# INFECTION PREVALENCE OF *BORRELIA BURGDORFERI* IN WHITE-FOOTED MICE (*PEROMYSCUS LEUCOPUS*) ACROSS THE NORTHWEST OF MICHIGAN'S LOWER PENINSULA

Keeley Kyra Brown

**A Thesis**

Presented to the Faculty of Miami University in partial
fulfillment of the requirements
for the degree of

Masters of Science

Department of Biology

The Graduate School
Miami University
Oxford, Ohio

2024

Dr. Susan Hoffman, Advisor
Dr. Tereza Jezkova, Reader
Dr. Brian Keane, Reader

## ABSTRACT

Lyme disease, caused by the bacterium *Borrelia burgdorferi*, is transmitted to humans through ticks, and it has become an increasing problem in the Midwest. In recent years, cases have been expanding from a hotspot in Wisconsin into Michigan's Lower Peninsula (LP) along the coastline of Lake Michigan. The expansion of cases coincides with increasing populations of the deer tick, *Ixodes scapularis*, and of the white-footed mouse, *Peromyscus leucopus*, which serves as the primary reservoir host for the bacterium. A study from 2010 testing the infection prevalence in both deer ticks and white-footed mice found no infections in either species in most of the northern LP. For this study, mice were trapped along a transect from the edge of the known range of infected mice northeastwards toward the tip of the LP. Infected mice and ticks were found more than 100km beyond the previous limit but were not found along the eastern part of the transect. The proportion of *P. leucopus* carrying ticks was correlated with higher infection prevalence in both ticks and mice.

## Table of Contents

## List of Tables

## List of Figures

## Dedication

To my parents, James and Wendy, and my sisters, Sophia and Shannon, and my aunt Kara, who have all given me so much love, support, and assistance. I couldn’t have gotten through this without any of you.

## Acknowledgements

First and foremost, I would like to thank my advisor Dr. Susan Hoffman for providing so much guidance and opportunity for growth during my time with her. I would also like to thank my committee members Brian Keane and Tereza Jezkova for their interest in my research and their flexibility. Also, thank you to Adam Livingston and Noelle Wittwer, who were a huge help in the completion of the research in the lab and made it a pleasant work environment. Another thank you goes to Dr. Jean Tsao for providing data that were necessary for the completion of this project, and to Dr. Suohong Wang for providing assistance with statistical analysis. Lastly, I would like to thank my fellow graduate students, who have been an invaluable source of support. This research was completed with funding from Miami University.

# INTRODUCTION

## Lyme Disease

Lyme disease first came into the public consciousness in the early 1970s, when many people, particularly children, were developing oligoarthritis near Lyme, Connecticut (Radolf et al., 2012). This arthritis was preceded by a "bull's-eye" skin lesion, which came to be known as erythema migrans (Radolf et al., 2012). This same lesion had been previously identified in Europe and was associated with tick bites, so in 1978 researchers investigated ticks as potential vectors for "Lyme arthritis" and found its distribution to be similar to the regional distribution of white-tailed deer and ticks (Elbaum-Garfinkle, 2011). The cause of Lyme disease, a spirochete bacterium, was isolated in 1982 by W. Burgdorfer, and subsequently named *Borrelia burgdorferi*. A decade later, in 1991, it became a nationally notifiable condition, and since then Lyme disease has become an increasing problem in North America.

While it is rare for a person to die from Lyme disease, symptoms can range in severity and persistence, and they may have a serious impact on a patient's quality of life. Symptoms may display themselves as early as 3 days after a tick bite, and they usually consist of fatigue, fever, headache, and joint aches (CDC, 2021). A common feature of Lyme disease is the erythema migrans, which appears at the site of the infection on average seven days after the bite; however, it occurs in only 80 percent of infected people and does not always have the "bull's-eye" appearance (CDC, 2021). While antibiotics like doxycycline and amoxicillin are successfully used to treat Lyme disease, symptoms can sometimes develop at a later point, even after treatment, and are usually more severe. This is referred to as Post Treatment Lyme Disease Syndrome and can include more severe versions of the earlier symptoms, such as arthritis and headaches, but can also include inflammation of various parts of the nervous system and heart, which can in turn result in conditions like facial palsy and Lyme carditis (CDC, 2021).

Besides its clinical impacts, Lyme disease is at the forefront of an increasing trend of human illnesses caused by zoonotic pathogens. Zoonotic diseases, or zoonoses, are defined as diseases that can be transmitted from either domestic or wild vertebrates to humans, and vice versa; currently, 61% of all human pathogens (bacterial, viral, parasitic, and fungal) have a zoonotic origin (Rahman et al., 2020). There are ways of becoming infected with zoonoses by contact with the source, such as through direct contact (e.g. tularemia), or via aerosols (e.g. hantavirus), but there are also indirect ways of becoming infected, like from eating food

contaminated with salmonella, or through third party vectors, as is the case for Lyme disease (Kruse et al., 2004). Along with multiple transmission methods, there are several factors that are contributing to the increased emergence of zoonoses like Lyme disease, such as the increase in population numbers for both humans and animal hosts, as well as the increased exposure of people to wildlife and animal products (Rahman et al., 2020; Kruse et al., 2004).

Currently there are more than 30,000 cases of Lyme disease per year in the United States, with an ongoing upward trajectory, making it the most reported vector-borne disease in the United States (CDC, 2021). Cases of Lyme disease have been reported in all U.S. states except Hawaii, and 15 states are classified as "high incidence", defined as having at least 10 confirmed cases per 100,000 people (CDC, 2021). States that are high incidence are concentrated in two regions, the northeastern U.S. along the East Coast down to Virgina, and the Upper Midwest, primarily in Wisconsin. In the Midwest over the last 20 years, there has been an expansion of the distribution of confirmed cases from Wisconsin into neighboring states, including Michigan, Illinois, and Indiana (Fig 1).

Over the past decade, the CDC has reported an increase in the number of confirmed cases in Michigan, from 76 in 2010 to more than double that in 2019, as well as an increase in incidence from 0.8 per 100,000 people in 2010 to 2.8 in 2019. This is the second highest incidence for Lyme disease cases out of all the states that are not currently classified as having a high incidence (CDC, 2021). A study that focused on the expansion of Lyme disease in Michigan between 2000 and 2014 showed an increase in the number of cases as well as an increase in the geographic range of cases (Lantos et al., 2017). That expansion is mainly occurring along the western shores of the Lower Peninsula (LP) of Michigan, from the southwest corner of the state northward along the coast of Lake Michigan (Lantos et al., 2017; Fig 2). This trend was apparent in the latest Lyme Disease Map from Michigan; most counties categorized as having a known risk are located along the western coast and in the southern half of the LP, where known risk is defined as having two confirmed Lyme disease cases acquired locally and/or the confirmed presence of infected deer ticks (Michigan Department of Health & Human Services, 2021). The only Michigan counties with no risk are in the central part of the LP.

## Enzootic Cycle of Lyme Disease

As previously stated, the causative agent of this disease is a spirochete bacterium from the genus *Borrelia*. *Borrelia* has several different species, but within the United States *Borrelia burgdorferi sensu stricto* is the species usually found, and this bacterium is passed to humans via infected ticks (Radolf et al., 2012). There are two species of ticks that transmit *B. burgdorferi* in the United States, depending on the location. *Ixodes scapularis*, the deer tick, is found along the East Coast and Upper Midwest, while the western blacklegged tick, *Ixodes pacificus*, is on the West Coast (CDC, 2021).

An *Ixodes scapularis* life cycle lasts for about two years and has four stages: egg, larva, nymph, and adult, with body mass increasing with each stage (CDC, 2020; Fig 3). In the spring of its first year, an egg hatches and a larva emerges; the tick will remain in the larval stage for the duration of the year. Early in its second year, it will molt and emerge as a nymph, spending some months in this stage before molting one last time into an adult. At the end of the life cycle, adult ticks mate, lay eggs, and die shortly thereafter.

Throughout its life, *I. scapularis* requires a vertebrate host to provide blood meals, with the host species differing in size at every stage, as well as differing in the role each plays in sustaining Lyme disease (CDC, 2021). One major host organism is the white-tailed deer, *Odocoileus virginianus*, which can be found through most of eastern North America. During their adult stage, *I. scapularis* will mate on their final blood meal host, which is usually *O. virginianus*, and will then detach from the host to lay eggs on the ground (Nguyen et al., 2019). The deer can also act as carriers, transporting adult *I. Scapularis* to new regions where there previously were no ticks, or moving infected *I. scapularis* to areas where the resident ticks are not infected. Evidence for the role deer play in transmitting Lyme disease to humans was found in a study that showed that a reduction in the local deer population corresponded with a reduction in reported Lyme disease cases (Kilpatrick et al., 2014). Birds can also act as carriers, especially ground-foraging species such as sparrows and quail, which are more likely to pick up young *I. scapularis* infected with *B. burgdorferi* (Loss et al., 2016). Thus, a person may become infected by a tick that was carried to the area because of deer or bird movement, even though the tick is not originally from the local population.

Both infected and uninfected ticks can also be spread to new areas by human carriers. With modern conveniences and globalization, it is easier for a person to travel long distances in a short period of time, but that also makes it possible for them bring pathogens or their vectors to

areas where historically that pathogen was not present. As people travel between the states, they may be bitten by a tick in one state but a develop clinical case of Lyme disease after they are in another state. For example, someone going from Texas, a low incidence state, to New York, a high incidence state, may be bitten by a deer tick there, and not show symptoms until after they have returned home, so that the case is reported as occurring in Texas even though the infection was actually acquired in New York. Unless a map or table explicitly states, "local confirmed cases," it is typically unclear where the infection was acquired. The CDC map for Lyme cases does not specify that the cases for each state are acquired locally, but it does include a reminder about the influence human travel may have on where the cases are reported versus where the infection took place. The only way to accurately reflect where the bacterium is endemic is to analyze the infection prevalence, or how common an infection is, in other host species.

Though the ticks are the vectors for Lyme disease, and though deer and other hosts carry the ticks, they generally do not sustain populations of the bacterium long term. Over the past few decades, researchers have confirmed that the white-footed mouse, *Peromyscus leucopus,* is the primary reservoir host for *B. burgdorferi* in eastern North America, which means that it can sustain an infection throughout its life and pass the bacterium on to a new generation of ticks. A 1989 study analyzing the potential reservoirs for *B. burgdorferi* in Massachusetts found that the prevalence of infection was highest, at 90%, in *P. leucopus*, compared to 75% in chipmunks and 5.5% in voles (Mather et al., 1989). Another study that took place over a 2-year period found that of 801 serum samples collected from 514 *P. leucopus*, 598 samples tested positive, and that rates of infection in the mice increased over the course of the summer (Bunikis et al., 2004). A recent study from 2020 also tested *P. leucopus* as a reservoir for *Borrelia mayonii,* another spirochete that can cause Lyme disease, and found that 21 of the 23 mice (90%) became infected and could pass on the infection to uninfected ticks (Pairse et al., 2020).

Because *I. scapularis* is unable to transmit *B. burgdorferi* transovarial, from adult to egg, each new generation of ticks must get the infection by biting a reservoir host (Radolf et al., 2012). During the summer of its first year, a tick larva will wait on a blade of grass for a blood meal host to pass by. At this stage the host is a smaller vertebrate, such as a rodent or bird. If the host carries *B. burgdorferi* the bacterium can be transmitted to the tick, and through transstadial (across molts) transmission the infection persists through the tick's later life stages (Radolf et al., 2012). Once infected, a tick can act as a vector and pass the bacterium along to other vertebrates,

such as deer and humans, most of which are dead-end hosts (Fig. 4). These other hosts do not usually pass the infection on to uninfected ticks, so each new generation of larval ticks picks up the infection primarily from its initial host, most often the white-footed mouse.

