HABITAT ASSOCIATIONS OF A POWASSAN VIRUS FOCUS IN SOUTHERN MAINE

A Thesis

Presented to the Faculty of the Graduate School

of Cornell University

In Partial Fulfillment of the Requirements for the Degree of

Master of Science

By

Lindsay Baxter

May 2021

ABSTRACT

Deer Tick virus (DTV) is a recently discovered lineage of Powassan virus and a member of the greater tick-borne encephalitis complex of viruses (TBEC) and causes significant morbidity and mortality to infected humans. DTV is associated with *Ixodes scapularis* ticks which are endemic to much of the Eastern and Midwestern United States and regularly bite humans. DTV, like other members of the TBEC, is thought to exist in small focal locations where transmission patterns are maintained over time. A field site was established on the Southern coast of Maine where high rates of DTV were found in questing ticks during 2018 and 2019. Host and tick abundance along with vegetation and microclimate conditions were measured in second growth forests across a gradient of infestation by invasive understory shrub species. DTV infection rate, was higher in the forest site highly invaded by Japanese barberry compared to forest sites moderately invaded or with native understory vegetation only. A higher density of *I. scapularis* was associated with Japanese barberry invasion, as well as a higher white-footed mouse abundance and lower saturation deficit. These findings are consistent with the theory of nidality (focality) of vector-borne infection in which zoonotic agents are maintained through an ideal assemblage of pathogen, hosts and habitat.

BIOGRAPHICAL SKETCH

The author, Lindsay Baxter, was born to parents Richard and Sandra Baxter in August 2nd, 1982 in Orange County California. She has one brother, Charles Baxter, who is two years her senior. Prior to beginning primary school, her family relocated to the Lake Tahoe region of Nevada. She completed her B.S. degree in Biology with a focus on molecular biology at Portland State University in Portland Oregon as a part time student over the course of 7 years while working in a hospital laboratory. Lindsay began working as a research technician in Dr. Laura Harrington's lab at Cornell in the fall of 2017 where she researched the mating biology of mosquito disease vectors. Her time working as a technician helped her realize her desire to continue research in vector biology. In 2019, she entered the Vector-Borne disease Entomology Master's Program funded through the Northeastern Center for Excellence in Vector Biology. This program has enabled Lindsay to explore and learn about public health entomology.

ACKNOWLEDGMENTS

I would like to thank my advisor, Dr. Laura Harrington for giving me so many opportunities to perform research and learn new skills, first as a research technician and finally as a student of medical entomology. She has allowed me to discover the many facets of this wonderful field and find my interests. Her support and guidance throughout the execution of my thesis has been invaluable. Furthermore, her support and dedication to all of her former students as they continue in their careers is a source of inspiration for me. Thank you to Emily Mader of the Northeast Regional Center for Excellence in Vector-borne Diseases for her administrative support, the benefit of which cannot be overstated.

I would also like to thank Dr. Rebecca Robich for her dedication that my field season move forward with precaution during the COVID-19 epidemic and the incredible opportunity that created. The scientists at the Maine Medical Center Research Institute Lyme and Vector-borne Disease Laboratory are some of the best most dedicated people I have ever had the pleasure to work with. I thank Charles Lubelczyk for the constant support through the field season, Molly Meagher and Jake Angelico for their hard work, fantastic ideas and perfectly timed laughs. I would like to thank Dr. Susan Elias and Dr. James Burtis for always being available for questions and advice and Erika Mudrak and the Cornell University Statistical Consulting Unit for all of the help and support.

I thank Sylvie Pitcher, Dr. Alex Amaro and Dr. Garrett League for their guidance, enthusiasm and patience as colleagues and mentors when I was a technician and new to the field of medical entomology. They made me feel at home and helped me find my footing and encouraged me to continue my education. Without them I would not be completing this thesis with nearly as much confidence.

Table of Contents

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1

LITERATURE REVIEW

Tick-Borne Disease

In the United States, tick-borne diseases present a larger threat than diseases transmitted by all other arthropods combined (Beard et al., 2016). Ticks have a long life span totaling in years rather than weeks as is common with other arthropods of medical importance such as mosquitoes and black flies. Tick-borne pathogens found in the United States include *Anaplasma phagocytophilum* (Baker et al., 2020), *Babesia microti* and *Babesia spp*. (Vannier & Krause, 2020), *Borrelia miyamotoi* (Wormser et al., 2019), *Borrelia burgdorferi* (Little et al., 2019), *Ehrlichia spp*. (Bakken and Dumler, 2000), *Francisella tularensis* (Farlow et al., 2005) and Powassan virus (POWV) (Ebel, 2010); which are all present in the Northeastern region. Heartland and Bourbon viruses (Savage et al., 2018), *Rickettsia rickettsii*: the causative agent of Rocky Mountain spotted fever (Gottlieb etal., 2018), Colorado tick fever virus (Williamson et al., 2019) and *Borrelia mayonii* (Wormser et al., 2019) are infections with human cases reported in other regions of the US.

Pathogens in ticks often undergo transstadial transmission and ticks can be exposed to new pathogens during each life stage during blood feeding and often are host to multiple pathogens at any given time (Diuk-Wasser et al., 2016). Bites from ticks infected with multiple pathogens can result in a coinfection of the human host, often resulting in more severe illness (Goldstein et al., 2001). Coinfection of ticks with multiple pathogens has been documented in the northeastern and upper Midwest regions of the United States where *B. burgdorferi* is endemic (Swanson et al., 2006). In cases of coinfection with *B. burgdorferi* and *B. microti*, *B. burgdorferi* promotes transmission of *B. microti* (Diuk-Wasser et al., 2016). In cases where a human is infected with *B. microti* while also diagnosed with Lyme disease they are more likely to have prolonged pain and severe flu-like symptoms (Krause et al., 2002). Coinfection of *Ixodes scapularis* ticks with *B.*

burgdorferi and either *B. microti* or *A. phagocytophilum* have been found in coastal Southern Maine (Holman et al., 2004). It is unclear how Powassan virus fits into the ecology of co-infected ticks or if pathogen interaction within the vector has an effect on POWV infection rates.

Tick-borne disease became an increasing public health concern in Maine in 1988 during which both locally acquired cases of Lyme disease and the vector, *I. scapularis* were identified for the first time (Ginsberg & Ewing, 1988). In response, a tick survey acquired from deer at twenty four tagging stations across Maine revealed *I. scapularis* presence in the Southern coastal and Midcoastal regions (Smith et al., 1990). Further surveillance of ticks parasitizing humans showed that *I. scapularis* was present in the southern half of the state (Smith et al., 1992). Since then, *I. scapularis* has been found in all counties in Maine and is responsible for the majority of human tick bites (Rand et al., 2007). *I. scapularis*-borne disease soon followed. In July, 2000, the first human case of Powassan virus disease was confirmed in a 25 year old patient followed closely by three others between 2000 and 2004 (Hinten et al., 2008). In Maine, the rates of POWV infection in questing ticks was between 0 and 3.5% from 2016-2017 with higher prevalence in the southern counties of York and Cumberland, at 2.5% and 3.5% respectively (Robich et al., 2019).

Powassan Virus and Deer Tick Virus

Powassan virus (POWV) was first isolated in 1958 from a tissue sample recovered during autopsy of a boy who died from viral encephalitis in Powassan, Ontario. (McLean & Donohue, 1959). *I. scapularis* vector competence for POWV was established in 1996 based on experimental transmission (Grayson and Costero, 1996). Shortly thereafter, a similar yet distinct virus was isolated from *I. scapularis* and named deer tick virus (DTV) (Telford et al., 1997). Prior to this discovery, Powassan virus was thought to exist exclusively in an enzootic cycle with

Ixodes cookei ticks and groundhog (*Marmota monax*) hosts, (Ko, 1972, McLean et al. 1964, 1968) or with *Ixodes marxi* ticks and red squirrels (*Sciurus vulgaris*) (McLean et al., 1968, McLean & Larke, 1963). Powassan virus phylogeny now includes two distinct lineages: lineage I (POWV) and lineage II (DTV) (Ebel et al., 2001). Both are zoonotic; however DTV poses a greater threat to human health due to its association with *I. scapularis* ticks. Despite evidence that *I. scapularis* is competent for Powassan virus lineage 1 in a laboratory setting it is not found in surveillance samples. While *I. cookei* is a nidiculous specialist- feeding primarily on groundhogs - the generalist feeding patterns of *I. scapularis* increases the risk of human bites (Xu et al., 2016). POWV is a member of the tick-borne encephalitis complex (TBEC) group of flaviviruses, all single stranded RNA viruses. Cross-neutralization among TBEC flaviviruses occurs due to envelope protein conservation (Subbotina & Loktev, 2012). Despite their differing ecology, POWV lineages are indistinguishable via traditional serological tests (Kuno et al., 2001). Brain tissue from the index case of Powassan virus in 1958 were compared to the DTV samples found in Connecticut and Massachusetts (Mclean & Donohue, 1959, Telford et al., 1997). The lineages can be differentiated with sequencing of the initiation sequence for the capsid protein and the envelope genes, with a 16% difference in nucleotide sequence (resulting in a 6% difference in amino acids) (Kuno et al., 2001). While there is variation within both lineages, DTV has greater sequence variation (Ebel et al., 2001). Different sampling locations in Connecticut showed stable, unique genetic sequence clusters indicating that DTV has existed through focal transmission over extended periods of time (Anderson & Armstrong 2012). Ebel and others (2001) suggested that DTV divergence from POWV lineage I is the result of positive natural selection stemming from the different ecological niches inhabited by these viruses. Below, I refer to DTV as lineage 2 virus, Powassan virus prototype refers to lineage 1 and

POWV refers to the overarching serogroup including both lineages.

DTV Virology and Clinical Symptoms

A human POWV infection can range in illness severity. An asymptomatic infection will typically go undetected and can be discovered during serological tests often during an investigation into pathogen prevalence (Ebel, 2010). The incubation period is difficult to identify because the exact time of exposure is not always known. Symptoms can take between 8 to 34 days to develop after a tick bite. (Ebel, 2010). Neuroinvasive Powassan virus disease refers to the suite of severe symptoms caused by human POWV infection. Symptoms during the onset of illness include fatigue, fever, and headaches of increasing severity. As the illness progresses, neurological symptoms develop such as cognitive impairment, muscle weakness, diplopia and dysphasia (Gholam et al., 1999, Tavakoli et al., 2009). The illness can progress to encephalitis, meningoencephalitis, and aseptic meningitis (Ebel, 2010).

POWV infection is deadly in approximately 10% of symptomatic cases with 50% of survivors suffering long-term, often irreversible, neurological problems. Two documented cases of Powassan virus disease in Connecticut involved children under one year of age. Both children survived and after a two-year recovery period, only one had remaining muscle weakness. In these cases, the tick was attached for 3 and 6 hours (Feder et al., 2020). While many tick-borne infections such as *B. burgdorferi* can be prevented by early tick removal (Piesman & Dolan, 2002), POWV has been shown to transmit within 15 minutes of tick attachment under laboratory conditions (Ebel & Kramer, 2004).

Transstadial transmission, where the virus is maintained from one tick life stage to the next, occurs for both POWV lineages (Telford et al., 1997, Costero & Grayson, 1996).

Transovarial (i.e. from mother to offspring) Powassan virus prototype transmission has been demonstrated in limited laboratory studies (Costero & Grayson, 1996), but it is unclear how important this is in nature. Humans and deer are considered dead-end hosts to POWV (Kemenesi & Bányai, 2018).

In an early experimental infection study, striped skunks (*Mephitis mephitis*), groundhogs, opossums (*Didelphis virginiana*), grey fox (*Urocyon cinereoargenteus*) and red fox (*Vulpes vulpes*) were given subcutaneous Powassan virus prototype inoculations. Striped skunks developed trace viremia for one day and opossums developed trace viremia for eight to 11 days. Researchers defined trace viremia as those titers in heparinized whole blood able to cause mortality in inoculated laboratory mice. Groundhogs, grey fox, and red foxes developed a more significant viral titer ($0.7 – 2.4 \log_{10}$). However, the length of viremia differed in these mammals, 8-11 days for groundhogs and 1-3 days for foxes (Kokernot et al., 1969). Consequently, the natural reservoir of POWV is still unclear. White footed mice (*Peromyscus leucopus*) have been implicated, but do not show signs of illness and restrict POWV replication (Telford et al., 1997, Mlera et al., 2017, Hermance & Thangamani, 2017). Until recently there had been no experimental infections of other small and medium-sized mammals with DTV (Ebel, 2010, Hermance & Thangamani, 2017). Recently, DTV and Powassan virus prototype were tested on wild groundhogs, striped skunks and fox squirrels (*Sciurus niger*). All animals seroconverted to both viruses by 21 days post injection. Groundhogs and fox squirrels showed a minimum viremia of $10^{1.7}$PFU/mL at 3 days post injection. Striped skunks and groundhogs showed signs of brain inflammation at 4 days post injection (Nemeth et al., 2021).

DTV Virus Detection and Estimated Distribution

DTV detection and distribution has been approximated by testing wild animals and human cases through serology (exposure) or PCR (active virus). Both methods have weaknesses. DTV serology studies are not conclusive due to the cross reactivity of DTV with other closely related members of the TBEC including Powassan virus prototype, which shares much of the same range as DTV (Subbotina & Loktev, 2012). Nevertheless, wildlife serosurveys have yielded important information about POWV presence and potential scale in the sampled region. Serosurvey results may be more descriptive when the animal tested has a smaller home range. In Connecticut deer, the prevalence of POWV neutralizing antibodies increased between 1979 and 2009 from 4% to 91%. This rapid increase in animals with POWV antibodies supports a growing prevalence in the Northeast over the 30 year span. The prevalence of POWV antibodies in Maine and Vermont deer from 2010, were lower than Connecticut at 17% and 14%, respectively (Nofchissey et al., 2013). Utilizing these comparisons, scientists can form generalized POWV distributions and inform surveillance efforts in new areas.

Serology studies of small and medium-sized mammals may provide an increasingly granular view of POWV distribution with limitations. Researchers have found POWV neutralizing antibodies in a number of these mammals across North America. In a New York study, serum samples from wild groundhogs, birds, and opossum showed prior POWV infection, while white footed mice and long-tailed weasels did not show evidence. Striped skunks were not bled for this study (Dupuis et al., 2013). Serum collected from meadow voles in Rhode Island and Massachusetts showed no prior infection with POWV, however, in this location approximately 3% of white footed mice captured at the same time were positive for POWV neutralizing antibodies (Goethert et al., 2009). In studies from Alaska and the southeastern US,

voles had serological evidence of POWV infection (Deardorff et al., 2013). Antibodies for POWV were also found in red squirrels, golden mantled ground squirrels, chipmunks, and snowshoe hares in southeastern British Columbia (Mclean et al., 1968).

