PREVALENCE OF POWASSAN VIRUS AND LYME DISEASE (*BORRELIA BURGDORFERI*) IN *IXODES SCAPULARIS* COLLECTED FROM NEW JERSEY AND PENNSYLVANIA BLACK BEARS (*URSUS AMERICANUS*)

By

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A Thesis Submitted in Partial Fulfillment of
The Requirements for the Degree of
Master of Science in Biology
To the office of Graduate and Extended Studies of
East Stroudsburg University of Pennsylvania

May 10, 2019

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ABSTRACT

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science in Biology to the office of Graduate and Extended Studies of East Stroudsburg University of Pennsylvania

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Title: Prevalence of Powassan Virus and Co-infection of Lyme Disease (*Borrelia burgdorferi*) in Ixodes scapularis from New Jersey and Pennsylvania Black Bears (*Ursus americanus*)

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Abstract

The blacklegged tick is the main vector for Lyme disease and Powassan virus Lineage II (Deer Tick Virus) in the United States. The objective of this study was to identify the prevalence of Powassan virus (DTV) and Lyme disease in adult and nymph blacklegged ticks collected in New Jersey (2015-2018) and Pennsylvania (2017-2018). All ticks were collected from lived trapped or hunter harvested black bears (*Ursus americanus*). A total of 2,713 ticks were collected, made up of four species. Only blacklegged ticks were analyzed in this study. Real-time Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) was used to amplify cDNA specific to the NS5 gene of POW Lineage II, and qPCR was used to amplify the 16s-23s intergenic spacer region rDNA of *Borrelia burgdorferi* (Lyme disease). A minimum infection rate (MIR) of 3.52% was determined for Powassan vitus and a MIR of 19.2% for Lyme disease. The findings in this study were similar to previous studies conducted for Powassan and Lyme prevalence in Lyme endemic region.

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Chapter I

Introduction

Tick-borne diseases affect thousands of people all over the globe every year. Ticks have been vectors of disease for thousands of years and the spread of tick-borne diseases has increased over the last decade, with over thirteen newly-recognized diseases discovered over the last two decades[48]. The organisms that cause these diseases range from bacterial to protozoan to viral, and each have their own unique transmission path and can have various effects on those infected. The most common vector-borne disease in the United States is Lyme disease, caused by the bacterium *Borrelia burgdorferi.* Lyme disease is one of the top ten most recorded diseases in the United States but is not the only tick-borne disease seen on the rise over the last decade[33]. Powassan virus is an emerging tick-borne virus and is broken into two lineages, lineage I vectored by the groundhog tick (*Ixodes cookei*) and lineage II (Deer Tick Virus) vectored by the blacklegged tick (*Ixodes scapularis*)[32]. Powassan virus can be asymptomatic or can cause encephalitis and severe neurological sequelae for those infected[36]. The blacklegged tick is the vector for many of these pathogens, including Lyme disease and Powassan virus, and plays a large part in tick-borne disease transmission to humans. As the blacklegged tick

population continues to grow and expand, tick-borne diseases are becoming a public health and safety crisis[21,22]. The increase in tick-borne diseases over the last decade has sparked a nationwide concern for education on treatment and prevention.

Lyme Disease

Lyme disease, also known as Lyme borreliosis was first discovered in Lyme, Connecticut in 1975 and since has had confirmed cases in North America, Europe and Asia. Lyme disease is caused by several genospecies of the *Borrelia burgdorferi* sensu lato group[6]. Of these, only two species of *Borrelia* bacteria, *B. burgdorferi and Borrelia mayonii,* cause Lyme disease in the United States. *Borrelia burgdorferi* is the leading cause of Lyme disease in the United States and Canada. In 2013, the Mayo Clinic discovered *B. mayonii*, a new species of *Borrelia* that was causing Lyme disease like symptoms in the upper Midwest[37]. Lyme disease is transmitted by the blacklegged tick (*Ixodes scapularis*) in the Northeast and upper Midwest and by the western blacklegged tick (*I. pacificus*) along the western coast of the U.S. The majority of Lyme disease cases are reported during the late spring, summer and fall when blacklegged ticks are actively seeking hosts.

Lyme disease is the fastest growing vector-borne disease in the United States. The CDC estimates reports approximately 30,000 confirmed cases each year. With accordance to the CDC and National Notifiable Diseases Surveillance System (NNDSS) confirmed cases must present an erythema migran (EM) with a single primary lesion that reaches greater than or equal to 5 cm in size across its largest diameter. The EM lesion is accompanied by other acute symptoms, particularly fatigue, fever, headache, mildly stiff

neck, arthralgia, or myalgia. As well, a Physician must confirm laboratory evidence in the form of a positive ELISA and western blot or a positive culture for *B. burgdorferi.* A probable case is defined by the CDC when a Physician diagnoses a positive laboratory test, but the patient lacks other evidence such as an erythema migran and no known exposure in a high incidence state. In addition to strict diagnostic guidelines, studies have shown only 10 percent of Lyme disease cases are reported. In 2008, Hinckley et, al. surveyed 2.4 million specimens from laboratories throughout the U.S. Following guidelines for testing Lyme disease recommended by the U.S. Public Health Service Agencies and the Infectious Diseases Society of America, the estimated percentage of true infection that year was upwards of 288,000 cases a year[33]. Nelson et al. (2015), evaluated the nationwide health insurance claims database from 2005-2010 identifying patients with clinician diagnosed Lyme disease. Positive cases were determined using ICD-9-CM codes for communicable diseases, along with a comprehensive analysis of the positive predictive values (PPVs), and the case definition for Lyme disease by the CDC[57]. The ICD-9-CM codes are used by hospitals to assign diagnosis in patient charts. The study determined based on clinical symptoms, there are approximately 329,000 cases of Lyme disease each year[1]. In 2016, there were 36,429 confirmed and probable cases of Lyme disease in the U.S. and 42,743 in 2017, an 8.5% increase[4]. Of these cases, 96% are reported in 14 states located in the Northeast and upper Midwest[35](Figure 1). The Mid-Atlantic states of Pennsylvania, New Jersey and New York have the highest number of reported cases. In 2017, Pennsylvania reported 9,250 confirmed cases and New Jersey reported 3,629 confirmed cases[4].

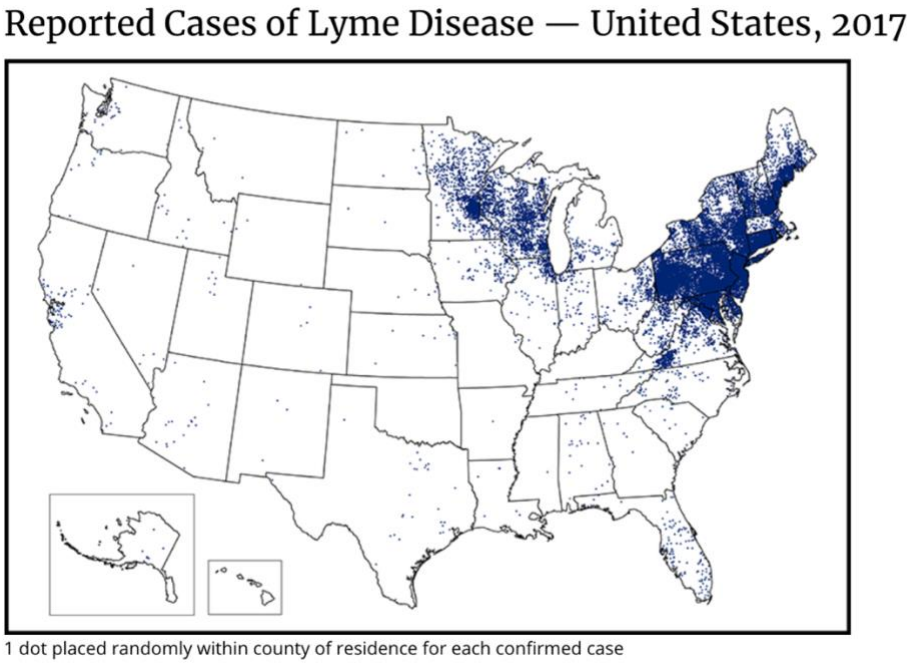

Figure 1. Reported cases of Lyme disease in the United States (2017). Each blue dot represents a confirmed case of Lyme disease. Endemic regions are illustrated within the Northeast and Mid-west. Massachusetts surveillance method does not match the national surveillance case definition set by the Council of State and Territorial Epidemiologist, information on most Lyme disease cases are not sent to the CDC and are not represented on this map. (Centers for Disease Control and Prevention)

Immune Response

Transmission time of Lyme disease from tick to host can be as short as 16 hours or up to 48 hours[15,44]. Transmission of Lyme disease from vector to host causes the bacterium to change its outer surface proteins to survive different environments. Within the vector, *B. burgdorferi* resides and replicates in the midgut. During this time Outer surface proteins are upregulated (OspA) allowing the bacterium to adhere to the tick's midgut until the tick feeds. As the tick attaches to a host and begins taking in a blood meal, the OspA gene is downregulated and the OspC gene is expressed as a result of the ticks midgut temperature and pH changes from the blood meal[64,44].

The bacterium will than migrate from the tick's midgut to the salivary glands; this allows access for the bacterium to enter the new host. At the initial infection site *B. burgdorferi* has several proteins that allow it to survive in the mammalian host and avoid destruction. The OspC gene allows for the bacterium to survive the warmer temperatures of the human body and basic pH level of the blood. The bacterium uses extra cellular matrix (ECM) binding proteins DbpA and DbpB, to bind to decorin, and protein BBk32 to bind to fibronectin, as well as Bgp to bind to proteoglycans and P66 which further binds to integrins[64]. These proteins may also play role in dissemination through mammalian tissue and persistence in joints[64]. The OspC gene has been found to play a possible role in dissemination by binding to human plasminogen[44]. These proteins at the initial infection site are recognized by the innate immune system, recognized by pathogen-associated molecular pattern (PAMP's) and signal Toll-like receptors (TLR's) to release signals to the rest of the immune system. At the erythema migran lesion sites, TLR2 has been found to play a specific role in releasing IFNγ, IL1β and IL6 cytotoxins[52]. TLRs signal an influx of macrophages, dendritic cells and neutrophils to the initial infection site. The immune system then produces pro-inflammatory cytokines such, TNF-α, IL-2, IL-6 and IFN's. Production of cytokines recruit T-cells to the initial site of infection which play an important role in activating complement and phagocytosis of the bacterium during early localized infection. After several days of infection, the immune system will begin to produce anti-inflammatory cytokine IL-10, this allows for surviving bacteria to disseminate throughout the body[66].

During early stage dissemination the surviving bacterium can travel through the body hidden in tissue and being motile through viscous media found in the body. The

spirochete shape and flagella transverse the whole body protected under the outer membrane, this helps the bacterium to penetrate host tissue and disseminate[64]. As the bacterium disseminates throughout the body the host immune system continues to produce pro-inflammatory cytokines damaging tissue, joints, muscles and the heart due to the inflammation caused by the host own immune system[52].

Lyme disease symptoms

Lyme disease is a multisystem illness that can affect the skin, nervous system, musculoskeletal system and the heart. Transmitted from a tick bite, symptoms may occur 3-30 days after exposure to the bacteria. There are three stages of symptomatology which include: early localized, early disseminated and late disseminated Lyme disease.
In early localized (stage 1) Lyme disease, 60-90% of patients may develop a rash known as an erythema migrans (EM)[50]. The rash tends to be localized in the area of the tick bite and has the characteristic bulls-eye shape. Some patients may develop an uncharacteristic rash that is patchy with no specific shape, and some patients may develop no rash at all during their infection. Early Lyme disease may also include flu-like symptoms of fatigue, malaise, fever, headache, arthralgias (joint pain), and myalgias (muscle pain)[31]. Lyme disease symptoms for *B. burgdorferi* and *B. mayonii* infections are similar with additional symptoms of *B. mayonii* including nausea, vomiting and diffuse rashes. Patients with *B. mayonii* tend to have higher concentrations of bacteria circulating in the bloodstream[21].
If early localized Lyme disease goes untreated, patient symptoms may progress into the early disseminated stage (stage 2). These symptoms can occur weeks or months after a

tick bite and symptoms may vary depending on the species of *Borrelia* causing the Lyme disease infection. Early disseminated symptoms can include neck stiffness, facial palsy (typically Bell's palsy), lymphocytic meningitis, progressing into the loss of motor and sensory function[31]. Lyme carditis may occur in this stage and cause an atrioventricular block.

If Lyme disease is continued to be left untreated, it can persist into late disseminated (stage 3) Lyme disease. This stage of Lyme disease can lead to severe arthritis synovitis, severe neurological problems, such as memory loss and black outs, and in rare cases cause encephalopathy[31].

Diagnosis and Treatment

In endemic regions, clinicians use characteristic signs of Lyme disease described and presented by patients for diagnosis. These characteristic signs include the patient reporting a known tick bite, developing an EM rash, or developing other symptoms common to early-localized Lyme disease. Not all patients develop an EM rash or remember a tick bite. These patients will present symptoms similar to other diseases such as fibromyalgia, multiple sclerosis and the flu. These patients may not receive the correct treatment resulting in symptoms developing into early and late disseminated Lyme disease[30].

Current testing recommended by the CDC is a two-tier serological test. The first step uses an ELISA (enzyme-linked immunosorbent assay) to detect IgG and IgM antibodies against *B. burgdorferi*, if positive, the second test is an immunoblot (Western

blot) to measure IgG and IgM[30,44]. During early Lyme disease serological testing can be inaccurate due to low antibody production and sensitivity from the serological test. If a patient continues to have symptoms after a negative ELISA, they can be retested 2-4 weeks after the initial exposure. IgM can be detected 2-4 weeks after initial infection and reaches peak antibody production at six weeks before the titer drops[66]. Studies are being conducting to develop new methods for diagnosing Lyme disease with higher sensitivity and accuracy[44].

Lyme disease can be treated with antibiotics. Doxycycline is the most commonly used antibiotic to treat Lyme disease; however, other antibiotics such as amoxicillin, or cefuroxime axetil can be used. Antibiotics are prescribed orally one to two times a day for 14-30 days[47].

Post Treatment Lyme Disease Syndrome

In most Lyme disease cases, patients clear the infection and no longer have symptoms following a course of oral antibiotics. However, 10 to 20% of treated patients continue to have symptoms for months to years following completion of their treatment[46]. This condition was previously referred to as Chronic Lyme Disease (CLD), and more recently referred to as Post Treatment Lyme Disease Syndrome (PTLDS) or Post Lyme disease Syndrome (PLDS)[23]. PTLDS received a case definition from the Infectious Disease Society of America (IDSA) stating that the individual must have a documented case of Lyme disease who has completed treatment but continues to show a relapse of symptoms including fatigue, musculoskeletal pain, and complaints of cognitive difficulties for a minimum of 6 months from treatment completion[55].

The cause of PTLDS is unknown but there are many studies with potential hypotheses as to the cause[55]. These theories include, the body is having an autoimmune response as a result of Lyme disease, the antibiotic course fails to clear the Lyme disease infection, the bacterium has the ability to change form and create biofilms during environmental stress and a secondary infection by a different pathogen with similar symptoms to Lyme disease[55].

The first theory focuses on the idea that PTLDS is a delayed autoimmune response to Lyme disease. Singh & Girschick (2004) reviewed the effects of T-cells on inflammation in the joints during Lyme disease. They found elevated levels of T-lymphocytes in synovial fluid and peripheral blood in adults who were showing symptoms of PTLDS[58]. Maccallini et al. (2018) evaluated the role of B cells and the similarity between human γ enolase and *Borrelia* enolase. They found that human γ and *Borrelia* enolase share a conformational B cell epitope which can cause a release of autoantibodies against enolase. These antibodies have been seen in other autoimmune diseases that affect the brain[41]. Further studies testing this theory must be conducted using several case studies with known Lyme disease patients and those who are suspected to have PTLDS[41].

Lyme disease is typically treated with a course of oral antibiotics between 14-28 days and is supposed to clear the infection. Cameron, Johnson & Maloney (2016) reviewed several studies conducted to determine the effectiveness of clearing Lyme disease after a course of antibiotics and found that those treated during early localized and early disseminated failed to bring 16% to 48% of the patients back to their pre-Lyme health status. A observation trial found that 33% of patients treated with a three week

course of doxycycline continued to have symptoms three to six months post-treatment[12]. A study conducted by Logigian, Kaplan & Steere (1990) observed patients with late disseminated Lyme disease and found that 63% of patients treated with intravenous (IV) ceftriaxone for 14 days showed improvement, 15% showed no health improvement and 22% showed initial improvement and relapsed with symptoms six months after treatment[39]. Failure to clear the infection with an oral or IV course of antibiotics can lead to these symptoms and be a possible cause for PTLDS. Patients were not tested for other tick-borne diseases, lasting symptoms may have been caused by a TBD that could not be treated with antibiotics.

Recent studies have found that the Lyme bacterium has the ability to change under environmental stresses (pH, temperature, immune attack, nutrient starvation) and form biofilms to protect itself. It was determined that *B. burgdorferi* has several forms, spirochete (stationary phase and log phase spirochete), round body and an aggregated microcolony that has the ability to form biofilms[17,28]. A study conducted by Feng, Tingting & Zhang (2018) determined that when studied *in vitro B. burgdorferi* can persist in variant forms and protect itself from antibiotics and, *in vivo* mice, the microcolony form and stationary phase caused more server inflammation in joints that log phase. Antibiotics were able to destroy log phase spirochete and some round body forms of the bacterium but failed to completely destroy stationary spirochete phase and the biofilm aggregates[17,28]. These forms can prevent the destruction of all *B. burgdorferi* bacterium in the body, leaving persistent bacteria behind and may play a role in PTLDS.

