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ABUNDANCE AND DISTRIBUTION OF MEDICALLY IMPORTANT HARD TICKS AND ASSOCIATED IXODES-BORNE PATHOGENS IN RHODE ISLAND

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ABUNDANCE AND DISTRIBUTION OF MEDICALLY IMPORTANT HARD TICKS AND ASSOCIATED *IXODES*-BORNE PATHOGENS IN RHODE ISLAND

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ABSTRACT

Rhode Island has one of the highest per capita rates of human tick-borne disease incidence in the United States. Novel pathogens previously unknown to occur in the Northeastern US, known as emerging tick-borne pathogens (TBP), have expanded beyond their previously established geographic ranges and have become a new threat to public health. These include *Borrelia burgdorferi* and *B. mayonii*, the etiological agent of Lyme disease, *B. miyamotoi* (tick-borne relapsing fever), *Anaplasma phagocytophilum* (Anaplasmosis), *Babesia microti* (Babesiosis), *Ehrlichia muris eauclairensis* (Ehrlichiosis), and Powassan virus. Additionally, hard tick vectors (Acari: Ixodidae) such as *Ixodes scapularis, Amblyomma americanum, Dermacentor variabilis*, and the invasive *Haemaphysalis longicornis* have expanded beyond their previously recognized ranges. Despite this, no attempt has been made to quantify their abundance and distribution in Rhode Island. Additionally, no active surveillance system has quantified the infection rate of emerging TBP's in Rhode Island. Using flag sampling we collected four species of tick vectors to understand their inter-seasonal abundance and distribution. For *I. scapularis*, we tested for 12 pathogen species to understand their prevalence and distribution in local nymphal *I. scapularis* populations. Finally, we analyzed if co-infection between certain pathogens was more likely to occur than singular pathogen rates would suggest. In 2022, we collected 1760 nymphal and 180 adult *I. scapularis*, 131 nymphal and 16 adult *A. americanum*, and zero nymphal and 372 adult *D. variabilis*. In 2023, we found 4803 nymphal and 403 adult *I. scapularis*, 403 nymphal and 40 adult *A. americanum*, two nymphal and 187 adult *D. variabilis,* and seven *H. longicornis* nymphs. Among *I. scapularis* nymphs in 2022, we found infection rates of 16.98% for *B. burgdorferi,* .89% for *B. miyamotoi,*

6.70% for *A. phagocytophilum*, 6.83% for *Ba. microti*, and .25% for Powassan virus. Additionally, we found a significant relationship in co-infection between *B. burgdorferi* and *Ba. microti* but no other significant association between co-infections of other pathogens.

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DEDICATIONS

From the bottom of my heart, from the sincerest and closed-off part of me, I struggle to think of all the people whose hope, joy, anger, pride, one-off gestures, cruelty, indifference, overflowing selflessness, and bottomless kindnesses have defined me as the person I am today. Without them, I know I would be a lesser person.

Thank you to my mother and father, whose support kept me going through school. Thank you to my Aunt Deanie and Uncle Clint for supporting my education. Thank you to Sammy Schofield and Peter Dear for feeding me. Thank you to my friends for listening to two years of constant venting. Thank you to everyone in my life. I don't have the words to say how much I love you.

PREFACE

This thesis is the original, unpublished, and independent work of the author.

TABLE OF CONTENTS

ABSTRACT ii
ACKNOWLEDGEMENTS iv
DEDICATIONS v
PREFACE vi
LIST OF TABLES viii
LIST OF FIGURES ix
CHAPTER 1 1
    BACKGROUND 1
CHAPTER 2 9
    METHODOLOGY 9
CHAPTER 3 13
    FINDINGS 13
CHAPTER 4 27
    CONCLUSIONS 27
BIBLIOGRAPHY 38

LIST OF TABLES

Table 1. Summary of nymphal abundance of *I. scapularis, A. americanum, D. variabilis, and H. longicornis* during Round 1 and Round 2 of 2022 and 2023... 13 -14

Table 2. Summary of adult abundance of *I. scapularis, A. americanum, D. variabilis, and H. longicornis* during Round 1 and Round 2 of 2022 and 2023 14

Table 3. Summary of infection type and occurrence rate of pathogens for *I. scapularis* nymphs in 2022. 19

Table 4. Infection rate of *I. scapularis* nymphs in Rhode Island 2022 for *B. burgdorferi, B. miyamotoi, A. phagocytophilum, Ba. microti,* and Powassan virus. .. 20

Table 5. Summary of probabilistic model for observed co-infections of *I. scapularis* nymphs in Rhode Island in 2022..... 26

LIST OF FIGURES

Figure 1. Encounter rate and distribution of adult and nymphal *I. scapularis* in Rhode Island in 2022 and 2023. Dot size symbology denotes per minute encounter rate..... 16

Figure 2. Encounter rate and distribution of adult and nymphal *A. americanum* in Rhode Island in 2022 and 2023. Dot size symbology denotes per minute encounter rate..... 18

