**Variations in *Borrelia burgdorferi* Prevalence and Risk between Two Distinct Forest Patches in Close Proximity**

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**Introduction**

 Arthropod vectors are invertebrates such as mosquitos, fleas, or ticks which carry and transmit pathogens to other organisms after feeding on blood, making them important transmission routes for disease. Lyme disease is the most common arthropod-borne disease in the United States. This disease makes up 95% of all vector-borne illnesses reported in the U.S. and it is estimated to infect 300,000 people annually (Ward & Brown, 2003). Infection results in a myriad of symptoms including a mild rash, flu-like illness, debilitating arthritis, and even heart attacks (Radolf et al. 2012). The disease is caused by the spirochete (spiral-shaped bacterium) known as *Borrelia burgdorferi* which is transmitted primarily by the black-legged tick (*Ixodes scapularis)* (Wright et al. 2012)*.* Different genotypes of this bacterium can cause more severe symptoms and are collectively known as Human Invasive Strains (HIS) (Earnhart et al. 2005). Understanding what is driving the prevalence of these severe strains is critical to better predict and prevent Lyme disease especially in areas of recent emergence.

 The emergence and spread of Lyme disease are linked to various environmental factors including climate change and fragmented reforestation (Millins et al. 2016). However, the influence of landscape management practices on Lyme disease risk has not been thoroughly examined. Landscape ecology can affect both animal host community structure and tick populations (Ostfeld et al. 2018). Therefore, landscape management strategies that modify forests may have a trickle-down effect on ticks, their hosts, and possibly Lyme disease risk. Lyme disease risk is a measurable parameter using the density of nymphal ticks and the nymphal infection prevalence in order to quantify a human’s risk of getting Lyme disease in a particular area (Vourch et al. 2016). Understanding how landscape ecology and landscape management influences Lyme disease risk can inform on the usage of various forest management strategies that could potentially reduce Lyme disease risk. For example, using invasive understory management, which has the potential to reduce risk by providing less habitat for ticks and less cover for small mammal disease reservoirs (Bergstrom et al. 2018). Because forest management has the potential to reduce Lyme risk, it is important to know what different forest characteristics are correlated with a higher prevalence of Lyme disease.

**Summary of Proposed Work**

The aim of this work was to understand how landscape management practices influence Lyme disease risk by examining two very distinct forests that are close in proximity (30 meters) and found on the Fullers Overlook Estate Foundation property in Waverly, PA. These sites differ dramatically in their forest composition; one is comprised of Northeastern mixed hardwoods while the other forest is a dying ash tree forest dominated by an invasive plant understory made up of multiflora rose and autumn olive. These two forests have different small mammal communities and tick populations, which is peculiar given their closeness. Because ticks pick up Lyme disease from various small mammals and different mammal species harbor different strains of the disease, differences in mammal communities may influence risk between the two sites. My objectives for this project include:

1. Sample small mammals, questing, and host-associated ticks in the two forests

2. Investigate *B. burgdorferi* infection prevalence in small mammals and ticks and compare between forests

3. Compare human disease risk between forests

**Work Completed**

Small mammals were trapped and ticks were collected over seven separate sampling events from May to August 2019. Small mammals were trapped using Sherman box traps baited with oats and a nestlet for thermal protection and bedding. The traps were placed in a grid made up of 100 traps spaced 10 meters apart in both the hardwood and ash forests. In general traps were left open for four days and three nights each session for a total of 54,925 total trap hours in the hardwood and 54,725 hours in the ash forest. Traps were checked in the morning and evenings making sure to not leave the traps unchecked for more than 12 hours. When animals were captured they were anesthetized in order to take measurements, apply a uniquely numbered ear tag for population estimates, obtain an ear tissue sample for Lyme testing, and remove ticks.

 Host-associated ticks were not the only ticks collected, questing ticks were also collected over the seven sessions in order to quantify risk. During each trapping session questing ticks were collected from each forest plot by dragging a 1m2 white cloth through the leaf litter. Questing ticks were not only collected from the hardwood and ash sites; ticks were also collected from 4 other sites on the property that were either highly trafficked or unique habitat areas. This was done to determine the risk of Lyme disease throughout the Overlook property. A total of 1000m2 were sampled at each habitat every session. In all, 6000m2 were searched for ticks during each session, totaling 42,000m2 or ~26 miles of transect surveyed from May to August 2019. The ticks were collected off the cloth using forceps and brought back to the lab for processing.