The last hypothesis states that PTLDS may be caused by a secondary infection masked by Lyme disease. One of the main co-infections that has been hypothesized to

cause PTLDS is Powassan virus. Powassan virus[63] has similar symptoms to those present in PTLDS such as fever, fatigue, myalgia, dizziness, confusion, memory loss and in severe cases, encephalitis and death[63]. Deer tick virus is not a well-known or studied virus but has been increasing in the U.S. over the last decade and can be hard to diagnosis and detect[29]. Frost *et al.* (2017) tested 41 patients with known Lyme disease for Powassan virus and identified 10 (4.1%) of those patients were also positive for Powassan virus. Furthermore, Thomm *et al*. (2018) tested 106 patients for Powassan virus which have been diagnosed with at least one tick-borne disease and identified 10 (9.4%) of these patients were also positive for Powassan virus[63].

Powassan Virus

Powassan virus (POW) is an emerging tick-borne virus that is on the rise in North America. It was first discovered in Powassan, Ontario in 1958 after a young boy died of encephalitis[49]. POW has since been found in the Great Lake Region and along the Northeast in the U.S., up into Canada and in the Primorsky region of Russia[51]. It is a ssRNA *Flavivirus*, part of Flavividae family[19]. It is part of the tick-borne encephalitis complex (TBC-E), and transmitted by the bite of an infected tick[51,63]. There are two lineages of POW; lineage I was first discovered in the 1958 Powassan, Canada case and, lineage II was discovered in 1996 and referred to as Deer Tick Virus (DTV). The two lineages are associated with having different vertebrate reservoirs and vectors[62]. Lineage I transmission cycle is maintained by the woodchuck tick (*Ixodes cookei*) and medium sized mammals such as the woodchuck (*Marmota monax*) whereas lineage II transmission cycle is maintained by the blacklegged tick (*Ixodes scapularis*) and the

white-footed mouse (*Peromyscus leucopus*)[51]. Although they are two distinct lineages, phylogenetic studies have found them to have 84% nucleotide similarity and an amino acid similarity of 93%[5].

POW lineage I is more aggressive with a higher possibility to be fatal than DTV. DTV is less aggressive[20] but studies over the last decade have found that DTV can cause fatal encephalitis or lasting neurological effects similar to lineage I[26,61,63]. The CDC has reported an increase in neuroinvasive POW cases from 6 in 2015 to 33 in 2017[60]. Neuroinvasive cases have been confirmed within 11 states from 2008 to 2017 (Figure 2). Since 2008, NJ has had 5 cases and PA has had 7. In 2017, PA and NJ both had 4 cases of confirmed POW, an increase from 0 diagnosed in 2016 in both states[60].

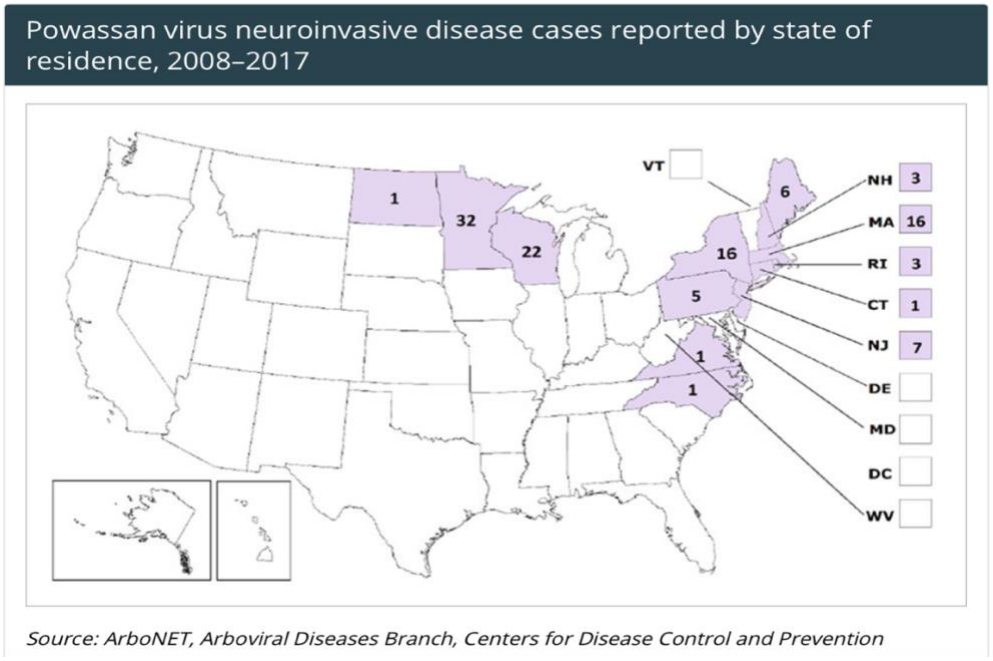

Figure 2. Map of neuroinvaisve Powassan virus cases reported in each US state, 2008-2017. (Centers for Disease Control and Prevention)

POW lineage II is typically asymptomatic, however it can cause life-threating conditions such as encephalitis and meningitis to those infected[49]. Symptoms occur

between 1-5 weeks after initial exposure by a tick bite[53]. They can range from headaches, drowsiness, nausea and disorientation during early infection and can progress into encephalitis, meningoencephalitis and coma during later stages of infection[32]. The mortality rate can be as high as 15% for patients infected, and 50% of the surviving patients are diagnosed with neurological sequelae[53]. Patients can be tested using their CSF or serum targeting POW IgM antibodies[29,53]. Current serological testing consist of using IgM ELISA, IgM immunofluorescence antibody assay (IFA), IgG ELISA and conformation testing with a $\geq$ 90% or $\geq$ 50% plaque neutralization test ($PRNT_{90}$ or $PRNT_{50}$) with a $\geq$4-fold increase in antibody titers from acute- and convalescent-phase sera[29,63]. Based on the sensitivity of current testing protocols, there may be more confirmed cases of POW then what is being reported[29,53]. Confirming lineage I vs lineage II POW requires using neutralization assays such as qPCR and sequencing following a positive serological test[49]. Transmission of POW from vector to host is as fast as 15 minutes following attachment as the virus resides within the salivary glands of the tick[18] .

Vector

There are hundreds of ticks worldwide which fall into one of three families: *Ixodidae* (hard ticks), *Argasidae* (soft ticks), and *Nuttalliellidae* (ticks of South Africa)[27]. The largest family *Ixodidae*, play a role in vectoring and transmitting a majority of tick-borne diseases globally. *Ixodes scapularis* (blacklegged tick or deer tick) is a medically-important tick as it contributes to many tick borne diseases in North America[38]. The blacklegged tick is distributed up into southeastern Canada down the

northeast coast and as far west as Texas, Oklahoma and parts of North and South Dakota (Figure 3).

The blacklegged tick is a 3-host tick with three life stages: larval, nymph and adult, that lives a two-year life cycle, molting between each life stage[38] (Figure 4). Larval ticks hatch in May and are active until August, feeding on small rodents such as the white-footed mouse (*Peromyscus leucopus*) and birds. Once a larval tick has fed to engorgement, it will drop off its host and molt into a nymph and enter diapause until the late spring and summer[24]. The nymph will quest for its second host which can be small to medium-sized animals such as rodents, lagomorphs, ungulates, cats, dogs and humans. Following engorgement, the nymph will molt into an adult. Adult females will use larger animals as a host before copulating with adult males and laying their eggs in the early spring[38], laying up to 3,000 eggs.

Figure 3. Distribution of blacklegged ticks in the United States as of 2018 (Centers for Disease Control and Prevention)

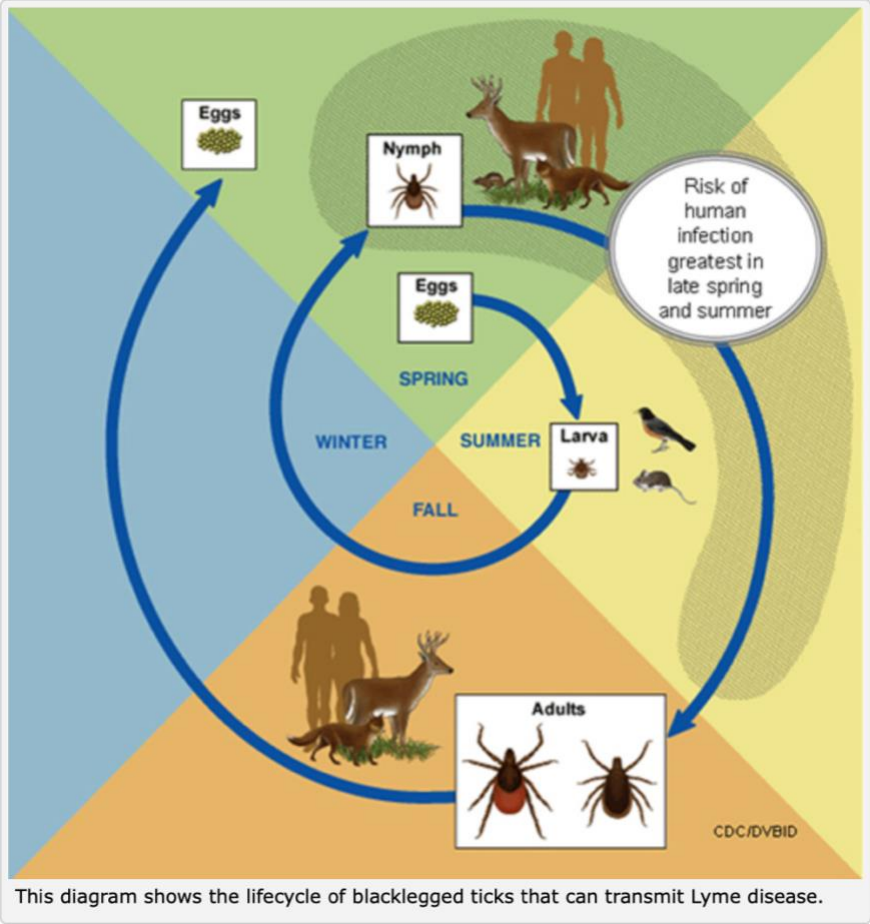

Figure 4. The two-year life cycle of the blacklegged tick. Broken down into when each life stage is most prevalent and the main host each life stage feeds on. (Centers for Disease and Control Prevention)

Blacklegged ticks acquire pathogens transovarially or transstadially depending on the pathogen. Transovarial pathogens are able to be passed on from an infected mother tick to the eggs. It is unknown if POW is transovarially passed down to the next generation of an infected tick. A transstadial pathogen is picked up by a vector when feeding on an infected reservoir. The bacterium that causes Lyme disease is a transstadial pathogen that will continue to live within the tick once acquired from a reservoir[40]. A reservoir host is able to survive living with a pathogen, without presenting symptoms or infection.

The white footed-mouse (*Peromyscus leucopus)* is the reservoir for several tick-borne pathogens that are vectored by the blacklegged tick. The most notable pathogen is *B. burgdorferi* (Lyme disease), in addition to *Anaplasma phagocytophilum* (Anaplasmosis), *Babesia microti* (Babesiosis), *Borrelia miyamotoi* and, DTV (Powassan virus lineage II)[16]. Other rodents, such as chipmunks, squirrels, shrews and woodchucks are competent reservoirs for tick-borne pathogens by the blacklegged tick[10]. Although only a few animals are reservoir host, other large animals play a role in the distribution and spread of infected ticks. Studies enumerating the number of ticks on black bears have identified on average 400 ticks[3]. A study conducted by LoGiudice, Ostfeld, Schmidt & Keesing (2003) identified the host diversity and community composition of ticks in Dutchess County, New York seen in Table 1. [40]. The average tick load on an animal can play a large role in pathogen and tick distribution depending on the animal. The white-footed mouse is a key reservoir host for many tick-borne pathogens and a high tick load can increase infected ticks in an area. While, other larger animals such as the white-tailed deer and black bear are not known reservoir host but can play crucial roles in carrying large tick loads and distributing them into and out of urban and rural areas. This mechanism of transport can result in introducing new tick species and diseases to an area inhabited by humans.

Table 1. Average tick load of small to large animals identified by LoGiudice, Satefeld, Scmidt & Keesing (2003) in Dutchess County, NY

Animal	Average tick load
White-footed mouse (*Peromyscus leucopus*)	27.8
Eastern chipmunk (*Tamias striatus)*	36.0
White-tailed deer (*Odocoileus virginianus*)	239

Short-tailed shrew (*Blarina brevicauda*)	62.9
Grey Squirrel (*Sciurus carolinensis*)	142

Black Bears

The American black bear (*Ursus americanus*) is medium-sized and historically found in the United States, Canada and Mexico. Black bears have great mobility, are generalist and have an omnivorous diet. This allows black bears to live in a wide range of habitats, from swamps to semi-desserts and dense forests. Black bear males (called boars) can weigh between 150 to 600lbs and females (called sows) can weigh from 150 to 400lbs[8]. Female black bears will typically have a home range between 2.5-10 square miles, while males can have a much larger home range between 10-59 square miles[9]. In the Northeast, they typically are black in color with a brown muzzle and can have a white blaze on their chest. Some bears are an atypical cinnamon color. Black bears are strong swimmers and good climbers due to their five toes and long curved claws[45]. In the wild, black bears can live up to 25 years. Although bears prefer to eat wild foods such as acorns, skunk cabbage, and blueberries they will also eat from human garbage and bird feeders. Merkle *et al.*(2013) surveyed black bears from 2009 and 2010 in Missoula, Montana where bears were 80% more likely to choose urban grounds for food than in the wild[42]. Black bears will do a wide range of damage to homes, sheds that have food stored in them, destroy garbage cans and bird feeders.

Black bears typically begin to mate around the age of three, but can start as early as two years old. Mating occurs late May through July. Females may enter their dens as early as the end of October and males may enter their dens as late as mid-December. During January, sows will give birth in their dens and can have a litter of one to five

cubs. Bears will begin to exit their dens in April, with the cubs following the sow for about a year to year and half before they go off on their own[8,45].

Figure 5. Sedated black bear cub, 2018 NJDFW research trapping (Photo credit Kristine Bentkowski)

Black bear populations in NJ, PA and NY have been increasing over the last decade. The New Jersey Division of Fish and Wildlife (NJDFW) has seen an expansion of the black bear population from the northeast region of the state in 1995 to sightings in every county to date[45]. Today in northwestern NJ, there are as many as three bears per square mile[45]. In PA, the Pennsylvania Game Commission (PAGC) has also seen an increase in the black bear population over the last decade. As a result of increased populations, both states have had significant increases in nuisance bear reports. Nuisance bears defined by the PAGC and NJDFW are black bears that are entering residential areas, destroying farmland or homeowner property. To control and monitor black bear

populations, the PAGC and NJDFW conduct annual research trappings, and an annual black bear harvest to control overpopulation (Figure 5). With the increase of interactions between black bears and humans, there is an increasing public health concern for zoonotic diseases. Very little research has been conducted to determine if black bears are reservoirs to some tick-borne diseases, however, these mammals play an important role in tick dispersal carrying ticks into residential areas.

Study Objectives

Monitoring the prevalence of Lyme disease and Powassan virus are important to public health and safety. This will be the first study conducted in Pennsylvania and New Jersey to determine the prevalence of Powassan virus and co-infection prevalence with Lyme disease. The goal of this study was to determine the prevalence of Lyme disease and Powassan virus in blacklegged ticks collected from black bears in New Jersey and Pennsylvania from 2015-2018. The objectives were to:

1. Determine the prevalence of Powassan virus (DTV) in blacklegged ticks from bears in NJ and PA from 2015-208
2. Determine the prevalence of Lyme disease in blacklegged ticks collected from black bears in NJ and PA from 2015-2018
3. Evaluate the co-infection rate of Powassan virus and Lyme disease in NJ and PA

Chapter II
Materials and Methods

Study Area and Sample Collection

From 2015-2018, ticks were collected from black bears (*Ursus americanus*) with assistance from the New Jersey Division of Fish and Wildlife (NJDFW). Ticks were collected from Hunterdon, Morris, Passaic, Sussex, and Warren Counties in New Jersey (Figure 6). They were collected three to four times throughout the year during research trapping and at the black bear hunt check stations. Biannual research trapping occurred May through June and again in the fall from August through September. Research trapping sites were selected based on bear activity, proximity to food sources such as cornfields, acorn mass, and reported bear sightings. Black bears were captured using Aldrich foot snares and culvert traps. Trained personnel used a mixture of ketamine and xylazine (ZooPharm Inc, Windsor, CO) to anesthetize captured animals. Morphological measurements were collected, and each animal was tagged, tattooed with corresponding tag number, and sexed. To determine age, the premolar of yearling and adult bears was removed. Ticks were also collected from hunter-harvested bears during NJDFWs hunting season. Segment A of the bear hunt occurred for one week in October, and segment B

occurred for one week in December. Harvested black bears were brought into check stations, where morphological measurements were collected, and tick searches were completed.

During the annual research trapping and black bear harvest, a 5 to 10-minute tick check focusing on the ears, around the eyes, neck, under the armpits and around the groin region was conducted. Ticks were collected with forceps and placed into 2mL screw top tubes labeled with the bear's ID number, date and location of capture. Ticks were stored in a cooler on ice until being brought back to the Northeast Wildlife DNA Lab (NEWDL), where they were stored at -20˚C.

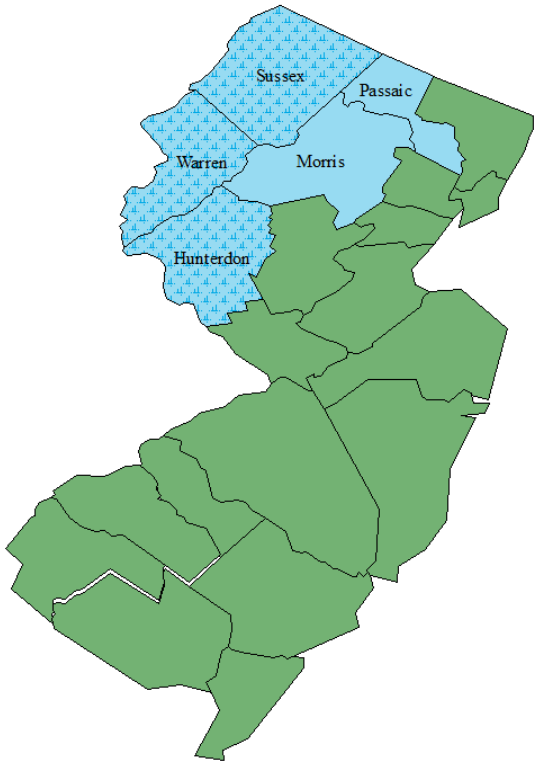

Figure 6. New Jersey counties where black bears were trapped or harvested, and ticks were collected. Ticks collected from blue stripped counties were analyzed for Powassan and *B. burgdorferi*.