Figure 3. Site level of prevalence of *B. burgdorferi* and *B. miyamotoi* in *I. scapularis* nymphs across round 1 and round 2 in 2022. Dot color denotes prevalence of pathogen, with white meaning no infection to dark red representing high pathogen prevalence and dot size symbology denotes # of *I. scapularis* nymphs detected..... 22

Figure 4. Site level of prevalence of *Ba. microti* and *A. phagocytophilum* in *I. scapularis* nymphs across round 1 and round 2 in 2022. Dot color denotes prevalence of pathogen, with white meaning no infection to dark red representing high pathogen prevalence and dot size symbology denotes # of *I. scapularis* nymphs detected..... 23

Figure 5. Site level of prevalence of Powassan virus in *I. scapularis* nymphs across round 1 and round 2 in 2022. Dot color denotes prevalence of pathogen, with white meaning no infection to dark red representing high pathogen prevalence and dot size symbology denotes # of *I. scapularis* nymphs detected. 24

Figure 6. Site level of prevalence of co-infections in *I. scapularis* nymphs across round 1 and round 2 in 2022. Dot color denotes prevalence of pathogen, with white meaning no infection to dark red representing high pathogen prevalence and dot size symbology denotes # of *I. scapularis* nymphs detected. 25

Figure 7. Historical data of annual I. scapularis nymphal abundance from 1994 to

2014. Includes abundance data we generated in 2022 and 2023. Only sites we sampled in 2022 and 2023 were used for comparison. 27

CHAPTER 1

BACKGROUND

Tick-borne pathogens (TBP) are the largest contributor to human vector-borne disease cases in North America. These includes a variety of zoonotic diseases such as Rocky Mountain Spotted Fever (RMSF), Tularemia, and Anaplasmosis with the single largest TBD burden, in human cases, being Lyme disease (*Borrelia burgdorferi*), vectored by *Ixodes scapularis* (Say) (Rodino et al. 2020). In 2019, the Centers for Disease Control and Prevention (CDC) reported that of the 50,865 cases of TBD recorded nationwide by health departments approximately 30,000 of those were Lyme disease (Schwartz et al. 2017). However, the CDC estimates more than over 400,000 people are treated annually for Lyme disease (Schwartz, et al. 2021). The expansion of Lyme disease is suspected to primarily be driven by the expansion of its principal host, *Ixodes scapularis* (Say).

The expansion and increased human burden of *I. scapularis* has been driven by large-scale land-use modification, climate variables, and increased host availability (Ogden et al. 2021, Ogden et al. 2013, Diuk-Wasser et al. 2021). In the last 100 years as the habitat of the American Northeast and Upper Midwest has switched from open fields to second-growth forests, it has created suitable microhabitat for *I. scapularis*. As forests have reclaimed these areas it has also led to an increase of available food resources allowing the principal hosts for both pathogens and *I. scapularis* to spread as well. These include the white-footed mouse (*Peromyscus leucopus)*, required for pathogen cycling in nature, and White-tailed deer (*Odocoileus virginianus*), important for successful reproduction for adult *I. scapularis* (Wilson et al. 1985, Goodwin et al.

2001). Climate variables such as ambient temperature and humidity have been found to have a positive correlation with *I. scapularis* nymphal and adult densities. In areas with high vegetation density and species variety, *I. scapularis* has been found to increase in both adult and nymph densities (Ginsberg et al. 2020). Ticks require high percent relative humidity (% RH) due to a thin cuticle to allow for engorgement when feeding. High temperatures are required to compensate for ectothermic physiology (Lindsay et al.1999, Lubelczyk et al. 2019). Temperature and % RH have been found to impact the abundance of nymphal *I. scapularis* that are host-seeking, i.e., 'questing' above the leaf litter as measured by flag or drag sampling (Ogden and Lindsay, 2016, Berger et al. 2014). This enables a tick to quest for longer periods before retreating into leaf litter, increasing its likelihood of finding a host. An increase in questing frequency has a direct impact on local disease ecology as it increases the chance an infected tick can spread a pathogen. Temporal changes in climate year to year change affect tick densities which is suspected to account for the annual variation in disease case prevalence (Nicholson and Mather 1996, Kilpatrick et al. 2014). Berger et al. found that increasing numbers of 8-hour periods below 82% RH in June generally decreased nymphal populations of nymphal *I. scapularis* within Rhode Island. This suggests that underlying variables for nymphal and adult tick density may be more complex and vary across spatial and temporal settings (Diuk-Wasser et al. 2014). Berger et al. named those 8-hour periods of non-optimal humidity Tick Adverse Moisture Events (TAMEs). Concerning pathogen spread and infection most cases of human tick-borne disease occurs in the summer months from May to July (Bratton and Corey, 2005). This closely mirrors the annual period with the highest abundance of

nymphal *I. scapularis* (Stafford et al 1998). While disease can occur throughout the year the high nymphal abundance and higher possibility of a nymph being infected given it has taken a bloodmeal means the summer is when most cases of tick-borne disease is reported (D Fish, 1993). Beyond *I. scapularis* acting as a vector for Lyme disease and it also vectors numerous other pathogenic microbes.

