, the northern part of the State falls in the Appalachian Piedmont Region and encompasses rolling hills and forest. Delaware forests are dominated by *Quercus* and *Carya spp.* (Lister et al. 2013), but with a long history of agricultural practices along with industrial and residential farming, most of the State's forested habitat has become fragmented (Northeastern Research Station 2002). Because Delaware is located in the northeastern United States and has substantial fragmented woody habitat, the state's high Lyme disease incidence rate is of no surprise (Schwartz et al. 2017).

DNREC collected nymphal *I. scapularis* ticks in seven locations across Delaware's three counties in the spring and summer of 2019. *I. scapularis* ticks were collected from fragmented areas where recreational activity is high, and people are most likely to come into contact with ticks. Delaware's most northern county, New Castle, is also the most urban. New Castle's urban and suburban areas are intermixed with fragmented forest patches with larger fragments seen in state parks. Kent and Sussex county represent the central and southern part of the state, respectively. These two counties are not as developed and urbanized as New Castle County, resulting in

areas with higher concentrations of forested land. The majority of Delaware's forested wetlands also occur across Kent and Sussex County (Lister et al. 2013).

Chapter 3

METHODS

Due to the current SARS-CoV-2 pandemic, we were unable to collect our own tick samples. Fortunately, DNREC let us use 111 *I. scapularis* samples that they collected in the spring and summer of 2019 in a variety of locations throughout Delaware's three counties. Tick collection methods included both the flag and drag method in which a 1 m^2 white cloth is attached to a dowel and dragged over the ground (dragging) or passed over vegetation by hand (flagging) and checked for ticks and equidistant intervals.

Three sets of samples were collected in New Castle County, with two sites located in Newark, DE and the other in Rockland (Wilmington), DE. Both samples taken in Newark occurred in field edge habitat. *I. scapularis* ticks were collected on a trail edge habitat in Rockland. The area sampled in Rockland was mostly composed of *Liriodendron tulipifera* and included a freshwater marsh.

Two separate *I. scapularis* samples were collected using the drag method in Leipsic, DE in Kent County in a field edge habitat. Samples were taken along the field edge in an area consisting of tidal salt marsh habitat. The last location sampled in Kent county was in Felton, DE in a forested edge habitat. The sampled area in Felton had a 66-acre millpond surrounded by hardwood forest.

Three different locations were sampled for *I. scapularis* in Sussex county. In Dagsboro, DE, samples were collected along a field edge habitat on the shores of the

inland bays. *I. scapularis* were collected along a trail edge habitat in Milton, DE that consisted of multiple habitat types including a back-barrier salt marsh, mid-Atlantic barrier island overwash, and grassland dunes. In Laurel, DE, samples were collected on a trail edge in a bald cypress tree habitat.

Individual ticks were given a specific identification consisting of a taxonomic abbreviation (Is) followed by a number indicating the order in which DNA was extracted. Each tick ID corresponds to the specific location and county in which the tick was collected.

We extracted DNA from each tick separately using a DNeasy Blood and Tissue kit from Qiagen. Preceding the DNA extraction, individual ticks were washed in a 10% bleach solution for 30 seconds followed by two separate 1 minute washes in ultrapure water to clean off any potential DNA on the outside of the tick. The tick was then bisected with a scalpel or razor blade to ensure its internal tissue would be thoroughly lysed. We conducted cell lysis and protein removal by adding both parts of the bisected tick to a 1.5-ml microcentrifuge tube containing 180 µl Buffer ATL. 20 µl of proteinase K was added and thoroughly mixed into the solution by vortexing. Samples were incubated overnight in a water bath at 56°C until only the exoskeleton remained. The following day, samples were quickly vortexed and 400 µl of a Buffer AL and ethanol mixture was added and the tube was thoroughly mixed by vortexing. Following cell lysis and protein removal, cellular debris was eliminated by pipetting the sample into a DNeasy mini spin column placed in a 2-ml collection tube. The sample was then centrifuged at 6000 x g (8000 rpm) in a Sorvall Legend centrifuge

(ThermoFisher Scientific) for 1 minute to separate the mixture from the DNA. The mixture flow-through and collection tube were discarded. The spin column was transferred a new 2-ml collection tube and 500 µl of Buffer AW1 was added. The previous steps of centrifugation and discarding the flow-through were repeated. Once again, the spin column was transferred to another 2-ml collection tube and 500 µl of Buffer AW2 was added. This time the sample was centrifuged for 3 minutes at 20,000 x g (14,000 rpm). The final step involved eluting the DNA by adding 200 µl of Buffer AE to the spin column membrane, incubating at room temperature for 1 minute and centrifuging for 1 minute at 6000 x g (8000 rpm). The Qiagen extraction procedure was followed as instructed except for one modification. To increase the total amount of DNA extract, we repeated the final step of the extraction procedure by adding an additional 200 µl of Buffer AE.