Ticks were collected from black bears in Pennsylvania between 2017-2018 with the assistance of the Pennsylvania Game Commission (PAGC) and collaborators at Penn State University. In 2017, ticks were collected from Monroe and Pike Counties, and in 2018 from Centre, Clearfield, Clinton, Huntingdon, Lycoming, Potter, and Tioga Counties (Figure 7). The PAGC collected ticks from nuisance and vehicle-strike bears between September and October of 2017. In 2018 ticks were collected throughout the year from June to December during the annual research trapping in the Fall and Spring, the bear hunt in October and December and bears caught throughout the time period with suspicion of mange infection from central Pennsylvania. Tick check methods were

designed by Hannah Greenberg from Pennsylvania State University from an ongoing study to determine the abundance and distribution of ticks on American black bears in Pennsylvania. Tick checks were conducted over 16 designated body regions on the black bear with 4" X 4" standardized tick square. There was no time constraint on tick checks. Ticks were placed in 2mL screw top tubes filled with 70% ethanol and had the black bear's age, sex, and date.

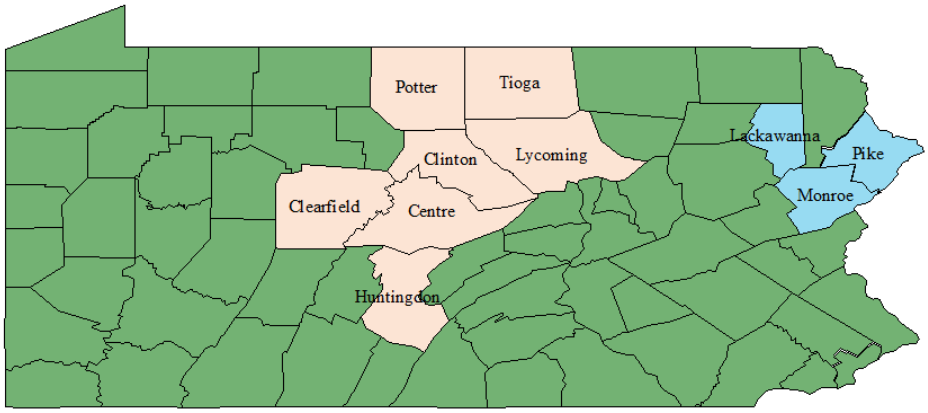

Figure 7. Pennsylvania counties where black bears were trapped or harvested, and ticks were collected 2017-18. Blue counties ticks were collected in 2017 and pink counties ticks were collected in 2018.

Identification and Extraction

All ticks were identified to species and life stage following Ward's Guide to North American Ticks (Ward's, Rochester, NY). This was done by examining their festoons, geographic location and scutum (shield) pattern, as each tick has their own unique pattern or color. The body of the *I. scapularis* begins to engorge and stretch as it feeds, while the hard scutum continues to keep its size and shape. A scutal index can be used to measure the engorgement level of the tick and estimate duration of attachment. The engorgement level of *I. scapularis* females and nymphs were determined using the

scutal index (SI = b/a) measuring the body length from the posterior edge to the basis capitulum and the maximum width of the scutum (Figure 8). Based on the SI, estimated engorgement hours were determined, and ticks were then labeled as unengorged (≤ 14 hours), semi-engorged (≤ 15-92 hours) or fully engorged (≥ 93 hours) (Figure 9).

A tick pool consisted of ticks of the same sex, life stage, engorgement level, individual black bear and county. Bears that had more than five ticks of the same engorgement, sex and life stage were pooled together but if the bear had less than five ticks each tick was analyzed individually. For example, a bear with three ticks collected from it had each tick analyzed alone, while a bear with five females of the same engorgement had one pool containing all five ticks. Pools typically ranged from 1-5 ticks. Ticks in the same pool were all placed into one tube for extraction of RNA and DNA and analyzed as one sample.

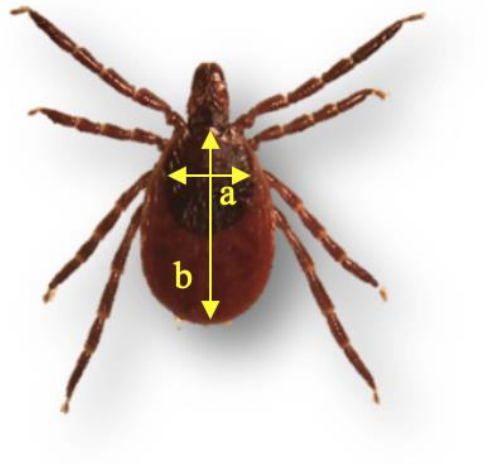

Figure 8. Measurement of a female blacklegged tick using the scutal index (SI = b/a), a = maximum width of scutum, b = length of tick from posterior to the basis capitulium

Figure 9. Examples of engorgement sizes of adult female blacklegged tick. Left to right: Fully engorged, semi-engorged, semi-engorged, unengorged, unengorged (Photo credit Kristine Bentkowski)

Powassan virus (Lineage II) SYBR Assay Optimization

To optimize a reverse transcriptase real-time PCR (RT- PCR) assay for Powassan virus Lineage II, a standard curve analysis was performed. Using a synthetic positive, serial 1:10 dilutions were created ranging from 10^{-1} to 10^{-10}. Each dilution was performed in triplicate to validate accuracy and a negative control of nuclease free water was used to confirm absence of contamination. A synthetic positive sample was created by GENEWIZ (South Plainfield, NJ) with the NS5 target primers. PCR was performed in 25µL reactions. Each reaction contained 0.2µM of forward primer 5'gaagctgggtgagtttggag 3' and 0.2µM reverse primer 5'cctgagcaaccaaccaagat 3' targeting a 318 base pair region of the NS5 gene (Knox et al. 2017). The PCR protocol followed manufacture guidelines with the modification of using 0.25µL of SYBR enzyme mix (Thermofisher, Waltham, MA). The 25µL RT-PCR reaction consisted of 1X SYBR Green Master Mix (Thermofisher, Waltham, MA), 1.0µL premixed forward and reverse primers, 6.25µL Qiagen nuclease free water, 0.25µL SYBR enzyme mix, and 5µL of synthetic positive. The standard curve was run on an Applied Biosystems StepOnePlus™

PCR system. Thermal cycling conditions for RT-PCR was performed at 50°C for 20 mins, 95°C for 5 minutes, followed by 45 cycles of 94°C for 10 seconds, 55°C for 5 seconds and 60°C for 25 seconds. A standard curve was created using the log dilution of copies (ng) by the CT (cycle threshold) value. Each CT value was determined by how many cycles it took for the fluorescent signal of the sample to cross the threshold.

RNA Analysis

RNA was extracted from tick pools following the Qiagen viral RNA extraction protocol (Qiagen, Germantown, MD). The protocol was modified to include an overnight incubation at room temperature. A blank was included in each analysis extraction to confirm absence of contamination during the extraction process.

Samples were analyzed for Powassan virus using the optimized Powassan virus Lineage II SYBR green assay. Specific primers were used to target the NS5 region of the Powassan virus genome (Table 2). Each analysis was run with a positive to validate the assay and negative to confirm the absence of contamination. All RT-PCR was conducted on an Applied Biosystems StepOnePlus™ PCR system. Positive samples were identified with CT values of 30-44 and a threshold of 1.725.

DNA analysis

DNA was extracted from tick pools along with RNA following the Qiagen viral RNA extraction protocol (Qiagen, Germantown, MD) and modifications. A blank was included in the extraction process to confirm the absence of contamination during extraction. To identify the presence of *Borrelia burgdorferi*, DNA was amplified using a

TaqMan real-time PCR protocol. A specific primer and probe targeting the 16s-23s intergenic spacer region of *Borrelia burgdorferi* was used (Table 2). A 25µL TaqMan real-time PCR (qPCR) reaction containing 1X TaqMan Master Mix (Thermofisher, Waltham, MA) was used for qPCR. The reaction was made up of 12.5µL TaqMan master mix, 5µL Qiagen nuclease free water, 4.48µL of premixed forward and reverse primer, 0.56µL *B. burgdorferi* probe, and 2.5µL of sample DNA. Thermal cycler conditions for qPCR were performed at 50°C for 2 minutes, an enzyme activation of 95°C for 10 minutes, followed by 50 cycles at 95°C for 15 seconds and 60°C for 1 minute. Positive samples were determined with CT values of 30-38 at a threshold of 0.047. A positive control was run with each analysis to validate the assay and a negative control to confirm the absence of contamination.

Table 2. Oligonucleotide primer used for PCR. SYBR green primer targeting the NS5 region of Powassan virus. Real-time primer targeting the 16S-23S intergenic spacer region for Lyme (*Borrelia* species) and specific probe for *Borrelia burgdorferi*

Target Organism	Gene Target	Sequence (5' → 3')	Amplicon Size (bp)
Powassan virus (DTV)	NS5	F 5'-AACATGATGGGAAAGAGAGAG-3'	318bp
Powassan Virus (DTV)		R 5' -CAGATCCTTCGGTACATGGAA-3'	
Borrelia species	16S-23S intergenic spacer	F 5'-GCTGTAAACGATGCACACTTGGT-3'	69bp
		R 5'-GGCGGCACACTTAACACGTTAG-3'	
Borrelia burgdorferi	Probe	6FAM-TTCGGTACTAACTTTTAGTTAA-QSY	

Statistical Analysis

The minimum infection rate (MIR) was determined by dividing the total number of positive individuals and pools by the total number of ticks analyzed. This value assumes only one tick per pool was infected; therefore, it is a conservative estimate of prevalence. The minimum infection rate calculated from the tested ticks was used as an estimate of the minimum prevalence of infected ticks in New Jersey and Pennsylvania. Prevalence rates were determined by life stage for adults and nymphs.

Statistical analysis was conducted using the statistical computing program R[34,56,67]. A Chi-square test was used to determine if there was significance between the proportion of tick species collected in New Jersey and Pennsylvania. A generalized linear model (GLM) was used to test for the effect of engorgement status (unengorged, semi-engorged or fully engorged) and life stage (nymph or adult) on the minimum infection rate. All factors were treated as fixed. A GLM was also used to test for the effect of the state (New Jersey/Pennsylvania) and year (2015-18 in New Jersey, 2017-28 in Pennsylvania) on infection rate. Lastly, a GLM was used to test for effect of New Jersey counties on infection rate. For all statistical analyses, the criterion for significance was set to $\alpha = 0.05$.

Chapter III
Results

Tick Collections

Appendix A presents the total number of ticks collected from 2015-2018 by collection season (Fall harvest and Fall or Spring research trapping), year, state, species and life stage. Overall, four species of ticks were collected: *Ixodes scapularis, Ixodes cookei, Amblyomma americanum* and *Dermacentor variabilis*. Larval, nymph and, adult life stages were collected of *I. scapularis* were collected. All other tick species were collected in their adult life stage. The most commonly collected tick was *Ixodes scapularis* (n=2,119) (78.1%), followed by *Dermacentor variabilis* (n = 590) (21.7%), *Amblyomma americanum* (n=2) (0.07%) and *Ixodes cookei* (n=2) (0.07%). The average tick load per black was 6.33. The total number of ticks collected from New Jersey between 2015-18 was 2,133 and in Pennsylvania from 2017-18 was 580. There was no significant difference between New Jersey and Pennsylvania in the relative abundance of tick species collected (chi-squared test; $X^2 = 8$, $p = 0.2381$, $df = 6$).

In New Jersey, four different tick species were collected throughout the year, with the majority of collected ticks being adult *I. scapularis* in October and *D. variabilis* in

June. Overall the majority of ticks were collected in October when adult *I. scapularis* are most active, then in June when *I. scapularis* nymphs and adult *D. variabilis* are most active (Figure 10). Fewer ticks were collected in August, most likely due to the hot, dry weather unfit for tick activity. Fig. 10 presents the total number of all ticks collected in the month of October between 2015-18, in June 2015-18 and August 2015-18. There were 1,554 *I. scapularis* collected between 2015-18 in New Jersey with 98% being adults, 1.6% being nymphs and 0.1% being larval. The majority of adults were collected each year in October (Figure 11).

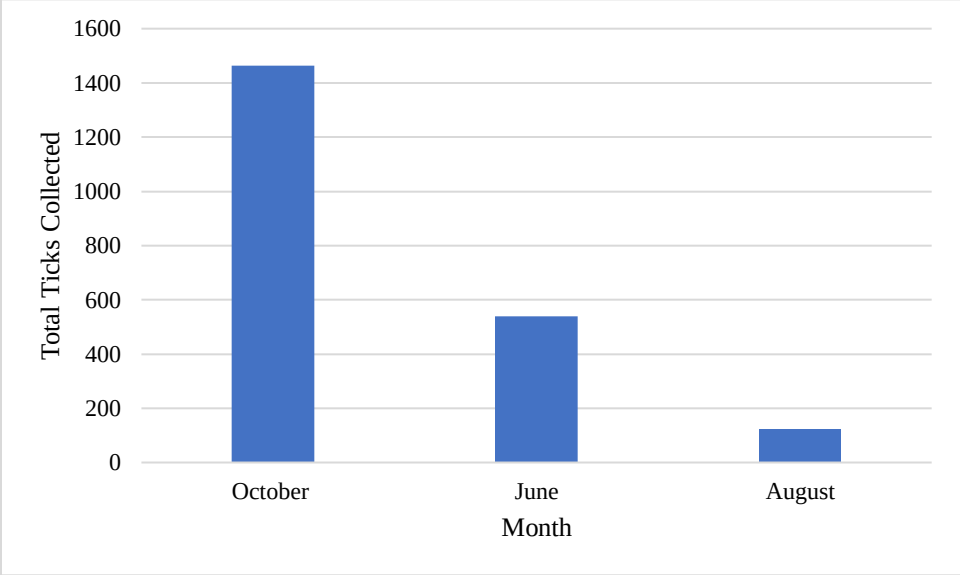

Figure 10. Total number of ticks collected from 2015-18 in New Jersey by month

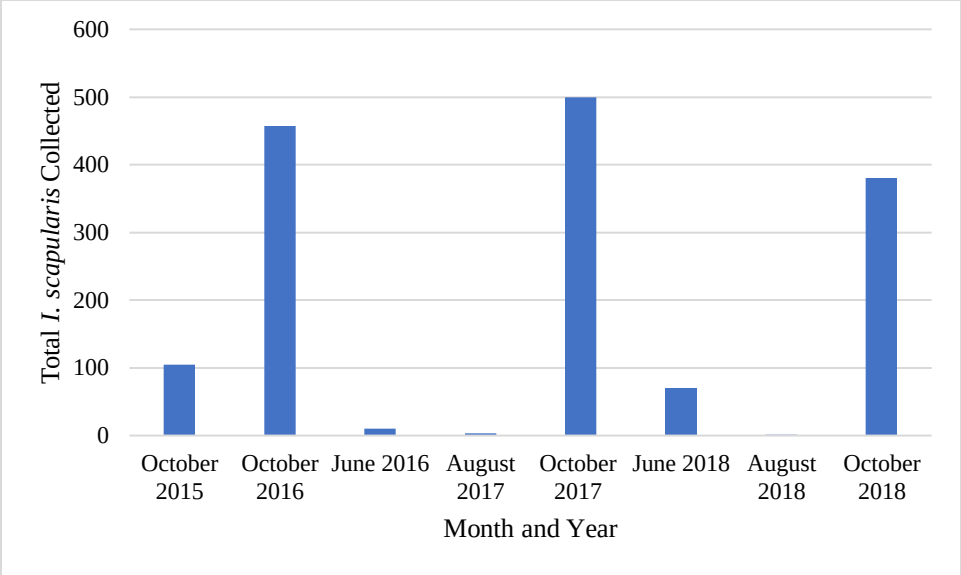

Figure 11. Total adult *I. scapularis* collected in the month throughout the year from New Jersey (2015-18)

Three tick species collected in Pennsylvania between 2017-18. The majority of ticks in Pennsylvania were collected in May, June, and November, active months for nymphs in the Spring and Summer, and adults in the Spring and Fall (Figure 12). The majority of ticks collected were *I. scapularis*, with 90.6% consisting of adults, 5.3% nymphs and 4.1% larval. Adult *I. scapularis* were collected through the majority of months but were most prevalent in May, June and November during periods that *I. scapularis* adults are known to be most active (Figure 13). Nymph *I. scapularis* were collected in five months out of the year, with May, June and August having the highest collection rate (Figure 14). Larval *I. scapularis* were collected four months out of the year with August being the peak month for larval collection (Figure 14).