Seroprevalence studies do not allow us to elucidate the patterns of DTV as a distinct lineage with a unique transmission cycle, due to cross reactivity with other members of the TBEC of which Powassan virus prototype and DTV both exist in North America. Indirect immunofluorescence assay (IFV) is needed to distinguish POWV positive samples from serum samples that may be positive with West Nile virus or dengue virus (Thomm et al., 2018). This presents a challenge when using wild animals for surveillance. Mammals infected with POWV exhibit short and low viremias if they become infective at all, thus collecting and testing blood and tissue studies from wild animals with PCR is not very informative, even in virus-endemic areas (Nemeth et al., 2021). An alternative is to test replete larval ticks collected from hosts for viral RNA, assuming a lack of vertical transmission to larvae. In the Hudson River Valley, a survey of replete *I. scapularis* larvae found DTV in ticks collected from opossums, striped skunks and raccoons (*Procyon lotor*); however, white footed mice, red squirrels, shrews, long tailed weasels, grey squirrels, and chipmunks did not have DTV-positive *Ixodes* larvae feeding on them despite hundreds of ticks sampled (Dupuis et al., 2013). Taken together, these results highlight variation and uncertainty for field studies investigating DTV reservoirs and underscore our lack of understanding about important reservoir animals that contribute to POWV infections, if they exist at all. Given the short-lived viremias for reservoir hosts, it is thought that transmission may occur during tick co-feeding (Randolph, 2004). Reasons for cofeeding may include small scale changes to phenology that result in larval and nymphal stages overlapping or plasticity in behavior with *I. scapularis* feeding on burrow dwelling mammals in areas with focal

DTV transmission. This pattern of transmission may be a major contributor to the focality of DTV.

Sampling and testing questing *Ixodes* ticks provides the clearest view of the DTV distribution as well as the ability to differentiate between the Powassan virus prototype and DTV. Unfortunately, collecting questing tick data on a large scale is time consuming and labor intensive. Questing DTV-positive ticks can be found in much of the Northeast and upper Midwest United States; however, the infection rate is low even in locations with stable prevalence, therefore, high tick sample numbers are required to pick up the pathogen. Additional difficulties with active tick surveillance include sample preservation. Ticks must be collected alive and maintained in a cold chain to preserve viral RNA, which differs from the ethanol collection methods used for other tick-borne pathogen surveillance (Robich et al., 2019).

Ixodes Scapularis Tick of Public Health Importance

Ixodes scapularis (the black legged tick) is a competent vector of many important disease agents in the Eastern United States. For example, *I. scapularis* is competent to transmit *B. burgdorferi, B. microti, A. phagocytophilum, B. miyamotoi, Ehrlichia spp*., and Deer Tick Virus (DTV) (Nelder et al., 2016).

During its 2-4 year life cycle *I. scapularis* develops through a larval, nymphal and adult stage. Engorged females overwinter or take a blood meal in spring and then drop off hosts to lay their eggs. The eggs hatch into larvae which host seek (quest) in the summer and then molt into nymphs. Unfed nymphs overwinter before feeding the following spring. Once blood-fed, the nymph will molt into an adult that will feed in the fall before overwintering and laying eggs the following spring (Yuval & Spielman, 1990).

I. scapularis is a 3-host tick; feeding on a different host each life stage, with well-

documented feeding patterns on rodent reservoirs of many of the above pathogens as well as a range of animals including humans (Apanaskevich & Oliver, 2014). The life stages of *I. scapularis can* be associated with different host species and it can feed on up to 100 different vertebrate host species throughout its range (Anderson & Magnarelli, 2008, Piesman & Spielman, 1979). The larval stage commonly feeds on small rodents, most commonly on the white-footed mouse (*Peromyscus leucopus*) (Main et al., 1982, Schulze et al., 1986, Anderson & Magnarelli, 1984). Mammals such as squirrels (*Sciurus carolinensis, Tamiasciurus hudsonicus*), chipmunks, striped skunk, woodchuck, opossum and raccoon are often host to *I. scapularis* nymphs (LoGiudice et al., 2003, Schulze 1986, Main et al., 1982, Fish & Dowler, 1989, McLean et al., 1968). Both larval and nymphal life stages can be found feeding on 11 families and 38 species of passerine birds (Dupuis et al., 2013, Anderson & Magnarelli, 1984). Voles including the Southern Red-backed vole (*Clethrionomys gapperi)* are less likely to host larval *I. scapularis* and are more likely than other animals of this size to host nymphal *I. scapularis* (Main et al., 1982). The adult life stage mostly feeds on large mammals including white tailed deer (*Odocoileus virginianus*) which can serve as a primary reproductive host for *I. scapularis* (Spielman et al. 1985). A study of meso mammals found that the majority of adults feeding on medium sized mammals (85%) were found on opossum (Fish & Dowler, 1989). Factors that impact Ixodid tick life span, successful feeding, and molting include temperature, diet, humidity, and saturation deficit (Randolph, 2004). *I. scapularis* has ample opportunity to bite humans as incidental hosts, often during the nymphal stage, and due to its small size can be difficult to detect (Eisen and Eisen, 2016). *I. scapularis* can be found feeding on humans often. The authors of a passive tick surveillance program study in the Northeast reported that the primary tick biting humans was *I. scapularis* (Xu et al., 2016).

In coastal Maine *I. scapularis* feeds on white-footed mouse, Eastern chipmunk (*Tamias striatus*), American Red Squirrel (*T. hudsonicus*) and Red Backed Vole (*Myodes gapperi*). *I. scapularis* also feeds on various mustelids in Maine including mink (*Neovison vison*) fishers, marten (*pennanti Erxleben*), and long-tailed weasel (*Mustela frenata*) (Lubelczyk et al., 2014) as well as a number of birds and reptiles (Anderson & Magnarelli., 2008).

There are two distinct biotypes of *I. scapularis* in the US, a southern clade in the Southeast and a northern clade that stretches north from Virginia into Canada and West to Oklahoma. *I. scapularis* was described by Say in 1821, but the northern form of the species was given a different species name (*Ixodes dammini*) until 1993, when it was found to be synonymous with *I. scapularis* (Keirans et al., 1996). Today, *I. scapularis* is one of eleven species of ticks in the *Ixodes ricinus* complex; a widely distributed paraphyletic group of ixodid ticks (Xu et al., 2003).

The two clades are no longer separated, overlapping in North and South Carolina, Alabama and Mississippi (Rich et al., 1995). Northern and southern types of *I. scapularis* can be distinguished using divergent mitochondrial DNA sequences although they are not reproductively isolated (Norris et al., 1996). The northern population of *I. scapularis* likely diverged from the southern population around 35,000 years ago with glaciers forming a physical barrier between the populations (Rich et al., 1995).

The once patchy distribution of the northern clade of *I. scapularis* has, over the past 2 decades, become endemic in most counties in the Northeast and upper Midwest (Eisen et al., 2017). Despite being members of the same species the north and south biotypes exhibit important differences in behavior that leads to them having a different impact on public health. Lyme disease is not as common in the southern states (CDC 2019). Ginsberg et al. (2021)

suggests this is related to a difference in host feeding. While the northern population feeds primarily on reservoir species for *Borrelia burgdorferi*, the southern population tends to feed on lizards which are inefficient reservoirs.

Identification and Collection Methods for *I. scapularis*

Ticks in the genus *Ixodes* are identified by an anal groove that extends anteriorly around the anus. *Ixodes* females have a scutum that covers the anterior portion of the dorsal side of the body and males have a scutum that covers the entire dorsum. Female *I. scapularis* have long palps and a rounded scutum. They have a large spur on the first coxa and external spurs on all coxae, the posterior margin of the basis capitulum is straight without a hump between the palps and the hypostome making the mouthparts appear long and narrow. Male *I. scapularis* have a smooth scutum absent of deep holes or rough texture. Their hypostome has denticles of varying size and they lack a spur like structure (cornua) at two sides of the distal end of the basis capitula (Keirans & Litwak, 1989).

Surveillance of *I. scapularis* is conducted by collecting ticks feeding on humans or animals, CO_2 traps and dragging methods. While most methods are useful to classify if the tick or a pathogen is present, dragging or flagging is acceptable to account for prevalence of viruses and density of nymphs (DON) or density of infected nymphs (DIN) and for phenology studies (CDC *I. Scapularis* surveillance guide). Dragging and flagging is the method by which a $1m^2$ textured weighted cloth is dragged behind or alongside the collector to capture host-seeking ticks. The time and distance the drag is on the ground is measured and ticks are removed at specific intervals and counted. Dragging or flagging is the most reliable method for quantitative analysis (Falco and Fish, 1992).

Phenology and Diapause

Phenology patterns in the *I. scapularis* life cycle can vary geographically. In the Northern regions adult tick activity is punctuated by the cold winter months causing two distinct peaks of activity in the fall and early spring. In Southern states ticks will remain active through the winter (Ogden et al., 2018). These variations can impact the transmission cycle of pathogens. In the Northeast the nymphal peak often falls before the larval peak, enabling naïve host species to be first introduced to pathogens by infected nymphs before encountering larvae (Ogden et al., 2007). *I. scapularis* exhibits behavioral diapause where they can become dormant during inopportune climate condition such as hot dry periods in the summer and a developmental diapause with decreasing day light hours (Gray et al., 2016).

I. scapularis response to desiccation has been reviewed by Stafford (1994). *I. scapularis* requires high humidity for survival and development through life stages, especially at high temperatures. In laboratory conditions, mortality was tested at constant temp of 27°C and relative humidity ranging from 65% to 100%. Mortality increases quickly in nymphs at 75% and lower RH. Larval survival differed between relative humidity of 93% and 100% (Stafford, 1994).

I. scapularis Distribution

Range expansion of *I. scapularis* over the past few decades is a primary cause of the increased incidence of tick-borne diseases such as Lyme disease, which has more than tripled in the years since 1991 (Bacon et al., 2008). During that time, the range for *I. scapularis* has expanded dramatically. In 1998, *I. scapularis* was considered established in 396 counties (32 states) mostly in the Northeastern states and the upper Midwest region (Dennis et al., 1998). As of 2016, they have been documented in 47.7% of all US counties and are established in 842

counties (37 states) in the eastern and Midwestern United States (Eisen et al., 2016). Regions that have seen the most change in *I. scapularis* range are the Northeastern and upper Midwestern states with these tick populations merging in Ohio. The range expansion in the Northeast extends beyond the border of the US into Canada. Active tick surveillance and National Lyme disease surveillance has been ongoing in Canada since 2009. From 2009 – 2012 incidence of Lyme disease, increased (Ogden et al., 2015) and the range of Lyme disease risk and *I. scapularis* population included the southern parts of Eastern Ontario, Quebec and Nova Scotia as well as Winnipeg and Northwestern Ontario (Ogden et al., 2014). Population movement northward into Canada is averaging 46km/year (Clow et al., 2017).

Much of the land in the Northeastern United States has gone through a process of massive deforestation, reforestation with second growth forests, and the introduction of a number of invasive ornamental plant species. Currently suburban communities make up much of the Northeast, interspersed with greenspace that allow wildlife to move between communities and close to urban centers. This combination of invasive species and room to roam for megafauna is a perfect situation for a high density of *I. scapularis* ticks. These factors in combination with climate changing, will likely cause the continuing expansion of *I. scapularis* range and human disease risk.

Landscape/environmental/climate Contributions to Prevalence of *I. scapularis*

While *I. scapularis* is widespread in the region, high abundance of *I. scapularis* is associated with a shrub layer and the presence of deciduous leaf litter. Forests with a shrub layer, moist soil ferns and >50% closed canopy have a greater abundance of questing adult ticks than more open canopy second growth forests (Lubelczyk et al., 2004). Japanese barberry stands can

host up to twice the abundance of adult *I. scapularis* ticks when compared to native forests (Elias et al., 2006). Removal of Japanese barberry results in a similar microclimate to native forests as well as 60% reduction of *B. burgdorferi* infected ticks. In one study of *I. scapularis*, tick density was highest in Japanese barberry stands, and was reduced significantly in areas where the plant was removed and injected with herbicide, and ticks were lowest in native forest conditions (Williams & Ward, 2010). Host availability may also be impacted by shrub communities. White footed mice infested with *I. scapularis* can be found in higher numbers in dense barberry stands (Piesman & Spielman, 1979).

High relative humidity is important for immature *I. scapularis* survival at warm temperatures. Ticks will desiccate if relative humidity is low. Mortality increases quickly in nymphs at 75% and lower RH. Larval survival dropped sharply with RH of less than 100% (Stafford, 1994). Weather station data on temperature and precipitation are not accurate predictors of nymphal black legged tick abundance (Schulze et al., 2009). Climate conditions recorded at the regional level often do not mirror the conditions at the ground level where ticks spend most of their life. Relative humidity is 24% higher on average in the leaf litter and more resistant to fluctuations over time than relative humidity measured at weather stations (Boehnke et al., 2017). Leaf litter and snow also contributes to greater tick survival in the winter where it can insulate ticks from harsh low temperatures (Linske et al., 2019).

Invasive Japanese barberry

The Northeastern region of the United States has experienced significant land use and population changes over the past few decades. Between 1980 and 2000, the population of the region grew by 10.5 million people (U.S. Forest Service 2005). During this time, land use in

many areas has shifted from dense city development to suburban sprawl: a landscape type that is a mixture of suburban, industrial, and other human-affected land covers (Valiela & Martinetto, 2007). Suburban sprawl has led to increased forest fragmentation, which is believed to be a major contributor to a low of biodiversity (Zuidema et al., 1996). Forest fragments often do not contain the resources necessary to support the wildlife they once held (U.S. Forest Service, 2005). Human land use in the region is linked to increased invasive, woody plant richness because of the increased edge habitat caused by roads and development (Allen et al., 2013).

Japanese barberry (*Berberis thunbergii*), native to China and Japan, was introduced to the United States in the 1920s as an ornamental plant. Planting benefits included its ease to prune and toleration of a wide range of moisture and temperature conditions. The invasive plant began to spread quickly through the Northeast by the 1960s (Silander & Klepeis, 1999). Unpruned barberry can grow tall, often over two meters in height. The plants tend to grow in thickets, as the seeds fall and germinate near each other. While Japanese barberry presence alone is not shown to affect species richness and diversity, a dense thicket is likely to have more severe impacts (Flinn et al., 2014). It provides a wide cover that prevents sunlight from reaching the ground, thereby negatively impacting the growing conditions of other plant life and can grow and set fruit in light conditions <4% full sunlight (Silander & Klepeis, 1999). The effects of Japanese barberry can be seen in arthropod communities as well. A comparison of low density barberry (12% of shrub community) to high density (52% of shrub community) show that Japanese barberry hosts a lower diversity of arthropods and fewer predator arthropod species than other native shrubs and may be experiencing trophic downgrading for this reason (Clark & Seewagen, 2019). Other invasive shrubs in the Northeast include Asiatic bittersweet (*Celastrus orbiculatus)*, and Eurasian honeysuckle *Lonicera* spp.