*Peromyscus leucopus* Life Cycle and Ecology

*P. leucopus* is one of the most common mammal species in North America. These mice occur in much of the eastern half of the continent, with a range stretching from southern Canada all the way to southern Mexico. They are typically found in deciduous forests, particularly those with oaks, but they can adapt to most brushy or wooded habitats as long as there is sufficient cover (Vessey et al., 2007). Even though individual mice can live to be 5-6 years old, this life span is mostly observed in captivity, while in the wild the average life span of *P. leucopus* is less than one year due to various factors like predation and food availability. In fact, one study found that less than 3% of a wild population lived for more than a year, and most females do not live long enough to reproduce (Vessey et al., 2007).

*P. leucopus* generally reach sexual maturity at about 8 to 9 weeks old, but the timing will depend on the population density and food supply (Schug et al., 1991; Vessey et al., 2007). Upon reaching sexual maturity, the mice disperse and establish territories, with the males generally traveling further than the females (Vessey et al., 2007; Keane, 1990). A *P. leucopus* male's territory will overlap with those of other males and of multiple females, but a female *P. leucopus* will not allow territorial overlap with other females (Vessey et al., 2007). Depending on photoperiod, the age of sexual maturation can be extended up to 140 days, and this impacts the age of females when they have their first litter; in turn, maternal age impacts litter size and the number of litters produced (Johnston & Zucker, 1980; Havelka & Millar, 2004). The litter sizes average 4 to 5 pups, and within a breeding season it is possible for a female to have between 2 and 4 litters, depending on when they had their first litter (Havelka & Millar, 2004; Vessey et al., 2007). In the northern part of their range *P. leucopus* only breed seasonally, from late spring to early autumn, which overlaps with when larval ticks are seeking a blood meal host (Bedford et al., 2015; Donnelly et al., 2015). Thus, an infected female mouse can pass *B. burgdorferi* infection via infected ticks to many offspring over the course of the summer breeding season, so that local populations of mice can have high rates of infection by early autumn.

Mouse populations fluctuate, and the pattern appears to be that their numbers are highest in late summer, at the tail end of the breeding season, and lowest in late winter, before the breeding season starts again (Vessey et al., 2007). This is in part due to severe winter mortality, especially for autumn-born mice, but also because there is a pause in breeding during the coldest months (Schug et al., 1991). Michigan, like Wisconsin, is at the northern edge of the range of *P. leucopus*, and a study conducted in Wisconsin found that *P. leucopus* experienced significant weight loss and high mortality rates during the winters (Long, 1996). This is because northern *P. leucopus* need to deal with extreme conditions that more southern *P. leucopus* do not.

Infection Prevalence in *P. leucopus*

Several studies have demonstrated the correspondence between *I. scapularis*, *P. leucopus* and the transmission of *B. burgdorferi*. As previously stated, there is much overlap of the geographic distribution as well as peak activity periods of ticks and mice, so that during the summer months there is an increase in the populations of both species, which increases their chances of encountering each other. With an increase in contact, there appears to be an increase in the prevalence of infection due to a higher frequency of transmission. As early as 1985, it was found that rates of *P. leucopus* and *I. scapularis* encountering each other were correlated with the prevalence of *B. burgdorferi* infection in the ticks (Levine et al., 1985). Later studies in places where there is frequent interaction between the two species have reaffirmed that there are high rates of infection in both. In an endemic area in southern Canada, adult male *P. leucopus* carried more ticks, 35% of the total ticks found, than any other species or any other demographic group of *P. leucopus* (Bouchard et al., 2011). The same 2020 study that tested *P. leucopus* as reservoirs for *B. mayonii* found that nymphs that fed on infected mice over 4 weeks had an average infection prevalence of 75%, starting at 56% with the first recording and increasing over time to 98% (Pairse et al., 2020).

Another study in Maryland found that the average infection prevalence in *P. leucopus* was 40%, and a similar infection prevalence of 36% was found in *I. scapularis* removed from mice; also, the highest prevalence of infection was where *I. scapularis* were most common (Poje et al., 2022). These frequencies of transmission can be tied not only to the presence of each species, but also to the physiological response of *P. leucopus*. When comparing two different species of rodents, it was found that guinea pigs had a more severe inflammatory response to a

tick bite that resulted in reduced tick feeding (Anderson et al., 2017). This contrasted with the *P. leucopus*, whose intact epidermal layer after they were bitten enabled ticks to keep feeding (Anderson et al., 2017). Since *P. leucopus* is important in sustaining and passing on *B. burgdorferi* in an area, it is worth monitoring the distribution of this species of mouse in relation to the expansion of Lyme disease cases.

Range Expansions

There has been an expansion in the range of Lyme disease cases over the past decades, which has coincided with an increase in the presence of both the reservoir host and the vector. This expansion is primarily driven by two factors: warmer temperatures and anthropogenic activity. At the northern edge of the range of *I. scapularis*, in southeastern Canada, the rate of its geographic expansion is currently projected to be about 46 km/yr (Clow et al., 2017). Also, another study in Canada surveying for tick presence found *I. scapularis* at 30 sites, 73% of which had no history of these ticks prior to 2017 (Robinson et al, 2022). In the United States, a county-scale survey for the presence of *I. scapularis* was done in 2016, classifying each county as either "Established" (having either 6 ticks or 2 ticks in the host-seeking stages) or "Reported" (1 tick found) (Eisen et al, 2016). In Michigan, 40 out of 83 counties were classified as either "Established" or "Reported", with 13 counties transitioning from "No Records" to either "Reported" or "Established" over an eight-year period (Eisen et al., 2016).

*P. leucopus* originally were common in the southernmost part of Michigan but relatively rare in northern parts of the LP. Over the last 30 years, however, they have become the dominant species of mice throughout the LP, in most areas replacing a more northern species of mouse, *Peromyscus maniculatis gracilis*. What historically kept *P. leucopus* from expanding at the northern edge of its range was the winter climate and the lack of cold weather adaptations these mice have compared to *P. m. gracilis* (Long, 1996). With changes in climate that include milder winters, *P. leucopus* have been able to become increasingly common to the north of their historic range in the Upper Midwest (Roy-Dufresne et al., 2013; Myers et al., 2009). The species they are replacing, *P. m. gracilis*, can be a competent reservoir host for *B. burgdorferi*, but whether it maintains and transmits the spirochete on the same scale as *P. leucopus* is unclear and requires further study (Myers et al., 2009; Peavey & Lane, 1995).

The expansions of both white-footed mice and reported cases of Lyme disease in Michigan leads to the question, what is the pattern of range expansion of the bacterium itself? What hosts are necessary for the *Borrelia* spirochete to become endemic in a region, and to what extent do these hosts need to be present? Three different scenarios for the expansion of Lyme disease were suggested in a 2010 study, based on the timing of invasion of the vector *I. scapularis* and the spirochete bacterium into the LP. The first scenario, "tick-first", proposes that uninfected ticks become established in new regions due to being carried there by deer, and the bacterium enters the region later via transport by white-footed mice (Hamer et al., 2010). A second potential scenario, "dual-invasion", suggests that mammalian or avian hosts carry infected ticks to a new region as they expand their own ranges. In the last scenario, "spirochete-first", the reservoir host is already present in an area and the bacterium is maintained via cryptic alternative hosts and vectors; later, *I. scapularis* spreads into the region and facilitates the spread of Lyme disease to humans (Hamer et al., 2010). As part of the same study, from 2004 to 2008 numerous *P. leucopus* were captured at a site in Manistee County, but they had low rates of infestation with *I. scapularis* and a low prevalence of *B. burgdorferi* infection compared to more southern sites along the Lake Michigan coast (Hamer et al., 2010). On top of that, coastal sites had a higher number of ticks present than inland sites, which reflects the coastal, northward geographic trend for the spread of Lyme disease cases in humans in the LP.

Research Objectives

My overall aim for this study was to examine the prevalence of infection of *B. burgdorferi* in both white-footed mice and deer ticks along a transect in northwestern Michigan. Doing so would enable me to address two research objectives. The first was to define the current edge of sustained *B. burgdorferi* populations and detect any further expansion of *B. burgdorferi*-infected reservoir hosts in the northern LP. Individuals of the reservoir host species, *P. leucopus*, were collected along the transect and tissue samples were taken for DNA extraction, then PCR was used to determine whether a mouse had *B. burgdorferi* DNA present in its system. I predicted that sustained bacterial populations in the mice would have expanded beyond the range defined by Hamer et al. (2010), but that the infection prevalence would decrease in mouse populations as one moves northwards along the transect.

The second research objective was to observe the pattern of disease expansion with regards to the three models suggested by Hamer et al. (2010), by analyzing the distributions of *P. leucopus*, *I. scapularis*, and *B. burgdorferi* along the transect. To do this, I combined my data on *B. burgdorferi* infections in the mice and data on infections in the ticks, which were PCR-screened by a colleague. I also compared the infection prevalence to the population numbers of both species at each site to determine whether my results reflected any of the distribution patterns predicted by the Hamer models. Based on the low rates of infection in the mice and their high population numbers in 2008 in Manistee and Benzie Cos., the "dual-invasion" scenario was unlikely, so I hypothesized that either the "tick-first" scenario or "spirochete-first" scenario would fit better. With the "tick-first" scenario I predicated that even if tick population numbers are high at certain sites, little to no *B. burgdorferi* would be detected in either the *I. scapularis* or *P. leucopus* because it had not expanded into that area yet. With the "spirochete-first" scenario I predicted that even if no or very few ticks (regardless of infection status), were present at a site, *B. burgdorferi* would still be detected in multiple mice. For both scenarios I predicted that I would observe a difference in the number of total *I. scapularis* present and the number of *B. burgdorferi* positive individuals present in a population, which would result in a lag in the infection prevalence along the transect relative to the expansion of mouse populations.

## MATERIALS AND METHODS

### Field Work

In the summer of 2021, *P. leucopus* were sampled from six locations in the northern half of the LP of Michigan, in Manistee, Benzie, Grand Traverse, and Antrim Counties (Fig 5). Each site consisted of typical *P. leucopus* habitat, with well grown mostly deciduous forest dominated by maple, oak, beech, birch, and pine trees, and all were located on publicly accessible land (Table 1). Since all three of the Benzie Co. sites are less than 10 km from each other, which is within typical *P. leucopus* dispersal distance (Maier, 2002), all the mice trapped in Benzie Co. were considered to be part of one population. There were thus four sites, each within a different county, spaced about 50 to 60 km apart, with the Manistee site being 53 km from the nearest site in Benzie, the Grand Traverse site being 49 km from the nearest Benzie site, and the Antrim site being 57 km from the Grand Traverse site. The selected sites form a transect that runs in a northeastern direction across the northern half of the LP of Michigan (Fig 5). The selected starting point was in Manistee Co. because that county was the most northern known location of a few *B. burgdorferi*-infected *P. leucopus* found by Hamer et al. (2010) in 2006 (except for a single infected mouse found at an isolated site on the Leelanau Peninsula). The transect extended northeast from Manistee Co. to Benzie Co., then to Grand Traverse and Antrim Cos., extending approximately 150 km northeast of where data were collected by Hamer et al. (2010). Antrim Co. was selected as the endpoint because in a nearby county to the northeast, Cheboygan Co., a large sample of *Peromyscus* trapped in 2013 all tested negative for *B. burgdorferi* (Freund, unpublished data). Thus, as of 2013 it was known that *P. leucopus* are common as far northeast as Cheboygan Co., but *B. burgdorferi* had not yet been detected there.