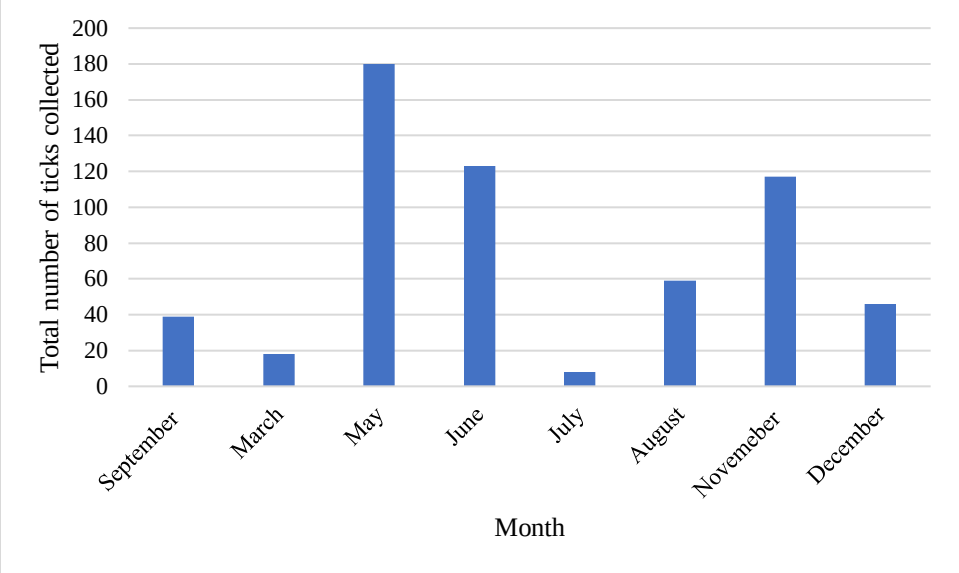

Figure 12. Total number of ticks collected from 2017-18 in Pennsylvania by month

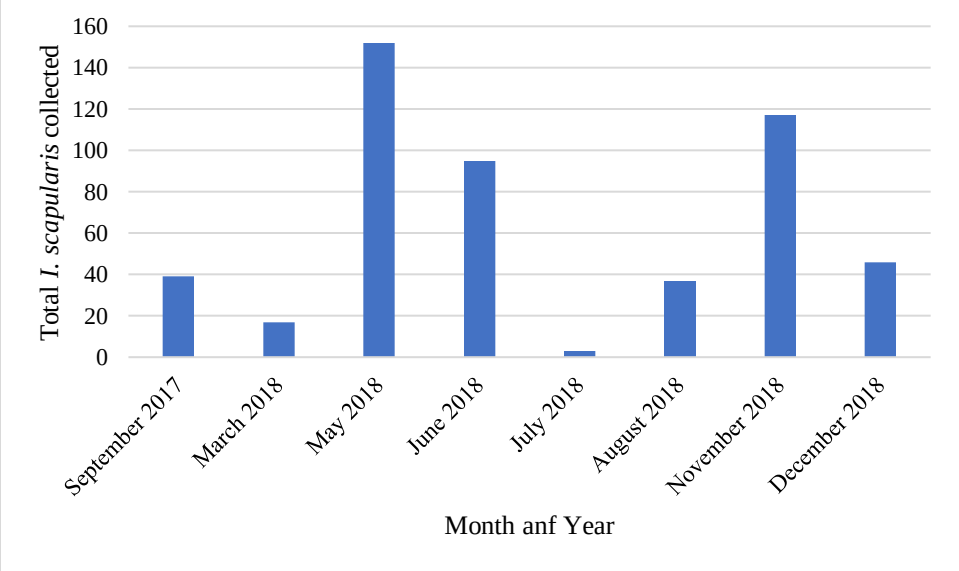

Figure 13. Total number of adult *I. scapularis* collected in Pennsylvania in 2017-18 by month

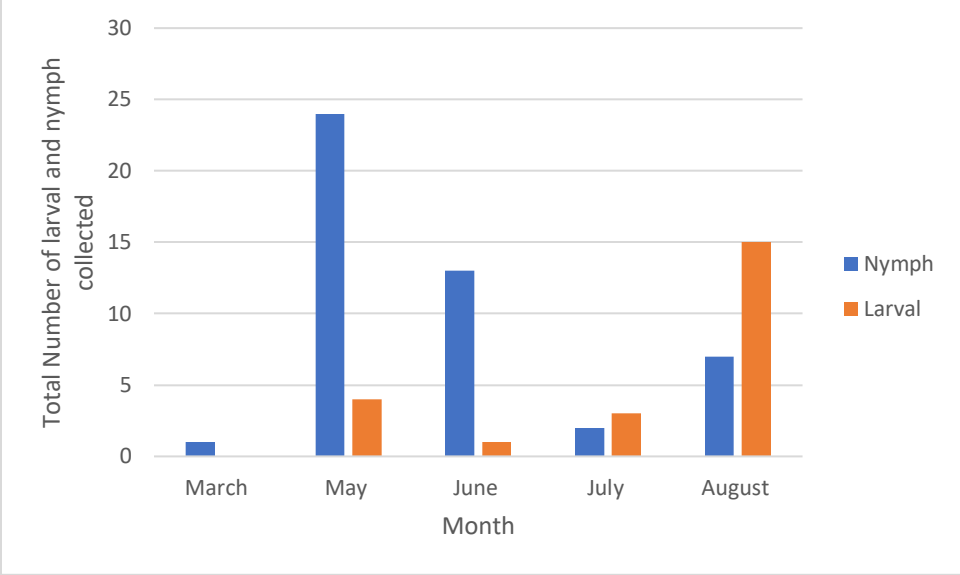

Figure 14. Total number of nymph and larval *I. scapularis* collected in Pennsylvania in 2017-18 by month

Optimization of Powassan Lineage II Assay

In order to standardize the Powassan virus Lineage II RT-PCR assay a standard curve was created using a synthetic positive created by GENEWIZ (South Plainfield, NJ). The synthetic positive control was created from the targeted NS5 primers used in the assay. Using 10 fold serial dilutions ranging from 10^{-1} to 10^{-10} with a starting concentration of 1.67^{11} copies/µL were used and performed in triplicate to validate accuracy. The 10^{-7} dilution was the last dilution to have a CT call for all three samples. CT values from dilutions 10^{-1} to 10^{-7} were inserted into Microsoft Excel and a linear regression was created using the log of number of copies per each dilution. The slope formula was determined to be $y = -5.036x + 74.524$, with an R^2 of 0.9853 (Figure 15). The CT cutoff value was determined to be 44.7 by plugging in the highest triplicate value

from the 10^{-7} dilution into the slope formula. A threshold generated by the Applied Biosystems StepOnePlus standard curve was 1.725 for Powassan virus (DTV)

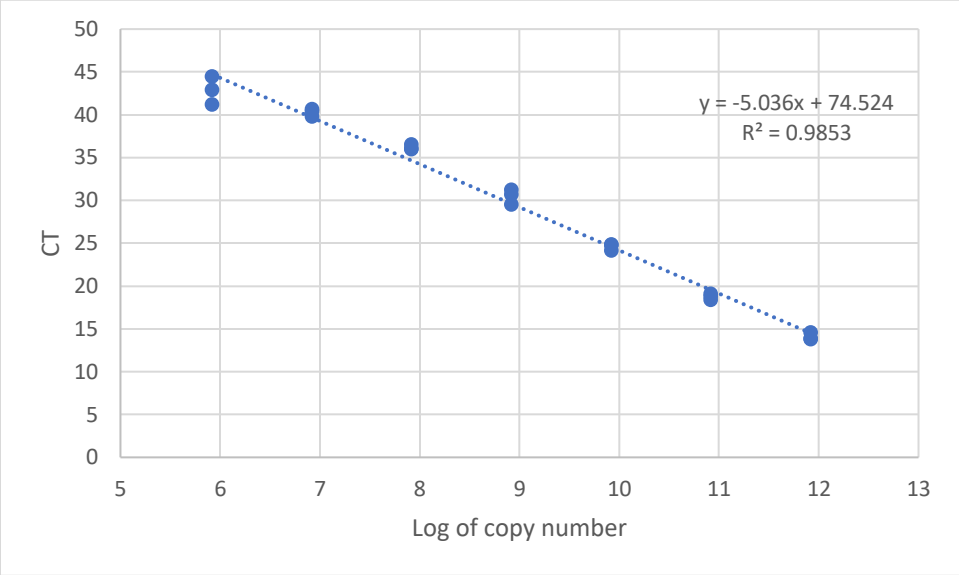

Figure 15. Plotted linear regression of the standard curve, using the log number of copies of each dilution by the CT call from each triplicate

Disease and Statistical Analysis

Ixodes scapularis were selected for analysis from three counites in New Jersey and nine counties in Pennsylvania because they are the known vectors of Powassan virus Lineage II. Samples that were selected to be analyzed were separated by state, county and year and each individual was accounted for (Table 3,Table 4). Counites in New Jersey were picked according to which had the highest yield of *I. scapularis* collected. Hunterdon County was also included as it has had a known human case of Powassan virus. All *I. scapularis* nymph and adult ticks from Pennsylvania were analyzed due to having only two years of ticks collected. A total of 1,277 blacklegged ticks were selected

to be analyzed for Powassan virus Lineage II and Lyme disease (*Borrelia burgdorferi*) using the polymerase chain reaction (PCR). A total 595 samples consisting of 344 individuals and 251 pools were analyzed (Appendix B). Of these, 831 (64.9%) were female, 384 (30.0%) were male and 64 (5.1%) were nymphs (Figure 16).

Table 3. Total number of blacklegged ticks analyzed from each county in New Jersey

YEAR	Hunterdon	Sussex	Warren	Total
2015	26	23	21	90
2016	0	184	47	231
2017	0	109	91	200
2018	0	108	117	225

Table 4. Total number of blacklegged ticks analyzed from each county in Pennsylvania

YEAR	Monroe	Pike	Centre	Clearfield	Clinton	Huntingdon	Lycoming	Potter	Toga	Total
2017	32	3	0	0	0	0	0	0	0	35
2018	0	0	10	22	304	23	111	5	23	498

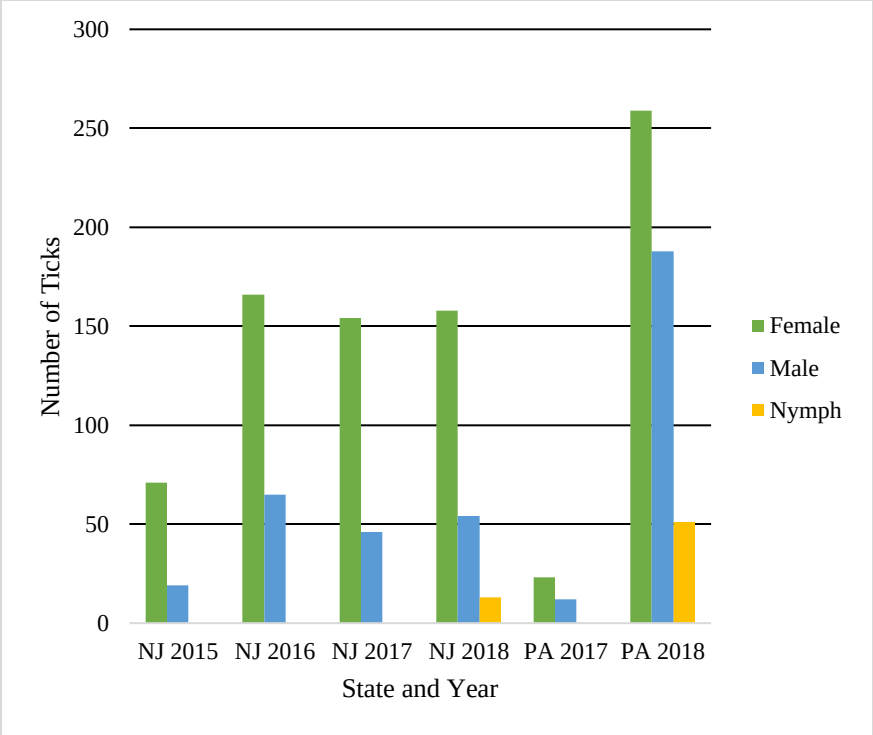

Figure 16. Total number of *I. scapularis* female, male and nymph ticks analyzed (n= 1,277) from NJ (2015-18) and (PA 2017-18)

Powassan Virus

The overall minimum infection (MIR) rate was determined for positive tick pools for Powassan virus as 3.52%. The overall minimum infection rate was determined for adult blacklegged ticks positive for Powassan virus as 3.54%. The MIR of adults *I. scapularis* infected in New Jersey ranged from 0-3.0% between 2015-18, with 2017 having the highest MIR with 3.0%. The MIR in northeast Pennsylvania counties was 5.7%, compared to 4.0% in central Pennsylvania counties (Figure 17). The minimum infection rate was determined for nymph blacklegged ticks positive for Powassan virus in New Jersey 0%, and Pennsylvania 3.92% for 2018 (Figure 18).

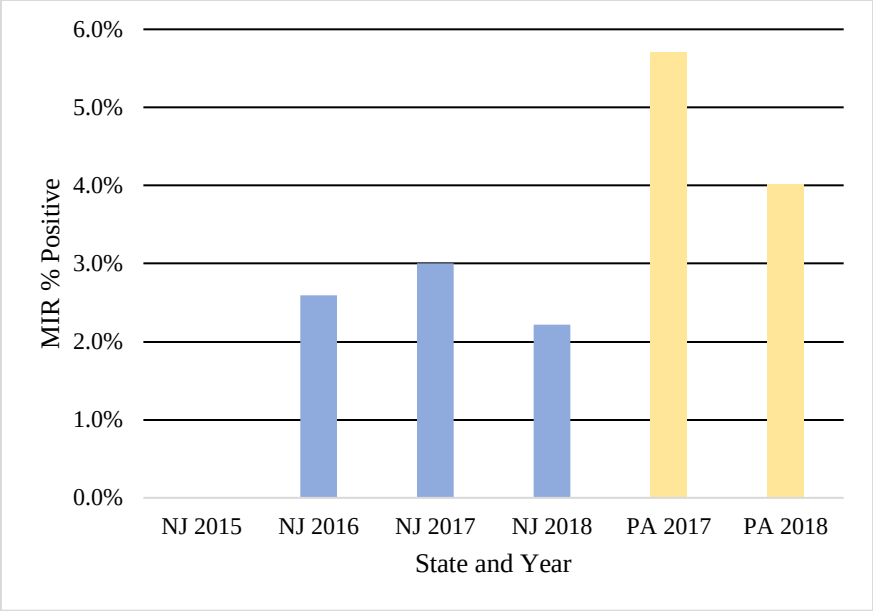

Figure 17. Minimum infection rate (MIR) percent of adult *I. scapularis* with POW (DTV) (n=1,213) from NJ (2015-18) and PA (2017-18)

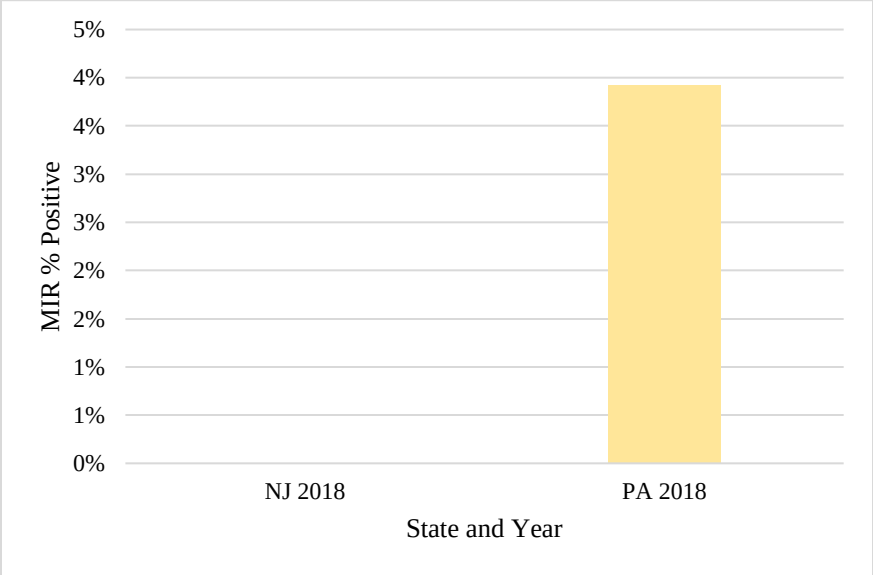

Figure 18. Minimum infection rate percent of nymph *I. scapularis* ticks with POW (DTV) (n=64), from NJ and PA 2018

Ixodes scapularis nymphs and adults did not differ in Powassan infection prevalence (GLM df = 2, p = 0.897). Powassan prevalence also did not vary with engorgement state in either adults (df = 3, p = 0.910) or nymphs (df = 1, p = 0.976). There was no significant difference between males and females in Powassan infection (df = 1, p = 0.836). There was no significant difference between New Jersey and Pennsylvania overall in Powassan infection (df = 1, p = 0.852). Appendix C consist of full statistical tables.

Borrelia burgdorferi

The overall minimum infection rate was determined as 19.2% for ticks positive for *B. burgdorferi*. The overall minimum infection rate was determined as 19.8%. for adult blacklegged ticks positive for *B. burgdorferi*. The MIR of adults *I. scapularis* infected in New Jersey ranged from 17.7-23.0% between 2015-18, increasing each year (Figure 19). The MIR in northeast Pennsylvania counties was 28.5%, compared to 18.1% in central Pennsylvania counties. The minimum infection rate was determined for nymph blacklegged ticks positive for *B. burgdorferi* in New Jersey 0%, and Pennsylvania 7.84 % for 2018 (Figure 20).