Once the DNA was isolated, we tested each sample for *B. burgdorferi* by amplifying the outer surface protein C gene in a standard PCR protocol. Our 25 µl PCR reaction included 21 µl of master mix with four components which are listed as followed: 12.5 µl of DreamTaq Hot Start Master Mix 2X (ThermoFisher cat# K9011), 1.25 µl of a 0.5 µM Oc-Fwd primer and 1.25 µl of a 0.5 µM Oc-Rev primer as described in Di et al. (2018), and 6 µl of ultrapure water. Each well in the PCR plate was brought up to 25 µl with the addition of 4 µl of *I. scapularis* DNA extract. The PCR thermal profile protocol involved 1 cycle of the initial denaturation (2 minutes at 95°C), followed by 35 cycles of denaturation (30 seconds at 95°C), annealing (30 seconds at 52°C), and extension (1 minute at 72°C). There was also a final extension

step at 72°C for 10 minutes, followed by the reaction being held at 4°C until the sample was collected.

To visualize what *I. scapularis* samples were infected with *B. burgdorferi* we loaded the PCR product onto a 1.5% agarose gel stained with Gel Red dye. We used a 1:5 ratio of 6X Tritrack DNA loading dye to PCR amplicon, and we also loaded a DNA ladder as a size standard consisting of 1 µl ladder, 1 µl 6X Tritrack DNA loading dye, and 4 µl ultrapure water. The gel was placed in a 1X TAE solution at 80 V for 40 minutes.

PCR products that were visible as a band on the gel at the expected size of the *ospC* target (*ca.* 720 bp) were sent to the University of Delaware's Genotyping and Sequencing Center at the Delaware Biotechnology Institute for library preparation and short-read Illumina sequencing.

Chapter 4

RESULTS

We found the average DNA concentration from *I. scapularis* extractions to be 0.7 ng/µl in a total volume of 400 µl. The final step of eluting the DNA by adding an additional 200 µl of Buffer AE lowered the overall concentration of the DNA in solution. After PCR amplification, DNA amplicon concentration averaged 9.33 ng/µl.

Table 1 shows the number of ticks collected and the number of *B. burgdorferi* – infected ticks. Kent County had the largest number of *I. scapularis* nymphs collected at 53 individual ticks and the largest number of infected nymphs with 20 infections. Sussex county had the lowest number of both collected and infected nymphs with 21 nymphs collected and 4 infected.

Unfortunately, the sequencing was not completed in time for the submission of this thesis. Without sequenced *ospC* genomes, we were unable to determine the prevalence of specific *ospC* genotypes at each sample site. With 111 nymphal *I. scapularis* samples screened for *B. burgdorferi*, we were able to determine an overall prevalence across sites and quantify variation in prevalence among sites as seen in Figure 1.

Kent County had the highest prevalence at 37.74% compared to New Castle (16.22%) and Sussex County (19.05%). Sussex County's prevalence was greatly influence by Laurel, DE, which had a 0% prevalence of *B. burgdorferi*, and Milton, DE which had 40% prevalence. Leipsic, DE in Kent County had a 45% prevalence

while Felton, DE had a 15.38% prevalence. Sites had mostly similar prevalences within New Castle County.

The prevalence across all sites was 27.03%, but it seemed that there was substantial variation in prevalence among sites, so we performed a chi-squared test to test the null hypothesis of no difference in prevalence among the sites. This resulted in a chi-squared statistic of 12.40 (degrees of freedom = 6) and a *P* value of 0.054. We therefore considered the null hypothesis to be rejected with the test suggested that there was a difference in prevalence among sites as suspected. In support of this conclusion, the *P* value is further lowered when the two sites with low sample sizes of ticks (Laurel, DE and Milton, DE) were removed (chi-squared statistic = 10.09, degrees of freedom = 4, *P* =0.04).