Future Directions

Deer tick virus is rare in humans; however, it is an increasing public health concern that requires further surveillance and study. This is challenging due to the scarcity of the virus in wildlife and questing ticks. The discovery of DTV foci that are maintained from year-to-year can support efforts to understand the ecology and epidemiology of the virus. Multiple surveillance methods may be employed in the search for DTV foci. Serosurveys of deer or investigations of reported human cases of Powassan virus disease can reveal regions where transmission is high. Then, a concentrated effort of questing tick and small mammal serology studies can provide a more precise understanding of the foci. A similar approach was employed in Italy to uncover a TBEV focus using goats as sentinel animals followed by targeted sampling of questing ticks (Alfano et al., 2021). Future research should focus on the environmental conditions, tick biology and putative reservoir/host animal assemblages surrounding locations where DTV is maintained.

References

Alfano, N., Tagliapietra, V., Rosso, F., Ziegler, U., Arnoldi, D., & Rizzoli, A. (2020). Tick-borne encephalitis foci in northeast Italy revealed by combined virus detection in ticks, serosurvey on goats and human cases. *Emerging Microbes & Infections*, *9*(1), 474–484. https://doi.org/10.1080/22221751.2020.1730246

Allen, J. M., Leininger, T. J., Hurd, J. D., Civco, D. L., Gelfand, A. E., & Silander, J. A. (2013). Socioeconomics drive woody invasive plant richness in New England, USA through forest fragmentation. *Landscape Ecology*, *28*(9), 1671–1686. https://doi.org/10.1007/s10980-013-9916-7

Anderson, J. F., & Magnarelli, L. A. (1984). Avian and mammalian hosts for spirochete-infected ticks and insects in a Lyme disease focus in Connecticut. *The Yale Journal of Biology and Medicine*, *57*(4), 627–641.

Anderson, John F., & Armstrong, P. M. (2012). Prevalence and Genetic Characterization of Powassan Virus Strains Infecting Ixodes scapularis in Connecticut. *The American Journal of Tropical Medicine and Hygiene*, *87*(4), 754–759. https://doi.org/10.4269/ajtmh.2012.12-0294

Anderson, John F., & Magnarelli, L. A. (2008). Biology of ticks. *Infectious Disease Clinics of North America*, *22*(2), 195–215, v. https://doi.org/10.1016/j.idc.2007.12.006

Apanaskevich, D. A. Oliver, Jr. J. H. (2014). Life cycles and natural history of ticks. In Sonenshine, D. E. and Roe, R. M. (Ed.) Biology of Ticks (2nd ed.). Oxford University Press (pp.59-72).

Bacon, R. M., Kugeler, K. J., Mead, P. S., & Centers for Disease Control and Prevention (CDC). (2008). Surveillance for Lyme disease—United States, 1992-2006. *Morbidity and Mortality Weekly Report. Surveillance Summaries (Washington, D.C.: 2002)*, *57*(10), 1–9.

Baker, A., Wang, H.-H., Mogg, M., Derouen, Z., Borski, J., & Grant, W. E. (2020). Increasing Incidence of Anaplasmosis in the United States, 2012 Through 2016. *Vector-Borne and Zoonotic Diseases*, *20*(11), 855–859. https://doi.org/10.1089/vbz.2019.2598

Bakken, J. S., & Dumler, J. S. (2000). Human Granulocytic Ehrlichiosis. *Clinical Infectious Diseases*, *31*(2), 554–560. https://doi.org/10.1086/313948

Beard, C. B., Eisen, R. J., Barker, C. M., Garofalo, J. F., Hahn, M., Hayden, M., Monaghan, A. J., Ogden, N. H., & Schramm, P. J. (2016). *Ch. 5: Vectorborne Diseases. The Impacts of Climate Change on Human Health in the United States: A Scientific Assessment*. U.S. Global Change Research Program. https://doi.org/10.7930/J0765C7V

Boehnke, D., Gebhardt, R., Petney, T., & Norra, S. (2017). On the complexity of measuring forests microclimate and interpreting its relevance in habitat ecology: The example of Ixodes ricinus ticks. *Parasites & Vectors*, *10*. https://doi.org/10.1186/s13071-017-2498-5

Clark, R. E., & Seewagen, C. L. (2019). Invasive Japanese Barberry, Berberis thunbergii (Ranunculales: Berberidaceae) Is Associated With Simplified Branch-Dwelling and Leaf-Litter Arthropod Communities in a New York Forest. *Environmental Entomology*, *48*(5), 1071–1078. https://doi.org/10.1093/ee/nvz095

Clow, K. M., Leighton, P. A., Ogden, N. H., Lindsay, L. R., Michel, P., Pearl, D. L., & Jardine, C. M. (2017). Northward range expansion of Ixodes scapularis evident over a short timescale in Ontario, Canada. *PLOS ONE*, *12*(12), e0189393. https://doi.org/10.1371/journal.pone.0189393

Costero, A., & Grayson, M. A. (1996). Experimental Transmission of Powassan Virus (Flaviviridae) by Ixodes scapularis Ticks (Acari:Ixodidae). *The American Journal of Tropical Medicine and Hygiene*, *55*(5), 536–546. https://doi.org/10.4269/ajtmh.1996.55.536

Deardorff, E. R., Nofchissey, R. A., Cook, J. A., Hope, A. G., Tsvetkova, A., Talbot, S. L., & Ebel, G. D. (2013). Powassan Virus in Mammals, Alaska and New Mexico, USA, and Russia, 2004–2007. Emerging Infectious Diseases, 19(12), 2012–2016. https://doi.org/10.3201/eid1912.130319

Dennis, D. T., Nekomoto, T. S., Victor, J. C., Paul, W. S., & Piesman, J. (1998). Forum: Reported Distribution of Ixodes scapularis and Ixodes pacificus (Acari: Ixodidae) in the United States. *Journal of Medical Entomology*, *35*(5), 629–638. https://doi.org/10.1093/jmedent/35.5.629

Diuk-Wasser, M. A., Vannier, E., & Krause, P. J. (2016). Coinfection by Ixodes Tick-Borne Pathogens: Ecological, Epidemiological, and Clinical Consequences. *Trends in Parasitology*, *32*(1), 30–42. https://doi.org/10.1016/j.pt.2015.09.008

Dupuis II, A. P., Peters, R. J., Prusinski, M. A., Falco, R. C., Ostfeld, R. S., & Kramer, L. D. (2013). Isolation of deer tick virus (Powassan virus, lineage II) from Ixodes scapularis and detection of antibody in vertebrate hosts sampled in the Hudson Valley, New York State. *Parasites & Vectors*, *6*(1), 185. https://doi.org/10.1186/1756-3305-6-185

Ebel, G. D. (2010). Update on Powassan Virus: Emergence of a North American Tick-Borne Flavivirus. *Annual Review of Entomology*, *55*(1), 95–110. https://doi.org/10.1146/annurev-ento-112408-085446

Ebel, G. D., & Kramer, L. D. (2004).Short report: Duration of tick attachment required for transmission of Powassan virus by deer ticks. *The American Journal of Tropical Medicine and Hygiene*, *71*(3), 268–271. https://doi.org/10.4269/ajtmh.2004.71.3.0700268

Ebel, G. D., Spielman, A., & Telford, S. R. (2001). Phylogeny of North American Powassan virus. *Journal of General Virology*, *82*(7), 1657–1665. https://doi.org/10.1099/0022-1317-82-7-1657

Eisen, L., & Eisen, R. J. (2016). Critical Evaluation of the Linkage Between Tick-Based Risk Measures and the Occurrence of Lyme Disease Cases. *Journal of Medical Entomology*, *53*(5), 1050–1062. https://doi.org/10.1093/jme/tjw092

Eisen, R. J., Eisen, L., & Beard, C. B. (2016). County-Scale Distribution of Ixodes scapularis and Ixodes pacificus (Acari: Ixodidae) in the Continental United States. *Journal of Medical Entomology*, *53*(2), 349–386. https://doi.org/10.1093/jme/tjv237

Elias, S. P., Lubelczyk, C. B., Rand, P. W., Lacombe, E. H., Holman, M. S., & Smith, R. P. (2006). *Deer browse resistant exotic-invasive understory: An indicator of elevated human risk of exposure to Ixodes scapularis (Acari: Ixodidae) in southern coastal Maine woodlands*. https://pubag.nal.usda.gov/catalog/715926

Falco, R. C., & Fish, D. (1992). A comparison of methods for sampling the deer tick,Ixodes dammini, in a Lyme disease endemic area. *Experimental & Applied Acarology*, *14*(2), 165–173. https://doi.org/10.1007/BF01219108

Farlow, J., Wagner, D. M., Dukerich, M., Stanley, M., Chu, M., Kubota, K., Petersen, J., & Keim, P. (2005). Francisella tularensis in the United States. *Emerging Infectious Diseases*, *11*(12), 1835–1841. https://doi.org/10.3201/eid1112.050728

Feder, H. M., Jr, Telford, S., III, Goethert, H. K., & Wormser, G. P. (2020). Powassan Virus Encephalitis Following Brief Attachment of Connecticut Deer Ticks. *Clinical Infectious Diseases*, *ciaa1183*. https://doi.org/10.1093/cid/ciaa1183

Fish, D., & Dowler, R. C. (1989). Host Associations of Ticks (Acari: Ixodidae) Parasitizing Medium-Sized Mammals in a Lyme Disease Endemic Area of Southern New York. *Journal of Medical Entomology*, *26*(3), 200–209. https://doi.org/10.1093/jmedent/26.3.200

Flinn, K., Bechhofer, J., & Malcolm, M. (2014). Little impact of the invasive shrub Japanese barberry (Berberis thunbergii DC) on forest understory plant communities 1. *The Journal of the Torrey Botanical Society*, *141*, 217–224. https://doi.org/10.3159/TORREY-D-14-00012.1

Gholam, B. I., Puksa, S., & Provias, J. P. (1999). Powassan encephalitis: A case report with neuropathology and literature review. *CMAJ: Canadian Medical Association Journal*, *161*(11), 1419–1422.

Ginsberg, H. S., & Ewing, C. P. (1988). Deer Ticks, Ixodes dammini (Acari: Ixodidae), and Lyme Disease Spirochetes, Borrelia burgdorferi, in Maine. *Journal of Medical Entomology*, *25*(4), 303–304. https://doi.org/10.1093/jmedent/25.4.303

Ginsberg, H. S., Hickling, G. J., Burke, R. L., Ogden, N. H., Beati, L., LeBrun, R. A., Arsnoe, I. M., Gerhold, R., Han, S., Jackson, K., Maestas, L., Moody, T., Pang, G., Ross, B., Rulison, E. L., & Tsao, J. I. (2021). Why Lyme disease is common in the northern US, but rare in the south: The roles of host choice, host-seeking behavior, and tick density. *PLOS Biology*, *19*(1), e3001066. https://doi.org/10.1371/journal.pbio.3001066

Goethert, H. K., & Iii, S. R. T. (2009). Nonrandom Distribution of Vector Ticks (Dermacentor variabilis) Infected by Francisella tularensis. *PLOS Pathogens*, *5*(2), e1000319. https://doi.org/10.1371/journal.ppat.1000319

Goldstein, E. J. C., Thompson, C., Spielman, A., & Krause, P. J. (2001). Coinfecting Deer-Associated Zoonoses: Lyme Disease, Babesiosis, and Ehrlichiosis. *Clinical Infectious Diseases*, *33*(5), 676–685. https://doi.org/10.1086/322681

Gottlieb, M., Long, B., & Koyfman, A. (2018). The Evaluation and Management of Rocky Mountain Spotted Fever in the Emergency Department: A Review of the Literature. *The Journal of Emergency Medicine*, *55*(1), 42–50. https://doi.org/10.1016/j.jemermed.2018.02.043

Gray, J. S., Kahl, O., Lane, R. S., Levin, M. L., & Tsao, J. I. (2016). Diapause in ticks of the medically important Ixodes ricinus species complex. *Ticks and Tick-Borne Diseases*, *7*(5), 992–1003. https://doi.org/10.1016/j.ttbdis.2016.05.006

Hermance, M. E., & Thangamani, S. (2017). Powassan Virus: An Emerging Arbovirus of Public Health Concern in North America. *Vector-Borne and Zoonotic Diseases*, *17*(7), 453–462. https://doi.org/10.1089/vbz.2017.2110

Hinten, S. R., Beckett, G. A., Gensheimer, K. F., Pritchard, E., Courtney, T. M., Sears, S. D., Woytowicz, J. M., Preston, D. G., Smith, Jr., R. P., Rand, P. W., Lacombe, E. H., Holman, M. S., Lubelczyk, C. B., Kelso, P. T., Beelen, A. P., Stobierski, M. G., Sotir, M. J., Wong, S., Ebel, G., … Marfin, A. A. (2008). Increased Recognition of Powassan Encephalitis in the United States, 1999–2005. *Vector-Borne and Zoonotic Diseases*, *8*(6), 733–740. https://doi.org/10.1089/vbz.2008.0022

Holman, M. S., Caporale, D. A., Goldberg, J., Lacombe, E. H., Lubelczyk, C. B., Rand, P. W., & Smith, R. P. (2004). Anaplasma phagocytophilum, Babesia microti, and Borrelia burgdorferi in Ixodes scapularis, Southern Coastal Maine. *Emerging Infectious Diseases journal CDC*. (10)4 https://doi.org/10.3201/eid1004.030566

Keirans, J. E., & Litwak, T. R. (1989). Pictorial key to the adults of hard ticks, family Ixodidae (Ixodida: Ixodoidea), east of the Mississippi River. *Journal of Medical Entomology*, *26*(5), 435–448. https://doi.org/10.1093/jmedent/26.5.435

Keirans, James E., Hutcheson, H. J., Durden, L. A., & Klompen, J. S. H. (1996). Ixodes (Ixodes) scapularis (Acari: Ixodidae): Redescription of all Active Stages, Distribution, Hosts, Geographical Variation, and Medical and Veterinary Importance. *Journal of Medical Entomology*, *33*(3), 297–318. https://doi.org/10.1093/jmedent/33.3.297

Kemenesi, G., & Bányai, K. (2018). Tick-Borne Flaviviruses, with a Focus on Powassan Virus. *Clinical Microbiology Reviews*, *32*(1). https://doi.org/10.1128/CMR.00106-17

Ko, R. C. (1972). Biology of Ixodes cookei Packard (Ixodidae) of groundhogs (Marmota monax Erxleben). *Canadian Journal of Zoology*, *50*(4), 433–436. https://doi.org/10.1139/z72-061

Kokernot, R. H., D.V.M., M.D., Dr. P. H.; Radivojevic, D.V.M.; Anderson, R. J. (1969). Susceptibility of wild and domesticated mammals to four arboviruses. *American journal of veterinary research*, 30, 2197-2203.