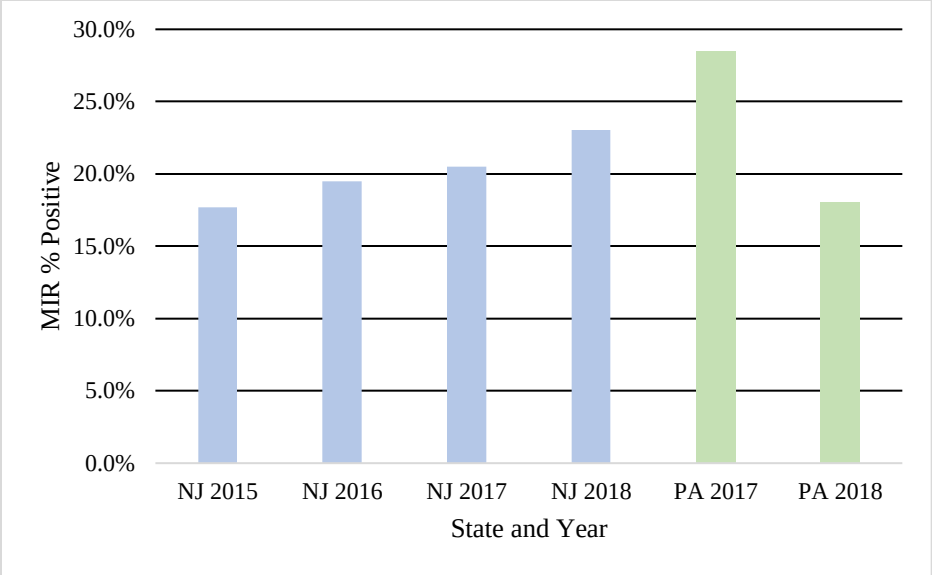

Figure 19. MIR (%) of adult I. scapularis ticks with B. burgdorferi (n=1,213) from NJ (2015-18) and PA (2017-28)

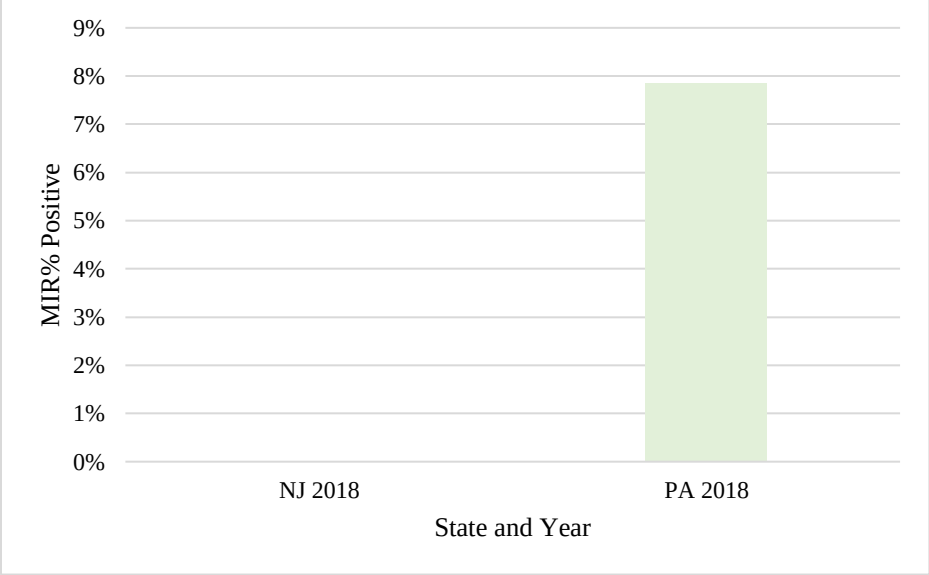

Figure 20. Minimum infection rate percent of nymph I. scapularis ticks with B. burgdorferi (n=64), from NJ and PA 2018

I. scapularis nymphs and adults did not differ in *Borrelia burgdorferi* infection prevalence (GLM df = 2, p = 0.725). *B. burgdorferi* prevalence also did not vary with engorgement state in either adults (df = 3, p = 0.799) or nymphs (df = 2, p = 0.815). There was no significant difference between males and females in *B. burgdorferi* infection (df = 1, p = 0.945). There was no significant difference between New Jersey and Pennsylvania in *B. burgdorferi* infection (df = 1, p = 0.986).

Co-infection

Overall of the 45 positive Powassan ticks, 25 of them were co-infected with *B. burgdorferi* (55.5%). Of these, 23 were adults (53.4%) and the two Powassan positive nymph pools were both co-infected with *B. burgdorferi* (100%). New Jersey had 11 co-infected tick pools and Pennsylvania had 14. There were 12 co-infected female pools, 11 co-infected male pools and 2 co-infected nymph pools. The majority of co-infected ticks came from central Pennsylvania in 2018, compared to New Jersey 2015-18 and Pennsylvania 2017.

County Data

Powassan

Positive pool samples of Powassan virus in New Jersey counties were consistent over the three years 2016-18. Sussex County had the most positive tick pools compared to Warren or Hunterdon Counties (Table 5). No counites in 2015 had a positive pool for

Powassan virus. Northeast Pennsylvania presented a lower positive pool sample of Powassan virus than central Pennsylvania (Table 6). Clinton County in central Pennsylvania had the highest amount of positive pools of Powassan virus throughout New Jersey or Pennsylvania with 16 positive tick pools.

New Jersey did not differ in Powassan infection prevalence between 2015- 2018 (GLM df = 2, p = 0.986). Pennsylvania did not differ in Powassan infection prevalence between 2017 and 2018 (df = 1, p = 0.906). There was no significant difference between Sussex and Warren county (df = 1, p = 0.917), Hunterdon county was excluded due to no positive ticks. No statistical test was run for Pennsylvania counties due to uneven distribution of ticks collected between counites.

Table 5. Total Powassan positive pools by year in each New Jersey county

COUNTY	2015	2016	2017	2018
HUNTERDON	0	0	0	0
SUSSEX	0	4	5	4
WARREN	0	2	1	1
TOTAL	0	6	6	5

Table 6. Total Powassan positive pools by year in each Pennsylvania county

COUNTY	2017	2018
PIKE	0	0
MONROE	2	0
CENTRE	0	0
CLEARFIELD	0	2
CLINTON	0	16
HUNTINGDON	0	1
LYCOMING	0	5
POTTER	0	0

TIOGA	0	2
TOTAL	2	26

Borrelia burgdorferi

The amount of positive tick pools overall and in Sussex County fluctuated over the years. In contrast, Warren had a continuous increase of positive tick pools between 2015-18 (Table 7). Northeast Pennsylvania had fewer positive pools than central Pennsylvania for *B. burgdorferi* (Table 8). Overall, Clinton County had the most positive tick pools in Pennsylvania and New Jersey for *B. burgdorferi*.

New Jersey did not differ in *B. burgdorferi* infection prevalence between 2015 and 2018 (GLM df = 3, p = 0.885). Pennsylvania did not have a significant difference in *B. burgdorferi* infection prevalence between 2017 and 2018 (df = 1, p = 0.955). There was no significant difference between counties in New Jersey 2015-18 (d=3, p = 0. 720). Statistical analysis were not conducted for Pennsylvania counties due to uneven distribution of tick collection.

Table 7. Total *B. burgdorferi* positive tick pools by year in New Jersey counties

COUNTY	2015	2016	2017	2018
HUNTERDON	4	0	0	0
SUSSEX	6	36	27	25
WARREN	6	9	14	22
TOTAL	16	45	41	47

Table 8. Total *B. burgdorferi* positive tick pools by year in Pennsylvania counties

	2017	2018
PIKE	0	0
MONROE	10	0
CENTRE	0	0
CLEARFIELD	0	2
CLINTON	0	55
HUNTINGDON	0	3
LYCOMING	0	19
POTTER	0	1
TIOGA	0	6
TOTAL	10	86

Chapter IV
Discussion

Powassan virus is an emerging tick-borne virus that has the potential to be debilitating and deadly to those infected. Lyme disease is one of the top vector-borne diseases in the United States and has been increasing in the amount of cases reported every year[1]. Conducting prevalence studies is an important tool to understanding tick-borne diseases in areas with high human and tick interactions. This is the first study identifying the prevalence of Powassan virus (DTV) and Lyme disease (*Borrelia burgdorferi*) co-infection in *Ixodes scapularis* collected from black bears in New Jersey and Pennsylvania.

Tick Collection Data

Tick distribution in the United States has been changing over the last decade. Ticks not established in regions are becoming established and populations of ticks in established regions are on the rise[59]. In 2014-2018, the distribution of *I. scapularis* has increased from the Northeast and Great Lake regions down south further into Texas and

west into the eastern part of Nebraska and Kansas. Eisen, Eisen & Beard (2017) examined the county-scale distribution of *I. scapularis* and noted that it had been documented in 1,420 of the 3,100 continental US counties as of 2016. This represented an increase of 44.7% when compared to the previous county-scale distribution map created in 1998[23]. Other tick species such as *Amblyomma americanum* (Lone star tick) have been increasing in geographic distribution. The Lone star ticks distribution has increased from established areas in southern states to newly established areas as far north as New York and Maine[25].

Several factors play a key role in the increased distribution of tick populations in the United States, with the two main hypotheses focused around climate change and habitat fragmentation. To survive, the majority of ticks need moderately warm temperatures. In fact, *I. scapularis* cannot survive temperatures over 40°C or under -10°C[59]. Additionally, ticks need a moderately humid climate to survive, as too not dry out or become overly saturated[24]. Climate change in the U.S. has caused warmer winters and wetter summers. This has allowed ticks to have a higher survival rate over the winter and summer[24]. In Warren County, NJ the average temperature in June 2015 was 24.6°C and in June 2018 increased to 24.8°C. June of 2015 in Warren county had a high average precipitation of 7.73 inches compared to June 2018 which had an average precipitation of 3.43 inches. In Monroe County the average temperature in June 2015 was 25.0°C and in June of 2018 it was 26.0°C with an average precipitation of 10.95 inches in 2015 and 3.62 inches in 2018. In Clinton County the average temperature was 25.6°C in 2015 and 25.2°C in 2018 with an above average precipitation in 2015 of 8.36 inches and an

average precipitation of 5.28 inches in 2018. Between 2015 and 2018 in these three counties temperatures tended to increase and had lower precipitation each year. The high precipitation in 2015 may of caused the tick population that year to become overly saturated with water, while in 2018 the precipitation was close to the known average for each county and could of created a more stable humid for ticks to survive in[14].

Changes due to human growth have not just impacted the climate but wildlife habitat too. Human growth and expansion have increased forest fragmentation and changed the micro-habitat of wildlife. Allan, Keesing & Ostfeld (2003) conducted a study to determine if forest fragmentation increased the white-footed mouse (*Peromyscus leucopus*) population and if it affected the infection rate of *B. burgdorferi* in larval and nymph *I. scapularis*. They concluded that in highly fragmented areas, the white-footed mouse population increased, possibly due to low predator abundance and low competition of resources. As the white-footed mouse is the main reservoir for many tick-borne pathogens, they noted larval and nymph infection rates of *B. burgdorferi* increased in highly fragmented areas[2]. Furthermore, forest fragmentations create edge habitats that are suitable for high populations of white-tailed deer (*Odocoileus virginianus*), a favorable host for *I. scapularis* adults to feed and reproduce on[11]. Forest fragmentations have increased the risk of human-tick interactions as many of these forest fragmentations border homes[11] and increased populations of ticks are present in them.

In this study, Lone star ticks were collected from black bears in 2018 in northern New Jersey and northeast and central Pennsylvania. Lone star ticks were not found on black bears in previous years in either states. Previous studies conducted by Zolink et al. (2015) and Chern, Bird & Frey (2016) collected ticks from black bears in New Jersey and

found the majority of ticks to be adult *D. variabilis* and adult and nymph *I. scapularis*[13,68]. Although, nymph and larval *I. scapularis* were only found in 2017 and 2018 on black bears during this study. The collection of larval and nymph *I. scapularis* from black bears could be due to an increase in the nymph and larval populations in 2017 and 2018 and choosing a larger mammal to feed on due to accessibility of feeding space. Ticks used in this study from 2015 and 2016 were collected by previous students and all nymphs collected those years may have been used for other studies. Lastly, another reason could be due to a more thorough search on the black bears by collectors, looking for all life stages of ticks, and not just the large adults easily visible to the eye. Black bears were combed over in 10-15-minute increments, a short period of time to search for ticks. The average bear had 6.7 ticks collected from it, with the low being 0 and the highest being 44 ticks. Al-Warid et al. (2017) determined an average tick community composition of 400 ticks on black bears in Missouri. Al-Warid did not state if there was a time restraint for each tick search. A longer search duration may yield a higher average of ticks on black bears in New Jersey and Pennsylvania and may more accurately describe the community composition of ticks on these bears.

Black bears may play an important role in the dilution of tick-borne diseases in an area. Black bears, like white-tailed deer, are not known reservoirs of many tick-borne pathogens but can host many ticks on their bodies. Huang et al. (2019) conducted a study on Block Island, RI and determined when nymphs and larval *I. scapularis* fed on white-tailed deer, the infection rate of *B. burgdorferi* in *I. scapularis* decreased the next year. Black bears may also help to decrease the infection rate of *I. scapularis* if larval and nymphs begin to feed on them as their first and second meals. Black bear populations in

the New York, New Jersey and Pennsylvania are on the rise and if they are becoming a primary host for larval, nymph and adult *I. scapularis* the infection rate of tick-borne disease may decrease in high-density black bear areas. It is unknown if black bears are reservoirs for Powassan virus and is unclear at this time if they play a role the transmission cycle.

Powassan (DTV) Prevalence

Few prevalence studies for Powassan virus lineage I and/or lineage II have been conducted over the last decade. A study conducted by Brackney et al. (2008) determined that 1.3% of *I. scapularis* adults tested were DTV positive in Wisconsin. Another study conducted in 2011-12 by Knox et al (2017) analyzed four quadrants of Wisconsin and determined a MIR range for DTV between the four quadrants to be 1.56-4.62%. Anderson and Armstrong (2012) analyzed adult and nymph *I. scapularis* in Connecticut and found 0.8% to 1.6% DTV-positive tick pools in Bridgeport and 0.4% to 3.9% DTV-positive pools in North Branford. A study conducted by Dupuis II et al. (2013) analyzed adult and nymph *I. scapularis* collected from several counties in Hudson Valley, NY between 2007 and 2012 and determined a Maximum Likelihood Estimate (MLE) range to be 0.2-6.0% for adults. Results from this study were similar to those found in previous studies, with a minimum infection rate (MIR) prevalence ranging from 0.0-3.0% in New Jersey (2015-18) and 4.0-5.7%% in Pennsylvania (2017-18). As the MIR was calculated to determine the prevalence of tick pools in New Jersey and Pennsylvania, the true prevalence rate of each state may be higher, as it was assumed only one tick was positive for Powassan virus in a pool and not multiple ticks in the pool being positive.

The prevalence of Powassan virus (DTV) in Warren and Sussex County, New Jersey appear to be stable, as each year the amount of positive pools either increased by one or decreased by one. It is unclear what the true prevalence of Powassan virus (DTV) is in Hunterdon County, as only one year had tick pool samples analyzed and zero were positive. The stability of Powassan virus (DTV) in Pennsylvania could not be determined by this study for reported counties as each county was only analyzed for one year. Although it was not found to be statistically significant Pennsylvania did have a higher prevalence rate than New Jersey in 2018 with 2.2% and 4.0% in Pennsylvania. These results could possibly be biased due to the sample size difference between the two states in 2018, with 102 more sample pools analyzed in Pennsylvania than New Jersey. Other biases may be seen in the results from Pennsylvania 2017 due to a small sample size of only 26 tick pools. An uneven number of ticks were collected from counties in Pennsylvania, creating an uneven distribution of tick pools per county. This could create biased results for counties with small tick pools such as Potter (5 ticks pools) and Centre (4 tick pools) compared to Clinton which had 203 tick pools. Although several of the counties had small sample sizes, Powassan positive tick pools came from eight of the twelve counties analyzed. This indicates a risk in several counties in New Jersey and Pennsylvania for humans to come into contact with Powassan infected ticks.

All positive pools for Powassan in New Jersey came from ticks collected in October, while the majority of positive ticks in Pennsylvania were collected in May and June. One possible explanation for this difference is that the population of the white-footed mouse, the primary reservoir of Powassan virus (DTV), may be different in New Jersey and Pennsylvania. If there are more white-footed mice in one area than another

ticks have a greater opportunity to feed on these mice as their first or second meal. This could cause some ticks to be infected earlier in the year and others to be infected later in the year. The majority of ticks analyzed were adults and 43 of the 45 positive tick pools were composed of adults. In New Jersey, adult *I. scapularis* ticks are most active in the Fall and as noted the majority of Powassan positive ticks were collected in October. The Fall months, notably October, in New Jersey are potential high-risk months for Powassan virus infection from *I. scapularis.* In Pennsylvania, ticks had the highest rates of Powassan infection in May and June. Although adults are not typically the most active in the Spring and Summer, they can be found questing for a host and will attach to a host if they find one. Nymphs are most active during this season and with two positive nymph pools, there is a risk that Powassan infection is present in these months in Pennsylvania. A concern with nymph ticks being positive for infection is the fact they are very small, and the majority of humans will not know to check or may miss them during a tick check, giving these ticks ample time to feed and transmit tick-borne diseases. As Powassan virus can transmit within 15 minutes adults and nymphs are both likely to be able to transmit the virus even if the tick is found within a short amount of time of attaching and feeding before being removed.

There was no significant effect of gender, life stage, or engorgement on Powassan infection of *I. scapularis.* This indicates there is no difference in the chance of contracting Powassan virus from a *I. scapularis* depending on its sex, life stage or engorgement level. There is also no significant difference in Powassan infection between 2015-18 in New Jersey or 2017-18 in Pennsylvania. Lastly, there was no significant difference between the overall positive pools in New Jersey and Pennsylvania.

Powassan virus (DTV) cases have been increasing over the last decade with 6 human cases reported in the US in 2015, 21 reported in 2016, and 33 reported in 2018. Prevalence research has been sparse for Powassan virus over the last decade, although it is on the rise. Most cases of Powassan virus have occurred in the Northeast and Great Lakes regions of the U.S., which are in Lyme endemic regions[63]. It is important for states with confirmed Powassan virus (DTV) cases to monitor the tick population to better understand the prevalence rate in high-risk areas for infection.

It is unclear how Powassan virus interacts with other tick-borne diseases, such as Lyme disease, or why some individuals are asymptomatic to the virus and others develop severe symptoms. This study did find *I. scapularis* adults and nymphs capable of being co-infected with Powassan virus and *B. burgdorferi*. Further research needs to be conducted on Powassan virus to better understand prevalence, transmission effects in presence of other tick-borne diseases and its ability to cause disease in those infected.