Additional pathogens and their associated disease have become new threats to global public health. Previously, these have been referred to as "emerging" pathogens. Emerging pathogens are pathogenic species that within the last 20 years have increased in human disease incidence, been discovered to be pathogenic in humans, or have expanded in their geographic distribution within vector or host populations (Tsao et al 2021). Numerous tick vectored pathogens are causative agents of current emerging human diseases, these include bacteria such as *B, burgdorferi* (Lyme disease), *B. miyamotoi* (tick-borne relapsing fever), *B. mayonii* (Lyme borreliosis), *Anaplasma phagocytophilum* (Anaplasmosis), *Ehrlichia muris eauclairensis* (Ehrlichiosis) (Madison-Antenucci et al. 2020). Protozoal parasites such as *Babesia microti* (Babesiosis) are also increasing in vectors, host, and human prevalence (P.J. Krause, 2019). Powassan virus, a previously rare pathogen, has increased its human disease burden and geographic range with infected *I. scapularis* being found as far Oklahoma, well beyond its recognized range (Hermance and Thangamani, 2017, Smalley IV et al. 2022). Rhode Island has one of the highest human incidence rates of Lyme disease, Babesiosis, and Anaplasmosis in the US (Couret & Garrett, 2021). While not widely spread, *B. miyamotoi* infections have been present since 2015 in RI by retrospective patient blood analysis (Fiorito et al. 2017). Additionally, Powassan

virus was recently confirmed to be present within hosts and humans in Rhode Island since 2016 (Goethert et al. 2021, Patel et al. 2020). Originally limited to the upper Midwest, *B. mayonii* and *E. muris eauclairensis* were thought to be limited to Michigan, Minnesota, and North Dakota based on patient blood and tick vector surveys (Pritt et al. 2016, Murphey, et al. 2017). However, it was recently confirmed in Massachusetts that both pathogens are present in *I. scapularis* and white-footed mice populations (Xu et al. 2023). The infection rate of *B. burgdorferi* in *I. scapularis* has a direct relationship with human disease incidence in RI but this relationship has not been demonstrated with other TBPs (Mather et al. 1996). While *B. burgdorferi* in *I. scapularis* has been studied extensively in RI (Nicholson & Mather, 2014), no attempt has been made to quantify the infection rate of other emerging TBPs in local *I. scapularis* populations. These pathogens occur in both the same vector, hosts, and spatial area leading to a high likelihood of co-infections. Co-infections have consequences for both transmissibility and human infection.

Co-infections of TBPs modulate transmissibility. The most well-studied interaction is that between *B. burgdorferi* and *Ba. microti*. In this system, *B. burgdorferi* infection is thought to disrupt the immune response in white-footed mice allowing the less efficiently transmitted pathogen, *Ba. microti*, an opportunity to infection host tissue (Diuk-Wasser et al. 2016, Dunn et al. 2014). Depletion of T-cells and helper cells by *B. burgdorferi, which* decreases immune system resources capable of controlling *Ba. microti* parasites, is thought to be the molecular process behind this (Djokic et al. 2019). However, the strength of this interaction can vary based on geographic location. In a survey of tick-borne pathogens in New Jersey, co-infection between *B.*

burgdorferi and *Ba. microti* was not more likely than random given singular infection rates, but co-infection was more likely between *B. burgdorferi* and *A. phagocytophilum* across both nymphs and adult *I. scapularis* (Narvaez et al. 2023). In a multi-year survey in Pennsylvania, *B. burgdorferi/Ba. microti* co-infection was more abundant than *B. burgdorferi/A. phagocytophilum* (Schwartz et al. 2022). However, within that study, only the human pathogenic strain (Ap-ha) was found to be associated with *B. burgdorferi* infection in *I. scapularis* nymphs. A New York survey conducted from 2011 to 2012 found both co-infections were significant across nymphs and adults of *I. scapularis* (Hersh et al. 2014). The first study for *B. burgdorferi/ Ba. microti* co-infection demonstrated this relationship in Connecticut and Block Island in RI. Even with Rhode Island's extensive background of TBP disease research, no state-wide survey has attempted to quantify associations between emerging TBPs. Co-infections aren't just relevant in interactions within a vector and reservoir host. When a person has both Lyme disease and babesiosis, symptom severity and duration have been found to be increased for both diseases (Knapp & Rice, 2015). Multiple pathogens have been found to be transferrable to a human by a bite from an infected tick (Belongia, 2002). The threat caused by not only singular pathogen infection and co-infections is clear. Beyond just emerging pathogens, tick vectors species have also become a growing public health issue.