Krause, P. J., McKay, K., Thompson, C. A., Sikand, V. K., Lentz, R., Lepore, T., Closter, L., Christianson, D., Telford, S. R., Persing, D., Radolf, J. D., Spielman, A., & Deer-Associated Infection Study Group. (2002). Disease-specific diagnosis of coinfecting tickborne zoonoses: Babesiosis, human granulocytic ehrlichiosis, and Lyme disease. *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America*, *34*(9), 1184–1191. https://doi.org/10.1086/339813

Kuno, G., Artsob, H., Karabatsos, N., Tsuchiya, K. R., & Chang, G. J. (2001). Genomic sequencing of deer tick virus and phylogeny of powassan-related viruses of North America. *The American Journal of Tropical Medicine and Hygiene*, *65*(5), 671–676. https://doi.org/10.4269/ajtmh.2001.65.671

Linske, M. A., Stafford, K. C., Williams, S. C., Lubelczyk, C. B., Welch, M., & Henderson, E. F. (2019). Impacts of Deciduous Leaf Litter and Snow Presence on Nymphal Ixodes scapularis (Acari: Ixodidae) Overwintering Survival in Coastal New England, USA. *Insects*, *10*(8). https://doi.org/10.3390/insects10080227

Little, E. A. H., Anderson, J. F., Stafford, K. C., Eisen, L., Eisen, R. J., & Molaei, G. (2019). Predicting spatiotemporal patterns of Lyme disease incidence from passively collected

surveillance data for Borrelia burgdorferi sensu lato-infected Ixodes scapularis ticks. *Ticks and Tick-Borne Diseases*, *10*(5), 970–980. https://doi.org/10.1016/j.ttbdis.2019.04.010

LoGiudice, K., Ostfeld, R. S., Schmidt, K. A., & Keesing, F. (2003). The ecology of infectious disease: Effects of host diversity and community composition on lyme disease risk. *Proceedings of the National Academy of Sciences of the United States of America*, *100*(2), 567–571. Scopus. https://doi.org/10.1073/pnas.0233733100

Lubelczyk, C. B., Elias, S. P., Rand, P. W., Holman, M. S., Lacombe, E. H., & Smith, R. P. J. (2004). *Habitat associations of Ixodes scapularis (Acari: Ixodidae) in Maine*. https://pubag.nal.usda.gov/catalog/675601

Lubelczyk, C., Lacombe, E. H., Elias, S. P., Beati, L., Rand, P. W., & Smith, R. P. (2014). Parasitism of mustelids by ixodid ticks (Acari: Ixodidae), Maine and New Hampshire, U.S.A. *Ticks and Tick-Borne Diseases*, *5*(4), 432–435. https://doi.org/10.1016/j.ttbdis.2014.02.001

Lyme Disease Maps: Historical Data | Lyme Disease | CDC. (2019, November 22). https://www.cdc.gov/lyme/stats/maps.html

Main, A. J., Carey, A. B., Carey, M. G., & Goodwin, R. H. (1982). Immature Ixodes Dammini (Acari: Ixodidae) on Small Animals in Connecticut, USA1. *Journal of Medical Entomology*, *19*(6), 655–664. https://doi.org/10.1093/jmedent/19.6.655

McLean, D. M., & Donohue, W. L. (1959). Powassan Virus: Isolation of Virus from a Fatal Case of Encephalitis. *Canadian Medical Association Journal*, *80*(9), 708–711.

McLean, D. M., Ladyman, S. R., & Purvin-Good, K. W. (1968). Westward extension of Powassan virus prevalence. *Canadian Medical Association Journal*, *98*(20), 946–949.

Mclean, D. M., & Larke, R. P. (1963). Powassan and Silverwater viruses: Ecology of two Ontario arboviruses. *Canadian Medical Association Journal*, *88*, 182–185.

McLean, D. M., Best, J. M., Mahalingam, S., Chernesky, M. A., & Wilson, W. E. (1964). Powassan Virus: Summer Infection Cycle, 1964. *Canadian Medical Association Journal*, *91*(26), 1360–1362.

Mlera, L., & Bloom, M. E. (2018). The Role of Mammalian Reservoir Hosts in Tick-Borne Flavivirus Biology. *Frontiers in Cellular and Infection Microbiology*, *8*. https://doi.org/10.3389/fcimb.2018.00298

Nelder, M. P., Russell, C. B., Sheehan, N. J., Sander, B., Moore, S., Li, Y., Johnson, S., Patel, S. N., & Sider, D. (2016). Human pathogens associated with the blacklegged tick Ixodes scapularis: A systematic review. *Parasites & Vectors*, *9*(1), 265. https://doi.org/10.1186/s13071-016-1529-y

Nemeth, N. M., Root, J. J., Hartwig, A. E., Bowen, R. A., & Bosco-Lauth, A. M. (2021). Powassan Virus Experimental Infections in Three Wild Mammal Species. *The American Journal of Tropical Medicine and Hygiene*, *104*(3), 1048–1054. https://doi.org/10.4269/ajtmh.20-0105

Nofchissey, R. A., Deardorff, E. R., Blevins, T. M., Anishchenko, M., Bosco-Lauth, A., Berl, E., Lubelczyk, C., Mutebi, J.-P., Brault, A. C., Ebel, G. D., & Magnarelli, L. A. (2013). Seroprevalence of Powassan Virus in New England Deer, 1979–2010. *The American Journal of Tropical Medicine and Hygiene*, *88*(6), 1159–1162. https://doi.org/10.4269/ajtmh.12-0586

Norris, D. E., Klompen, J. S. H., Keirans, J. E., & Black, W. C., IV. (1996). Population Genetics of Ixodes scapularis (Acari: Ixodidae) Based on Mitochondrial 16S and 12S Genes. *Journal of Medical Entomology*, *33*(1), 78–89. https://doi.org/10.1093/jmedent/33.1.78

Ogden, N. H., Bigras-Poulin, M., O'callaghan, C. J., Barker, I. K., Kurtenbach, K., Lindsay, L. R., & Charron, D. F. (2007). Vector seasonality, host infection dynamics and fitness of

pathogens transmitted by the tick Ixodes scapularis. *Parasitology*, *134*(2), 209–227. https://doi.org/10.1017/S0031182006001417

Ogden NH, Koffi JK, Lindsay LR, Fleming S, Mombourquette DC, Sanford C, et al. Surveillance for Lyme disease in Canada, 2009 to 2012. Can Commun Dis Rep. 2015; 41: 1±14.

Ogden, N. H., Koffi, J. K., Pelcat, Y., & Lindsay, L. R. (2014). Environmental risk from Lyme disease in central and eastern Canada: A summary of recent surveillance information. *Canada Communicable Disease Report = Releve Des Maladies Transmissibles Au Canada*, *40*(5), 74–82. https://doi.org/10.14745/ccdr.v40i05a01

Ogden, N. H., Pang, G., Ginsberg, H. S., Hickling, G. J., Burke, R. L., Beati, L., & Tsao, J. I. (2018). Evidence for Geographic Variation in Life-Cycle Processes Affecting Phenology of the Lyme Disease Vector Ixodes scapularis (Acari: Ixodidae) in the United States. *Journal of Medical Entomology*, *55*(6), 1386–1401. https://doi.org/10.1093/jme/tjy104

Ostfeld, R. S., Schauber, E. M., Canham, C. D., Keesing, F., Jones, C. G., & Wolff, J. O. (2001). Effects of Acorn Production and Mouse Abundance on Abundance and Borrelia burgdorferi Infection Prevalence of Nymphal Ixodes scapularis Ticks. *Vector-Borne and Zoonotic Diseases*, *1*(1), 55–63. https://doi.org/10.1089/153036601750137688

Piesman, J., & Dolan, M. C. (2002). Protection against lyme disease spirochete transmission provided by prompt removal of nymphal Ixodes scapularis (Acari: Ixodidae). *Journal of Medical Entomology*, *39*(3), 509–512. https://doi.org/10.1603/0022-2585-39.3.509

Piesman, J., & Spielman, A. (1979). Host-Associations and Seasonal Abundance of Immature Ixodes dammini1 in Southeastern Massachusetts2. *Annals of the Entomological Society of America*, *72*(6), 829–832. https://doi.org/10.1093/aesa/72.6.829

Rand, P. W., Lacombe, E. H., Dearborn, R., Cahill, B., Elias, S., Lubelczyk, C. B., Beckett, G. A., & Smith, R. P. (2007). Passive surveillance in Maine, an area emergent for tick-borne diseases. *Journal of Medical Entomology*, *44*(6), 1118–1129. https://doi.org/10.1603/0022-2585(2007)44[1118:psimaa]2.0.co;2

Randolph, S. E. (2004). Tick ecology: Processes and patterns behind the epidemiological risk posed by ixodid ticks as vectors. Parasitology, 129 Suppl, S37-65. https://doi.org/10.1017/s0031182004004925

Rich, S. M., Caporale, D. A., Telford, S. R., Kocher, T. D., Hartl, D. L., & Spielman, A. (1995). Distribution of the Ixodes ricinus-like ticks of eastern North America. *Proceedings of the National Academy of Sciences*, *92*(14), 6284–6288. https://doi.org/10.1073/pnas.92.14.6284

Robich, R. M., Cosenza, D. S., Elias, S. P., Henderson, E. F., Lubelczyk, C. B., Welch, M., & Smith, R. P. (2019). Prevalence and Genetic Characterization of Deer Tick Virus (Powassan Virus, Lineage II) in Ixodes scapularis Ticks Collected in Maine. *The American Journal of Tropical Medicine and Hygiene*, *101*(2), 467–471. https://doi.org/10.4269/ajtmh.19-0281

Savage, H. M., Godsey, M. S., Jr., Tatman, J., Burkhalter, K. L., Hamm, A., Panella, N. A., Ghosh, A., & Raghavan, R. K. (2018). Surveillance for Heartland and Bourbon Viruses in Eastern Kansas, June 2016. *Journal of Medical Entomology*, *55*(6), 1613–1616. https://doi.org/10.1093/jme/tjy103

Schulze, T. L., Bowen, G. S., Lakat, M. F., Parkin, W. E., & Shisler, J. K. (1986). Seasonal Abundance and Hosts of Ixodes Dammini(Acari: Ixodidae) and Other Ixodid Ticks from an Endemic Lyme Disease Focus in New Jersey, USA. *Journal of Medical Entomology*, *23*(1), 105–109. https://doi.org/10.1093/jmedent/23.1.105

Silander, J. A., & Klepeis, D. M. (1999). The Invasion Ecology of Japanese Barberry (Berberis thunbergii) in the New England Landscape. *Biological Invasions*, *1*(2), 189–201. https://doi.org/10.1023/A:1010024202294

Smith, R. P., Lacombe, E. H., Rand, P. W., & Dearborn, R. (1992). Diversity of tick species biting humans in an emerging area for Lyme disease. *American Journal of Public Health*, *82*(1), 66–69.

Smith, R. P., Rand, P. W., & Lacombe, E. H. (1990). Potential for Lyme disease in Maine: Deer survey of distribution of Ixodes dammini, the tick vector. *American Journal of Public Health*, *80*(3), 333–335.

Spielman, A., Wilson, M. L., Levine, J. F., & Piesman, J. (1985). Ecology of Ixodes Dammini-Borne Human Babesiosis and Lyme Disease. *Annual Review of Entomology*, *30*(1), 439–460. https://doi.org/10.1146/annurev.en.30.010185.002255

Stafford, K. C. (1994). Survival of Immature Ixodes scapularis (Acari: Ixodidae) at Different Relative Humidities. *Journal of Medical Entomology*, *31*(2), 310–314. https://doi.org/10.1093/jmedent/31.2.310

Subbotina, E. L., & Loktev, V. B. (2012). Molecular evolution of the tick-borne encephalitis and Powassan viruses. *Molecular Biology*, *46*(1), 75–84. https://doi.org/10.1134/S0026893311060148

Surveillance for Ixodes scapularis and pathogens found in this tick species in the United States. (n.d.). 34.

Swanson, S. J., Neitzel, D., Reed, K. D., & Belongia, E. A. (2006). Coinfections Acquired from Ixodes Ticks. *Clinical Microbiology Reviews*, *19*(4), 708–727. https://doi.org/10.1128/CMR.00011-06

Tavakoli, N. P., Wang, H., Dupuis, M., Hull, R., Ebel, G. D., Gilmore, E. J., & Faust, P. L. (2009). Fatal case of deer tick virus encephalitis. *The New England Journal of Medicine*, *360*(20), 2099–2107. https://doi.org/10.1056/NEJMoa0806326

Telford, S. R., Armstrong, P. M., Katavolos, P., Foppa, I., Garcia, A. S., Wilson, M. L., & Spielman, A. (1997). A new tick-borne encephalitis-like virus infecting New England deer ticks, Ixodes dammini. *Emerging Infectious Diseases*, *3*(2), 165–170.

U.S. Forest Service (2005). Snapshot of the Northeastern Forests. Forest Service USDA https://www.fs.usda.gov/naspf/sites/default/files/ss.pdf

Thomm, A. M., Schotthoefer, A. M., Dupuis, A. P., Kramer, L. D., Frost, H. M., Fritsche, T. R., Harrington, Y. A., Knox, K. K., & Kehl, S. C. (2018). Development and Validation of a Serologic Test Panel for Detection of Powassan Virus Infection in U.S. Patients Residing in Regions Where Lyme Disease Is Endemic. *MSphere*, *3*(1). https://doi.org/10.1128/mSphere.00467-17

Valiela, I., & Martinetto, P. (2007). Changes in Bird Abundance in Eastern North America: Urban Sprawl and Global Footprint? *BioScience*, *57*(4), 360–370. https://doi.org/10.1641/B570410

Vannier, E., & Krause, P. J. (2020). 105—Babesiosis. In E. T. Ryan, D. R. Hill, T. Solomon, N. E. Aronson, & T. P. Endy (Eds.), *Hunter's Tropical Medicine and Emerging Infectious Diseases (Tenth Edition)* (pp. 799–802). Elsevier. https://doi.org/10.1016/B978-0-323-55512-8.00105-8

Williams, S. C., & Ward, J. S. (2010). Effects of Japanese barberry (Ranunculales: Berberidaceae) removal and resulting microclimatic changes on Ixodes scapularis (Acari: Ixodidae) abundances

in Connecticut, USA. *Environmental Entomology*, *39*(6), 1911–1921. https://doi.org/10.1603/EN10131

Williamson, B. N., Fischer, R. J., Lopez, J. E., Ebihara, H., & Schwan, T. G. (2019). Prevalence and Strains of Colorado Tick Fever Virus in Rocky Mountain Wood Ticks in the Bitterroot Valley, Montana. *Vector-Borne and Zoonotic Diseases*, *19*(9), 694–702. https://doi.org/10.1089/vbz.2018.2407

Wormser, G. P., Shapiro, E. D., & Fish, D. (2019). Borrelia miyamotoi: An Emerging Tick-Borne Pathogen. *The American Journal of Medicine*, *132*(2), 136–137. https://doi.org/10.1016/j.amjmed.2018.08.012

Xu, G., Fang, Q. Q., Keirans, J. E., & Durden, L. A. (2003). Molecular phylogenetic analysis indicate that the *Ixodes ricinus* complex is a paraphyletic group. *Journal of Parasitology*, *89*(3), 452–457. https://doi.org/10.1645/0022-3395(2003)089[0452:MPAITT]2.0.CO;2

Xu, G., Mather, T. N., Hollingsworth, C. S., & Rich, S. M. (2016). Passive Surveillance of Ixodes scapularis (Say), Their Biting Activity, and Associated Pathogens in Massachusetts. *Vector-Borne and Zoonotic Diseases*, *16*(8), 520–527. https://doi.org/10.1089/vbz.2015.1912

Yuval, B., & Spielman, A. (1990). Duration and Regulation of the Developmental Cycle of Ixodes dammini (Acari: Ixodidae). *Journal of Medical Entomology*, *27*(2), 196–201. https://doi.org/10.1093/jmedent/27.2.196

Zuidema, P. A., Sayer, J. A., & Dijkman, W. (1996). Forest fragmentation and biodiversity: The case for intermediate-sized conservation areas. *Environmental Conservation*, *23*(4), 290–297.