Borrelia burgdorferi **Prevalence Data**

Previous studies have been conducted on the prevalence of *B. burgdorferi* in *I. scapularis* ticks in Lyme disease endemic regions of the U.S.. Courtney et al. (2003) conducted a prevalence study in Northwestern and Southeastern Pennsylvania and determined a prevalence of 61.6% and 13.1%, respectively. A study conducted by Steiner et al. (2008) collected adult *I. scapularis* from Indiana, Maine, Pennsylvania and Wisconsin and found prevalence rates that ranged between to 35% and 70%. In central New Jersey, Schulze et al. (2005) analyzed adult *I. scapularis* and determined an infection prevalence for *B. burgdorferi* to be 50.3%. Prusinski et al. (2014) collected *I.*

scapularis adults and nymphs from eight New York Counties from 2003 to 2006 and determined an overall prevalence rate of *B. burgdorferi* 14.4% in adults and 45.7% in nymphs.

The current study determined a MIR prevalence range of 17.7-22.6% in New Jersey 2015-18 for *B. burgdorferi.* The MIR prevalence in Pennsylvania in 2017 and 2018 was 28.5% and 18.9%. These findings are comparable to other previous studies conducted for *B. burgdorferi* prevalence in Lyme endemic regions. In a thesis study conducted by Bird (2014) found a MIR of 0% of *I. scapularis* larval pools positive and 41% adult *I. scapularis* pools positive for *B. burgdorferi*[7]. The 9.6% difference between 2017 and 2018 could be caused by the large sample size difference between 2017 (26 tick pools) and 2018 (203 tick pools). Additionally, different regions of Pennsylvania may have different prevalence rates of Lyme disease, as seen with 2017 ticks collected from the Northeast region of Pennsylvania and 2018 ticks collected from the Central region of Pennsylvania.

Overall, out of 245 positive pools 98.3% were adults and 1.63% were nymphs. Similar to Powassan, the majority of positive *B. burgdorferi* tick pools in New Jersey were ticks collected in October. These may be due to the increased number of adult *I. scapularis* out questing during this time of the year. Female adult *I. scapularis* accounted for 71.8% of positive ticks pool, males made up 26.5% of the positive pools and nymphs made up 1.63% positive pools. The Fall is a high-risk season for Lyme disease with large populations adults questing, especially females in New Jersey. Pennsylvania had the majority of *B. burgdorferi* positive tick pools from ticks collected in May (23 positive tick pools), June (22 positive tick pools) and November (17 positive tick pools). All other

positive tick pools were from ticks collected in March, July, August, and December. Risk for Lyme disease in Pennsylvania is high throughout the Spring, Summer and Fall due to the high percentage of nymphs questing in the Spring and Summer and the adults questing in the Fall. Human contact with ticks increases in the Spring, Summer, and Fall as the warmer weather increases outdoor activities such as hiking and camping increase.

There was no significant difference between life stage, or engorgement and *B. burgdorferi* positive ticks. There is no difference in chance of contracting *B. burgdorferi* from a *I. scapularis* depending on its life stage or engorgement level. There was no significant difference between 2015-18 positive tick pools in New Jersey or 2017-18 in Pennsylvania. There was no significant difference between the amount of overall positive tick pools between New Jersey and Pennsylvania.

As nymph ticks play a crucial role in causing Lyme disease infections in humans, this study is not consistent with other studies with regards to prevalence rates in nymph *I. scapularis* ticks. This can be due to the small sample size of nymphs. Also, the MIR was calculated for prevalence and these numbers may be underestimating the true percentage of positives ticks, as it was assuming only one out of the number of ticks in the pool was positive for adult and nymph pools analyzed.

The CDC reported 12,801 confirmed cases of Lyme disease in 1997. Just20 years later, the CDC reported 29,513 confirmed cases and 13,230 probable cases. The rise of Lyme disease in the Northeastern and Midwest United States is a cause for alarm and a need for continuous prevalence and surveillance studies to monitor high risk.

Co-infection Prevalence Data

I. scapularis is the vector to many different pathogens and is able to harbor more than one pathogen at once. Many of these pathogens such as *B. burgdorferi* and Powassan virus (DTV) cause infection in humans. Studies that have examined the ability of *I. scapularis* to have co-infections and found them to be able to harbor up to four or more pathogens[65]. Studies have focused on the infection rate and co-infection rate of *B. burgdorferi* with the other tick-borne pathogens that include *Anaplasma phagocytophilum, Babesia microti*, Powassan virus (DTV), and *Borrelia miyamotoi*[65]. Many of these pathogens share the reservoir host of small mammals, specifically the white-footed mouse (*Peromyscus leucopus*)[16]. Larval and nymphs can become infected when they feed off of the reservoir host during their first or second meal. The larval and nymph ticks will continue to carry these pathogens between molting to the next life stage and can transmit the pathogen(s) to its next host during feeding.

Tokarz et al. (2010) conducted a study in New York where 70% of *I. scapularis* had one pathogen and 30% had a polymicrobial infection. Specifically, they found that 24% were co-infected with *B. burgdorferi/A. phagocytophilum*, 31% were co-infected with *B. burgdorferi/Babesia microti,* and five ticks were Powassan virus (DTV) positive, with two being co-infected with Powassan (DTV)/*B. burgdorferi* (40%). Knox et al. (2017) analyzed adult *I. scapularis* in Wisconsin and found that eight were positive for Powassan virus (DTV) and four of the eight were co-infected Powassan (DTV)/*B. burgdorferi* (50%).

This current study had similar co-infection rates to Knox et al. (2017) and Tokarz et al. (2010) in adult *I. scapularis,* with 53.4% Powassan virus (DTV) positive adults

being co-infected with *B. burgdorferi*. Frost et al. (2015) tested patients with one known tick-borne disease for Powassan and 17.1% of patients showed serological evidence that they were infected with both *B. burgdorferi* and Powassan virus. Three patients (7.3%) were laboratory confirmed (PCR) to have a Powassan and *B. burgdorferi* co-infection.

Co-infections increase the difficulty for accurately diagnosing and treating tick-borne diseases[43]. Pathogens can range from bacterial to protozoan to viral and need different treatment methods. Symptoms of one tick-borne disease can be masked by another when a co-infection occurs. This allows one pathogen to be treated but the other to continue causing symptoms and illness. Some studies, such as those conducted by Thomm et al (2018), have suggested Post Treatment Lyme Disease Syndrome (PTLDS) may be caused by an undiagnosed co-infection. Thomm et al (2018) has worked to develop and validate a serological test panel for the detection of Powassan virus and has suggested those who have been treated for Lyme disease but have persistent symptoms consistent with PTLDS should be tested for Powassan virus due to a possible co-infection. With co-infections as high as 28% in *I. scapularis* ticks in Lyme disease endemic regions in the U.S., surveillance studies are important to monitor co-infection rates in these areas.

Conclusion

The overall increase in tick populations and distribution can have severe impacts on human infection and tick-borne diseases. Ticks established in new areas increase the risk of tick-borne disease brought into areas that they were not present in before. The increased distribution of *I. scapularis* may be a possible cause of the increase in Lyme

disease infections in the U.S. and may lead to an increase of other tick-borne diseases such as Powassan virus (DTV). Lone star ticks are known to carry the tick-borne pathogens *Ehrlichia chaffeensis, E. ewingii, Borrelia lonestari* (STARI), and tularemia. Human and pet populations in New Jersey and Pennsylvania can be at risk of being bitten by Lone star ticks and becoming infected with these diseases, as they are now known to inhabit these areas.

Tick surveillance studies such as this one help to determine the prevalence rate and high-risk areas for tick-borne diseases. Pennsylvania and New Jersey are the top two states for Lyme disease cases in the US[4]. This is the first study to determine the MIR of Powassan virus in New Jersey and Pennsylvania and found the MIR to be similar to rates determined in previous studies. This indicates that these states are at risk for human tick-borne infections and should be continued to be monitored for human, pet and wildlife health. Tick surveillance is important to continue to monitor populations of new and established ticks and diseases for the safety and health of those living in tick populated areas.

Future Study

As this was the first study in New Jersey or Pennsylvania to survey the prevalence of Powassan virus (DTV) and co-infection with Lyme disease (*B. burgdorferi*), there is ample opportunity to continue monitoring the prevalence rates in these states. With Powassan virus, as an emerging tick-borne virus capable of causing deadly disease in humans, it is important to monitor the prevalence rate of it to determine if risk to humans increases. This allows for physicians to be aware should a patient exhibit symptoms of it.

Additionally, only three of the twenty-one counties in New Jersey had ticks analyzed for Powassan virus (DTV), leaving a need for other counties in New Jersey to be analyzed in future work. Pennsylvania had nine out of sixty-seven counites analyzed with uneven tick samples from each county, future studies can conduct more thorough analysis of counties.

Blacks bears in this study had several different ticks' species and life stages attached to them. Future studies can conduct a more thorough search on black bears to better determine what the tick community composition consists of on black bears in New Jersey and Pennsylvania. Another study can be done using serological test (ELISA, IFA or $PRNT_{50 \text{ or } 90}$) with blood to determine if black bears have the capability to be reservoirs of Powassan virus, Lineage I or Lineage II, as they were found to be host to *I. scapularis* and *I. cookei*.

There has not been a study conducted to determine the prevalence rate of infected white-footed mice with Powassan virus (DTV) in Pennsylvania or New Jersey. As the reservoir host to many tick-borne pathogens, observing the prevalence of these pathogens in these mice can help to determine the prevalence rate of DTV and the chances of ticks obtaining the infection when feeding on wild mice.

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Appendix A: Tick Collection Raw Data

Tick Collection New Jersey 2015	
County	*Ixodes scapularis*
	Adult
Hunterdon	25
Morris	6
Passaic	0
Sussex	23
Warren	51
Total	105

Tick Collection New Jersey 2016		
County	*Ixodes scapularis*	*Dermacentor variabilis*
	Adult	Adult
Hunterdon	0	0
Morris	28	0
Passaic	10	14
Sussex	309	48
Warren	54	145
Total	401	207

Tick Collection New Jersey 2017					
County	*Ixodes scapularis*			*Dermacentor variabilis*	*Ixodes cookie*
	Adult	Nymph	Larval	Adult	Adult
Morris	54	0	0	0	0
Passaic	20	0	0	0	0
Sussex	262	0	0	1	0
Warren	93	5	1	72	1
Total	429	5	1	73	1

Tick Collection New Jersey 2018						
County	*Ixodes scapularis*			*Dermacentor variabilis*	*Ixodes cookei*	*Amblyomma americanum*
	Adult	Nymph	Larval	Adult	Adult	Adult
Morris	19	0	0	1	0	0
Passaic	12	1	0	0	0	0
Sussex	199	6	0	3	0	0
Warren	149	9	1	121	1	1
Total	379	16	1	125	1	1

Tick Collection Pennsylvania 2017	
County	*Ixodes scapularis*
	Adult
Lackawanna	1
Monroe	35
Pike	3
Total	39

Tick Collection Pennsylvania 2018					
County	*Ixodes scapularis*			*Dermacentor variabilis*	*Amblyomma americanum*
	Adult	Nymph	Larval	Adult	Adult
Centre	1	9	0	0	0
Clearfield	19	3	1	0	0
Clinton	276	28	22	1	1
Huntingdon	23	0	0	0	0
Lycoming	102	9	0	13	0
Potter	3	2	0	0	0
Tioga	23	0	0	0	0
Total	447	51	23	14	1

Appendix B: Powassan and Lyme Raw Data

ID	Bear ID	Sex	Pooled Ticks	Engorgement	POW	Lyme	Year	County	State
1	86	F	1	Semi	-	-	2017	Sussex	NJ
2	86	M	1	Un	-	-	2017	Sussex	NJ
3	86	F	1	Un	-	-	2017	Sussex	NJ
4	92	F	1	Un	-	-	2017	Sussex	NJ
5	111	F	1	Un	-	+	2017	Sussex	NJ
6	111	M	1	Un	-	-	2017	Sussex	NJ
7	291	F	1	Un	-	-	2017	Sussex	NJ
8	453	F	1	Semi	-	-	2017	Sussex	NJ
9	453	F	1	Semi	-	-	2017	Sussex	NJ
10	453	F	1	Fully	-	-	2017	Sussex	NJ
11	10	F	1	Semi	-	-	2017	Sussex	NJ
12	10	F	5	Semi	-	+	2017	Sussex	NJ
13	32	F	1	Semi	-	+	2017	Sussex	NJ
14	32	F	5	Semi	-	-	2017	Sussex	NJ
15	605	F	5	Semi	-	+	2017	Sussex	NJ
16	318	F	1	Fully	-	-	2017	Sussex	NJ
17	318	F	1	Semi	-	-	2017	Sussex	NJ
18	318	F	1	Un	-	+	2017	Sussex	NJ
19	106	M	1	Un	-	-	2017	Sussex	NJ
20	106	M	1	Un	-	-	2017	Sussex	NJ
21	103	F	1	Semi	-	+	2017	Sussex	NJ
22	69	F	1	Un	-	+	2017	Warren	NJ
23	69	F	1	Un	-	+	2017	Warren	NJ
24	69	M	1	Un	-	-	2017	Warren	NJ
25	41	F	1	Semi	-	+	2017	Warren	NJ
26	41	F	1	Semi	-	-	2017	Warren	NJ
27	41	F	1	Semi	-	+	2017	Warren	NJ
28	41	F	1	Semi	-	+	2017	Warren	NJ
29	41	M	1	Un	-	-	2017	Warren	NJ
30	296	F	5	Semi	-	-	2017	Warren	NJ
31	9273	F	1	Semi	-	-	2018	Warren	NJ
32	9273	F	1	Un	-	+	2018	Warren	NJ
33	9273	M	1	Un	-	-	2018	Warren	NJ
34	10202	F	1	Semi	-	+	2018	Warren	NJ

35	10202	F	1	Semi	-	+	2018	Warren	NJ
36	10202	F	1	Un	-	-	2018	Warren	NJ
37	10202	F	1	Un	-	-	2018	Warren	NJ
38	10202	M	2	Un	-	+	2018	Warren	NJ
39	9473	F	4	Semi	-	+	2018	Warren	NJ
40	9473	F	1	Fully	-	-	2018	Warren	NJ
41	9473	M	1	Un	-	-	2018	Warren	NJ
42	9194	F	1	Fully	-	-	2018	Sussex	NJ
43	9194	N	1	Fully	-	-	2018	Sussex	NJ
44	10041	M	1	Un	-	-	2018	Sussex	NJ
45	10041	N	1	Semi	-	-	2018	Sussex	NJ
46	10041	F	1	Semi	-	-	2018	Sussex	NJ
47	6573	M	1	Un	-	-	2018	Warren	NJ
48	6573	F	5	Un	-	+	2018	Warren	NJ
49	10003	M	1	Un	-	+	2018	Sussex	NJ
50	10003	F	1	Semi	-	-	2018	Sussex	NJ
51	10003	F	1	Semi	-	-	2018	Sussex	NJ
52	10003	F	1	Semi	-	-	2018	Sussex	NJ
53	10004	F	1	Semi	-	-	2018	Sussex	NJ
54	9149	N	1	Un	-	-	2018	Sussex	NJ
55	9887	F	1	Semi	-	-	2018	Sussex	NJ
56	9198	M	1	Un	-	+	2018	Sussex	NJ
57	9198	F	1	Semi	-	-	2018	Sussex	NJ
58	9848	F	1	Fully	-	-	2018	Sussex	NJ
59	9848	M	1	Un	-	-	2018	Sussex	NJ
60	10204	F	1	Un	-	+	2018	Warren	NJ
61	10204	F	1	Semi	-	+	2018	Warren	NJ
62	10204	F	1	Semi	-	+	2018	Warren	NJ
63	9776	F	1	Fully	-	-	2018	Sussex	NJ
64	9848	M	4	Un	-	+	2018	Sussex	NJ
66	19	F	1	Un	-	-	2018	Sussex	NJ
67	19	F	1	Un	-	-	2018	Sussex	NJ
68	7900	M	5	Un	-	-	2018	Warren	NJ
69	7900	F	3	Un	-	-	2018	Warren	NJ
70	7900	F	1	Semi	-	-	2018	Warren	NJ
71	7900	N	1	Fully	-	-	2018	Warren	NJ
72	9848	N	1	Fully	-	-	2018	Warren	NJ
73	9848	F	1	Un	-	-	2018	Warren	NJ

74	9848	F	3	Semi	-	-	2018	Warren	NJ
75	9848	F	4	Semi	-	-	2018	Warren	NJ
76	8814	F	1	Fully	-	-	2018	Warren	NJ
77	8844	M	5	Un	-	+	2018	Warren	NJ
78	8844	F	4	Semi	-	-	2018	Warren	NJ
79	8844	F	4	Semi	-	-	2018	Warren	NJ
80	8844	F	2	Fully	-	-	2018	Warren	NJ
81	8844	N	3	Fully	-	-	2018	Warren	NJ
82	295	M	3	Un	-	+	2017	Warren	NJ
83	632	F	1	Fully	-	-	2015	Warren	NJ
84	632	F	1	Fully	-	+	2015	Warren	NJ
85	631	F	1	Semi	-	-	2015	Warren	NJ
86	631	F	1	Semi	-	-	2015	Warren	NJ
87	631	F	1	Fully	-	+	2015	Warren	NJ
88	631	M	1	Un	-	-	2015	Warren	NJ
89	633	F	3	Semi	-	-	2015	Hunterdon	NJ
90	633	F	4	Semi	-	+	2015	Hunterdon	NJ
91	633	F	1	Fully	-	-	2015	Hunterdon	NJ
92	633	M	1	Un	-	-	2015	Hunterdon	NJ
93	630	F	1	Semi	-	+	2015	Warren	NJ
94	616	F	1	Semi	-	-	2015	Hunterdon	NJ
95	616	F	1	Semi	-	-	2015	Hunterdon	NJ
96	628	F	1	Semi	-	-	2015	Warren	NJ
97	628	F	1	Semi	-	-	2015	Warren	NJ
98	628	F	1	Fully	-	-	2015	Warren	NJ
99	628	F	1	Un	-	-	2015	Warren	NJ
100	628	M	1	Un	-	-	2015	Warren	NJ
101	627	M	1	Un	-	-	2015	Warren	NJ
102	627	F	1	Semi	-	-	2015	Warren	NJ
103	627	F	1	Semi	-	-	2015	Warren	NJ
104	625	F	1	Fully	-	-	2015	Warren	NJ
105	625	F	5	Semi	-	+	2015	Warren	NJ
106	625	M	3	Un	-	+	2015	Warren	NJ
107	624	F	1	Semi	-	-	2015	Warren	NJ
108	624	F	1	Un	-	-	2015	Warren	NJ
109	623	F	1	Semi	-	-	2015	Warren	NJ
110	623	F	1	Semi	-	-	2015	Warren	NJ
111	623	F	1	Un	-	-	2015	Warren	NJ