Amblyomma americanum (Linnaeus), while primarily found in the southern US, has been suggested to previously been present in the northeastern US before anthropogenic and land use changes extirpated it from the Northeast (Rochlin et al. 2022). Increased average humidity and mean ambient temperature along with a return

of second-growth forests has aided its re-introduction into the Northeast (Rochlin et al. 2022). Jordan et al. (2019) demonstrated that seasonality of nymphal *A. americanum* is similar to *I. scapularis* in New Jersey. *A. americanum* nymphal population is highest from May to August where they are most likely to infect a human (Kollars et al. 2000). *Amblyomma americanum* is recognized as the principal vector for alpha-gal, a carbohydrate that when transferred into a human can elicit a potentially life-threatening allergy to red meat known as alpha-gal syndrome, and (Goddard & Varela-Stokes, 2009). It was recently shown between 2017 and 2022, 90,018 people were positive for alpha-gal anti-bodies in the US (Thompson, 2023). Prudence Island in Rhode Island has harbored a population of *A. americanum* for decades, but no published work has calculated their abundance and distribution across mainland Rhode Island (Mather and Mather, 1990). *Dermacentor variabilis* (Say), the vector of RMSF and tularemia, has been noted for its northward expansion into northern new England and southern Canada from the Midwestern and Southern US (Minigan et al. 2018, Dergousoff et al. 2013). An invasive species of tick vector from Asia, *Haemaphysalis longicorni*s (Neumann), is also present in RI since 2018 (Tufts et al. 2021). First identified on a single sheep in New Jersey, *H. longicornis* has quickly expanded throughout the eastern seaboard of the US, now found as far south as Arkansas and as north as Massachusetts (Raghavan et al. 2019). Vector competency of *H. longicornis* is not similar to *I. scapularis, A. americanum,* and *D. variabilis*. Experimental studies show that *A. phagocytophilum* and *B. burgdorferi* are incapable of being transmitted from an *H. longicornis* bite (Breuner et al. 2020, Levin et al. 2021). Only severe fever with thrombocytopenia syndrome virus (Dabie bandavirus) is

associated with *H. longicornis* but this virus has only been found in Asia, with no confirmation in the Northern Hemisphere (Luo et al. 2015).

As tick vectors and their associated pathogens continue to expand, they become an increasingly more relevant threat to public health. Our ability to quantify risk to humans relies on knowing where a vector is and what proportion of a population carries pathogens. This can only be determined by surveying tick populations coupled with extensive pathogen testing. Rhode Island has a high tick-borne disease burden, but infection rates for pathogens beyond *B. burgdorferi* are not well documented. Additionally, little is known of populations of tick vectors besides *I. scapularis*.

We have several objectives that this survey for medically important hard ticks and associated *Ixodes*-borne pathogen will achieve. First, we want to determine how abundance and distribution of nymphs and adults for hard ticks vary over space and time. However, what this will ultimately look like depends on the individual hard tick species. Rhode Island was previously demonstrated to have a high degree of inter-annual variability primarily determined by TAMEs events (Berger et al. 2014). Given that no attempts have been made since 2014 to determine state-wide and site-level abundance we expect to see similar trends as has been demonstrated formerly for *I. scapularis*. As *A. americanum* is only recently re-emerging into the Northeast we have much less historical data to rely on to inform hypothesis on abundance and distribution. However, we do know that *A. americanum* and *I. scapularis* can survive in similar habitats and environmental conditions. *A. americanum* is more tolerant of lower humidity and open environments (Ginsberg and Zhioua, 1996). With that we expect *A. americanum* to have a similar state-wide distribution but lower abundances

when compared to *I. scapularis* across both adults and nymphs. Additionally, we expect *A. americanum* to experience a similar inter-annual variability as *I. scapularis.* For *H. longicornis,* we aim to understand if populations are increasing similar to Pennsylvania and New York or if they remain at an overall low abundance. Secondly, among pathogens found in *I. scapularis* nymphs we aim to understand the difference in their geographic distribution and how their prevalence varies within a nymphal season. As Rhode Island has confirmed human cases and previous confirmation in reservoir hosts populations we expect *B. burgdorferi, B. miyamotoi, A. phagocytophilum, Ba. microti*, and Powassan virus to be widely distributed throughout the state. As for intra-seasonal variation, we expect that prevalence with be similar across the nymphal season for all pathogens. Finally, we aim to quantify significant relationships between co-infection such as *B. burgdorferi/Ba. microti* or *B. burgdorferi/A. phagocytophilum* among infected *I. scapularis*. As this relationship is driven primarily by *P. leucopus* and has been noted to occur in other studies, we expect to notice a significant trend among *B. burgdorferi/Ba. microti* but not among other co-infection pathogen pairs (Dunn et al 2014).

CHAPTER 2

METHODOLOGY

Field Collection

To record abundance of hard ticks in RI we conducted surveillance sampling for adults and nymphs of *I. scapularis*, *A. americanum*, *D. variabilis*, and *H. longicornis*. Field collections were conducted during the summer (late May – late July) of 2022 and 2023. This seasonal window is commonly recognized as representing peak nymphal abundance in the Northeastern US for the nymphal and adult life stages of multiple hard tick species (Ginsberg & Zhioua, 1996, Kollars et al. 2000).