CHAPTER 2

Investigation of a Powassan Virus Focus in Southern Maine, USA

Introduction

Powassan virus is a member of the tick-borne encephalitis complex of flaviviruses that causes severe illness and death in up to 30% of human cases and prolonged impairment in up to 30% of survivors (Kemenesi & Bányai 2018). Although rare, human cases of Powassan encephalitis in the United States have been increasing over the past decade (Hermance & Thangamani, 2017). Two distinct lineages of Powassan virus are recognized: lineage I (prototype) and lineage II, known as deer tick virus (DTV) (Ebel et al., 2000, Telford et al., 1997). Greater genetic variation in the DTV clade is thought to be due to differing ecology and host associations (Ebel et al., 2000). Both clades are maintained in nature; however, DTV poses a greater threat to human health because it can be transmitted by *Ixodes scapularis,* a generalist feeder that frequently attaches and feeds on humans in the northeastern region (Ebel et al., 1999, Telford et al., 1997, Xu et al., 2016).

Key reservoir hosts for DTV have not been clearly demonstrated and more research is required to identify which mammals amplify the virus in nature (Nemeth et al., 2021). DTV may circulate among populations of white-footed mice (*Peromyscus leucopus*) which make up a large portion of the host population for sub-adult *I. scapularis* (Apanaskevich et al., 2014). Researchers collecting serum from a small number (3-4%) of white-footed mice collected in Rhode Island, Wisconsin, Nantucket Island and Massachusetts claimed to show prior exposure to DTV (Ebel et al., 2000); however this serological result should be considered with caution due to the issues of cross reactivity with other flaviviruses (Subbotina & Loktev, 2012, Kuno et al., 2001). While the presence of DTV in questing *I. scapularis* ticks implicates white footed mice as a potential reservoir host due frequent feeding of this species on mice, virus isolation has not

been demonstrated from wild caught white footed mice (Mlera & Bloom, 2018, Telford et al., 1997). Replete nymphs and adult ticks removed from woodchucks (*Marmota monax*) and skunks (*Mephitis mephitis*) in Ontario were positive for POWV infection, with few clinical symptoms (McLean et al., 1964, McLean & Larke, 1963). Other collections of replete *I. scapularis* larvae in New York State reported DTV positive ticks from Virginia opossum (*Didelphis virginiana*), striped skunk (*Mephitis mephitis*), and raccoon (*Procyon lotor*) (Dupuis et al., 2013). However, DTV was not detected from 848 larval ticks collected from 161 individual white footed mice in that same study (Dupuis et al., 2013).

DTV is found in questing ticks throughout much of the Northeast and upper Midwest of the United States and in Eastern Canada (Robich et al., 2019, Dupuis et al., 2013, Knox et al., 2017, Smith et al., 2018). The virus was detected in 0-5% of questing adult ticks (Anderson & Armstrong, 2012, Robich et al., 2019, Dupuis et al., 2013, Ebel et al., 2000). Focal and persistently positive locations may be found during routine pathogen surveillance, but the factors that lead to this persistence are not understood. In 2016 and 2017, a study was conducted to determine the prevalence of DTV in *I. scapularis* collected from different counties in Maine (Robich et al., 2019). Those results were consistent with neighboring states and showed a DTV infection rate in *I. scapularis* nymphs and adults between 0-5%. However, many of the DTV positive ticks were from a single site in the Wells National Estuarine Research Reserve (WNERR). Additional research was conducted to understand factors that may drive DTV focality within the reserve, such as habitat types beneficial to *I. scapularis* (Robich et al., in preparation). In that study, the greatest number of ticks was collected from a forest habitat with a non-native invasive shrub understory, potentially because these plants tend to be resistant to deer browsing, act as good habitat for small mammals, and provide shade and optimal

microclimate for ticks. Robich et al. (in preparation) detected DTV in 6.5% of adults from their study in 2018 and 3.2 % positive in 2019.

In this study I tested the hypothesis that DTV is located in small microhabitats (foci) consisting of an optimal mixture of environmental factors such as tick hosts and habitat that sustain virus persistence over time. I systematically collected ticks and measured microclimate and vegetation features from three different habitats representing a gradient of invasive understory species (1. forest with a high amount of invasive species in the understory, 2. forest with a moderate invasive shrub species in the understory and 3. forest with sparse, native shrub species in the understory) and tested them for virus. Small mammals were trapped in the two invasive habitat types, measured tick burden by animal species and tested ticks for infection with DTV.

Materials and Methods:

Study site

This study was conducted within the Wells National Estuarine Research Reserve at Laudholm Farm [WNERR] (43.339349 °N, 70.551008°W) in three forest stands with different physical and biological characteristics. WNERR is located on the Maine coast, with an estuary that runs through the reserve. Average summer temperatures reach approximately 27°C in July and August, with evening lows averaging around 15.5°C (UMaine 2020). Average precipitation at the preserve is 10 cm per month (National Weather Service averages for York County). Dominant forest overstory tree species were red oak (*Quercus rubra* L.), red maple, (*Acer rubrum* L.), yellow birch (*Betula lutea* Michx.) and white pine, (*Pinus strobus* L.) (Lubelczyk et al. 2004). Forest understory at WNERR could be categorized as either native understory or invaded by non-native shrub species. Native understory shrub species included high bush

blueberry (*Vaccinium corymbosum*), bayberry (*Myrica spp*.), and huckleberry (*Gaylussacia baccata*). Typically, where the forest shrub layer was native, shrub and ground vegetation density was sparse with a layer of deciduous or deciduous/coniferous leaf litter. However, in some portions of WNERR, invasive shrub species, mainly Japanese barberry *Berberis thunbergii*, Eurasian honeysuckle *Lonicera* spp., and the vine Asiatic bittersweet *Celastrus orbiculatus* thrive in a dense layer beneath the tree canopy. Researchers at Laudholm Farm have demonstrated that forest stands with Japanese barberry had a higher density of *I. scapularis* ticks (Lubelczyk et al. 2004, Elias et al. 2006) and higher prevalence of DTV than forest stands with native understory shrub species (Robich et al., in preparation).

Sampling grids

In 2020, one grid was established in each of three forest stand habitat types at WNERR: highly invasive understory, invasive understory, and native understory (Figure 1) (Figure 2). The siting of the grids was based on transect sampling in 2018-2019 which yielded more DTV-positive ticks from transects that ran through invasive understory than native understory. The highly invasive and invasive grids were in forest stands comprised of invasive barberry and honeysuckle. The native grid served as a reference grid. The highly invasive and native grids were 70 m x 70m grids, each containing 49 10m x 10m plots with flags at plot centers. Due to space constraints the invasive grid was a 70m x 60m grids, containing 42 10m x 10m plots. Plots were designated according to a letter/number system, e.g., A1, G7.

Figure 1: 2020 tick sampling grid locations within the Wells National Estuarine Research Reserve (WNERR) in Wells, ME. The highly invasive understory habitat is outlined in red, in invasive understory habitat in yellow and the native understory habitat in green. Blue dots represent locations of Hobo data loggers.

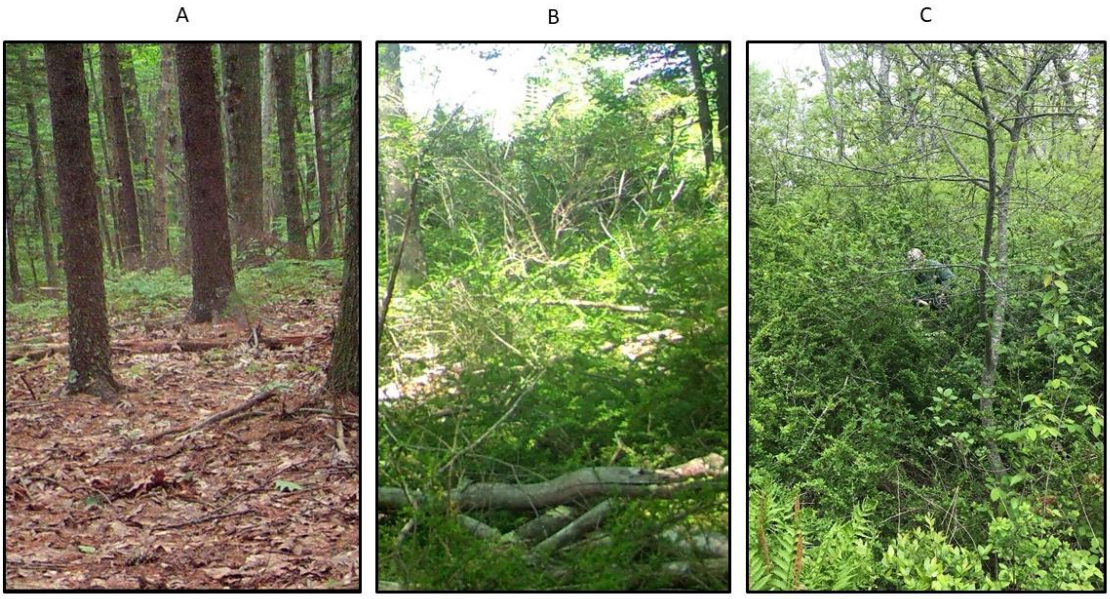

Figure 2: (A) Native grid: understory consists of lowbush blueberries and saplings (B) Invasive grid: medium sized fallen branches for reference (C) Highly invasive grid: human of average height in the center for height reference.

Plant survey and site description

A plant survey was conducted on 29 August 2020 to identify and characterize tree and shrub species in each plot of all three grids. Observations were made from each flag looking inward to the 10m/10m section of the grid from the Southeast corner. In each grid plot, I measured the average height of each shrub species and checked for dominance (i.e., > 70% of vegetation present in the space). Height of Japanese barberry was considered in one of 4 categories: <0.5m, 0.5-1.0m, 1.0-1.5m and >1.5m. Leaf litter at each station was recorded as either deciduous, coniferous, mixed, or bare. Duff layer depth was measured in cm and soil moisture was measured with a Lincoln Soil Moisture Meter inserted 2-3 cm into the ground. For the vegetation survey I identified the three most prevalent shrub species in each location. Trees in all stations were cataloged. Percent canopy cover was obtained by taking images of the tree canopy at 109 cm with an LQ G7 Think phone at the four cardinal directions at each station and then averaged. Care was taken to ensure that the camera was held level for each picture. The images were analyzed using Fiji ImageJ software (Schindelin et al., 2019). Each image was converted to black and white, and the percent black was recorded, with black assigned to the tree canopy and white to the sky.

Microclimate

Temperature and humidity data were collected each hour using Hobo data loggers (Onset Pro v2 #U23-001, Cape Cod, MA) placed in 4 locations within each grid at ground level and 1 m. A data logger was placed at the center of each grid (plot D4) and at 3 other locations based on

the most representative vegetation (Figure 1). The highly invasive grid had loggers set at plots D4, B7, C5, and between plots C6 and C7. The invasive grid had loggers set up a plots D4, D1, A4, and F3 and the native grid had loggers at plots D4, B2, C6 and F4. Loggers recorded data from 20 June 2020 through 7 Jan 2021. Logger data were downloaded and imported into R for further analysis. Saturation deficit was calculated from temperature and relative humidity (Randolph & Storey, 1999).

Questing tick survey

Each grid was sampled once per week, weather permitting, and all transects were sampled between 0800 and 1500 hrs. Host seeking *I. scapularis* and *Dermacentor variabilis* ticks were collected using a $1m^2$ white corduroy flag. Flags were connected to a 1 m wooden pole and pulled along the shrubs at a steady rate ensuring maximum contact between the cloth and the ground. Flags were checked for ticks at intervals of 10 m and 30 sec flag time. Ticks were collected alive and stored in labelled vials with plaster of Paris bases to retain humidity. Ticks were held at 4 °C until species was identified according to published keys (Keirans & Durden, 2005). After identification, ticks were frozen at -80°C individually in 2 mL sterile micro centrifuge tubes. Larvae were pooled in groups of up to 50 larvae per tube. All *I. scapularis* samples were labeled with life stage, drag, location and date. I used G-power to estimate a sample size for a pairwise Fishers exact test to compare the infection rates between grids. I am anticipating a focality to have roughly a 3% infection rate and a control site to have roughly a 1% infection rate. I will need 1,332 ticks of each life stage at an 80% power.

Small mammal abundance and feeding tick survey

All animals were captured and handled according to animal use protocols approved by Main Medical Centers Institutional Animal Care and Use Committee (#1604). Small mammals

were trapped in both invasive forest grids and checked for ticks using Sherman traps (3310A H.B. Sherman Traps, Inc. Tallahassee, Fl) baited with peanuts as well as apples on warmer days. All traps were placed within 1 m of a flag marker to provide stable footing for the entering animal and cover from the elements. Traps were removed from the field between 0700 and 0900 hrs. and returned to a shaded work station for processing. Pregnant animals were handled first and returned to their exact collection location immediately after processing. Traps were then set between 1400 and 1600 hrs for the following trap night. Trapping was completed on two consecutive traps nights every other week. Traps were not set on days where the high temperature was forecasted to be above 30°C. All mammals were processed for body length and weight, age, and sex. Ticks were removed with forceps by grasping the mouthparts and pulling straight out. Animals were tagged in the ear and released within 5 m of where they were captured. Ticks were collected and held at 4°C for identification to species and then stored at -80°C until RNA extraction as described above.