 Extensive lab methods were employed throughout the summer to determine Lyme disease prevalence in the sites. Mammal tissue samples and ticks were processed to determine if they harbored *B. burgdorferi*. The DNA was extracted from mammal tissue, host-associated ticks, and questing ticks using commercially available column-based extraction kits following manufacturer’s instructions. PCR was used to amplify specific Lyme disease primers and the product was run on agarose gels to determine Lyme positivity. Positive samples were recorded and saved for downstream genotyping in order to determine which samples have HIS. The procedure of genotyping the positive samples is still being perfected and will hopefully be completed by January 2020. PCR was also done to determine what species of mice (white-footed or deer mouse) is present on the property.

**Results**

During the summer of 2019, 132 small mammals were trapped a total of 381 times among both sites. 71 small mammal individuals were trapped in the hardwood forest a total of 238 times and 61 small mammals in the ash forest were trapped a total of 143 times. In the hardwood site 65% of captures were white-footed mice, 19% were red-backed voles, and 16% were chipmunks. In the ash site 50% of the animals captured were white-footed mice, 49% were red-backed voles and 1% were woodland jumping mice. These results show that the two properties, even though close in proximity, have differing small mammal communities.

 Figure 1 below shows the results of Lyme disease infection testing in the small mammals captured. In the hardwood site 130 tissue samples were tested for Lyme disease and 56% tested positive (n=73). In the ash site 80 tissues samples were tested and 46% tested positive (n=37). In the hardwood site 66% (27/41) of the white-footed mice captured tested positive. In the ash site 62% (13/21) of the white-footed mice captured tested positive. Ten percent (2/19) of the voles captured in the hardwood site were positive for Lyme and 26% (10/39) of the voles from the ash site tested positive. In the hardwood site 73% (8/11) of the chipmunks were positive for Lyme disease. In the ash site 100% (1/1) of the jumping mice were positive for Lyme disease. These results demonstrate that *Borrelia* infection in small mammals is higher in the hardwood site than the ash site. The differences in forest patch composition may be contributing to the variations in infection rates.

Figure 1. *B. burgdorferi* % relative abundance in small mammals from the hardwood (HW) and ash (ASH) plots.

 Small mammal infestation was determined by the number of host-associated ticks taken off of mammals and the number of those ticks positive for Lyme disease. Table 1 shows the number of sampling events for each small mammal species and the number of those species captured that were infested with ticks. Table 1 also show the general range of the number of ticks that were collected off of each species. White-footed mice in the ash site had more ticks on them than any other species in the ash or hardwood site, even though there were more encounter events of white-footed mice in the hardwood site. Chipmunks were feeding a significant amount of ticks in the hardwood site but are completely absent in the ash, which is peculiar. Some small mammal individuals were more severely infested than others, one chipmunk accounted for 31% of all the host associated ticks collected in the hardwood site and one white-footed mouse in the ash site accounted for 11% of all the host-associated ticks collected there. 73% (n=53) of the host-associated ticks in the ash site tested positive for Lyme and 62% (n=37) tested positive in the hardwood site. These results show that the ash site had more host-associated ticks collected than the hardwood site and a higher proportion of those ticks tested positive in the ash site than in the hardwood site.



Table 1. Small mammal infestation results in the HW and ASH plots.

 Tables 2 and 3 shown below describe results from the Lyme disease risk assessments done at each site (HW and ASH). Lyme disease risk is determined by multiplying the density of nymphs (DON) by the nymphal infection prevalence (NIP). Lyme disease risk can be estimated using the density of infected risk (DIN) and is shown in Tables 2 and 3 surrounded by the red boxes. Nymphal questing ticks are used to quantify risk because they typically go unnoticed on a human’s body and are therefore most likely to transmit Lyme (larva do not have Lyme disease until after their first blood meal and adults are typically large enough for humans to remove before infection). As shown in the tables, the ash site had a higher risk of Lyme disease (DIN) for both June and July, when the nymphal ticks are most active for questing. The hardwood site had a higher density of questing ticks in June and July when compared to the ash site (8 and 12/1000m2 in hardwood vs. 7 and 5.5/1000m2 in the ash) but the ash site had a higher infection rate in both June and July compared to the hardwood site (71% and 55% positive in the ash vs. 50% and 21% in the hardwood). These results show that there is a higher risk of contracting Lyme disease is the ash forest during nymphal questing peak months than the hardwood site.



Table 2. Lyme disease risk results from the June flagging session in the HW and ASH



Table 3. Lyme disease risk results from July flagging sessions in the HW and ASH.

**Future Work**

In the coming year I hope to determine the different strains of Lyme disease present in both sites. This knowledge will help to determine what landscape characteristics might influence the presence of HIS. I also hope to complete another abbreviated field season in Overlook for the summer of 2020 to gather more data to further support conclusions I will make. I hope to determine the small mammal abundance numbers in each forest plot using different population parameter models in order to definitively know the difference in small mammal communities between the sites. I also hope to model ecological factors that may help explain the differences in ticks and small mammal abundance and infection between the two forest plots.

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