Across mainland RI, we sampled all possible local environments where ticks are understood to be including rural, suburban, and urban areas and a gradient of forest types from deciduous forests to purely coniferous forests. We sampled only within forested woodland areas with a preference to sample more interior to a forest patch rather than focusing on trails or other edge habitats. To account for inherent seasonal variability of nymph populations across the sampling period, we sampled every site twice, once earlier in the period (1st Round) then later in the sampling period (2nd Round). This was to collect at both high and low local abundance to get close to the true average for an individual site. Each round of sampling required ~1 month in both years.

To sample for hard ticks, we used the established method of flagging to collect for actively questing nymphs and adults (Ginsberg & Ewing, 1989). Tick flags were a .5 X .5 M^2 piece of white flannel cloth attached to a ~3 ft wooden pole, referred to

hereafter as a “Flag”. Flags were dragged across the ground in 30-second intervals, repeated 90 times for each site, with each flag covering a new transect. After each flagging event. the flag was laid flat on the ground and then inspected for ticks. If found, ticks were identified down to species and life stage using established taxonomic keys (Keirans and Clifford 1978, Durden and Keirans 1996) and deposited into 90% ethanol for molecular pathogen identification.

Once all specimens were collected, counted, and identified an encounter rate was calculated for each for each species at each life stage. Encounter rate is defined here as the number of collected specimens for each species and life stage divided by 45 to generate a value that represents the # of ticks encountered per minute. Both overall Rhode Island and site-specific encounter rate is the # of ticks encountered per minute.

Historical Data

To compare our recorded abundances to historical data we used data provided by Tick Encounter, based at the University of Rhode Island, which maintained an active surveillance system from 1994 – 2014 for *I. scapularis* nymphs. While they used 60 sites to determine nymph abundance, we only used 40 sites. Data displayed is only for the 40 sites we used for 2022 and 2023. Hard tick sampling methods used for historical data generation matched our sampling techniques exactly. For 20 years of historical survey data, we generated an overall abundance of *I. scapularis* nymphs for each year. Historical data was collected from the same sampling period we used (late May – late July).

Climatalogical Data

Local Climatological Data (LCD) was pulled the National Oceanic and Atmospheric Administration (NOAA) (https://www.ncdc.noaa.gov/cdo-web/datatools/lcd) for June and July of 2022 and 2023. We pulled data for hourly relative humidity (RH) across seven weather stations in Rhode Island. We calculated Median relative humidity for both overall sampling period and for individual months (June and July).

Molecular Pathogen Identification

I. scapularis nymphs were tested for *B. burgdorferi, B. miyamotoi, B. mayonii, Ba. microti, A. phagocytophilum* (undifferentiated between human pathogenic Ap-ha or unpathogenic Ap-v1 strains)*, E. muris eauclairensis*, Powassan virus, Covid-19-2, Heartland virus, Bourbon virus, Colorado tick fever virus, Tick-borne encephalitis virus, and Severe fever with thrombocytopenia syndrome virus. All nymphs were tested as individuals rather than being pooled.

DNA extraction was performed with Epicenter Master Complete DNA and RNA Purification Kits (Epicenter Technologies, Madison, WI, USA) following manufacturer protocols. All bacterial, protozoa, and viral pathogens were detected by a multiplex Taqman real-time PCR assay 16 µl reaction volumes using the Brilliant III qPCR Master Mix (Agilent, La Jolla, CA, USA) in an Agilent MX3000P qPCR System. See Sack et al. 2023 and Xu et al. 2016 for more information on molecular pathogen identification.

Statistical analysis

An unequal variances *t*-test was performed to compare significance of overall pathogen prevalence and co-infections between round 1 and round 2. An individual

unequal variances *t*-test was performed for each observed pathogen excluding Powassan virus due to low sample size. unequal variances *t*-test were used since # of pathogen positive sites varied between round 1 and round 2.

We used the cooccur() function from the *cooccur* package to analyze rates of co-infection among pathogens (Griffith et al. 2016). The package *cooccur* applies a probabilistic model of species co-occurrence used to determine if observed co-infection rates of pathogens occurred more frequently than expected if infection rates occurred independently of each other (i.e., randomly).

All statistical analysis was performed using Microsoft excel® and R 4.2.3 (R Team Core 2023). ArcGIS Pro® (Esri, Redmond, California) was used for map-based data visualization.

CHAPTER 3

FINDINGS

Hard Tick Encounter Rate and Geographic Distribution

During the 2022 and 2023 sampling period, we collected for four species of nymph and adult life stages. All *I. scapularis* nymphs collected during the 2022 nymphal season were tested for 12 pathogens confirmed or suspected to be present within RI nymphal populations.