112	622	F	4	Fully	-	-	2015	Warren	NJ
113	622	F	3	Semi	-	-	2015	Warren	NJ
114	622	M	1	Un	-	-	2015	Warren	NJ
115	619	F	2	Semi	-	+	2015	Hunterdon	NJ
116	619	F	3	Fully	-	-	2015	Hunterdon	NJ
117	619	M	3	Un	-	-	2015	Hunterdon	NJ
118	618	F	1	Semi	-	+	2015	Hunterdon	NJ
119	618	F	1	Fully	-	+	2015	Hunterdon	NJ
120	618	M	1	Un	-	-	2015	Hunterdon	NJ
121	617	F	1	Fully	-	-	2015	Hunterdon	NJ
122	617	F	1	Fully	-	-	2015	Hunterdon	NJ
123	617	F	1	Fully	-	-	2015	Hunterdon	NJ
124	617	M	1	Un	-	-	2015	Hunterdon	NJ
125	21	F	1	Fully	-	-	2015	Warren	NJ
126	20	F	1	Fully	-	-	2015	Warren	NJ
127	15	F	1	Semi	-	+	2015	Warren	NJ
128	15	F	1	Semi	-	-	2015	Warren	NJ
129	41	F	1	Fully	-	-	2015	Sussex	NJ
130	39	F	1	Semi	-	-	2015	Sussex	NJ
131	39	F	1	Semi	-	-	2015	Sussex	NJ
132	35	F	1	Fully	-	-	2015	Sussex	NJ
133	34	F	1	Fully	-	+	2015	Sussex	NJ
134	34	M	1	Un	-	-	2015	Sussex	NJ
135	31	F	1	Fully	-	-	2015	Sussex	NJ
136	31	M	1	Un	-	-	2015	Sussex	NJ
137	30	F	1	Fully	-	+	2015	Sussex	NJ
138	30	M	1	Un	-	-	2015	Sussex	NJ
139	28	F	3	Fully	-	+	2015	Sussex	NJ
140	28	F	1	Semi	-	-	2015	Sussex	NJ
141	28	M	1	Un	-	+	2015	Sussex	NJ
142	22	F	1	Un	-	+	2015	Sussex	NJ
143	22	F	1	Fully	-	-	2015	Sussex	NJ
144	18	F	1	Semi	-	-	2015	Sussex	NJ
145	18	M	1	Un	-	-	2015	Sussex	NJ
146	13	F	1	Semi	-	-	2015	Sussex	NJ
147	13	M	1	Un	-	-	2015	Sussex	NJ
148	12	F	1	Fully	-	+	2015	Sussex	NJ
149	11	F	1	Semi	-	-	2015	Sussex	NJ

150	2	M	3	Un	-	-	2016	Sussex	NJ
151	2	F	4	Semi	-	+	2016	Sussex	NJ
152	2	F	4	Semi	-	-	2016	Sussex	NJ
153	2	F	1	Fully	-	+	2016	Sussex	NJ
154	3	M	4	Un	-	-	2016	Sussex	NJ
155	3	F	1	Un	-	-	2016	Sussex	NJ
156	3	F	5	Semi	-	-	2016	Sussex	NJ
157	3	F	4	Semi	-	+	2016	Sussex	NJ
158	3	F	2	Fully	-	+	2016	Sussex	NJ
159	4	F	1	Semi	-	-	2016	Sussex	NJ
160	5	M	1	Un	-	-	2016	Sussex	NJ
161	5	M	1	Un	-	-	2016	Sussex	NJ
162	5	F	1	Semi	-	+	2016	Sussex	NJ
163	5	F	1	Semi	-	-	2016	Sussex	NJ
164	6	M	1	Un	-	-	2016	Sussex	NJ
165	6	F	1	Semi	-	-	2016	Sussex	NJ
166	6	F	1	Semi	-	-	2016	Sussex	NJ
167	6	F	1	Semi	-	+	2016	Sussex	NJ
168	8	F	1	Semi	+	+	2016	Sussex	NJ
169	8	F	1	Semi	-	+	2016	Sussex	NJ
170	8	F	1	Semi	-	-	2016	Sussex	NJ
171	8	F	1	Semi	-	-	2016	Sussex	NJ
172	9	M	1	Un	-	-	2016	Sussex	NJ
173	9	F	1	Semi	-	-	2016	Sussex	NJ
174	10	M	4	Un	-	+	2016	Sussex	NJ
175	10	F	2	Un	-	+	2016	Sussex	NJ
176	10	F	4	Semi	-	+	2016	Sussex	NJ
177	10	F	4	Semi	-	+	2016	Sussex	NJ
178	11	M	5	Un	-	-	2016	Sussex	NJ
179	11	F	5	Un	-	+	2016	Sussex	NJ
180	11	F	5	Semi	-	-	2016	Sussex	NJ
181	11	F	1	Fully	-	-	2016	Sussex	NJ
182	13	M	3	Un	-	+	2016	Sussex	NJ
183	13	F	5	Semi	-	+	2016	Sussex	NJ
184	13	F	5	Semi	-	-	2016	Sussex	NJ
185	13	F	3	Fully	-	-	2016	Sussex	NJ
186	14	M	2	Un	-	-	2016	Sussex	NJ
187	14	F	3	Semi	-	+	2016	Sussex	NJ

188	15	M	1	Un	+	-	2016	Sussex	NJ
189	15	F	3	Un	-	+	2016	Sussex	NJ
190	15	F	2	Semi	-	+	2016	Sussex	NJ
191	17	M	3	Un	-	+	2016	Sussex	NJ
192	17	F	5	Semi	-	-	2016	Sussex	NJ
193	20	M	7	Un	-	+	2016	Sussex	NJ
194	20	F	1	Un	-	-	2016	Sussex	NJ
195	20	F	5	Semi	-	+	2016	Sussex	NJ
196	20	F	5	Semi	-	+	2016	Sussex	NJ
197	20	F	5	Semi	-	+	2016	Sussex	NJ
198	22	M	2	Un	-	-	2016	Sussex	NJ
199	22	F	4	Semi	-	-	2016	Sussex	NJ
200	16	M	2	Un	-	-	2016	Warren	NJ
201	16	F	2	Un	+	+	2016	Warren	NJ
202	16	F	5	Semi	-	+	2016	Warren	NJ
203	30	M	3	Un	-	-	2016	Warren	NJ
204	30	F	3	Un	-	+	2016	Warren	NJ
205	30	F	4	Semi	-	+	2016	Warren	NJ
206	154	F	1	Fully	-	+	2016	Warren	NJ
207	162	M	2	Un	-	+	2016	Warren	NJ
208	162	F	4	Semi	-	+	2016	Warren	NJ
209	167	M	2	Un	-	-	2016	Warren	NJ
210	167	F	1	Un	-	-	2016	Warren	NJ
211	167	F	3	Semi	-	+	2016	Warren	NJ
212	169	M	1	Un	+	-	2016	Warren	NJ
213	169	F	3	Semi	-	-	2016	Warren	NJ
214	169	F	2	Fully	-	-	2016	Warren	NJ
215	8819	M	1	Un	-	-	2016	Warren	NJ
216	8819	F	1	Semi	-	-	2016	Warren	NJ
217	8819	F	1	Fully	-	-	2016	Warren	NJ
218	8819	F	1	Fully	-	-	2016	Warren	NJ
219	9209	F	1	Semi	-	-	2016	Warren	NJ
220	9553	M	1	Un	-	-	2016	Warren	NJ
221	9553	M	1	Un	-	-	2016	Warren	NJ
222	9553	F	1	Fully	-	-	2016	Warren	NJ
223	9553	F	1	Fully	-	+	2016	Warren	NJ
224	28	M	3	Un	-	-	2016	Sussex	NJ
225	28	F	1	Un	-	-	2016	Sussex	NJ

226	28	F	5	Semi	-	+	2016	Sussex	NJ
227	29	M	3	Un	-	+	2016	Sussex	NJ
228	29	F	3	Un	-	+	2016	Sussex	NJ
229	31	M	1	Un	+	+	2016	Sussex	NJ
230	31	F	1	Un	-	+	2016	Sussex	NJ
231	31	F	4	Semi	-	+	2016	Sussex	NJ
232	144	M	3	Un	+	+	2016	Sussex	NJ
233	144	F	5	Semi	-	-	2016	Sussex	NJ
234	145	M	1	Un	-	-	2016	Sussex	NJ
235	145	M	1	Un	-	-	2016	Sussex	NJ
236	145	F	1	Un	-	-	2016	Sussex	NJ
237	145	F	1	Semi	-	+	2016	Sussex	NJ
238	145	F	1	Semi	-	-	2016	Sussex	NJ
239	147	F	1	Semi	-	-	2016	Sussex	NJ
240	147	F	1	Semi	-	+	2016	Sussex	NJ
241	148	M	1	Un	-	-	2016	Sussex	NJ
242	148	F	1	Un	-	+	2016	Sussex	NJ
243	148	F	3	Semi	-	-	2016	Sussex	NJ
244	149	M	1	Un	-	-	2016	Sussex	NJ
245	149	F	1	Un	-	-	2016	Sussex	NJ
246	149	F	1	Semi	-	-	2016	Sussex	NJ
247	249	F	1	Semi	-	+	2016	Sussex	NJ
248	379	F	1	Semi	-	+	2016	Sussex	NJ
249	379	F	1	Semi	-	+	2016	Sussex	NJ
250	91	M	4	Un	-	-	2017	Warren	NJ
251	91	F	1	Un	-	-	2017	Warren	NJ
252	91	F	5	Semi	-	+	2017	Warren	NJ
253	91	F	5	Semi	-	+	2017	Warren	NJ
254	91	F	3	Semi	-	-	2017	Warren	NJ
255	105	M	2	Un	-	-	2017	Warren	NJ
256	105	F	2	Semi	-	+	2017	Warren	NJ
257	105	F	1	Fully	-	-	2017	Warren	NJ
259	110	M	7	Un	-	+	2017	Warren	NJ
260	110	F	2	Un	+	+	2017	Warren	NJ
261	110	F	3	Semi	-	+	2017	Warren	NJ
262	6	M	5	Un	-	-	2017	Warren	NJ
263	6	F	1	Un	-	-	2017	Warren	NJ
264	6	F	5	Semi	-	+	2017	Warren	NJ

265	6	F	5	Semi	-	+	2017	Warren	NJ
266	6	F	5	Semi	-	-	2017	Warren	NJ
267	6	F	5	Semi	-	-	2017	Warren	NJ
268	6	F	5	Semi	-	-	2017	Warren	NJ
269	6	F	5	Semi	-	-	2017	Warren	NJ
270	90	M	1	Un	-	-	2017	Sussex	NJ
271	90	F	2	Un	-	+	2017	Sussex	NJ
272	90	F	2	Semi	-	+	2017	Sussex	NJ
273	93	M	4	Un	-	+	2017	Sussex	NJ
274	93	F	1	Un	+	-	2017	Sussex	NJ
275	93	F	1	Semi	-	+	2017	Sussex	NJ
276	94	M	1	Un	-	+	2017	Sussex	NJ
277	94	F	1	Semi	-	+	2017	Sussex	NJ
278	94	F	1	Fully	-	+	2017	Sussex	NJ
279	30	M	1	Un	-	-	2017	Warren	NJ
280	30	F	1	Semi	-	-	2017	Warren	NJ
281	30	F	1	Semi	-	-	2017	Warren	NJ
282	30	F	1	Semi	-	-	2017	Warren	NJ
283	101	M	1	Un	-	-	2017	Sussex	NJ
284	101	F	1	Semi	-	-	2017	Sussex	NJ
285	101	F	1	Semi	-	-	2017	Sussex	NJ
286	104	F	1	Un	-	-	2017	Sussex	NJ
287	9	M	1	Un	-	+	2017	Sussex	NJ
288	9	F	6	Semi	-	-	2017	Sussex	NJ
289	9	F	1	Fully	-	+	2017	Sussex	NJ
290	55	F	1	Semi	-	-	2017	Sussex	NJ
291	55	F	1	Semi	-	+	2017	Sussex	NJ
292	55	F	1	Semi	-	-	2017	Sussex	NJ
293	55	F	1	Semi	-	+	2017	Sussex	NJ
294	64	M	1	Un	+	-	2017	Sussex	NJ
295	64	F	2	Un	-	-	2017	Sussex	NJ
296	64	F	2	Semi	-	-	2017	Sussex	NJ
297	67	M	2	Un	-	+	2017	Sussex	NJ
298	67	F	2	Un	-	+	2017	Sussex	NJ
299	67	F	2	Semi	-	-	2017	Sussex	NJ
300	68	M	1	Un	-	+	2017	Sussex	NJ
301	252	M	1	Un	+	-	2017	Sussex	NJ
302	252	F	1	Semi	-	-	2017	Sussex	NJ

303	252	F	1	Semi	-	-	2017	Sussex	NJ
304	302	M	1	Un	-	-	2017	Sussex	NJ
305	302	F	1	Un	-	-	2017	Sussex	NJ
306	302	F	1	Un	-	-	2017	Sussex	NJ
307	302	F	1	Fully	-	-	2017	Sussex	NJ
308	306	F	1	Un	+	+	2017	Sussex	NJ
309	306	F	1	Un	-	-	2017	Sussex	NJ
310	608	M	4	Un	-	-	2017	Sussex	NJ
311	608	F	1	Un	+	-	2017	Sussex	NJ
312	608	F	4	Semi	-	+	2017	Sussex	NJ
313	608	F	5	Fully	-	+	2017	Sussex	NJ
314	608	F	5	Fully	-	-	2017	Sussex	NJ
315	299	F	1	Un	-	+	2017	Sussex	NJ
316	299	F	1	Fully	-	+	2017	Sussex	NJ
317	299	F	1	Un	-	+	2017	Sussex	NJ
318	304	F	1	Un	-	-	2017	Sussex	NJ
319	304	F	1	Un	-	+	2017	Sussex	NJ
320	14	M	1	Un	+	-	2017	Monroe	PA
321	38	F	1	Semi	-	-	2017	Monroe	PA
322	37	M	1	Un	-	+	2017	Monroe	PA
323	37	M	1	Un	-	+	2017	Monroe	PA
324	37	F	1	Semi	-	-	2017	Monroe	PA
325	37	F	1	Semi	-	-	2017	Monroe	PA
326	37	F	1	Semi	-	+	2017	Monroe	PA
327	44	F	1	Un	+	+	2017	Monroe	PA
328	44	F	1	Semi	-	-	2017	Monroe	PA
329	44	F	1	Semi	-	-	2017	Monroe	PA
330	44	M	1	Un	-	-	2017	Monroe	PA
331	55	F	1	Fully	-	-	2017	Pike	PA
332	55	M	1	Un	-	-	2017	Pike	PA
333	43	F	1	Fully	-	-	2017	Pike	PA
334	1723665	M	2	Un	-	-	2017	Monroe	PA
335	1723665	F	2	Semi	-	+	2017	Monroe	PA
336	1723665	F	1	Fully	-	-	2017	Monroe	PA
337	1723667	M	1	Un	-	+	2017	Monroe	PA
338	1723667	F	4	Semi	-	+	2017	Monroe	PA
339	1723667	F	1	Fully	-	-	2017	Monroe	PA
340	1723668	M	2	Un	-	-	2017	Monroe	PA

341	1723668	F	2	Un	-	+	2017	Monroe	PA
342	172366	F	1	Semi	-	-	2017	Monroe	PA
343	172366	M	2	Un	-	-	2017	Monroe	PA
344	172366	F	1	Un	-	+	2017	Monroe	PA
345	172366	F	2	Semi	-	+	2017	Monroe	PA
346	51100	F	1	Un	-	-	2018	Clinton	PA
347	51100	N	1	Fully	-	-	2018	Clinton	PA
348	52030	F	1	Un	-	-	2018	Lycoming	PA
349	35998	M	5	Un	-	+	2018	Clinton	PA
350	35998	M	5	Un	-	+	2018	Clinton	PA
351	35998	F	1	Un	-	+	2018	Clinton	PA
352	35998	F	2	Semi	-	-	2018	Clinton	PA
353	35998	F	2	Fully	-	-	2018	Clinton	PA
354	27518	F	1	Un	-	-	2018	Clinton	PA
355	27518	F	1	Un	+	-	2018	Clinton	PA
356	27518	F	1	Semi	-	-	2018	Clinton	PA
357	51039	M	5	Un	-	+	2018	Clinton	PA
358	51039	M	5	Un	-	+	2018	Clinton	PA
359	51039	M	3	Un	-	+	2018	Clinton	PA
360	51039	F	5	Semi	-	+	2018	Clinton	PA
361	51039	F	5	Semi	-	+	2018	Clinton	PA
362	51039	F	5	Semi	-	+	2018	Clinton	PA
363	51039	F	1	Fully	-	+	2018	Clinton	PA
364	48076	N	1	Fully	-	-	2018	Lycoming	PA
365	48076	F	1	Semi	+	-	2018	Lycoming	PA
366	48076	F	1	Semi	-	-	2018	Lycoming	PA
367	51660	F	1	Un	-	+	2018	Potter	PA
368	51049	M	1	Un	-	-	2018	Clinton	PA
369	51049	F	1	Un	+	-	2018	Clinton	PA
370	51049	F	1	Semi	-	+	2018	Clinton	PA
371	52028	F	1	Un	-	+	2018	Clinton	PA
372	52028	F	4	Semi	-	+	2018	Clinton	PA
373	52028	F	1	Fully	-	+	2018	Clinton	PA
374	51032	M	5	Un	-	+	2018	Clinton	PA
375	51032	M	5	Un	+	+	2018	Clinton	PA
376	51032	M	2	Un	-	+	2018	Clinton	PA
377	51032	F	3	Un	+	+	2018	Clinton	PA
378	51032	F	3	Semi	-	+	2018	Clinton	PA