Medium and large mammal survey

Camera traps were used to determine the diversity of medium and large mammals in the grids (Linske et al., 2018). Motion triggered camera traps (Moultrie M-880, #MCG-12691, Calera, AL) were placed 1 m above ground in four locations of each grid facing a clearing and at the edge of the salt marsh pointing inward to capture anything moving into and out of the forest. Camera traps were operated for a total of 30 trap nights during the summer.

Virus testing

Individual adults, nymphs, and pools of larval ticks were first crushed using a sterile paperclip (5cm size, ACCO Brands, Booneville, MS). Samples were homogenized in 200 ml minimum essential medium (MEM) reagent by pulse vortexing with 3 stainless steel BBs.

Aliquots (14 ml) of each individual homogenate was combined in pools of up to 10 questing ticks or ticks from 10 animals (140ml). Ten ticks were removed from -80°C, processed into pools and then returned to the freezer. RNA was extracted using a QIAmp Viral RNA Mini Kit (Qiagen, Germantown MD). RNA was stored at -20°C for same day RT-PCR or returned to the -80°C for reverse transcription polymerase chain reaction (RT-PCR) on another day. RT-PCR was used to detect POWV complementary DNA (from either Powassan virus prototype or DTV) and then submitted to Anne Piantadosi, M.D., Ph.D at the Department of Pathology and Laboratory Medicine at Emory University in Atlanta, GA for full genome sequencing for confirmation and lineage typing. POW-blue f [5'AATCCTGTGTGACATCGGGG3'] and POW-blue r [5'CCAGAGCTGCGTTGGATCTC3'] primers (Robich et al., 2019) were used to amplify an 806 bp region of the nonstructural protein gene (*NS-5*) using the Superscript III Taq Polymerase One-Step RT-PCR System (Thermo Fisher). Cycling conditions included 50°C reverse transcription for 30 min, 94°C initial denature for 2 min, 94°C denature for 15 sec, 56°C annealing for 30 sec, 68°C elongation for 55 sec and a final extension at 68°C for 5 min. Steps 3-5 were repeated 39 times. PCR products were run on a 1% agarose gel for 1.5 hrs at 100 Hz and then imaged. The positive control for the RT-PRC was a 1:100 dilution of DTV isolated from *I. scapularis* collected in North Branford, CT and provided by the Connecticut Agricultural Experiment Station.

Data Analysis

Data analyses were conducted in R (R Core Team 2021) including the following packages: ggplot2 (Wickham, 2016), lubridate (Grolemund & Wickham, 2011), dplyr (Wickham et al., 2021) for data management, emmeans (Lenth, 2020) for post hoc comparisons, and brglm2 (Kosmidis, 2021). The basis sampling unit was the plot. Ticks per hour was summarized across

the entire study period (June 13- November 5) by species (*I. scapularis*, *D. variabilis*), life stage (nymph/adult), and plot within habitat type (grid) and graphed. Questing *I. scapularis* nymphal and adult abundance was calculated for 2-week intervals for each plot for their respective peak season (nymphs, June-July, and adults, October-November). A Poisson regression model was used to make pairwise comparisons in questing nymphal and adult *I. scapularis* abundance by habitat (grid); the model included a categorical fixed effect of 2-week interval, and used an offset of the log of the minutes of sampling time to adjust for slight variation in sampling effort.

A logistic model was used to compare the infection rate of adult ticks during the fall adult peak (October-November) between the highly invasive grid and invasive grid. Since none of 19 ticks collected from the native forest habitat grid were positive for DTV, we used bias-reduced logistic regression via the brglm2 package (Kosmidis, 2021). Small mammal abundance was determined as minimum number alive per 100 trap nights by grid. Attached larvae and nymph infestation rates as well as attached tick burden by grid and season are listed in table 1 and table 2. To determine the diversity and variation of mammal populations collected from each grid, the overall proportion of mammal species caught by grid were compared using a chi-squared test to the ratios of chipmunks, white footed mice and red-backed voles.

Analysis of variance was used to compare the mean effect of grid for several vegetation and abiotic factors including height of invasive barberry, percent canopy cover, variation in saturation deficit, and dominance of invasive species. Significance was determined at the $\alpha = 0.05$ level. A linear model was used to compare the percent canopy cover at the grid level with each individual station as a replicate measurement. Post-hoc pairwise tests with a Tuckey correction were used to compare differences by grid.

Saturation deficit was calculated from the temperature and relative humidity as a single

measure of the drying power of the atmosphere according to Randolph 1999. The data were divided into nymphal peak (6/17/2020 – 8/15/2020) and adult peak (10/01/2020 – (11/15/2020) time periods. A linear model was used to compare the mean saturation deficit at the ground level in the center of each grid with date as a covariate. Post-hoc pairwise tests with a Tukey correction were used to compare differences by grid. Stations in the salt marsh with no canopy cover were not included in this analysis.

Results

Questing ticks

Overall, I collected 474 *I. scapularis* and 16 *Dermacentor variabilis* ticks from 13 June to 5 November 2020 by flagging technique (Figure 4). This included 20 *I. scapularis* larvae, 77 nymphs, 174 female adults and 203 male adults as well as 2 *D. variabilis* nymphs, 8 female adults and 6 male adults. The abundance of questing *I. scapularis* nymphs and adults in the native grid was significantly lower than in either the highly invasive or invasive grids; nymphs ($Z = 8.517$, $P < 0.0001$, and $Z = 7.487$, $P < 0.0001$ respectively) adults ($Z = 80517$, $P < 0.0001$, and $Z = 7.487$, $P < 0.0001$ respectively). The abundance of questing *I. scapularis* nymphs and adults was not different between the highly invasive and the invasive grids, ($Z = -0.818$, $P = 0.413$ and $Z = 1.476$, $P = 0.3027$). Questing adult ticks in the highly invasive grid had higher rates of DTV (5.67%) than ticks from the invasive grid (1.01%), ($Z = 2.035$, $P = 0.0419$) (Figure 3).

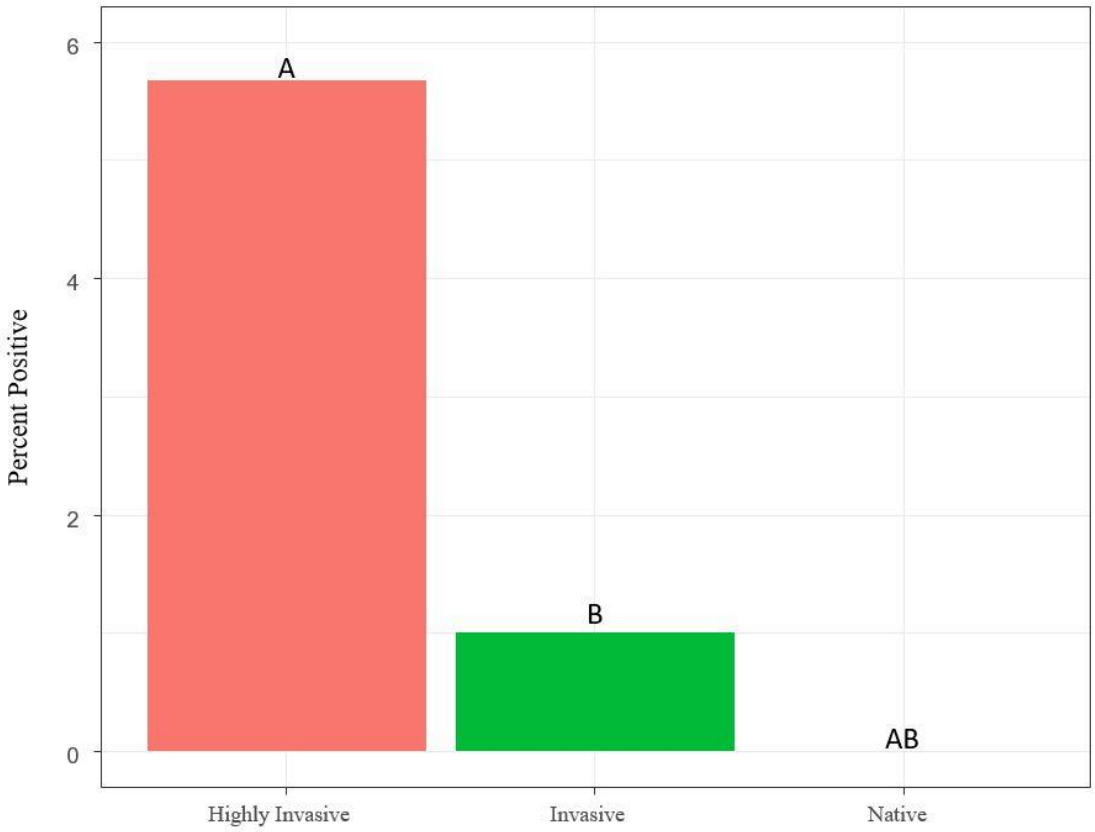

Figure 3: Percent of adult questing *I. scapularis* ticks positive with DTV RNA by grid location.

Highly invasive (n=213) – Invasive (n=111) Z= 2.035, P=0.0419.

Table 1: Location and sex of infected adult *I. scapularis* ticks

Tick ID	Grid	Station	Sex
299P1	Highly Invasive	D6	F
299P2	Highly Invasive	D6	F
299P3	Highly Invasive	D6	M
299S1	Highly Invasive	E6	F
338L1	Highly Invasive	C3	F
338L2	Highly Invasive	C3	F
339M1	Highly Invasive	C5	F
338S2	Highly Invasive	E4	F
338BB2	Highly Invasive	G6	F
422F1	Highly Invasive	B2	F
422F3	Highly Invasive	B2	F
422I1	Highly Invasive	B5	F
422Q2	Highly Invasive	E3	M
337X5	Invasive	G5	M

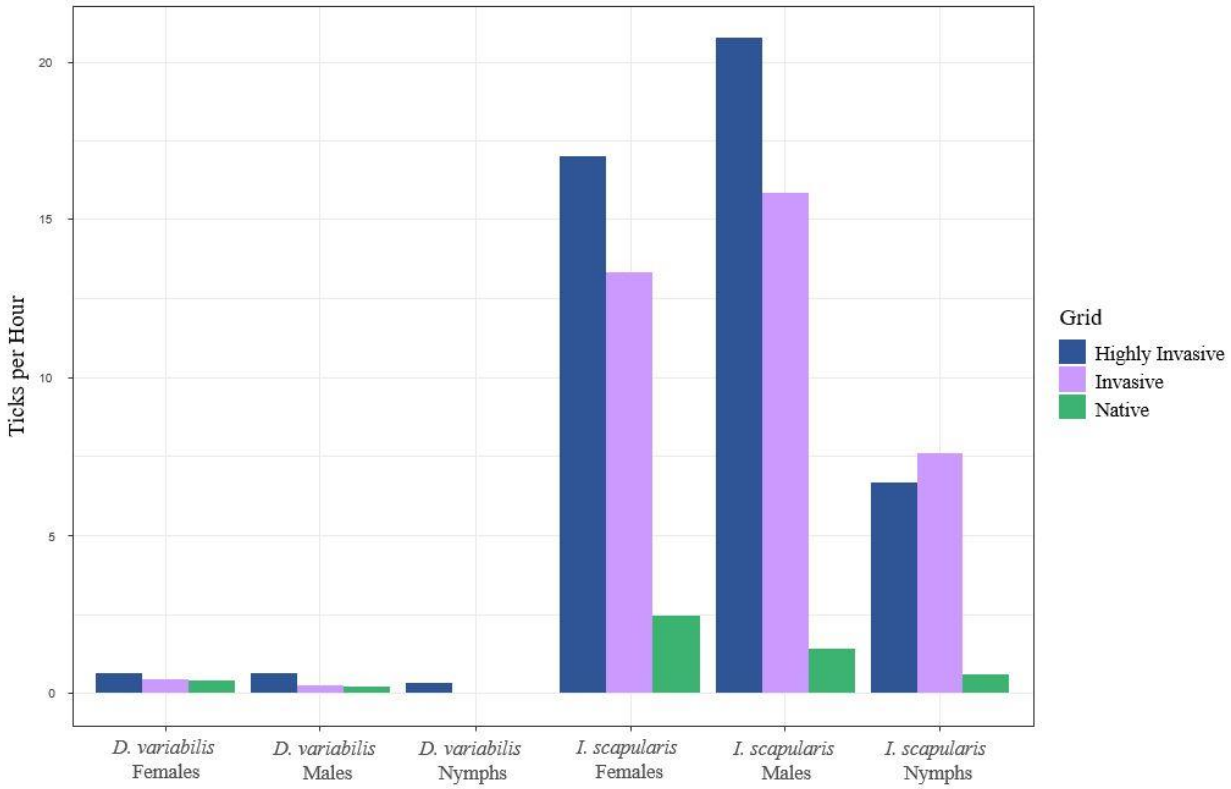

Figure 4. Questing ticks per hour collected by flagging from 13 June to 5 November 2020. *D. variabilis (*n=8 female, 6 male, 2 nymph); *I. scapularis* (n= 174 female, 203 male, 77 nymph, 20 larvae)

Small mammal abundance, and tick infestation rate and burden

Comparing the small mammal host abundance in the highly invasive grid and the invasive grid, there were significantly more chipmunks captured in the invasive grid ($\chi 2 = 28.3$, df = 1, P-value < 0.0001), more white-footed mice in the highly invasive grid, ($\chi 2 = 5.9$, df = 1, P-value = 0.01) and no difference in voles caught between grids ($\chi 2 = 0.0006$, df = 1, P-value = 0.98). Ratio of infested animals was calculated as the number of a species with ≥ one tick divided by the total number of that species in each study site. Tick burden was calculated as the number of individuals of the mammals divided by the number of ticks from each life stage

removed (Rehman et al., 2017). DTV was not detected in any of the 711 larvae collected from 185 individual mammals or the 73 nymphs collected from 50 individual animals.

Table 2. Species of mammals captured between June 10th and September 16th 2020 in the highly invasive trapping grid at WNERR

*Percent of animals found to have ≥ 1 tick feeding on them at the time of capture.

Species	No. individuals	*I. scapularis* larva burden	*I. scapularis* nymph burden	Percent infested*	No. DTV positive pools
Short-tailed shrew *Blarina brevicauda*	7	0	0	0%	0
Southern flying squirrel *Glaucomys Volans*	1	–	–	0%	0
Meadow Vole *Mictrotus pennsylvanicus*	1	10.00	0	100%	0
Ermine *Mustela erminea*	1	2.00	0	100%	0
Long-tailed weasel *Mustela frenata*	1	–	–	–	0
Red backed vole *Myodes gapperi*	40	0.20	0.18	25%	0
White-footed mouse *Peromyscus Leucopus*	157	1.54	0.07	48%	0
Norway rat *Rattus norvegicus*	1	0	0	0%	0
Masked shrew *Sorex cinereus*	3	0	0	0%	0
Red Squirrel *Tamiasciurus hudsonicus*	1	3	1	100%	0
Chipmunk *Tamias striatus*	2	9	1.50	100%	0

Table 3. Species of mammals captured between June 10th and September 16th 2020 in the invasive trapping grid at WNERR

*Percent of animals found to have ≥ 1 tick feeding on them at the time of capture.