Trapping occurred at each site for one to three nights, until a sample size of at least 20 mice was captured (except for Antrim Co.--see below). At each site, 100 to 200 Sherman Live (TM) traps baited with whole oats were placed about 10m apart in lines of varied lengths, depending on the forest patch. Traps were set in the evening and all animals caught were processed early the next morning. Animals were handled following the standards set by the Miami University IACUC and the American Society of Mammologists (Sikes et al., 2016). The species, age (determined by the pelage), gender, reproductive status, weight, trap ID, and presence of parasites was recorded for each animal caught, including recaptures, with the ear length also recorded for each *Peromyscus*. Each mouse was examined for ticks, and any ticks

present were removed with forceps that had been flame-sterilized, and placed in a tube filled with 95% ethanol. All ticks were sent to Dr. Jean Tsao's lab at Michigan State University for further analysis.

Once the ticks were removed from the mouse, a sample of epithelial tissue was cut from the right ear using flame sterilized scissors, and the mouse was released. Skin has been shown to have a relatively high concentration of *B. burgdorferi* in infected mammals and is thus the most effective host tissue to use when testing for infection (Zawada et al., 2020). Each ear sample was placed in SET Buffer (1% SDS, 10mM Tris-HCL pH 7.5, 5mM EDTA) and kept on ice until it could be frozen.

DNA Extraction

DNA was extracted from each tissue sample using the E.Z.N.A Tissue DNA Kit (OmegaBio-Tek, GA) according to the manufacturer's instructions, except as modified below. After mincing, tissue samples were shaken at 55 degrees for at least 48 hours to maximize breakdown of the tissue. During the wash stage, all centrifuging times were doubled. Lastly, before adding the elution buffer, the Hi-Bind columns were left open for two minutes so that any remaining ethanol would evaporate. Once the DNA was extracted, it was stored at -20 degrees. All the equipment that touched the tissue during processing was rinsed three times between each sample, once with 20% bleach and twice with sterile water, in order to prevent cross contamination. Benches were wiped down with 70% ethanol after each sample was processed.

Before PCR was performed, the concentration of each DNA sample was measured with a NanoDrop spectrophotometer. Most of each DNA sample was expected to consist of mouse DNA, so a minimum total DNA concentration of 10 uL/ng was necessary to try to ensure that any bacterial DNA present in the sample could be amplified. If the total concentration (including both mammalian and bacterial DNA) fell below 10 uL/ng, the DNA was ethanol-reprecipitated to achieve a higher concentration.

Nested PCR and Gel Electrophoresis

To amplify *Borrelia* DNA from each mouse tissue sample, a nested PCR was performed using standard primer sets for the bacterial Outer Surface Protein A (OspA) (Clark et al, 2005; Table 3). For a nested PCR, two sequential PCRs are run, with the products of the first, outer

PCR acting as the template for the second, inner PCR; the primers for the inner amplification are known to uniquely match sequences within the fragment amplified using the outer oligonucleotide pair (Green et al., 2019). OspA primers were utilized because of their reliability in detecting *B. burgdorferi* DNA in samples from a number of different vertebrate species (Moter et al., 1994; Radzijevskaja et al., 2011). The final product from the second amplification is a 352 bp segment of the *ospA* gene (Clark et al, 2005; Guy et al., 1991; Table 3). Fragments produced from *Peromyscus* samples by this PCR procedure in our lab have been sequenced to confirm that they are in fact *Borrelia* OspA DNA (R. Freund, unpublished data).

At the start of the PCR for each set of DNA samples, master mixes were made for both outer and inner amplifications. The standard recipe per sample is shown below (Table 2). All primers were stored at -20 degrees in 2.5 uL aliquots of the 100 ug/uL stock; for each experiment, fresh aliquots were diluted to 0.5 ug/uL by adding 47.5 uL of sterile water before the primers were added to the PCR master mix. Sets of aliquots were also made for all other reagents and renewed frequently. The negative control for each PCR was sterile water and the positive control was a previous DNA sample that had repeatedly produced an appropriate 352 bp band on the resulting gel. Both controls were run with every PCR, and for any gel where either of the controls did not appear as expected, a second PCR was run on the same set of samples, while the original gel results were not included in the data.

At the start of each PCR, all the ingredients for the master mix were set on ice, and all equipment (micropipettes, PCR tubes, PCR lids, labeled 0.5 microcentrifuge tubes, and racks) was sterilized under UV light for four minutes. Master mixes for both the outer and inner PCR were assembled simultaneously, following the order of reagents listed in Table 2. Each reagent was thawed on ice and vortexed before being added to each of the master mixes. Once the master mixes were assembled, the inner master mix was set aside at -20 degrees until the second amplification. For the outer PCR, 11 uL of master mix was added to each tube and then four uL of the appropriate DNA template was added, with all DNA samples and controls being fully thawed in a separate ice bin and briefly vortexed before addition. To prevent cross contamination between samples, the experimenters' gloves were wiped down with ethanol after each DNA sample was added. Once the PCR tubes were assembled and placed in the thermocycler, the racks that held the tubes were washed with bleach and the benchtop was wiped down.

When the outer PCR was finished, the inner master mix was thawed and vortexed, and a new set of labeled PCR tubes, lids, racks, and micropipettes was sterilized under UV light for 4 minutes. The outer PCR products and the inner PCR tubes were kept separate to prevent cross contamination. For the inner PCR, 12.5 uL of the master mix was combined with 2.5 uL of the outer PCR product for each tube. As with the outer PCR, the experimenters' gloves were wiped down each time the outer products were handled to prevent cross contamination.

After completing the second PCR, an ethidium bromide stained 1.5% agarose gel with TAE buffer was used to electrophorese the PCR products. A 100 bp DNA ladder (New England Bio Labs, MA) was added to each row that contained a sample. Each product was mixed with one uL XC loading dye, and eight uL of the PCR product was loaded onto the gel. Three uL of ethidium bromide was mixed with the TAE buffer at the positive end of the electrophoretic rig, then the gel was run at 106 volts for 30 minutes and imaged with a ChemiDoc Touch Imaging System (BioRad). If a 352 bp band appeared in any of the experimental lanes, this was considered a demonstration of the presence of *Borrelia* DNA in that mouse. Band intensity was not considered, as the amount of potential *Borrelia* DNA could vary widely between samples; only the presence of an appropriately sized band was considered when determining the infection status of each specimen. A mouse that displayed 2 consistently positive amplifications was considered to be infected, while two negative amplifications indicated a lack of detectable infection. Any mouse that gave inconsistent results from the first two rounds of PCR was reamplified until at least 3 consistently negative or consistently positive amplifications were obtained.

Statistical Analysis

All comparisons of *B. burgdorferi* infection rates between populations of mice and all *I. scapularis* populations to determine the significance in difference were calculated using the Fisher's Exact Test, except for between *P. leucopus* in Benzie and Grand Traverse which used a chi-square test for independence because the sample size was large enough. Alpha value was established at 0.005 and all calculations were performed in RStudio, using scripts written by Dr. Suohong Wang from University of Toledo.

# RESULTS

## Trapping

Forty *P. leucopus* were trapped from the Grand Traverse population and 42 *P. leucopus* were trapped from the Benzie population, while 24 mice were trapped at Manistee and 15 mice at Antrim (Table 4). Based on morphological characteristics (pelage color and ear size) as well as temperament *P. leucopus* was the only mouse species that was caught at all sites. One other small mammal was caught, the eastern chipmunk, *Tamias striatus*, with one to three individuals trapped at every site. The proportion of *P. leucopus* carrying *I. scapularis* ticks was similar across 3 of the 4 populations, with 46.7% of mice in Antrim, 54.2% in Manistee and 59.5% in Benzie having at least one tick. In the Grand Traverse population, however, only 7.5% of the captured mice carried *I. scapularis* ticks (Table 4). The proportion of mice carrying multiple ticks was the highest at Benzie (31%), followed by Manistee at 16.7%, Antrim at 6.7%, and lastly Grand Traverse at 0% (Table 4). None of the chipmunks trapped were carrying ticks.

The Benzie population had the highest number of *I. scapularis* collected, with a total of 56 ticks, and the Grand Traverse population had the lowest number at three ticks (Table 4). The Antrim and Manistee populations had intermediate total numbers of *I. scapularis*, with 21 collected from the Manistee mice and 8 from the Antrim mice (Table 4). Most of the ticks collected were at the larval stage, but at least one nymph was collected from each population except Antrim, and no adults were collected. In the Grand Traverse and Benzie populations, another species of tick, *Dermacentor variabilis*, was found on a few mice (one from Benzie and two from Grand Traverse), but this tick species is not known to transmit Lyme disease (Hamer et al., 2010).

## PCR Results

The Manistee population, in the most southwestern county of the transect, had the highest prevalence of infection, with 66.7% of the captured mice testing positive for *B. burgdorferi*, compared to Benzie's 57.1 %, though the prevalence of infection between the two populations was not significantly different ($p = 0.601$; Table 5; Table 6). Moving eastwards, there was a significant decrease in infection prevalence, with a steep drop-off between infection prevalence in the Benzie population and the next population to the east, in Grand Traverse County, where

only 2.5% of the *P. leucopus* (p = 2.86e-7; Table 5; Table 6) tested positive. None of the mice collected in Antrim Co., the most northeastern population, tested positive (Table 5).

As for the *I. scapularis*, the Benzie population had the highest number of infected ticks (seven), five infected ticks from the Manistee population, and none from either the Grand Traverse or Antrim populations. The Manistee ticks had an infection prevalence of 23.8%, which was higher than the Benzie ticks with a 12.5% infection prevalence. In the eastern two populations, Grand Traverse and Antrim, there was an infection rate of 0%, which coincided with a low number of ticks (Table 4, Table 5). Despite the decrease in infection prevalence there was no significant difference between any tick populations which contrasts with the significant difference between western and eastern populations of mice (Table 6). Both infected larvae and nymphs were collected from the Manistee and Benzie populations, but Manistee had more infected larval *I. scapularis* (four larvae versus one nymph) and Benzie had more infected nymphs (three larvae versus four nymphs) (Table 5). The rates of infection for both *I. scapularis* and *P. leucopus* are displayed graphically in Figure 6. For both the Manistee and Benzie sites, a higher proportion of the mice than of their ticks was infected with *B. burgdorferi*; that is, a number of infected mice had not yet transferred the infection to the ticks they carried at the time of capture.

Tick/Mouse Infection Correlation

Over half of the *P. leucopus* in both the Manistee and Benzie populations that were infested, i.e. carrying *I. scapularis*, were also infected with *B. burgdorferi* (Table 7). It was observed that in both the more-western populations an infested mouse is more likely to be infected, with 84.7% of the infested population being infected in Manistee and 52% in Benzie (Table 7). Infected mice that were infested compromised more than half of both the Manistee and Benzie samples and about a third of the entire Manistee population, 31.2%, and a quarter of the entire Benzie population, 24%, were infected mice with infected ticks (Table 7).

Fewer than half of the infected mice from both populations had multiple ticks on them, and the highest proportion of those came from the Benzie population (29.2%) rather than the Manistee population (12.5%) (Table 7). The few mice in Manistee that were carrying multiple *I. scapularis* collectively had only one infected tick. Seven infected mice from the Benzie population carried multiple *I. scapularis*, and six ticks from four of these mice were infected,

with at least one mouse having two infected ticks on it. All infected *I. scapularis* from the Manistee population came from infected mice, while 85.7% of the infected *I. scapularis* from the Benzie population came from infected mice (Table 7). There was one nymph that was infected, but it was collected from an uninfected mouse.