Table 1 Ixodes scapularis ticks collected and infected with Borrelia burgdorferi across Delaware

Site	Nymphs collected	Nymphs infected with *B. burgdorferi*
New Castle County	**37**	**6**
Newark, DE	23	4
Rockland, DE	14	2
Kent County	**53**	**20**
Leipsic, DE	40	18
Felton, DE	13	2
Sussex County	**21**	**4**
Dagsboro, DE	11	2
Milton, DE	5	2
Laurel, DE	5	0
Totals across Delaware	**111**	**30**

Table 1: The table above shows the number of nymphal *I. scapularis* ticks DNREC collected in the spring and summer of 2019. Number of ticks are separated by county, collection site and the state of Delaware as a whole. The number of collected ticks that screened positive for *B. burgdorferi* by PCR are also shown.

Figure 1 Borrelia burgdorferi prevalence in nymphal Ixodes scapularis across Delaware

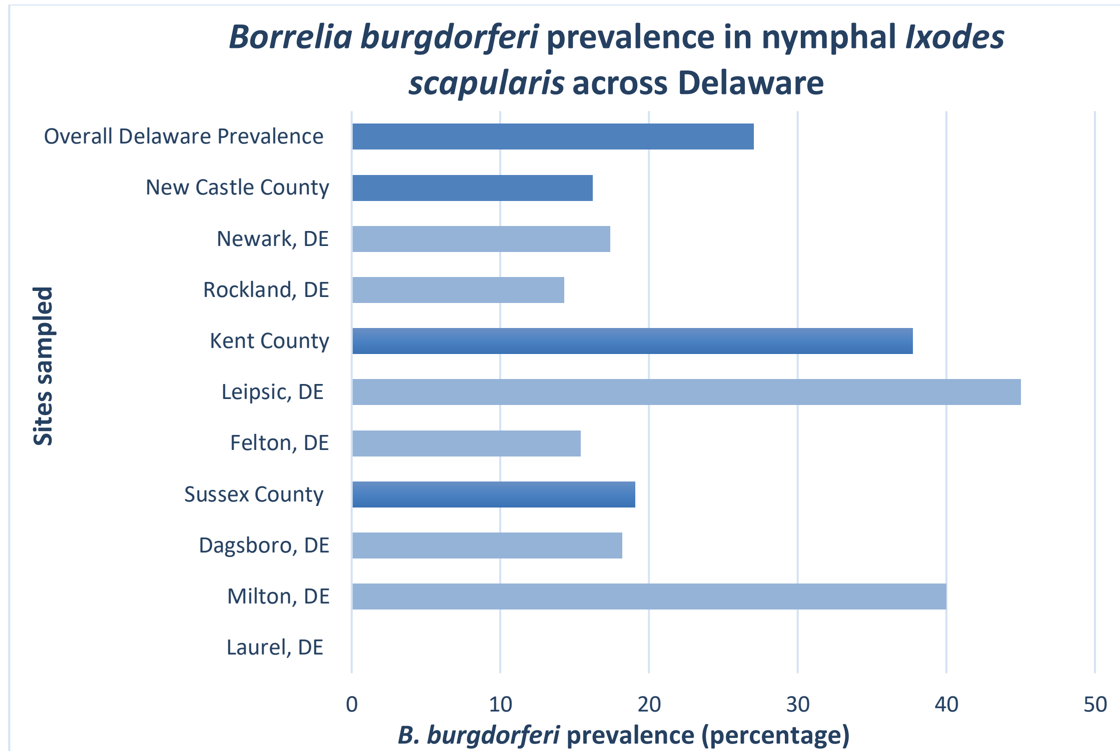

Figure 1: The prevalence of *B. burgdorferi* in nymphal *I. scapularis* ticks across the state of Delaware. Overall *B. burgdorferi* prevalence in Delaware was 27.03%. Prevalence is shown for each sample site and for the three counties and Delaware overall (counties and overall prevalence are shown with dark blue bars, while site-level prevalence is shown with light blue bars).

Chapter 5

DISCUSSION

We found that *B. burgdorferi* prevalence in *I. scapularis* nymphs varied substantially across the state of Delaware. Specifically, Kent County had a much higher prevalence than New Castle and Sussex County. There was also large variation between sites sampled in Kent and Sussex County, with Sussex County showing a prevalence range from 0% to 40% (Figure 1). Some of the variation in prevalence may be due sample size of sites along with the habitat and wildlife composition of each location. Importantly, the prevalence data likely included ospC lineages that do not typically infect humans. Sequencing results from our study will further clarify the prevalence of ospC lineages to *B. burgdorferi* that are known to infect humans and further elucidate human Lyme disease risk across the state.