During the 2022 nymphal season, we only found two species of nymphs and three species of adults. For nymphs, we found 1760 (22 ± 31.48 per site) (mean encounter rate: .98 per minute) *I. scapularis* and 131 (1.64 ± 7.46) *A. americanum* (mean encounter rate: .072) *(*table 1). For adults, we found 180 (2.25 ± 6.44) (mean encounter rate: 0.1) *I. scapularis*, 16 (0.20 ± 0.81) (mean encounter rate: .008) *A. americanum*, and 372 (4.65 ± 13.42) (mean encounter rate: 0.21) *D. variabilis.* We found no *D. variabilis* or *H. longicornis* nymphs (Table 1).

Table 1. Summary of nymphal abundance, including total number of detections, means per site (±SD), and encounter rate of *I. scapularis, A. americanum, D. variabilis*, and *H. longicornis* across 40 sites during round 1 and round 2 of 2022 and 2023.

Year	Species	Total	Mean (per site)	SD
2022	*I. scapularis*	1760	22	31.49
	A. americanum	131	1.64	7.46
	D. variabilis	0	0	0
	H. longicornis	0	0	0
2023	*I. scapularis*	4803	60.04	52.73
	A. americanum	403	5.03	22.27
	D. variabilis	2	-	-
	H. longicornis	7	-	-

During the 2023 nymphal season, we found nymphs across four species and adults for three species. For nymphs, we found 4803 (60.04 ± 52.73) (mean encounter rate: 2.66) *I. scapularis*, 403 (5.03 ± 22.27) (mean encounter rate: .22) *A. americanum*, seven *H. longicornis*, two *D. variabilis*. For adults, 107 (2.64 ± 2.45) (mean encounter rate: 0.05) *I. scapularis*, 40 (0.99 ± 1.99) (mean encounter rate: .02) *A. americanum*, 187 (4.61 ± 3.71) (mean encounter rate: .10) *D. variabilis* (Table 2).

Table 2. Summary of adult abundance, including total number of detections, means per site (±SD), and encounter rate *of I. scapularis, A. americanum, D. variabilis*, *and H. longicornis* across 40 sites during round 1 and round 2 of 2022 and 2023.

Year	Species	Total	Mean (per site)	SD
2022	*I. scapularis*	180	2.25	6.44
	A. americanum	16	0.2	0.81
	D. variabilis	372	4.65	13.42
2023	*I. scapularis*	107	2.64	2.45
	A. americanum	40	0.99	1.99
	D. variabilis	187	4.61	3.71

Geographic distribution differed within Rhode Island depending on the tick species and life stage. Distribution was similar among *I. scapularis* nymphs for 2022 and 2023. Of 40 sampled sites in 2022, we discovered at least one *I. scapularis* nymph at every site (Figure 1). Their presence among sites was different from Round 1 and Round 2. We found *I. scapularis* nymphs present at every site during round 1 (late May/June). By round 2, *I. scapularis* nymphs were only found at 32 sites. In 2023, *I. scapularis* nymphs were present at all sites during both round 1 and round 2 of sampling.