There was an infected *I. scapularis* found on a mouse the second time it was captured from the Benzie population, either because it was overlooked the first time or because it was acquired by the mouse during the day between the captures. In the Grand Traverse population, there was only one infected mouse, and it was not carrying any *I. scapularis* at the time of capture, while none of the mice or ticks at Antrim were infected (Table 4, Table 7).

## DISCUSSION

Over the past 30 years, *P. leucopus* has expanded its range so that it is now the dominant species of mouse in all parts of Michigan's LP. After 2006 *P. leucopus* were still captured in Manistee and Benzie Counties in Michigan, but those mice had low rates of tick infestation compared to the mice along the southern coast of Lake Michigan, and in 2007 and 2008 neither the ticks nor the mice from those counties tested positive for *B. burgdorferi* (Hamer et al., 2010). The current expansion of *I. scapularis* in Michigan is moving from southwest to northeast along the lake coastline, with fewer ticks being detected inland, and this follows the trend of Lyme disease case reports. My two research objectives for this project were to define the current limit of *B. burgdorferi* presence in its reservoir host in the northern LP of Michigan, and to better understand the enzootic cycle for Lyme disease in newly colonized regions.

### Current Edge of Sustained *B. burgdorferi* Range

Based on the data, the range of sustained *B. burgdorferi* infection has expanded beyond what was previously known in north-central Michigan. In 2006, a few infected mice were found in Manistee County but in 2007 and 2008 no infected mice were detected in either Benzie or Manistee Counties, suggesting that the range edge of sustained populations of the bacterium was in the southern part of the LP (Hamer et al., 2010). As of 2021, mouse populations in those counties have become heavily infected, with infection rates of 66.7% in Manistee and 57.1% in Benzie (Table 5). At Grand Traverse, the next site along the transect, there was only one infected mouse and a low tick count in relation to the number of mice, indicating that there is not yet a sustained *B. burgdorferi* population in that area. This significant decrease in infection rate defines the edge for sustained bacterial populations as being between northeastern Benzie County and western Grand Traverse County, with the recent expansion roughly paralleling the coastline as it curves northeastwards.

### Model of Expansion

Out of the three models suggested by Hamer at al. (2010), my data conflicts with both the "dual-invasion" and "spirochete-first" models. The "dual-invasion" model requires that mouse populations are continuing to expand their range into new areas, which is not true in this region, and that the rates of infection in both mice and ticks would be roughly equivalent, which is not

what my data show (Myers et al., 2009; Table 5). The "spirochete-first" model requires that infected mice be present in an area before there are ticks present, which is contradicted by the presence of *I. scapularis* on uninfected mice in both Antrim and Cheboygan Counties, at the far northeast end of the transect (Table 4; Table 5; Freund, unpublished data). Instead, my data fit best with the "tick-first" scenario for expansion of *B. burgdorferi*, where the bacteria spread into new areas that already have established tick populations on mouse hosts. The prevalence of infection should be higher in *P. leucopus* than in their ticks since they are carrying the bacteria to a new region, and that is true for all populations except Antrim, where *B. burgdorferi* was not detected in either mice or ticks. This model also requires some movement of *P. leucopus*, and while the population numbers of *P. leucopus* have remained fairly stable over the past dozen years across the northern LP, mice will still disperse upon reaching adulthood, so there is the potential for a gradual immigration of infected mice into uninfected populations. Infections in both the ticks and mice follow the same geographic patterns, with rates decreasing (though not significantly) between the Manistee site and the Benzie site, then dropping off sharply from the Benzie site to the next site along the transect, Grand Traverse (Figure 6).

Where the data in this study differ from the criteria for the "tick-first" model is that, even though the ticks are spreading ahead of the bacterium, as evidenced by the uninfected *I. scapularis* caught in Antrim Co., tick numbers dropped off moving eastward along the transect, while the mice are well established at all locations (Table 4). Thus, my data support a slightly modified scenario from the "tick-first" model of Hamer et al. (2010), with uninfected *P. leucopus* establishing themselves in a region first, followed by adult *I. scapularis* being carried in on deer or birds, reproducing, and spreading onto mouse hosts. Finally, the bacterium is brought in by infected mice or birds and becomes well established in *P. leucopus* hosts, setting up a sustained *B. burgdorferi* population.

Support for Previous Research

The data also support previously identified trends of expansion for both Lyme disease and ticks in the LP, with a decrease in the number of ticks and the proportion of infected mice as one moves northwards and eastwards from the south end of Lake Michigan, and a time lag between the range expansion of ticks and of the bacterium (Nguyen et al, 2019; Clow et al, 2017). The infection prevalence of *B. burgdorferi* for *P. leucopus* was highest in Manistee

County, the most southwestern population, and decreased moving northeastwards; also, fewer ticks were collected from the two more northeastern sites, which were further away from the west coastline of Michigan (Table 5). There was a dramatic turnover in infection rates between the Benzie *P. leucopus* population and the Grand Traverse population. The more southwestern populations had the majority of mice test positive for *B. burgdorferi*, while the Grand Traverse and Antrim populations had only a single infected mouse between them (Table 5). Also, there was a difference between the infection prevalence in *I. scapularis* versus *P. leucopus* in both the Manistee and Benzie populations, with the infection rates in ticks consistently lower than in their mouse hosts, presumably because many young ticks had not been feeding long enough to acquire the bacterium from the mice at time of capture.

In terms of maintaining the enzootic cycle for Lyme disease, both *P. leucopus* and *I. scapularis* need to be present in sufficient numbers, and a certain fraction of each need to be infected. Looking at the correspondence between infected *P. leucopus* and *I. scapularis*, with at least a quarter of infected mice carrying infected ticks in Manistee and Benzie Counties and all but one of the infected ticks between Manistee County and Benzie County coming from infected mice, the data support the finding that *P. leucopus* is a primary reservoir for *B. burgdorferi*. However, it is unknown whether the ticks received the infection exclusively from the mice, because a timeline of infection and infestation cannot be established and no other local animals were tested. In the Manistee population, every single infected *I. scapularis* came from an infected *P. leucopus*, and a little more than two-thirds of the infected mice carried ticks (Table 5; Table 6). In Benzie County, all infected ticks came from an infected mouse except for one (Table 6), while only about half of the infected *P. leucopus* were carrying *I. scapularis*.

Rate of Lyme Disease Expansion

There are several factors that may explain why the expansion of *B. burgdorferi* lags behind expansions of the host and vector populations. Carriers such as birds and deer are able to distribute ticks further than any mouse could in its lifetime (which is possibly how a single infected tick appeared in the Grand Traverse mouse population), but movement patterns and tick life stages may limit the distribution of the bacteria. White tailed deer are important in the life cycle of *I. scapularis* because they are the end stage hosts where ticks will mate, reproduce, then die. While deer may carry ticks into a new region, they have no role in maintaining the bacterial

infection because they are carrying primarily adult ticks. These adults are unlikely to feed on mice, as evidenced by my data, where adult ticks were the only mobile stage not caught on mice, and they cannot transmit the infection transovarially, so all their offspring start out uninfected (Table 4; Radolf et al., 2012). As for birds, some species, such as the American robin (*Turdus migratorius*) and song sparrow (*Melospiza melodia*), are competent reservoir hosts, but their infectivity wanes more rapidly than it does in *P. leucopus*, starting at 2 months and clearing altogether after 6 months (Richter et al., 2000). Many bird species in Michigan migrate south for the winter then return when temperatures rise; however, the Hamer paper (2010) suggests that migratory birds travel parallel to Lake Michigan, making periodic stops on the coast during the night (Hamer et al, 2010; Gesicki et al, 2019). This may explain why ticks in general are spread along the coastline, with only a single infected tick captured more than 66 km inland from the coast.

The spread of *B. burgdorferi* from coastal to more inland sites is likely to be more reliant on the dispersal of small mammals, such as *P. leucopus*. While it is possible for individually infected mice to disperse far enough to introduce the bacterium into a previously uninfected population, this does not generally occur over long distances, and even if it does occur it is very gradual unless mice are accidentally transported by humans (Myers et al. 2009). Also, due to being at the northern limits of their range, *P. leucopus* in this region suffer high winter mortality because of nesting behaviors, scarcity of food resources, and the presence of intestinal parasites (Long, 1996; Pederson & Greives, 2007). As a result of the limited breeding season and high winter mortality, only a small fraction of the previous year's *P. leucopus* population will be present in the spring to breed the following season. Therefore, there must be a high rate of infection in the fall mouse population in order for some spring breeders to be infected, so that they can then spread the infection to young mice and ticks during the summer.

Drivers of Lyme Disease Expansion

Recent climate trends may continue to increase local *P. leucopus* population numbers in the northern Midwest by lowering their winter mortality rates. Milder winters would negate the lack of adaptations that *P. leucopus* have for surviving colder winters, because the average temperature during winters would increase and there would be less frost (Myers et al., 2009). Also, an increase in the number of females able to reproduce early in the breeding season would

increase the population numbers, and of the autumn-born mice, the females are more likely to survive to the next spring (Schug et al., 1991). *I. scapularis* also benefits from milder winters. The ticks have earlier and prolonged feeding periods in warmer climates, which increases the chance of their becoming infected and passing the infection on (Nguyen et al., 2019). There is also less of a chance of inoculative freezing, which requires long term direct exposure to ice at -5C to -3C, and this is an important factor in the overwinter mortality in ticks (Burks et al., 1996). While *B. burgdorferi* infection has no known impact on the overwinter survival of *P. leucopus,* it does seem to have a positive impact on the survival of *I. scapularis,* possibly because it triggers the production of an antifreeze protein (Nabbout et al., 2023).

Another factor behind the increased presence of *B. burgdorferi* in new regions is driven by anthropogenic activity. The dilution effect suggests that a loss of local biodiversity caused by changing human land use may increase the spread of *B. burgdorferi* (Cook et al., 2012). The reason for this is twofold--there can be a decrease in the population of predators that control the populations of *P. leucopus,* and there is also a decrease in the populations of some dead-end hosts that would not sustain the infection (Cook et al., 2012). One study has shown an association between vertebrate species richness and infection prevalence in nymphs, with fewer nymphs being infected when there was a higher species richness (Werden et al, 2014).

Many species of birds have territories that overlap with the generalist *P. leucopus,* especially ground foraging species that are more likely to encounter ticks. Two species in particular, the American robin and the song sparrow, are regarded as competent hosts for *B. burgdorferi sensu lato* and are able to pass the infection on to feeding ticks. A 2005 study showed 81.8% of ticks fed on *T. migratorius* and 21.1% of ticks fed on *M. melodia* testing positive for *B. burgdorferi* in the lab, though the prevalence of infected ticks that fed on birds decreased in the wild to 16% and 4.8% (Ginsberg et al, 2005). Furthermore, ticks that fed on infected *T. migratorius* were able to pass the infection to *P. leucopus*, which then infected the ticks that fed on them (Richter et al., 2000), showing that the bacterium can pass from avian to mammalian species and remain infective to ticks. In terms of the life stage of ticks that infest their blood meal host, birds will have roughly the same number of larvae and nymphs, while mice typically have more larvae feeding on them then nymphs, though nymphs can still infest mice (Richter et al., 2000; Table 4). Also, of the three mobile life stages of ticks, nymphs appear to be the most cold resistant (Vandyk et al., 1996); which might increase their survival on mouse

hosts in the northern part of their range. Thus, the expanding range and increased population sizes of *P. leucopus* are likely to be a major factor in the continued expansion of Lyme disease in the Greak Lakes region.