Species	No. individuals	*I. scapularis* larvae burden	*I. scapularis* nymph burden	Percent infested*	No. DTV positive pools
Short-tailed shrew *Blarina brevicauda*	4	0.50	0	25%	0
Southern flying squirrel *Glaucomys Volans*	3	0	0.50	25%	0
Meadow Vole *Mictrotus pennsylvanicus*	0	–	–	–	0
Ermine *Mustela erminea*	1	1	1	100%	0
Long-tailed weasel *Mustela frenata*	0	–	–	–	0
Red backed vole *Myodes gapperi*	41	0.27	0.02	24%	0
White-footed mouse *Peromyscus Leucopus*	140	2.88	0.14	69%	0
Norway rat *Rattus norvegicus*	0	–	–	–	0
Masked shrew *Sorex cinereus*	1	0	0	0%	0
Red Squirrel *Tamiasciurus hudsonicus*	1	0	0	0%	0
Chipmunk *Tamias striatus*	35	0.27	0.73	51%	0

Camera traps

I captured white tailed deer in all three grids and moving in and out of the highly invasive grid via the salt marsh. Coyotes were only captured in the native grid. Because deer were present in all grids, they were not included in my final analysis.

Microclimate and forest structure

Mean saturation deficit (Figure 5) varied between the grids during the nymphal peak with the native grid being the highest (model predicted $\bar{x}$ =2.1, SE = 0.056) followed by the invasive

grid (model predicted $\bar{x}$ = 1.5, SE = 0.056) and the highly invasive grid had the lowest (model predicted $\bar{x}$ = 0.8, SE = 0.057). All grids were significantly different from each other (P<0.0001 for all) during the adult peak, the mean saturation deficit of the native grid (model predicted $\bar{x}$ = 0.8, SE = 0.043) was significantly higher than highly invasive grid ($\bar{x}$ = 0.6, SE = 0.043; t_{88}=-3.316, p=0.0038). The moderately invasive grid did not vary from the other two ($\bar{x}$ = 0.7, SE = 0.043). Percent canopy cover differed among grids (Figure 6) (P<0.001 for all) with the native grid having the highest most consistent cover (model predicted $\bar{x}$ = 72.7%, SE = 1.49), the invasive grid in the middle (model predicted $\bar{x}$ = 64.1%, SE = 1.61), and the highly invasive grid with the lowest percent canopy cover (model predicted $\bar{x}$ = 51.6%, SE = 1.49).

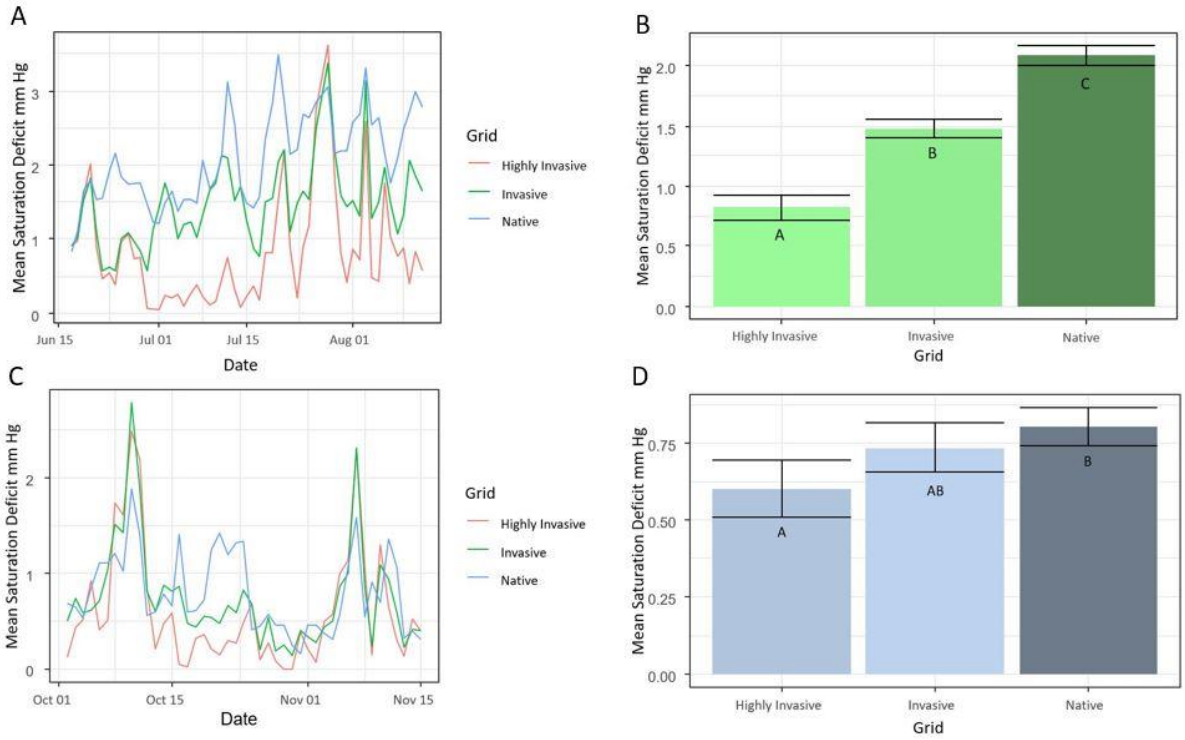

Figure 5: Mean saturation deficit measured at the center station 0m (D4) ground level of all three sampling grids. **A.** Daily average shown over time throughout the nymphal peak (6/17/2020 – 8/15/2020). **B.** Mean daily saturation deficit average from 6/17/2020 – 8/15/2020

ANOVA, 106 df, p <.0001. **C.** Daily average shown over time throughout the adult peak (10/01/2020 – (11/15/2020). **D.** Mean daily saturation deficit average for adult peak, ANOVA 88 df, p > 0.05, highly invasive - native, p= 0.0038.

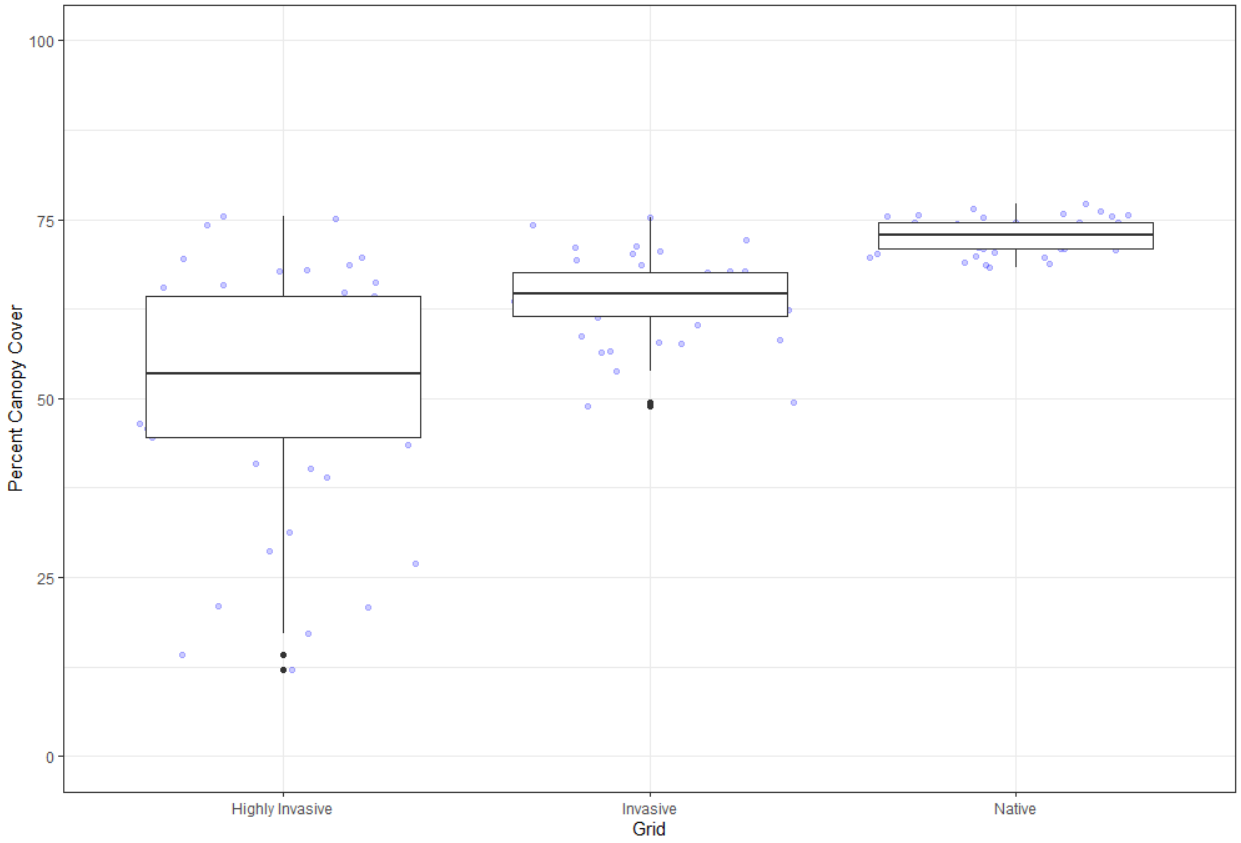

Figure 6: Percent canopy cover by grid. All comparisons P<0.001. Highly invasive grid (model predicted $\bar{x}$ = 51.6%, SE = 1.49). Invasive grid (model predicted $\bar{x}$ = 64.1%, SE = 1.61). Native grid (model predicted $\bar{x}$ = 72.7%, SE = 1.49).

Discussion

In my study I tested the hypothesis that DTV is maintained in small microhabitats (foci) where environmental factors such as invasive shrub understory and abundant tick hosts sustain virus persistence over time. To test this hypothesis, I collected data on tick and host species abundance, vegetation characteristics, and microclimate. Consistent with my hypothesis, I found

that DTV infection rate was higher in the forest grid highly invaded by Japanese barberry compared to forest grids moderately or with native understory vegetation only. Higher density of vector *I. scapularis* was associated with Japanese barberry invasion and higher white-footed mouse abundance and lower saturation deficit were associated with the highly invaded site. I demonstrated a difference in host species assemblages among the grids with more white footed mice and fewer chipmunks harbored in the grid with high invasive shrub and DTV infections. These findings are consistent with the theory of nidality (focality) of vector-borne infection in which zoonotic agents are maintained through an ideal assemblage of pathogen, hosts and habitat (Goethert and Telford, 2009).

Japanese barberry was the most dominant species in both invasive shrub grids. Prior work in coastal Maine has shown that Japanese barberry supports a greater abundance of questing *I. scapularis* ticks (Lubelczyk et al., 2004). Nymphs of this species were twice as abundant and adults between 2.8 and 16.3 times more abundant in barberry than in other native forest types (Elias et al., 2006). Abundance of *I. scapularis* from 2020 at WNERR is consistent with these past studies. Abundance in the native grid was significantly lower than the invasive grids and the invasive grids did not differ significantly from each other for either nymphal or adult peaks. This is evidence that tick abundance alone may not determine DTV focality, but that host and habitat configuration is also important.

The microclimate within a dense Japanese barberry stand can maintain a more suitable microclimate for tick survival. This insulating effect may also impact viral replication and ultimately, degree of vector competence of *I. scapularis*. The effect of temperatures in the high range can impact *I. scapularis* competence to transmit *Borrelia* (Estrada-Peña et al., 2011, Shih et al., 1995). Temperature has also been identified as an important factor in vector competence in

multiple mosquito-virus transmission dynamics (Reisen et al., 1993, Vogels et al., 2016, Carrington et al., 2013). A key result of my study was the difference in saturation deficit over time between invasive grids. The DTV focality grid had higher moisture levels as evidence by saturation deficit readings than the other two grids, especially during the nymphal peak and to a lesser extent during the adult peak. This highlights the potential microclimate characteristics of tall impenetrable barberry and the impact it may have on DTV prevalence. It is notable that despite lower percent canopy cover, which one might think would lead to desiccating conditions, Japanese barberry maintained lower saturation deficit. Future studies are warranted to investigate the modulating impact of invasive shrub microhabitats during weather extremes on *I. scapularis* viability and DTV infection rates.

Japanese barberry may also provide structure and protection to important amplifying host species such as white footed mice (Piesman & Spielman, 1979). Invasive plants have been shown to alter the behavior of primary consumers because of the protection provided (Dutra et. al., 2011). In our study larger carnivores such as coyotes were trapped by camera only in the native grid with no protective understory. Smaller carnivores such as ermine and long tailed weasel were captured in Sherman traps and present in both invasive grids. While we did not capture enough carnivores to assess their population size, it is possible that predator species such hawks and owls have less access to small carnivores in dense barberry. These animals are difficult to capture but may play a role in amplifying DTV through cofeeding by hosting multiple life stages of *I. scapularis* at once.

Cofeeding is thought to be important factor in multiple viral and bacterial pathogen transmission cycles (Randolph et al., 1996). The mechanisms of transmission through cofeeding as reviewed by Randolph 2011 include leukocytes carrying infective particles between feeding

sites and efficient transmission of TBE through cofeeding has been demonstrated previously (Labuda et al., 1993). Microclimate could play a role in enhancing in the likelihood that multiple tick life stages cofeeding on small host animals. For example ratios of larvae to nymphs feeding on animals can be affected by humidity (Randolph et al., 1999). This may impact pathogen amplification in micro sized habitats. While I did not see a significant difference in vole populations between invasive grids, the voles from the high DTV grid had a higher burden of *I. scapularis* nymphs. In addition, dominant barberry may create an impenetrable barrier for larger host species creating a situation where nymphal and adult ticks are more likely to feed together on small-medium sized hosts.

DTV RNA was not detected in replete *I. scapularis* larvae collected from trapped animals. Thus, we can conclude only that none of the trapped animals were positive for DTV at the time of capture and cannot infer past infections. Testing ticks attached to hosts is not a sensitive method of reservoir animal detection as the detectible window of DTV viremia is short (3-10 days) and shorter than our sampling intervals of every two weeks.

No DTV positive questing ticks were found in the native grid and tick abundance was too low in this location to make conclusions about the potential infection level. The combination of limited shrub cover and the presence of coyotes in the native grid may have created an unfavorable environment for *I. scapularis* ticks. A substantial understory would provide small host species refuge from larger carnivores. It is known that tick survival drops significantly in the absence of humidity (Stafford, 1994). Thus, the absence of an insulating shrub-cover could mean an irregular level of humidity needed for questing ticks. In dry conditions fewer larvae can be found feeding on small animals preferring instead to stay in the soil where moisture is maintained (Randolph et al., 1999).