379	51032	F	2	Fully	-	+	2018	Clinton	PA
380	27516	M	5	Un	-	+	2018	Clinton	PA
381	27516	M	5	Un	+	+	2018	Clinton	PA
382	27516	M	1	Un	-	-	2018	Clinton	PA
383	27516	F	1	Un	-	-	2018	Clinton	PA
384	27516	F	5	Semi	-	+	2018	Clinton	PA
385	27516	F	5	Semi	-	+	2018	Clinton	PA
386	27516	F	1	Fully	-	+	2018	Clinton	PA
387	27516	F	1	Fully	-	-	2018	Clinton	PA
388	27516	F	1	Fully	-	-	2018	Clinton	PA
389	27516	N	1	Fully	-	-	2018	Clinton	PA
390	41630	M	5	Un	-	+	2018	Tioga	PA
391	41630	M	1	Un	+	+	2018	Tioga	PA
392	41630	F	5	Un	+	+	2018	Tioga	PA
393	41630	F	2	Semi	-	+	2018	Tioga	PA
394	41638	M	4	Un	-	+	2018	Tioga	PA
395	41638	F	5	Semi	-	+	2018	Tioga	PA
396	41638	F	1	Fully	-	-	2018	Tioga	PA
397	23095	F	1	Un	-	-	2018	Lycoming	PA
398	23095	N	1	Fully	-	+	2018	Lycoming	PA
399	51047	M	1	Un	-	-	2018	Clinton	PA
400	51047	F	1	Semi	+	-	2018	Clinton	PA
401	33138	M	2	Un	-	+	2018	Clinton	PA
402	33138	F	2	Un	-	-	2018	Clinton	PA
403	33138	F	1	Fully	-	-	2018	Clinton	PA
404	51942	M	1	Un	-	-	2018	Clinton	PA
405	51942	F	1	Un	-	+	2018	Clinton	PA
406	41688	N	1	Fully	-	-	2018	Potter	PA
407	33142	M	3	Un	-	+	2018	Clinton	PA
408	33142	F	3	Semi	-	-	2018	Clinton	PA
409	33142	F	1	Fully	-	-	2018	Clinton	PA
410	35414	M	2	Un	-	-	2018	Clinton	PA
411	35414	F	1	Un	-	+	2018	Clinton	PA
412	35414	F	2	Semi	-	+	2018	Clinton	PA
413	51636	F	1	Fully	-	+	2018	Clinton	PA
414	51172	F	1	Semi	-	+	2018	Clinton	PA
415	51172	F	1	Un	-	+	2018	Clinton	PA
416	51172	F	1	Un	-	-	2018	Clinton	PA

417	51172	F	1	Fully	-	-	2018	Clinton	PA
418	51949	M	4	Un	+	+	2018	Clinton	PA
419	51949	F	2	Un	-	+	2018	Clinton	PA
420	1	M	3	Un	+	+	2018	Warren	NJ
421	1	F	2	Semi	-	-	2018	Warren	NJ
422	2	M	1	Un	-	+	2018	Warren	NJ
423	2	F	6	Semi	-	+	2018	Warren	NJ
424	2	F	3	Un	-	+	2018	Warren	NJ
425	2	F	1	Fully	-	+	2018	Warren	NJ
426	2	N	2	Fully	-	-	2018	Warren	NJ
427	3	M	1	Un	-	-	2018	Warren	NJ
428	3	F	5	Semi	-	-	2018	Warren	NJ
429	3	F	5	Semi	-	+	2018	Warren	NJ
430	4	M	1	Un	-	-	2018	Sussex	NJ
431	4	F	2	Un	-	-	2018	Sussex	NJ
432	4	F	1	Semi	-	+	2018	Sussex	NJ
433	4	F	1	Fully	-	-	2018	Sussex	NJ
434	44	M	3	Un	-	+	2018	Sussex	NJ
435	44	F	2	Un	+	+	2018	Sussex	NJ
436	44	F	3	Semi	-	+	2018	Sussex	NJ
437	6	F	3	Un	+	+	2018	Sussex	NJ
438	6	F	5	Semi	-	-	2018	Sussex	NJ
439	7	M	5	Un	-	+	2018	Warren	NJ
440	7	F	2	Un	-	+	2018	Warren	NJ
441	7	F	2	Semi	-	+	2018	Warren	NJ
442	7	N	1	Fully	-	-	2018	Warren	NJ
443	8	M	1	Un	+	+	2018	Sussex	NJ
444	8	F	1	Un	-	+	2018	Sussex	NJ
445	8	F	3	Semi	-	+	2018	Sussex	NJ
446	8	N	1	Semi	-	-	2018	Sussex	NJ
447	9	M	1	Un	-	-	2018	Warren	NJ
448	9	F	3	Un	-	+	2018	Warren	NJ
449	9	F	6	Semi	-	+	2018	Warren	NJ
450	9	F	5	Semi	-	+	2018	Warren	NJ
451	45	M	5	Un	-	-	2018	Sussex	NJ
452	45	F	5	Un	-	+	2018	Sussex	NJ
453	45	F	2	Un	-	-	2018	Sussex	NJ
454	45	F	1	Semi	-	+	2018	Sussex	NJ

455	45	F	2	Fully	-	+	2018	Sussex	NJ
456	56	F	1	Fully	-	-	2018	Warren	NJ
457	15	M	5	Un	-	+	2018	Sussex	NJ
458	15	F	6	Semi	-	+	2018	Sussex	NJ
459	15	F	6	Semi	-	+	2018	Sussex	NJ
460	14	M	4	Un	-	+	2018	Sussex	NJ
461	14	F	4	Un	-	+	2018	Sussex	NJ
462	14	F	4	Semi	-	+	2018	Sussex	NJ
463	13	M	1	Un	-	+	2018	Sussex	NJ
464	13	F	4	Semi	-	+	2018	Sussex	NJ
465	13	F	4	Semi	-	+	2018	Sussex	NJ
466	13	F	2	Fully	+	+	2018	Sussex	NJ
467	44	F	1	Fully	-	+	2018	Sussex	NJ
468	56	N	1	Un	-	-	2018	Sussex	NJ
469	33168	F	1	Un	-	-	2018	Clinton	PA
470	33168	F	1	Semi	-	-	2018	Clinton	PA
471	51357	F	1	Semi	-	-	2018	Potter	PA
472	51357	F	1	Fully	-	-	2018	Potter	PA
473	51357	N	1	Semi	-	-	2018	Potter	PA
474	35676	M	3	Un	-	+	2018	Clinton	PA
475	35676	F	4	Un	-	+	2018	Clinton	PA
476	35676	F	2	Semi	-	+	2018	Clinton	PA
477	35676	F	3	Fully	-	-	2018	Clinton	PA
478	51370	N	3	Semi	-	+	2018	Clinton	PA
479	52026	N	2	Un	-	-	2018	Centre	PA
480	52026	N	5	Semi	-	-	2018	Centre	PA
481	52026	N	2	Semi	-	-	2018	Centre	PA
482	52026	F	1	Fully	-	-	2018	Centre	PA
483	35918	N	1	Un	-	-	2018	Clinton	PA
484	35918	N	1	Semi	-	-	2018	Clinton	PA
485	35918	N	1	Fully	-	-	2018	Clinton	PA
486	35918	N	1	Fully	-	-	2018	Clinton	PA
487	41479	N	2	Un	-	-	2018	Lycoming	PA
488	41479	N	5	Semi	-	-	2018	Lycoming	PA
489	41479	F	2	Semi	-	+	2018	Lycoming	PA
490	51486	M	5	Un	-	+	2018	Clinton	PA
491	51486	M	4	Un	-	+	2018	Clinton	PA
492	51486	F	1	Un	-	-	2018	Clinton	PA

493	51486	F	3	Semi	-	-	2018	Clinton	PA
494	51486	F	2	Fully	-	-	2018	Clinton	PA
495	51366	N	3	Un	+	+	2018	Clearfield	PA
496	36361	N	2	Un	-	-	2018	Clinton	PA
497	35017	N	5	Un	-	-	2018	Clinton	PA
498	35017	N	5	Semi	+	+	2018	Clinton	PA
499	35017	N	6	Fully	-	-	2018	Clinton	PA
500	35017	M	1	Un	+	-	2018	Clinton	PA
501	35017	F	2	Un	-	-	2018	Clinton	PA
502	51234	M	1	Un	-	-	2018	Clinton	PA
503	51234	M	5	Un	+	+	2018	Clinton	PA
504	51234	F	4	Un	-	+	2018	Clinton	PA
505	51234	F	2	Semi	-	+	2018	Clinton	PA
506	51234	F	1	Fully	-	-	2018	Clinton	PA
507	51034	M	4	Un	-	+	2018	Clinton	PA
508	51034	F	4	Un	-	+	2018	Clinton	PA
509	51034	F	5	Semi	-	-	2018	Clinton	PA
510	51034	F	5	Semi	-	-	2018	Clinton	PA
511	51034	F	2	Fully	+	-	2018	Clinton	PA
512	35017	M	5	Un	+	-	2018	Clinton	PA
513	35017	M	5	Un	-	-	2018	Clinton	PA
514	35017	M	5	Un	-	+	2018	Clinton	PA
515	35017	F	5	Un	+	+	2018	Clinton	PA
516	35017	F	2	Semi	-	-	2018	Clinton	PA
517	35017	F	4	Semi	-	-	2018	Clinton	PA
518	35017	F	1	Fully	-	-	2018	Clinton	PA
519	1805571	M	1	Un	-	+	2018	Huntingdon	PA
520	1805571	M	5	Un	-	+	2018	Huntingdon	PA
521	1805571	F	2	Semi	-	-	2018	Huntingdon	PA
522	1805571	F	5	Semi	-	+	2018	Huntingdon	PA
523	1805571	F	1	Fully	-	-	2018	Huntingdon	PA
524	1805574	F	5	Semi	-	-	2018	Huntingdon	PA
525	1805574	F	2	Fully	-	-	2018	Huntingdon	PA
526	51044	M	3	Un	+	+	2018	Clinton	PA
527	51044	M	5	Un	+	+	2018	Clinton	PA
528	51044	F	1	Un	-	-	2018	Clinton	PA
529	51044	F	1	Semi	-	-	2018	Clinton	PA
530	51044	F	3	Fully	-	+	2018	Clinton	PA

531	51178	N	1	Fully	-	-	2018	Clinton	PA
532	51178	F	4	Un	+	+	2018	Clinton	PA
533	51178	F	5	Un	-	+	2018	Clinton	PA
534	51178	F	5	Semi	-	-	2018	Clinton	PA
535	51178	F	1	Fully	-	-	2018	Clinton	PA
536	51036	M	4	Un	-	+	2018	Clinton	PA
537	51036	M	5	Un	-	-	2018	Clinton	PA
538	51036	F	1	Un	-	-	2018	Clinton	PA
539	51036	F	1	Semi	-	-	2018	Clinton	PA
540	51036	F	5	Semi	-	-	2018	Clinton	PA
541	51036	F	5	Semi	-	-	2018	Clinton	PA
542	51036	F	1	Fully	-	-	2018	Clinton	PA
543	1804200	M	1	Un	-	-	2018	Lycoming	PA
544	1804200	F	1	Semi	-	+	2018	Lycoming	PA
545	1804200	F	1	Semi	-	+	2018	Lycoming	PA
546	1804201	M	1	Un	-	-	2018	Lycoming	PA
547	1804201	M	1	Un	-	-	2018	Lycoming	PA
548	1804202	M	3	Un	-	+	2018	Lycoming	PA
549	1804202	F	1	Semi	-	+	2018	Lycoming	PA
550	1804202	F	1	Fully	-	-	2018	Lycoming	PA
551	1804204	F	1	Semi	+	-	2018	Lycoming	PA
552	1804203	M	5	Un	-	-	2018	Lycoming	PA
553	1804203	M	5	Un	-	-	2018	Lycoming	PA
554	1804203	M	5	Un	-	-	2018	Lycoming	PA
555	1804203	M	5	Un	-	-	2018	Lycoming	PA
556	1804203	F	5	Un	-	-	2018	Lycoming	PA
557	1804203	F	5	Un	-	+	2018	Lycoming	PA
558	1804203	F	5	Semi	-	-	2018	Lycoming	PA
559	1804203	F	5	Semi	-	-	2018	Lycoming	PA
560	1804203	M	2	Un	-	-	2018	Lycoming	PA
561	1804203	F	2	Un	-	-	2018	Lycoming	PA
562	1804207	F	1	Un	-	+	2018	Lycoming	PA
563	1804207	F	1	Semi	-	-	2018	Lycoming	PA
564	1804207	F	1	Fully	-	+	2018	Lycoming	PA
565	1804208	M	2	Un	-	+	2018	Lycoming	PA
566	1804208	M	5	Un	-	+	2018	Lycoming	PA
567	1804208	F	5	Un	-	+	2018	Lycoming	PA
568	1804208	F	5	Semi	-	+	2018	Lycoming	PA

569	1804208	F	4	Semi	-	+	2018	Lycoming	PA
570	1804208	F	1	Fully	-	-	2018	Lycoming	PA
571	1804209	M	2	Un	-	+	2018	Lycoming	PA
572	1804209	F	5	Un	-	+	2018	Lycoming	PA
573	1804209	F	2	Semi	-	+	2018	Lycoming	PA
574	1803379	M	1	Un	-	-	2018	Clearfield	PA
575	1803379	M	1	Un	-	-	2018	Clearfield	PA
576	1803379	F	1	Semi	-	-	2018	Clearfield	PA
577	1803380	M	1	Un	-	-	2018	Clearfield	PA
578	1803380	M	1	Un	-	-	2018	Clearfield	PA
579	1803380	M	1	Un	-	-	2018	Clearfield	PA
580	1803380	M	1	Un	-	-	2018	Clearfield	PA
581	1803378	F	1	Un	-	-	2018	Clearfield	PA
582	1803384	M	1	Un	-	-	2018	Clearfield	PA
583	1803384	F	1	Semi	-	-	2018	Clearfield	PA
584	1803388	M	5	Un	-	-	2018	Clearfield	PA
585	1803388	F	1	Semi	-	+	2018	Clearfield	PA
586	1803390	M	1	Un	+	-	2018	Clearfield	PA
587	1803390	M	1	Un	-	-	2018	Clearfield	PA
588	1803390	M	1	Un	-	-	2018	Clearfield	PA
589	1804278	M	1	Un	+	-	2018	Lycoming	PA
590	1804278	F	1	Fully	-	-	2018	Lycoming	PA
591	1804281	M	1	Un	-	+	2018	Lycoming	PA
592	1804281	F	1	Fully	-	+	2018	Lycoming	PA
593	1805549	M	1	Un	+	-	2018	Huntingdon	PA
594	1805549	F	1	Fully	-	-	2018	Huntingdon	PA
595	1810780	F	1	Semi	-	-	2018	Lycoming	PA
596	1810784	M	1	Un	+	-	2018	Lycoming	PA
597	1810787	M	1	Un	+	-	2018	Lycoming	PA

Appendix C: Statistical Raw Data

Powassan statistical data

	Estimated	p- value	Degrees of freedom	Z value	Std. error	Fisher Scoring iterations
Sex (male vs female)	-0.5594	0.836	1	-0.207	2.7060	6
Adult engorgement	-0.5783	0.920	3	-0.113	5.1210	7
Nymph engorgement	-0.06677	0.976	1	-0.030	2.1948	5
Life stage (adult vs nymph)	0.6374	0.897	2	-0.129	3.4835	6

Borrelia burgdorferi statistical data

	Estimated	p- value	Degrees of freedom	Z value	Std. error	Fisher Scoring iterations
Sex (male vs female)	0.09929	0.945	1	0.069	1.4355	3
Adult engorgement	-0.4213	0.799	3	-0.254	1.6578	4
Nymph engorgement	-0.6931	0.815	2	-0.234	1.6578	5
Life stage (adult vs nymph)	-0.4500	0.725	2	0.352	1.8119	5

Powassan State data

	Estimated	p- value	Degrees of freedom	Z value	Std. error	Fisher Scoring iterations
New Jersey 2015 / 2016 / 2017 / 2018	0.06077	0.986	2	0.017	3.48817	6
Pennsylvania 2017 / 2018	-0.2834	0.906	1	-0.917	2.3999	5
New Jersey vs Pennsylvania	-0.5254	0.852	1	-0.186	2.8238	6

B. burgdorferi State data

	Estimated	p- value	Degrees of freedom	Z value	Std. error	Fisher Scoring iterations
New Jersey 2015 / 2016 / 2017 / 2018	-0.70299	0.720	3	-0.358	1.96366	4
Pennsylvania 2017 / 2018	-0.08109	0.955	1	-0.056	1.44226	3
New Jersey vs Pennsylvania	-0.02498	0.986	1	-0.017	1.43616	3

Chi-Square tick collection data for difference between tick species collected from New Jersey vs Pennsylvania

X^2	Degrees of freedom	P-value
8	6	0.2381

Powassan county data to determine for significant difference between Sussex and Warren County

	Estimated	p-value	Degrees of freedom	Z value	Std. error	Fisher Scoring iterations
Sussex vs Warren	0.3791	0.917	1	0.105	3.6248	6

B. burgdorferi county data to determine for significant difference between Sussex and Warren County

	Estimated	p-value	Degrees of freedom	Z value	Std. error	Fisher Scoring iterations
Sussex vs Warren	0.1333	0.926	1	0.092	1.4428	3