Limitations & Future Research

One limitation to this study was that only one year of trapping was conducted, so that these data provide a snapshot of a single time point. There was also the issue of having small numbers of infected mice in Grand Traverse and Antrim populations along with a low number of infected ticks in all populations which impacted the statistical analysis. Also, no birds were trapped and no ticks were found on other trapped small mammals, so I have no information on alternative hosts along this transect. In the future, I would recommend continual trapping and monitoring across the northern tip of the LP, in particular along the edge of the range of established *B. burgdorferi* as defined by this study. Monitoring ticks found on birds would also be important, since that is likely how the bacterium is going to be introduced over longer distances.

There are ways to help mitigate the impact of *B. burgdorferi* and prevent the spread of Lyme disease. One such strategy that has been suggested is the immunization of *P. leucopus*, though this seems impractical due to the large numbers of mice present in any suitable habitat. OspA immunization testing found that when mice were given a vaccine based on the OspA protein, the infectivity of their ticks was greatly reduced (Tsao et al, 2001; Radolf et al, 2012). In fact, Valneva and Pfizer are currently working on a Lyme disease vaccine for humans that targets OspA (CDC, 2021).

Conclusions

Lyme disease has continued to spread across parts of the United States, along with its vector, *I. scapularis*, and reservoir host, *P. leucopus*. The Upper Midwest is a region of concern, as there has been an increase in the incidence of cases in states like Michigan and Ohio. Since it is an increasingly common and widespread disease, it is important to know where and how the bacterium becomes established in a region. Past studies showed that infection prevalence of *B. burgdorferi* was not high enough in the northern half of Michigan's LP to be considered

sustained, and that there was a decreasing trend in infection prevalence moving both north and east from the southwest corner of the state.

I have found that in the past decade there has been an expansion of the region where sustained populations of *B. burgdorferi* can be found in LP mice; between 2008 and 2021, the bacterium has spread along the western coastline in Michigan, with established populations extending from the southern half of the LP to as far north as eastern Benzie County. This was likely due to long distance carriers, such as migratory birds, increasing the number of both infected and uninfected ticks in an area. Moving into more inland counties, where the infection prevalence is much lower, deer may have more of a role as carriers, but while they do transport greater number of ticks, they do not enable *B. burgdorferi* becoming endemic in an area. Carriers help get the enzootic cycle for Lyme disease started and assist with maintaining it, but both *I. scapularis* and *P. leucopus* need to present in sufficient numbers to establish *B. burgdorferi* in an area. While Lyme disease remains a major issue across the nation, it is important to be vigilant about the locations of infected *P. leucopus* in order to direct preventative measures towards those areas where the infection is truly endemic.

REFERENCES

2021. 2021 Michigan Lyme Disease Map. Michigan Department of Health & Human Services. https://www.michigan.gov/-/media/Project/Websites/emergingdiseases/Folder2/Lyme_risk_map2020.pdf?rev=c578ab550b864851a88c542bf0494963

Anderson, J.M., Moore, I.N., Nagata, B.M., Ribeiro, J.M.C., Valenzuela, J.G., and Sonenshine, D.E. 2017. Ticks, *Ixodes scapularis*, Feed Repeatedly on White Footed Mice Despite Strong Inflammatory Response: An Expanding Paradigm for Understanding Tick-Host Interactions. Frontiers in Immunology. 8:1784. https://www.frontiersin.org/articles/10.3389/fimmu.2017.01784/full

Barbour, A.G. and Fish, D. 1993. The Biological and Social Phenomenon of Lyme Disease. Science. 260(5114):1610-1616. https://www.science.org/doi/10.1126/science.8503006

Bedford, N.L and Hoekstra, H.E. 2015. The Natural History of Model Organisms: *Peromyscus* mice as a Model for Studying Natural Variation. eLife. https://elifesciences.org/articles/06813

Bouchard, C., Beauchamp, G., Nguon, S., Trudel, L., Milord, F., Lindsay, L.R., Belanger, D., and Ogden, N.H. 2011. Associations Between *Ixodes scapularis* ticks and Small Mammal Hosts in a Newly Endemic Zone in Southeastern Canada: Implications for *Borrelia burgdorferi* transmission. Tick and Tick-borne Diseases. 2(4):183-190. https://pubmed.ncbi.nlm.nih.gov/22108010/

Bunikis, J., Tsao, J., Luke, C.J., Luna, M.G., Fish, D., and Barbour, A.G. 2004. Borrelia burgdorferi Infection in a Natural Population of *Peromyscus leucopus* Mice: A Longitudinal Study in an Area Where Lyme Borreliosis is Highly Endemic. The Journal of Infectious Diseases. 189(8):1515-1523. https://academic.oup.com/jid/article/189/8/1515/821767?login=true

Burks, C.S., Stewart, R.L., Needham, G.R., Lee, R.E. 1996. The Role of Direct Chilling Injury and Inoculative Freezing in Cold Tolerance of *Amblyomma americanum*, *Dermacenter variabilis* and *Ixodes scapularis*. Physiological Entomology. 22(1): 44-50. https://resjournals.onlinelibrary.wiley.com/doi/abs/10.1111/j.1365-3032.1996.tb00833.x

CDC. 2020. Lyme Disease: Transmission. https://www.cdc.gov/lyme/transmission/index.html

CDC. 2021. Lyme Disease: Signs and Symptoms of Untreated Lyme Disease. https://www.cdc.gov/lyme/signs_symptoms/index.html

CDC. 2021. Lyme Disease Data Tables: Historical Data. https://www.cdc.gov/lyme/stats/tables.html

CDC. 2021. Lyme Disease Maps: Historical Data. https://www.cdc.gov/lyme/stats/maps.html

CDC, 2021. Lyme Disease Vaccine. https://www.cdc.gov/lyme/prev/vaccine.html#:~:text=VLA15%20is%20a%20multivalent%2C%20protein,of%20the%20Lyme%20disease%20bacterium.

CDC. 2021. Reported Cases of Lyme Disease-United States, 2019. https://www.cdc.gov/lyme/datasurveillance/maps-recent.html
CDC. 2022. Lyme Disease. https://www.cdc.gov/lyme/index.html

Clark, K., Hendricks, A., and Burge, D. 2005. Molecular Identification and Analysis of *Borrelia burgdorferi* Sensu Lato in Lizards in the Southeastern United States. Applied and Environmental Microbiology. 71(5):2616-2625. https://journals.asm.org/doi/10.1128/AEM.71.5.2616-2625.2005

Clow, K.M., Leighton, P.A., Ogden, N.H., Lindsay, L.R., Michel, P., Pearl, D.L., and Jardine, C.M. 2017. Northward Range Expansion of *Ixodes scapularis* Evident Over a Short Timescale in Ontario, Canada. PLoS ONE.
12(12). https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0189393

Cook, R.A. and Karesh, W.B. 2012. *Emerging Diseases at the Interface of People, Domestic Animals, and Wildlife.* Fowler's Zoo and Wild Animal Medicine. 136-146. https://www.sciencedirect.com/science/article/pii/B9781437719864000184?via%3Dihub

Donnelley, T.M., Bergin, I., and Ihrig, M. 2015. Biology and Disease of Other Rodents. Laboratory Animal Medicine. 285-349. https://www.sciencedirect.com/science/article/pii/B9780124095274000079

Eisen, R.J., Eisen, L., and Beard, C.B. 2016. County Scale Distribution of *Ixodes scapularis* and *Ixodes pacificus* (Acari: Ixodidae) in Continental United States. https://academic.oup.com/jme/article/53/2/349/2459744

Elbaum-Garfinkle, S. 2011. Close to Home: A History of Yale and Lyme Disease. Yale Journal of Biology and Medicine. 84(2):103-108. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3117402/

Gesicki, D. V., Cech, E. L., Bingman, V. P. 2019. Detoured Flight Direction Responses Along the Southwest Coast of Lake Erie by Night Migrating Birds. The Auk. 136 (3). https://academic.oup.com/auk/article/136/3/ukz018/5471136

Ginsburg, H.S., Buckley, P.A., Balmforth, M.G., Zhioua, E., Mitra, S., Buckley, F.G. 2005. Reservoir Competence of Native North American Birds for the Lyme Disease Spirochete, *Borrelia burgdorferi*. Journal of Medical Entomology. 42(3): 445-449. https://academic.oup.com/jme/article/42/3/445/850298

Green, M.R. and Sambrook, J. 2019. Nested Polymerase Chain Reaction (PCR). Cold Spring Harbor Protocols. http://cshprotocols.cshlp.org/content/2019/2/pdb.prot095182

Guy, E.C. and Stanek, G. 1991. Detection of *Borrelia burgdorferi* in Patients with Lyme disease by the Polymerase Chain Reaction. Journal of Clinical Pathology. 44(1):610-611. https://pubmed.ncbi.nlm.nih.gov/1856296/

Hamer, S.A., Tsao, J.I., Walker, E.D., and Hickling, G. 2010. Invasion of Lyme Disease Vector *Ixodes scapularis*: Implications for *Borrelia burgdorferi* Endemicity. Ecohealth. 7:47-63. https://link.springer.com/article/10.1007/s10393-010-0287-0

Havelka, M. A., Millar, J.S. 2004. Maternal Age Drives Seasonal Variation in Litter Size of *Peromyscus leucopus*. Journal of Mammalogy. 85(5): 940- 947. https://academic.oup.com/jmammal/article/85/5/940/860900

Jonston, P.G., Zucker, I. 1980. Photoperiodic Regulation of Reproductive Development in White-footed Mice (*Peromyscus leucopus*). Biology of Reproduction. 22(4): 983-989. https://academic.oup.com/biolreprod/article/22/4/983/2768163

Keane, B. 1990. Dispersal and Inbreeding Avoidance in the White-footed Mouse, *Peromyscus leucopus*. Animal Behavior. 40 (1):143-152. https://www.sciencedirect.com/science/article/abs/pii/S0003347205806748

Kilpatrick, H.J., Labonate, A.M., and Stafford, K.C. 2014. The Relationship Between Deer Density, Tick Abundance, and Human Cases of Lyme Disease in a Residential Community. Journal of Medical Entomology. 51(4):777-784. https://academic.oup.com/jme/article/51/4/777/894869?login=false

Kruse, H., Kirkemo, A.M., and Handeland, K. 2004. Wildlife as Source of Zoonotic Infections. Emerging Infectious Diseases. 10(12):2067-2072. https://wwwnc.cdc.gov/eid/article/10/12/04-0707_article

Lantos, P.M., Tsao, J., Nigrovic, L.E., Auwaerter, P.G., Fowler, V.G., Ruffin, F., Foster, and E., Hickling, G. Geographic Exanpsion of Lyme Disease in Michigan, 2000-2014. Open Forum Infectious Diseases. 4(1):1-5. https://academic.oup.com/ofid/article/4/1/ofw269/2871218?login=false

Levine, J.F., Wilson, M.L., and Spielman, A. 1985. Mice as Reservoirs of the Lyme Disease Spirochete. The American Journal of Topical Medicine and Hygiene. 34(2):355-360. https://www.ajtmh.org/view/journals/tpmd/34/2/article-p355.xml

Long, A.D., Baldwin-Brown, J., Tao, Y., Cook, V.J., Balderrama-Gutierrez, G., Corbett-Detig, R., Mortazavi, A., Barbour, A.G. 2019. The Genome of *Peromyscus leucopus*, Natural History for Lyme Disease and Other Emerging Infections. Science Advances. 5(7):1-9. https://www.science.org/doi/10.1126/sciadv.aaw6441