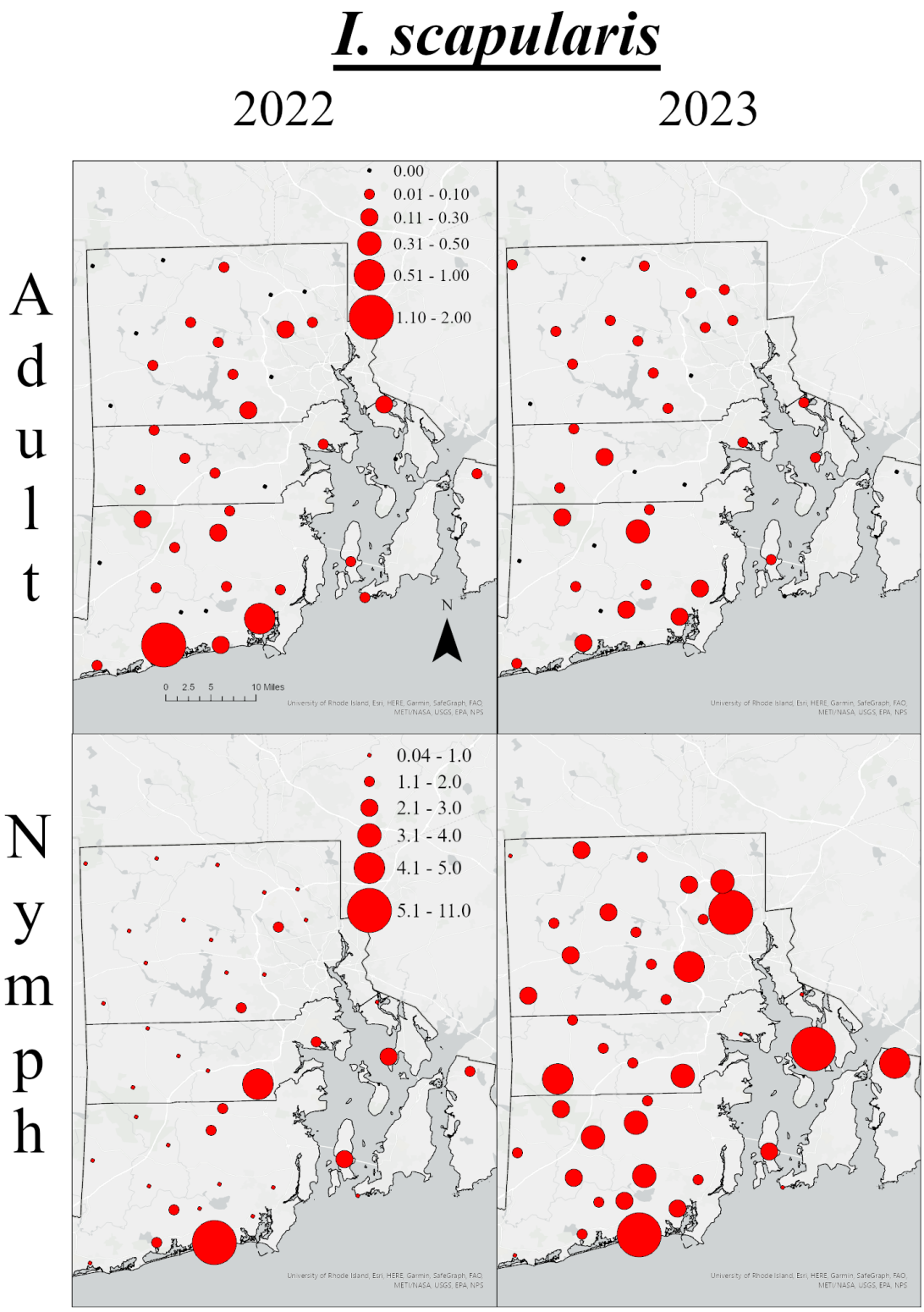


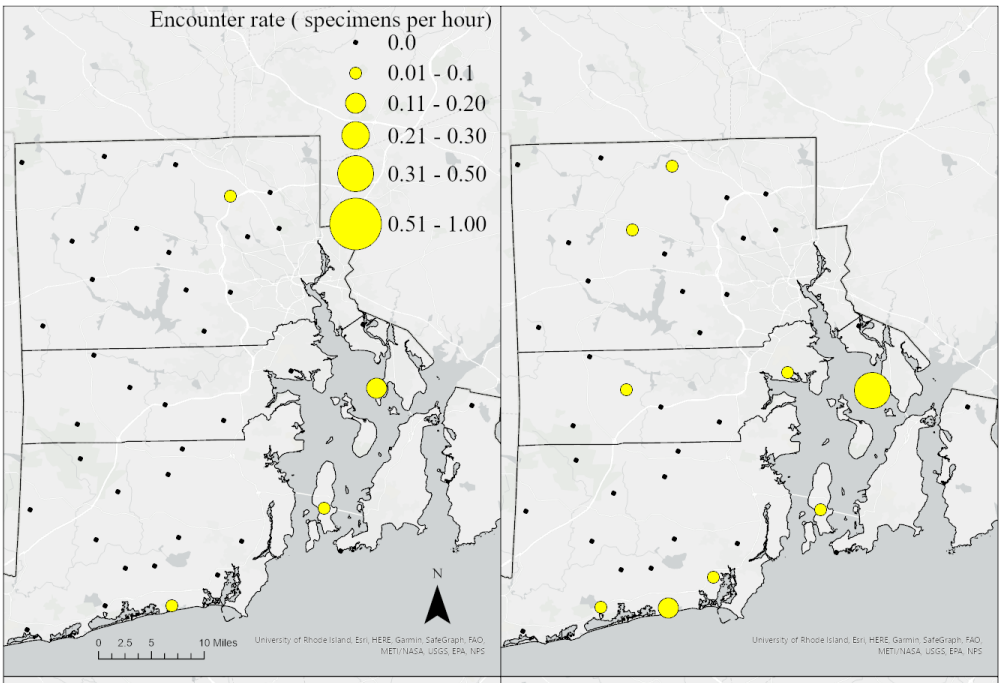
Figure 1. Encounter rate and distribution of adult and nymphal *I. scapularis* in Rhode Island in 2022 and 2023. Dot size symbology denotes per minute encounter rate.

Amblyomma americanum was found to occupy fewer sites than *I. scapularis*. In 2022, we found *A. americanum* nymphs in 11 sites across both round 1 and round 2 (Figure 2). However, in 2023 we found *A. americanum* nymphs in only six sites. Distribution of *A. americanum* also depended on life stage, we noted that *A. americanum* adults were more widely distributed in seasons where *A. americanum* nymphs' geographic distribution was limited. In 2022, *A. americanum* adults were collected within four sites. In 2023, *A. americanum* adults were present within nine sites.