We do recognize that our study had serval limitations. County level prevalence was determined by each county's sampled sites with both New Castle and Kent County having two sample sites, and Sussex County having three. While overall County prevalence of *B. burgdorferi* in *I. scapularis* nymphs is important for public health decisions, these county level estimates only took into account a small proportion of each county. Sussex County's prevalence was largely influenced by the site in Laurel, Delaware, demonstrating that this study likely had too few sample locations per county. Adding more sample units to each of Delaware's three counties that are collected across a broader range will allow sampled location to better

represent individual counties. The number of sample units within certain study areas was also low. This resulted in a single infected or non-infected nymph having major influence on prevalence. This was seen at the Milton, Delaware site which had a sampling unit of five *I. scapularis* nymphs with two of the nymphs being infected with *B. burgdorferi*. This low sample unit caused the site to have a 40% prevalence of *B. burgdorferi* in nymphal *I. scapularis*. It is plausible to expect that if the sampling unit at Milton, Delaware increased the prevalence would likely change.

The number of *I. scapularis* ticks collected in sites across Sussex County was far less than the number of ticks collected in the other two counties. Two sites in Sussex County, Milton and Laurel, had a total of five ticks collected. For both sites, this low capture number could have possibly been affected by that day's weather or vegetation dampness. While the weather in Milton may not have been the limiting factor, the vegetation was recorded as being dry. Sampling for ticks when vegetation is too dry will impair tick attachment to sampling cloth and overall tick activity (Salomon et al. 2020). The day sampling occurred in Laurel had strong winds and wet vegetation, both factors that will negatively impact tick capture and should be avoided (Eisen et al. 2019).

Our results show a higher prevalence of *B. burgdorferi* in *I. scapularis* ticks when compared to similar studies done in the state of Delaware. Curran et al. (2000) sampled adult *I. scapularis* ticks taken from white-tailed deer in each of Delaware's three counties in 1998. Curran et al. (2000) found a 12% prevalence of *B. burgdorferi*

in *I. scapularis* in New Castle County and an 8% prevalence in both Kent and Sussex County. Adalsteinsson et al. (2018) sampled nymphal *I. scapularis* in New Castle County in the spring of 2013 and 2014, finding a very similar *B. burgdorferi* prevalence as Curran et al. (2000) at 11.2%. While the average *B. burgdorferi* prevalence in nymphal *I. scapularis* was 11.2% in the Adalsteinsson et al. (2018) study, they found a 15.9% prevalence at sample sites invaded by non-native multiflora rose. This prevalence more closely resembled our findings for New Castle County, and further data should be taken at our sample sites to see if multiflora rose was present and if it possibly played a role in the increased prevalence. Our findings for both Kent and Sussex county were substantially higher than the findings of Curran et al. (2000), with a 37.74% prevalence in Kent County and a 19.05% prevalence in Sussex County. Curran et al. (2000) noted that the proportion of ticks that were *I. scapularis* increased significantly from 93% in 1988 to 100% in 1998. Future studies should see how the relative frequency of *I. scapularis* in the overall tick population in Delaware has changed since 1998 to see if there is any correlation with the increase in *B. burgdorferi* prevalence.

Our findings of *B. burgdorferi* prevalence in *I. scapularis* ticks varying within a state has been supported in multiple studies. Adelson et al. (2004) found a 33.6% prevalence in nymphal and adult *I. scapularis* infected with *B. burgdorferi* in Union County New Jersey while Courtney et al. (2003) found a 43% prevalence in both nymphs and adults in Hunterdon County New Jersey, just 48 miles away. Courtney et al. (2003) also documented *B. burgdorferi* prevalence in nymphal and adult *I.*

scapularis ticks in Pennsylvania, finding a 61.6% prevalence in northwestern Pennsylvania and a 13.1% prevalence in southeastern Pennsylvania. Brisson and Dykhuizen (2004) found a 35% prevalence of *B. burgdorferi* in *I. scapularis* nymphs collected in Millbrook, New York while 56 miles away, Telford et al. (1996) found a 53% prevalence in nymphal and adult *I. scapularis* in Westchester County, New York. Overall, *B. burgdorferi* prevalence in *I. scapularis* is lower in the state of Delaware as a whole and across the state's counties compared to other Northeastern states. Many of these studies sampled for both nymphal and adults *I. scapularis* which may factor into the increased prevalence rates seen in these studies. Adults should have a higher prevalence of *B. burgdorferi* than nymphs because they have had an additional blood meal and therefore more chances to become infected.