Long, C.A. 1996. Ecological Replacement of the Deer Mouse, *Peromyscus maniculatus*, by the White-Footed Mouse, *P. leucopus*, in the Great Lakes Region. Canadian Field Naturalist. 110(2):271-277. https://www.biodiversitylibrary.org/item/106979#page/283/mode/1up

Loss, S.R., Noden, B.H., Hamer, G.L., and Hamer, S.A. 2016. A Quantitative Synthesis of the Role of Birds in Carrying Ticks and Tick-borne Pathogens in North America. Oecologia. 182:947-959. https://link.springer.com/article/10.1007/s00442-016-3731-1

Maier, T. J. 2002. Long-distance Movements by Female White-Footed Mice, *Peromyscus leucopus*, in Extensive Mixed-wood Forest. Canadian Field Naturalist. 116(1):108-111. https://www.fs.fed.us/ne/newtown_square/publications/other_publishers/OCR/ne_2002_maier001.pdf

Mather, T.N., Wilson, M.L., Moore, S.I., Ribeiro, J. M. C., and Spielman, A. 1989. Comparing The Relative Potential of Rodents as Reservoirs Of The Lyme Disease Spirochete (*Borrelia Burgdorferi*). American Journal of Epidemiology. 130(1):143-150. https://link.springer.com/article/10.1007/s10144-008-0094-4

Moter, S.E., Hofmann, H., Wallich, R., Simon, M.M., and Kramer, M.D. 1994. Detection of *Borrelia burgdorferi* Sensu Lato in Lesional Skin Patients with Erythema Migrans and Acrodermatitis Chronica atrophicans by ospA-specific PCR. Journal of Clinical Microbiology. 32(12):2980-2988. https://journals.asm.org/doi/epdf/10.1128/jcm.32.12.2980-2988.1994

Myers, P., Lundrigan, B.L., Hoffman, S.M.G., Haraminac, A.P., and Seto, S. H. 2009. Climate-Induced Changes in the Small Mammal Communities of the Northern Great Lakes Region. Global Change Biology. 15(6):1434-1454. https://onlinelibrary.wiley.com/doi/epdf/10.1111/j.1365-2486.2009.01846.x

Nabbout, A.E., Ferguson, L.V., Miyashita, A., Adamo, S.A. 2023. Female ticks (*Ixodes scapularis*) infected with *Borrelia burgdorferi* have increased overwintering survival, with implications for tick population growth. Insect Science. https://onlinelibrary.wiley.com/doi/full/10.1111/1744-7917.13205

Nguyen, A., Mahaffy, J., and Vaidya, N.K. 2019. Modeling Transmission Dynamics of Lyme Disease: Multiple Vectors, Seasonality, and Vector Mobility. Infectious Disease Modelling. 4:28-43. https://www.sciencedirect.com/science/article/pii/S2468042718300538?via%3Dihub

Parise, C.M., Breuner, N.E., Hojgaard, A., Osikowicz, L.M., Replogle, A.J., Eisen, R.J., and Eisen, L. 2020. Experimental Demonstration of Reservoir Competence of the White Footed Mouse, *Peromyscus leucopus* (Rodentia: Cricetidae), for the Lyme Disease Spirochete, *Borrelia mayonii* (Spirochetales: Spirochetaceae). Journal of Medical Entomology. 57(3):927-932. https://academic.oup.com/jme/article/57/3/927/5670886?login=false

Peavey, C.A., Lane, R.S. 1995. Transmission of *Borrelia burgdorferi* by *Ixodes pacificus* Nymphs and Reservoir Competence of Deer Mice (*Peromyscus maniculatus*) Infected by Tick-

bite. The Journal of Parasitology. 81(2): 175- 178. https://www.jstor.org/stable/3283916?origin=crossref

Pederson, A.B., Grieves, T.J. 2008. The Interaction of Parasites and Resources Cause Crashes in Wild Mouse Population. Journal of Animal Ecology. 77(2): 370- 377. https://besjournals.onlinelibrary.wiley.com/doi/10.1111/j.1365-2656.2007.01321.x

Poje, J.E., Azevedo, J.F., Nair, N., Mahachi, K., Frank, L.E., Sherpa, P., Krizek, R.S., Baccam, T., Gomes-Solecki, M., and Peterson, C.A. 2022. *Borrelia burgdorferi* (Spirochaetales: Spirochaetaceae) Infection Prevalence and Host Associations of Ticks Found on *Peromyscus* spp in Maryland. Journal of Medical Entomology. 59(2):752-757. https://academic.oup.com/jme/article-abstract/59/2/752/6490744?redirectedFrom=fulltext&login=false

Radolf, J.D., Caimano, M.J., Stevenson, B., and Hu, L.T. 2012. Of Ticks, Mice, and Men: Understanding the Dual-host Lifestyle of Lyme Disease Spirochaetes. Nature Reviews Microbiology. 10:87-99. https://www.nature.com/articles/nrmicro2714

Radzijevskaja, J., Paulauskas, A., and Rosef, O. 2011. Molecular Detection and Characterization of *Borrelia burgdorferi* Sensu Lato in Small Rodents. Veterinarija ir Zootechnika. 55(77):40-46. https://www.researchgate.net/profile/Olav-Rosef/publication/228827340_Molecular_detection_and_characterization_of_Borrelia_burgdorferi_sensu_lato_in_small_rodents/links/00463529a6bf29028f000000/Molecular-detection-and-characterization-of-Borrelia-burgdorferi-sensu-lato-in-small-rodents.pdf

Rahman, T., Sobur, A., Islam, S., Ievy, S., Hossain, J., El Zowalaty, M.E., Rahman, T., and Ashour, H.M. 2020. Zoonotic Diseases: Etiology, Impact, and Control. Microorganisms. 8(9):1-34. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7563794/

Richter, D., Spielman, A., Komar, N., Matuschka, F.R. 2000. Competence of American Robins as Reservoir Hosts for Lyme Disease Spirochetes. Emerging Infectious Diseases. 6(2): 133-138. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2640847/

Robinson, E.L., Jardine, C.M., Koffi, J.K., Russell, C., Lindsay, L.R., Dibernardo, A., and Clow, K.M. 2022. Range Expansion of *Ixodes scapularis* and *Borrelia burgdorferi* in Ontario, Canada, from 2017 to 2019. Vector Borne Zoonotic Diseases. 22(7):361-369. https://www.liebertpub.com/doi/10.1089/vbz.2022.0015

Roy-Dufresne, E., Logan, T., Simon, J.A., Chumra, G.L., and Millien, V. 2013. Poleward Expansion of the White Footed Mouse (*Peromyscus leucopus*) under Climate Change: Implications for the Spread of Lyme Disease. PLoS ONE. 8(11). https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0080724#:~:text=A%20prime%20example%20of%20a,Michigan's%20Upper%20Peninsula%20%5B13%5D.

Schug, M.D., Vessey, S.H., and Korytko, A.I. 1991. Longevity and Survival in a Population of White-Footed Mice (*Peromyscus leucopus*). Journal of Mammalogy. 72(2):360-366. https://academic.oup.com/jmammal/article-abstract/72/2/360/838384

Sikes, R.S. 2016. 2016 Guidelines for the American Society of Mammalogists for the Use of Wild Mammals in Research and Education. Journal of Mammalogy. 97(3):663-688. https://academic.oup.com/jmammal/article/97/3/663/2459909

Tsao, J., Barbour, A.G., Luke, C.J., Fikrig, E., and Fish, D. 2004. OspA Immunization Decreases Transmission of *Borrelia burgdorferi* Spirochetes from Infected *Peromyscus leucopus* Mice to Larval *Ixodes scapularis* Ticks. Vector-Borne and Zoonotic Diseases. 1(1)65-74. https://www.liebertpub.com/doi/10.1089/153036601750137705

Vandyk, J.K., Bartholomew, D.M., Rowley, W.A., Platt, K.B. 1996. Survival of *Ixodes scapularis* (Acari: Ixodidae) Exposed to Cold. Journal of Medical Entomology. 33(1): 6-10. https://academic.oup.com/jme/article-abstract/33/1/6/883174

Vessey, S.H. and Vessey, K.B. 2007. Linking Behavior, Life History and Food Supply with the Population Dynamics of White-footed Mice (*Peromyscus leucopus*). Integrative Zoology. 123-130. https://onlinelibrary.wiley.com/doi/epdf/10.1111/j.1749-4877.2007.00053.x

Werden, L., Barker, I.K., Bowmann, J., Gonzales, E.K., Leighton, P.A., Lindsay, L.R., and Jardine, C. Geography, Deer, and Host Biodiversity Shape the Pattern of Lyme Disease Emergence in the Thousand Islands Archipelago of Ontario, Canada. PLoS ONE. 9(1). https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0085640

Wolff, J.O. and Durr, D.S. 1986. Winter Nesting Behavior of *Peromyscus leucopus* and *Peromyscus maniculatus*. Journal of Mammalogy. 67(2):409-412. https://academic.oup.com/jmammal/article-abstract/67/2/409/861666?redirectedFrom=fulltext

Zawada, S.G., von Fricken, M.E., Weppelmann, T.A., Sikaroodi, M., and Gillevet, P.M. 2020. Optimization of Tissue Sampling for *Borrelia burgdorferi* in White-footed Mice (*Peromyscus leucopus*). PLoS ONE. https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0226798

TABLES

Table 1: List of sites in MI where trapping occurred. Sites start with the most southern one, then are listed as they progress along the south-north transect. Includes county each site is found in and GPS coordinates.

| County | Site | GPS Coordinates |
|---|---|---|
| Manistee | Bayou | 44.2695, -86.274 |
| Benzie | Betsie River Campground | 44.5868, -86.0044 |
| | Alysworth Rd 2 | 44.60164, -85.9887 |
| | Alysworth Rd | 44.60199, -85.9922 |
| Grand Traverse | Brown Bridge | 44.64238, -85.4819 |
| Antrim | Grass River | 44.90959, -85.2292 |

Table 2: Standard master mix recipe for both the outer and inner PCRs, using OspA primers for one reaction.

| Reagent | Outer PCR (uL per sample) | Inner PCR (uL per sample) |
|---|---|---|
| Water | 3 | 4 |
| MgCl2 | 1.5 | 1.5 |
| Buffer | 1.5 | 1.5 |
| dNTP | 2.4 | 2.4 |
| OspA Forward primer | 1.5 | 1.5 |
| OspA Reverse primer | 1.5 | 1.5 |
| Taq polymerase | 0.1 | 0.1 |
| BSA | 0.15 | 0.15 |

Table 3: Oligonucleotide primer sequences for the *B. burgdorferi* target gene OspA (Guy et al, 1991).

| Primer | Primer Sequence (5'-3') | Base Position |
|---|---|---|
| Outer Forward (N1) | GAG-CTT-AAA-GGA-ACT-TCT-GAT-AA | 334-356 |
| Outer Reverse (C1) | GTA-TTG-TTG-TAC-TGT-AAT-TGT | 894-874 |
| Inner Forward (N2) | ATG-GAT-CTG-GAG-TAC-TTG-AA | 362-381 |
| Inner Reverse (C2) | CTT-AAA-GTA-ACA-GTT-CCT-TCT | 713-693 |

Table 4: Total number of both white footed mice, *P. leucopus*, and deer ticks, *I. scapularis*, obtained at each county. Along with the number of *P. leucopus* with *I. scapularis* found on them, and the life stage of each *I. scapularis* collected (L = larval, N = nymph, A = adult). Infested mice were labeled as "TcM" for tick carrying mice.