Limitations of my study include the fact that I was only able to investigate one DTV focality during the summer of 2020. In that year, the region experienced an abnormally hot dry summer and low nymphal abundance (UMaine, 2020). Multiyear studies are necessary to determine if my results reflect consistent patterns of DTV in Maine. Another limitation was that I did not capture medium sized mammals such as striped skunks and groundhogs, although they may represent important hosts. However, the Wells National Estuarine Research Reserve is an important refuge for the vulnerable New England cottontail (*Sylvilagus transitionalis*) so efforts to trap and process larger mammals would need to be undertaken with an abundance of caution. Future efforts to study medium sized mammals could rely on baited camera trapping or burrow disruption to avoid unwanted impacts to the rehabilitation of cottontails.

Phylogenetic analysis of the samples collected at WNERR will be important to determine if the DTV strains are consistent with what would be expected for long term DTV focality in this location. Prior phylogenetic analysis has supported DTV focality in WNERR and in Wisconsin and Connecticut (Robich et al., 2019, Brackney et al. 2008, Anderson & Armstrong, 2008); suggesting that this is a broad feature of DTV.

Altogether, my research highlights key habitat features, abiotic conditions, and animal associations of a consistent DTV focus in Maine. Understanding environmental and landscape features that support high infection rates could lead to the identification of high risk habitats for contracting this emerging tick borne virus.

References

Anderson, J. F., & Armstrong, P. M. (2012). Prevalence and Genetic Characterization of Powassan Virus Strains Infecting Ixodes scapularis in Connecticut. *The American Journal of Tropical Medicine and Hygiene*, *87*(4), 754–759. https://doi.org/10.4269/ajtmh.2012.12-0294

Apanaskevich, D., & Oliver Jr., J. H. (2014). Life Cycles and Natural History of Ticks. In Daniel Sonenshine & Michael Roe (Eds.), *Biology of Ticks* (Second Edition). Oxford University Press.

Brackney, D. E., Nofchissey, R. A., Fitzpatrick, K. A., Brown, I. K., & Ebel, G. D. (2008). Stable Prevalence of Powassan Virus in Ixodes scapularis in a Northern Wisconsin Focus. *The American Journal of Tropical Medicine and Hygiene*, *79*(6), 971–973. https://doi.org/10.4269/ajtmh.2008.79.971

Carrington, L. B., Seifert, S. N., Armijos, M. V., Lambrechts, L., & Scott, T. W. (2013). Reduction of Aedes aegypti Vector Competence for Dengue Virus under Large Temperature Fluctuations. *The American Journal of Tropical Medicine and Hygiene*, *88*(4), 689–697. https://doi.org/10.4269/ajtmh.12-0488

Dupuis II, A. P., Peters, R. J., Prusinski, M. A., Falco, R. C., Ostfeld, R. S., & Kramer, L. D. (2013). Isolation of deer tick virus (Powassan virus, lineage II) from Ixodes scapularis and detection of antibody in vertebrate hosts sampled in the Hudson Valley, New York State. *Parasites & Vectors*, *6*(1), 185. https://doi.org/10.1186/1756-3305-6-185

Dutra, H. P., Barnett, K., Reinhardt, J. R., Marquis, R. J., & Orrock, J. L. (2011). Invasive plant species alters consumer behavior by providing refuge from predation. *Oecologia*, *166*(3), 649–657. https://doi.org/10.1007/s00442-010-1895-7

Ebel, G. D., Campbell, E. N., Goethert, H. K., Spielman, A., & Telford, S. R. (2000). Enzootic transmission of deer tick virus in New England and Wisconsin sites. *The American Journal of*

Tropical Medicine and Hygiene, *63*(1–2), 36–42. https://doi.org/10.4269/ajtmh.2000.63.36

Ebel, G. D., Foppa, I., Spielman, A., & Telford, S. R. (1999). A focus of deer tick virus transmission in the northcentral United States. *Emerging Infectious Diseases*, *5*(4), 570–574.

Ebel, Gregory D. (2010). Update on Powassan Virus: Emergence of a North American Tick-Borne Flavivirus. *Annual Review of Entomology*, *55*(1), 95–110. https://doi.org/10.1146/annurev-ento-112408-085446

Elias, S. P., Lubelczyk, C. B., Rand, P. W., Lacombe, E. H., Holman, M. S., & Smith, R. P. (2006). Deer browse resistant exotic-invasive understory: An indicator of elevated human risk of exposure to Ixodes scapularis (Acari: Ixodidae) in southern coastal Maine woodlands. *Journal of Medical Entomology*, *43*(6), 1142–1152. https://doi.org/10.1603/0022-2585(2006)43[1142:dbreua]2.0.co;2

Estrada-Peña, A., Ortega, C., Sánchez, N., DeSimone, L., Sudre, B., Suk, J. E., & Semenza, J. C. (2011). Correlation of Borrelia burgdorferi Sensu Lato Prevalence in Questing Ixodes ricinus Ticks with Specific Abiotic Traits in the Western Palearctic ▿ . *Applied and Environmental Microbiology*, *77*(11), 3838–3845. https://doi.org/10.1128/AEM.00067-11

Garrett Grolemund, Hadley Wickham (2011). Dates and Times Made Easy with lubridate. Journal of Statistical Software, 40(3), 1-25. URL https://www.jstatsoft.org/v40/i03/.

Goethert, H. K., & Telford, S. R. (2009). Nonrandom Distribution of Vector Ticks (Dermacentor variabilis) Infected by Francisella tularensis. *PLoS Pathogens*, *5*(2), e1000319. https://doi.org/10.1371/journal.ppat.1000319

H. Wickham. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York, 2016

Hadley Wickham, Romain François, Lionel Henry and Kirill Müller (2021). dplyr: A Grammar of Data Manipulation. R package version 1.0.4. https://CRAN.R-project.org/package=dplyr

Hermance, M. E., & Thangamani, S. (2017). Powassan Virus: An Emerging Arbovirus of Public Health Concern in North America. *Vector-Borne and Zoonotic Diseases*, *17*(7), 453–462. https://doi.org/10.1089/vbz.2017.2110

Keirans, J. E., & Durden, L. A. (2005). Tick Systematics and Identification. In *Tick-Borne Diseases of Humans* (pp. 123–140). John Wiley & Sons, Ltd. https://doi.org/10.1128/9781555816490.ch7

Kemenesi, G., & Bányai, K. (2018). Tick-Borne Flaviviruses, with a Focus on Powassan Virus. *Clinical Microbiology Reviews*, *32*(1). https://doi.org/10.1128/CMR.00106-17

Knox, K. K., Thomm, A. M., Harrington, Y. A., Ketter, E., Patitucci, J. M., & Carrigan, D. R. (2017). Powassan/Deer Tick Virus and Borrelia Burgdorferi Infection in Wisconsin Tick Populations. *Vector-Borne and Zoonotic Diseases*, *17*(7), 463–466. https://doi.org/10.1089/vbz.2016.2082

Kosmidis I (2021). _brglm2: Bias Reduction in Generalized Linear Models_. R package version 0.7.1, <URL: https://CRAN.R-project.org/package=brglm2>.

Kuno, G., Artsob, H., Karabatsos, N., Tsuchiya, K. R., & Chang, G. J. (2001). Genomic sequencing of deer tick virus and phylogeny of powassan-related viruses of North America. *The American Journal of Tropical Medicine and Hygiene*, *65*(5), 671–676. https://doi.org/10.4269/ajtmh.2001.65.671

Labuda, M., Jones, L. D., Williams, T., Danielova, V., & Nuttall, P. A. (1993). Efficient Transmission of Tick-Borne Encephalitis Virus Between Cofeeding Ticks. *Journal of Medical Entomology*, *30*(1), 295–299. https://doi.org/10.1093/jmedent/30.1.295

Linske, M. A., Stafford, K. C., Williams, S. C., Lubelczyk, C. B., Welch, M., & Henderson, E. F. (2019). Impacts of Deciduous Leaf Litter and Snow Presence on Nymphal Ixodes scapularis (Acari: Ixodidae) Overwintering Survival in Coastal New England, USA. *Insects*, *10*(8), 227.

https://doi.org/10.3390/insects10080227

Lubelczyk, C. B., Elias, S. P., Rand, P. W., Holman, M. S., Lacombe, E. H., & Smith, R. P. J. (2004). *Habitat associations of Ixodes scapularis (Acari: Ixodidae) in Maine*. https://pubag.nal.usda.gov/catalog/675601

McLean, Donald M., Best, J. M., Mahalingam, S., Chernesky, M. A., & Wilson, W. E. (1964). Powassan Virus: Summer Infection Cycle, 1964. *Canadian Medical Association Journal*, *91*(26), 1360–1362.

McLean, Donald M., & Larke, R. P. B. (1963). Powassan and Silverwater Viruses: Ecology of Two Ontario Arboviruses. *Canadian Medical Association Journal*, *88*(4), 182–185.

Mlera, L., & Bloom, M. E. (2018). The Role of Mammalian Reservoir Hosts in Tick-Borne Flavivirus Biology. *Frontiers in Cellular and Infection Microbiology*, *8*. https://doi.org/10.3389/fcimb.2018.00298

Nemeth, N. M., Root, J. J., Hartwig, A. E., Bowen, R. A., & Bosco-Lauth, A. M. (2021). Powassan Virus Experimental Infections in Three Wild Mammal Species. *The American Journal of Tropical Medicine and Hygiene*, *104*(3), 1048–1054. https://doi.org/10.4269/ajtmh.20-0105

Piesman, J., & Spielman, A. (1979). Host-Associations and Seasonal Abundance of Immature Ixodes dammini1 in Southeastern Massachusetts2. *Annals of the Entomological Society of America*, *72*(6), 829–832. https://doi.org/10.1093/aesa/72.6.829

R Core Team (2019). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Russell V. Lenth (2020). emmeans: Estimated Marginal Means, aka Least-Squares Means. R package version 1.5.3. https://CRAN.R-project.org/package=emmeans

Randolph, S. E., & Storey, K. (1999). Impact of Microclimate on Immature Tick-Rodent Host

Interactions (Acari: Ixodidae): Implications for Parasite Transmission. *Journal of Medical Entomology*, *36*(6), 741–748. https://doi.org/10.1093/jmedent/36.6.741

Randolph, S. E., Gem, L., & Nuttall, P. A. (1996).Co-feeding Ticks: Epidemiological Significance for Tick-Pathogen Transmission. *Parasitology Today, 12(12)* https://doi.org/10.1016/S0169-4758(96)10072-7.

Randolph, S. E. (2011). Transmission of tick-borne pathogens between co-feeding ticks: Milan Labuda's enduring paradigm. *Ticks and Tick-Borne Diseases*, *2*(4), 179–182. https://doi.org/10.1016/j.ttbdis.2011.07.004

Rehman, A., Nijhof, A. M., Sauter-Louis, C., Schauer, B., Staubach, C., & Conraths, F. J. (2017). Distribution of ticks infesting ruminants and risk factors associated with high tick prevalence in livestock farms in the semi-arid and arid agro-ecological zones of Pakistan. *Parasites & Vectors*, *10*(1), 190. https://doi.org/10.1186/s13071-017-2138-0

Reisen, W. K., Meyer, R. P., Presser, S. B., & Hardy, J. L. (1993). Effect of Temperature on the Transmission of Western Equine Encephalomyelitis and St. Louis Encephalitis Viruses by Culex tarsalis (Diptera: Culicidae). *Journal of Medical Entomology*, *30*(1), 151–160. https://doi.org/10.1093/jmedent/30.1.151

Robich, R. M., Cosenza, D. S., Elias, S. P., Henderson, E. F., Lubelczyk, C. B., Welch, M., & Smith, R. P. (2019). Prevalence and Genetic Characterization of Deer Tick Virus (Powassan Virus, Lineage II) in Ixodes scapularis Ticks Collected in Maine. *The American Journal of Tropical Medicine and Hygiene*, *101*(2), 467–471. https://doi.org/10.4269/ajtmh.19-0281

Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., Schmid, B., Tinevez, J.-Y., White, D. J., Hartenstein, V., Eliceiri, K., Tomancak, P., & Cardona, A. (2012). Fiji: An open-source platform for

biological-image analysis. *Nature Methods*, *9*(7), 676–682. https://doi.org/10.1038/nmeth.2019

Shih, C. M., Telford, S. R., & Spielman, A. (1995). Effect of ambient temperature on competence of deer ticks as hosts for Lyme disease spirochetes. *Journal of Clinical Microbiology*, *33*(4), 958–961.

Smith, K., Oesterle, P. T., Jardine, C. M., Dibernardo, A., Huynh, C., Lindsay, R., Pearl, D. L., Bosco-Lauth, A. M., & Nemeth, N. M. (2018). Powassan Virus and Other Arthropod-Borne Viruses in Wildlife and Ticks in Ontario, Canada. *The American Journal of Tropical Medicine and Hygiene*, *99*(2), 458–465. https://doi.org/10.4269/ajtmh.18-0098

Stafford, K. C. (1994). Survival of Immature Ixodes scapularis (Acari: Ixodidae) at Different Relative Humidities. *Journal of Medical Entomology*, *31*(2), 310–314. https://doi.org/10.1093/jmedent/31.2.310

Subbotina, E. L., & Loktev, V. B. (2012). Molecular evolution of the tick-borne encephalitis and Powassan viruses. *Molecular Biology*, *46*(1), 75–84. https://doi.org/10.1134/S0026893311060148

Telford, S. R., Armstrong, P. M., Katavolos, P., Foppa, I., Garcia, A. S., Wilson, M. L., & Spielman, A. (1997). A new tick-borne encephalitis-like virus infecting New England deer ticks, Ixodes dammini. *Emerging Infectious Diseases*, *3*(2), 165–170. https://doi.org/10.3201/eid0302.970209

University of Maine Tick Surveillance Program—Annual Report—2020. (2020). *Annual Report*, 18.

Vogels, C. B. F., Fros, J. J., Göertz, G. P., Pijlman, G. P., & Koenraadt, C. J. M. (2016). Vector competence of northern European Culex pipiens biotypes and hybrids for West Nile virus is

differentially affected by temperature. *Parasites & Vectors*, *9*(1), 1–7. https://doi.org/10.1186/s13071-016-1677-0

Xu, G., Mather, T. N., Hollingsworth, C. S., & Rich, S. M. (2016). Passive Surveillance of Ixodes scapularis (Say), Their Biting Activity, and Associated Pathogens in Massachusetts. *Vector-Borne and Zoonotic Diseases*, *16*(8), 520–527. https://doi.org/10.1089/vbz.2015.1912