The ecology of *B. burgdorferi* is far from simple. The ecology requires understanding the factors that determine the distribution and abundance of the specific organisms involved in the maintenance and transmission of *B. burgdorferi* (Ostfeld 2011). While there is still a lot of unknown in this particular disease system, it is understood that habitat fragmentation can lead to both increased densities of *B. burgdorferi*-infected ticks and *B. burgdorferi* prevalence in nymphal *I. scapularis* ticks (Allan et al. 2003). As forests are fragmented, many vertebrate species are lost (Rosenblatt et al. 1999). Conversely, the white-footed mouse, a highly competent *B. burgdorferi* reservoir (Schmidt and Ostfeld 2001), thrives in fragmented habitats (Nupp and Swihart 1998). However, degree of forest fragmentation alone is not likely

to be adequate to predict *B. burgdorferi* prevalence given its large degree of spatial variation (Ostfeld and Keesing 2000).

There are many factors, most involving *I. scapularis* host abundance and diversity, that may play a role in this high spatial variation in *B. burgdorferi* prevalence. Diuk-Wasser et al. (2020) emphasized the importance of the vertebrate host species of *I. scapularis* for aiding in *I. scapularis* dispersal and *B. burgdorferi* transmission. With an infected host playing a large role in the dispersal of a tick and its pathogens, it is possible that populations of small, highly competent mammals are isolated in fragmented forest patches making these patches become independent in terms of their *B. burgdorferi* prevalence. This independence in prevalence may explain the substantial variation among sampled sites. To gain a better understanding of this concept, future studies should sequence host DNA and check population genetic structure for the small mammals.

The most common hypothesis to explain high spatial variation in *B. burgdorferi* prevalence among regions and habitats is the Dilution Effect hypothesis. The Dilution Effect hypothesis states that infection risk to an infectious disease will decline with increasing biodiversity if the disease is transmitted by a generalist vector, the increased biodiversity is seen with non-competent or weakly-competent reservoir species, and the increased biodiversity decreases the abundance of competent reservoirs (Ostfeld and Keesing 200). Van Buskirk and Ostfeld (1995) supported this idea by finding that habitats with higher biodiversity of non-competent hosts had

decreased *B. burgdorferi* prevalence due to the decreased chances of an *I. scapularis* tick feeding on a competent host and contracting the bacterium.

With *B. burgdorferi* prevalence in *I. scapularis* ticks varying across and within states, it is important to know infection rates on an event finer spatial scale. Local tick infection rates are vital for residents and physicians to make prevention and treatment decisions (Piesman and Eisen 2008), especially when sampled areas are of high recreational use like the sites in this study. Determining specific *ospC* genotypes at sampled sites will also allow for more accurate estimates of Lyme disease risk to humans in Delaware. Lyme disease risk is frequently represented by *B.* burgdorferi prevalence alone, when that is not always an accurate representation. Human Lyme disease risk can be difficult to calculate with factors involving human encroachment into tick habitat (Hamer et al. 2010) and *ospC* human infection establishment. There are 22 major groups of *ospC* alleles with 4 groups, A,B,I, and K, typically associated with invasive infection in humans (i.e., a pathogenic strain that is able to overcome the human immune response and cause disease) and groups C, D, and N less frequently associated with invasive infection (Earnhart et al. 2005). By determining the prevalence of specific *ospC* alleles known to infect humans, human Lyme disease risk can be more accurately predicted. Given that Delaware is a high incidence state for Lyme disease, it is important to continue to conduct molecular surveys across the state for *B. burgdorferi* prevalence. Long term molecular surveys will allow for a better understanding of what *B. burgdorferi* prevalence looks like across Delaware and how it may be changing over time. *B. burgdorferi* prevalence trends should be related to

reported human infection data in hopes of better understanding why Delaware has such a high Lyme disease incidence rate. This study is a step in that direction.

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