| Population | Number of Mice | Number of Ticks | Number of TcM | TcM (%) | Mice w/ Multiple Ticks (%) | Life Stage (L/N/A) |
|---|---|---|---|---|---|---|
| Manistee | 24 | 21 | 13 | 54.2 | 16.7 | 20/1/0 |
| Benzie | 42 | 56 | 25 | 59.5 | 31 | 47/9/0 |
| Grand Traverse | 40 | 3 | 3 | 7.5 | 0 | 1/2/0 |
| Antrim | 15 | 8 | 7 | 46.7 | 6.7 | 8/0/0 |

Table 5: Number and proportion of both *P. leucopus* and *I. scapularis* that tested positive for *B. burgdorferi* in each population, and the life stage of the infected ticks (L = larval, N = nymph, A = adult).

| Population | Number of Infected Mice | Infected Mice (%) | Number of Infected Ticks | Infected Ticks (%) | Life Stage Infected Ticks (L/N/A) |
|---|---|---|---|---|---|
| Manistee | 16 | 66.7 | 5 | 23.8 | 4/1/0 |
| Benzie | 24 | 57.1 | 7 | 12.5 | 3/4/0 |
| Grand Traverse | 1 | 2.5 | 0 | 0 | 0/0/0 |
| Antrim | 0 | 0 | 0 | 0 | 0/0/0 |

Table 6: Statistical data based on the comparisons between proportion of infected mice (A) and infected ticks (B) in populations at different sites.

| Populations (Mice) | P value | 95% Confidence Intervals |
|---|---|---|
| Manistee/ Benzie | 0.6013 | 0.4723966, 4.9706780 |
| Manistee/ Grand Traverse | 2.16E-08 | 8.846082, 3275.972331 |
| Manistee/ Antrim | 2.52E-05 | 5.095566, Inf |
| Benzie/ Grand Traverse | 2.86E-07 | 0.3647, 0.7281 |
| Benzie/ Antrim | 5.00E-05 | 3.969449, Inf |
| Grand Traverse/ Antrim | 1.00E+00 | 0.009630859, Inf |

| Populations (Ticks) | P value | 95% Confidence Intervals |
|---|---|---|
| Manistee/ Benzie | 0.291 | 0.4723544, 9.2616919 |
| Manistee/ Grand Traverse | 1 | 0.09968377, Inf |
| Manistee/ Antrim | 0.2832 | 0.3553859, Inf |
| Benzie/ Grand Traverse | 1 | 0.05110359, Inf |
| Benzie/ Antrim | 0.5819 | 0.1912339, Inf |
| Grand Traverse/ Antrim | 1 | 0, Inf |

Table 7: Correspondence of *Borrelia* positive *P. leucopus* with any *I. scapularis* collected off them. Infested mice were labeled as "TcM" for tick carrying mice.

| **Population** | **Total # of Mice** | **TcM that were Infected (%)** | **Infected Mice that were TcM (%)** | **Infected Mice w/ Infected Ticks (%)** | **Infected Ticks on Infected Mice (%)** |
|---|---|---|---|---|---|
| Manistee | 24 | 84.6 | 68.7 | 31.2 | 100 |
| Benzie | 42 | 52 | 54.2 | 24 | 85.7 |
| Grand Traverse | 40 | 0 | 0 | 0 | 0 |
| Antrim | 15 | 0 | 0 | 0 | 0 |

FIGURES

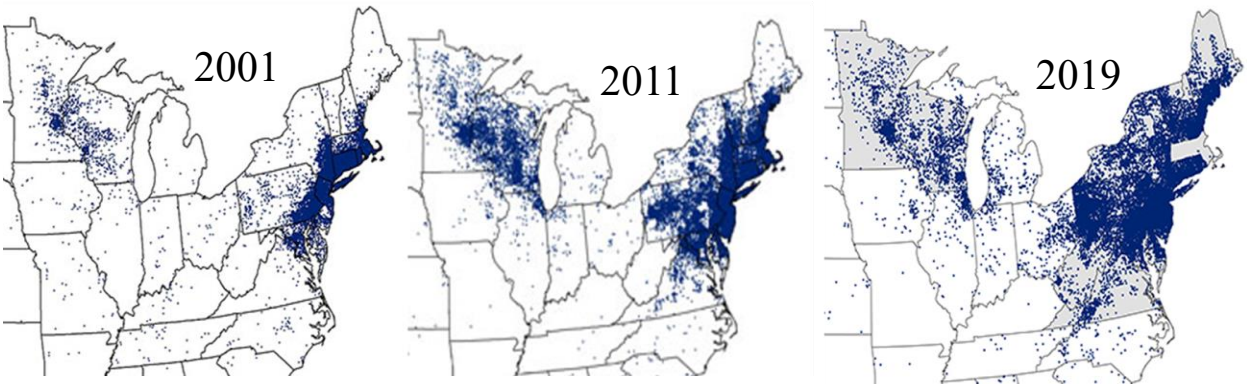


Figure 1: Comparison of the number of reported cases for Lyme disease over an 18 year period in the northeastern quarter of the United States, with 2001 cases map on left, 2011 cases map in the middle, and 2019 cases map occupying the right. In the 2019 map white states are considered low incidence and gray states are considered high incidence (CDC, 2021).

Figure 2: Cases of Lyme disease by county in Michigan over a 14 year period. Highest concentration of cases were in the southwest corner of the LP and the southern tip of the UP (Lantos et al., 2017).

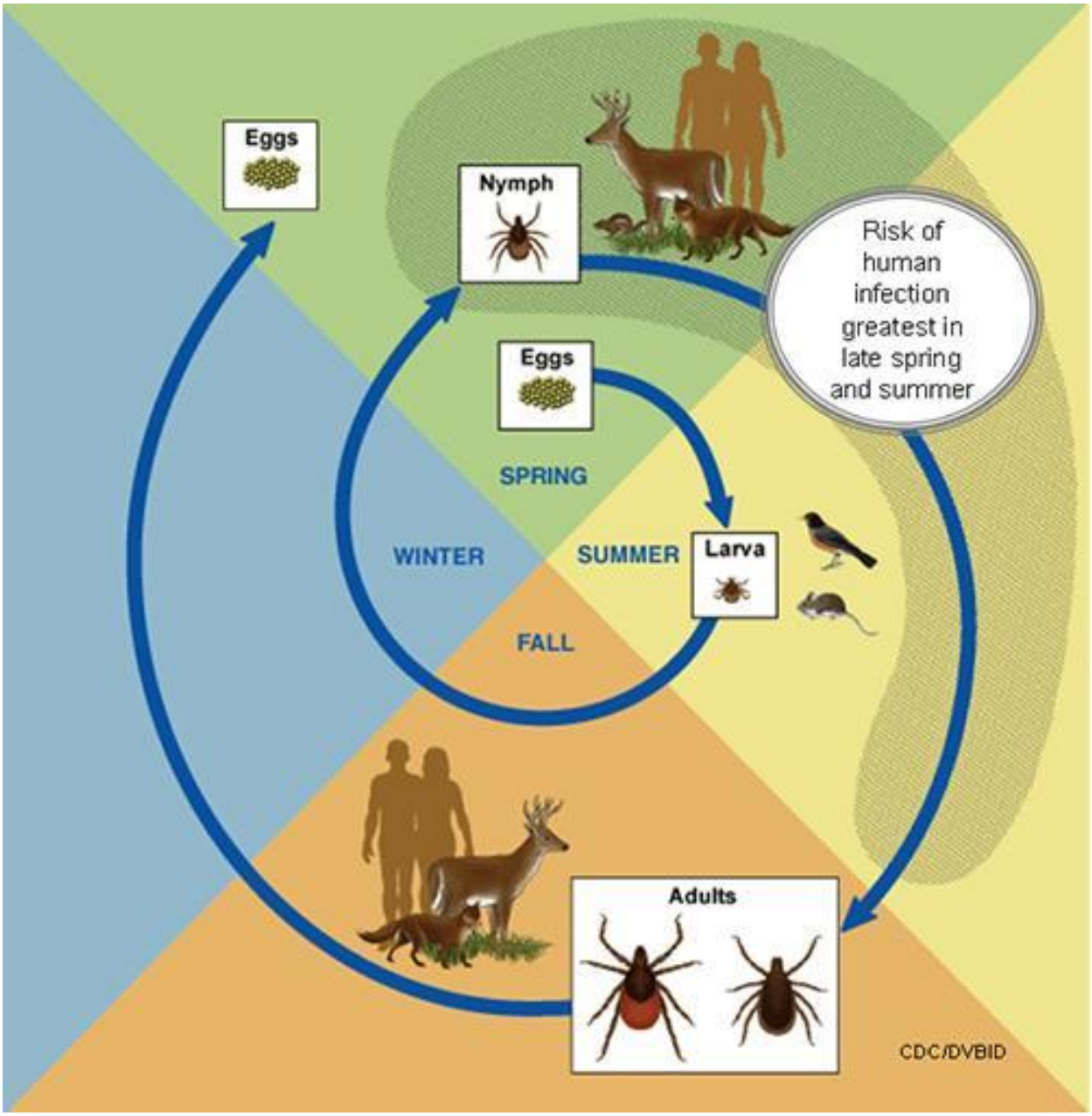


Figure 3: Life cycle of *Ixodes scapularis* along with the hosts they usually parasitize at each stage (CDC, 2020).

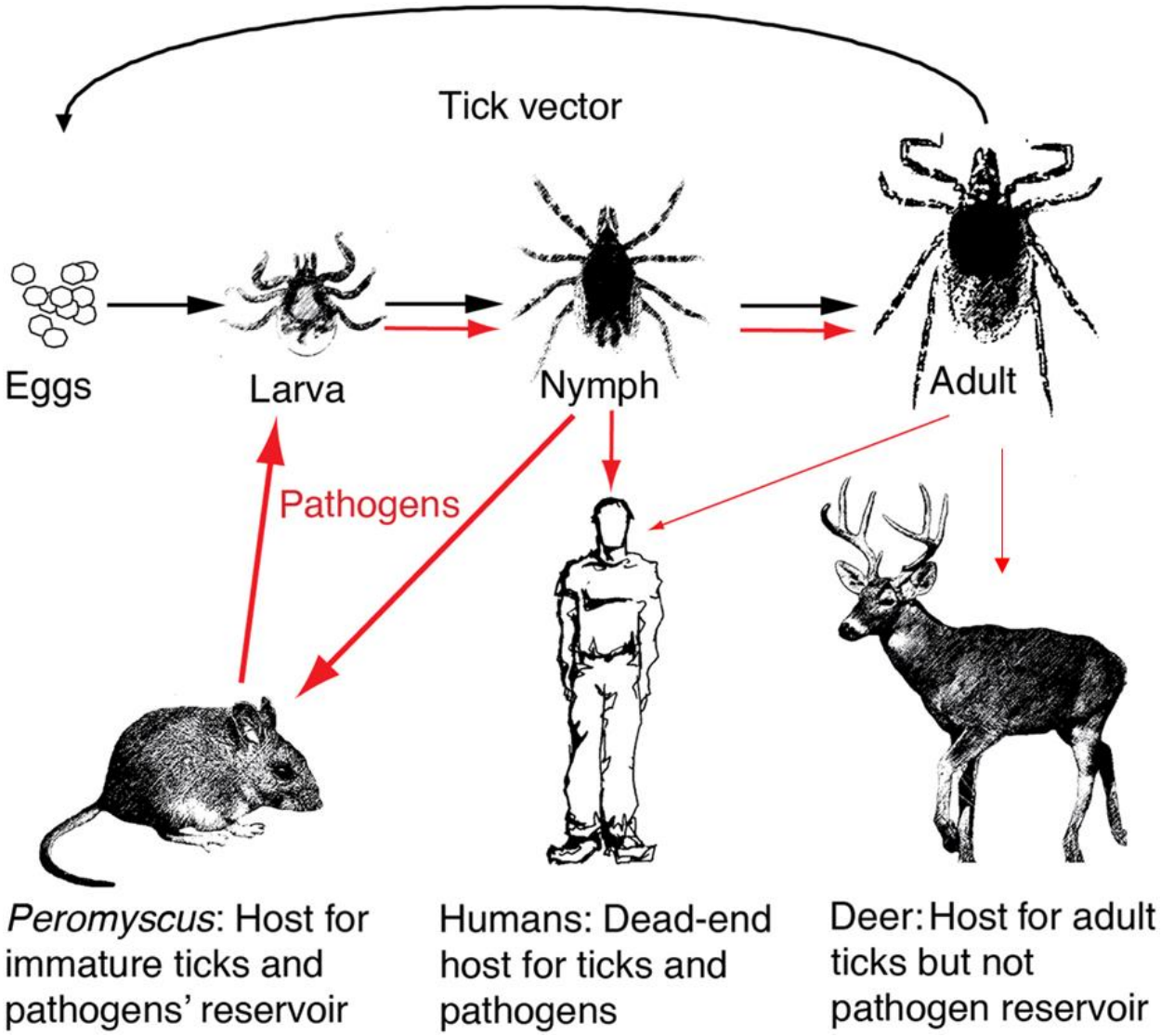


Figure 4: Life cycle of *I. scapularis* and its relation to the enzootic cycle of *B. burgdorferi* infection (Long et al, 2019). Red arrows indicate transmission of pathogen and display the movement of the bacteria between ticks and hosts and different life stages of the ticks.

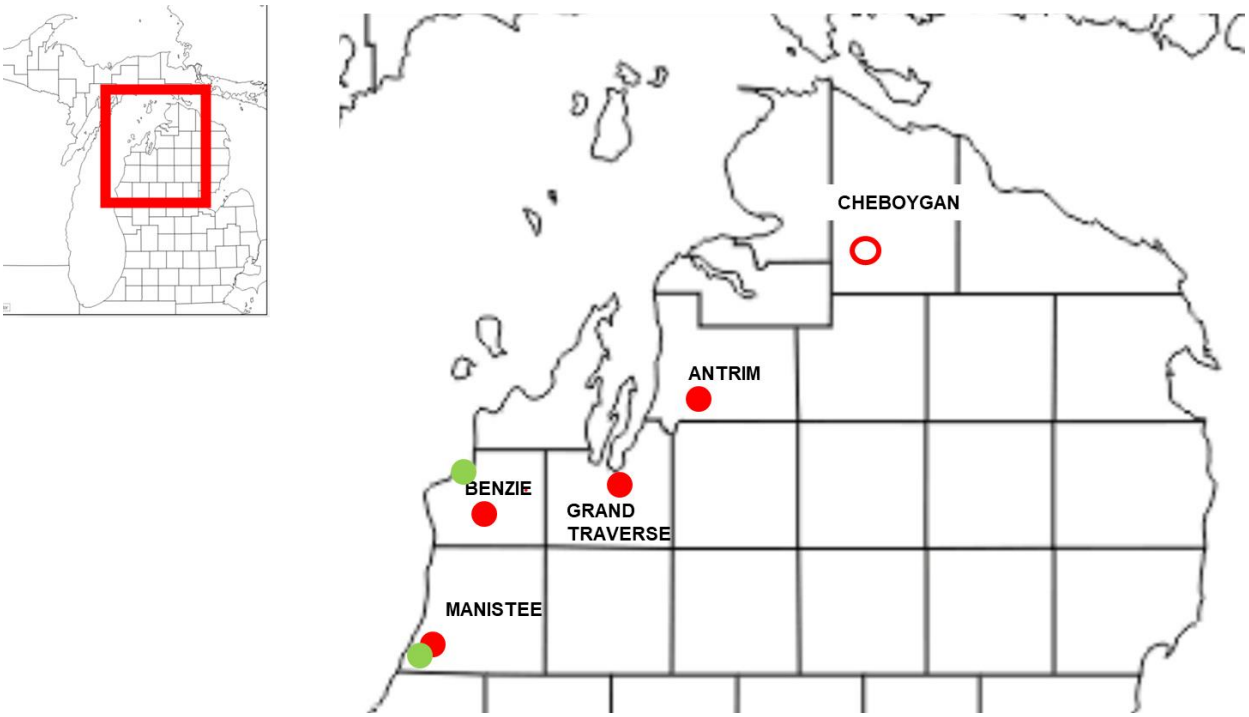


Figure 5: Red dots indicate the location of each of the trapping sites in the LP; the sites are named by the counties that form the transect. The open circle in Cheboygan Co. indicates that *P. leucopus* and ticks are present but that none of the mice or ticks have tested positive as of 2013 (Freund, unpublished data). Green dots represent the sites from a previous study that tested the infection prevalence of *B. burgdorferi* in both mice and ticks (Adapted from Hamer et al., 2010).

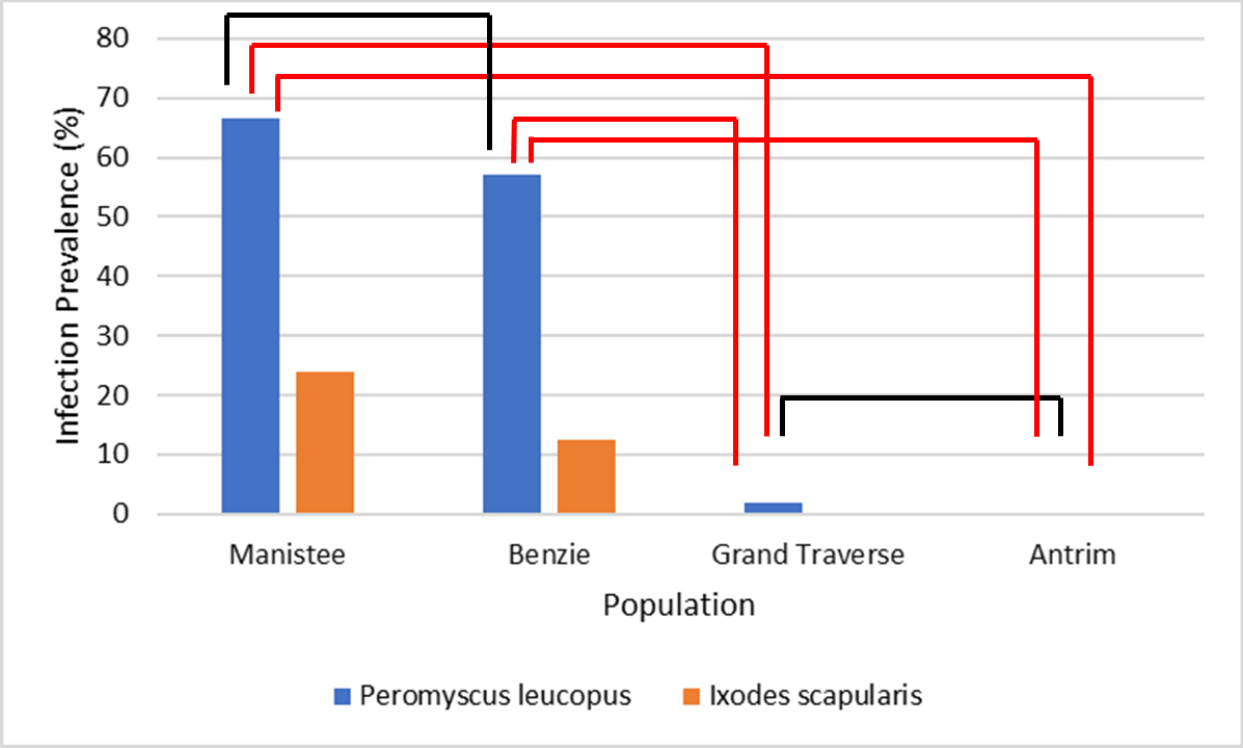


Figure 6: Comparison of prevalence of infection by population for *Peromyscus leucopus* (blue) and *Ixodes scapularis* (orange). The brackets only display significant difference for the mice because all tick comparisons were not significantly different. The key for significant difference displayed on the graph is as follows: the black brackets are non-significant and the red brackets stands for a P value of less than 0.005.

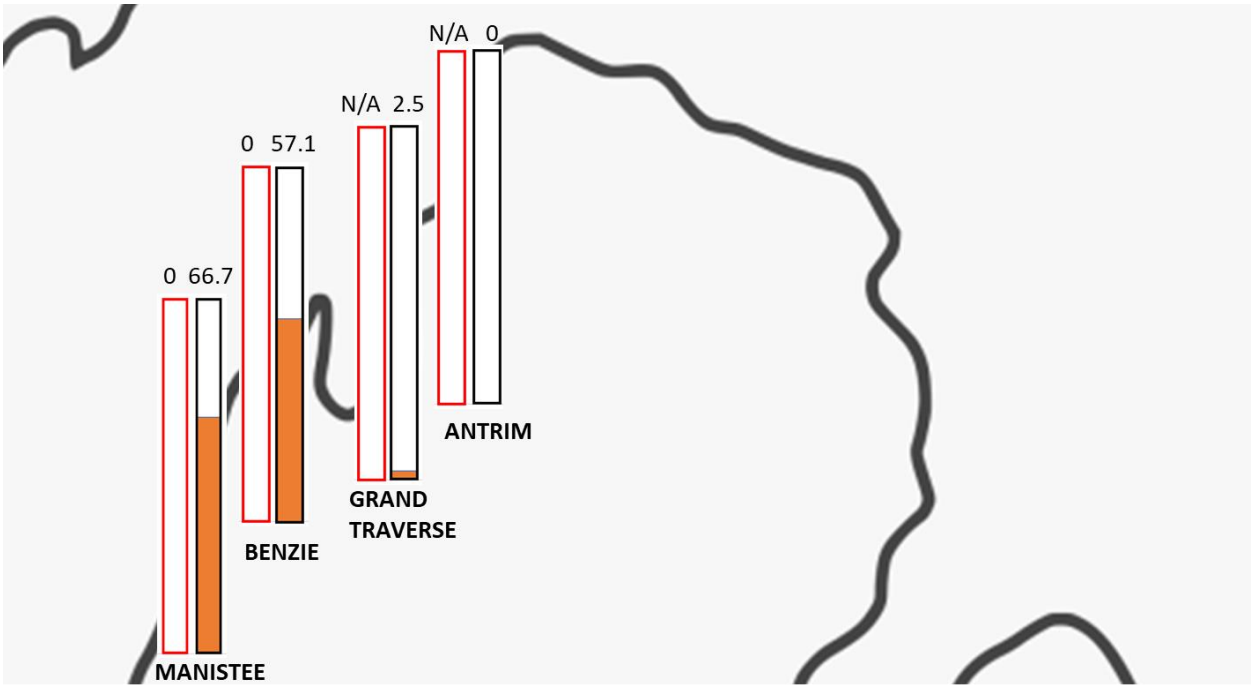


Figure 7: Current infection prevalence in *P. leucopus* (black bars) compared to prevalence of infection in 2008 (red bars). Numerical values of each proportion, as a percentage, are above each bar, and the location of each population is below each set of bars. The proportion for 2008 in Grand Traverse and Antrim is labeled as N/A because no trapping was done at either site in that year (adapted from Hamer et al., 2010).