A. americanum

2022 2023

Adult



Nymph

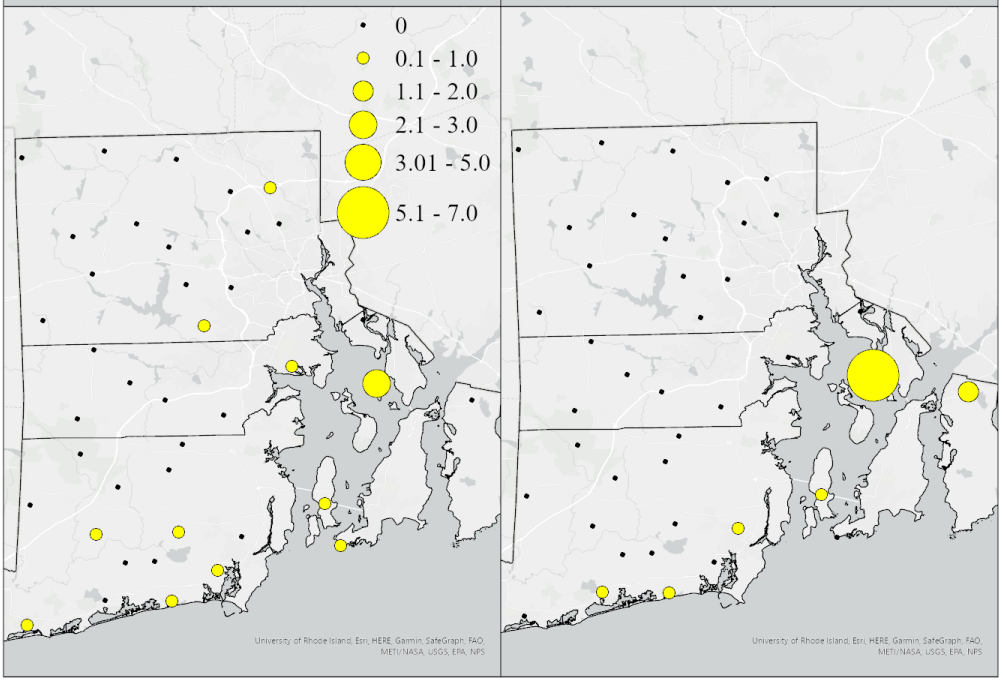


Figure 2. Encounter rate and distribution of adult and nymphal *A. americanum* in Rhode Island in 2022 and 2023. Dot size symbology denotes per minute encounter rate.

Few *H. longicornis* were found and of those exclusively detected in 2023. In 2023, 90% of *H. longicornis* nymphs were collected in 2 sites in southern RI. At a single site located in northern RI, we found one *H. longicornis* nymph.

Pathogen Prevalence of TBP's in *I. scapularis* Nymphs

A total of 1566 *I. scapularis* nymphs of the 1760 collected in 2022 were tested for pathogens. None of the nymphs tested were found to be infected with *B. mayonii*, *E. muris eauclairensis,* Heartland virus, Bourbon virus, Colorado Tick Fever virus, Tick-borne encephalitis virus, Severe Fever with Thrombocytopenia Syndrome Virus, or severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Overall, 436 nymphs (27.84%) were infected with at least one pathogen; 367 nymphs (23.43%) were infected with only one pathogen (singular infection), 63 nymphs (4.02%) were infected with two pathogens (double infection), and two nymphs (.12%) were infected with three pathogens (triple infection) (Table 3).

Table 3. Summary of infection type and occurrence rate of pathogens for *I. scapularis* nymphs in 2022.

Infection Type	% Infection	95% Confidence Interval	
		Lower	Upper
Uninfected	72.92	70.65	75.11
Single Infection	23.43	21.35	25.61
Double Infection	4.02	3.10	5.17
Triple Infection	0.12	.015	0.46

Overall, 266 nymphs were infected (16.98%) with *B. burgdorferi*, 107 nymphs (6.83%) with *Ba. microti*, 105 nymphs (6.70%) with *A. phagocytophilum*, 17 nymphs (1.08%) with *B. miyamotoi*, and 4 nymphs (0.25%) with Powassan virus (Table 4).

Table 4. Infection rate of *I. scapularis* nymphs in Rhode Island 2022 for *B. burgdorferi, B. miyamotoi, A. phagocytophilum, Ba. microti,* and Powassan virus.

Pathogen	% Infection Rate (Total tested nymphs = 1566)	95% Confidence Interval	
		Lower	Upper
B. burgdorferi	16.98	15.16	18.94
B. miyamotoi	0.89	.48	1.49
A. phagocytophilum	6.70	5.52	8.07
Ba. microti	6.83	5.63	8.19
Powassan virus	0.25	0.07	0.65

A total of 65 nymphs were infected with more than one pathogen simultaneously. Rates of double infections are as recorded: 40 nymphs (61%) infected with *B. burgdorferi*/*Ba. microti*, 15 nymphs (23.07%) with *B. burgdorferi/ A. phagocytophilum*, four nymphs (6.15%) with *B. burgdorferi/B. miyamotoi*, two nymphs (3.07%) with *A. phagocytophilum/Ba. microti*, one nymph (1.54%) with *A. phagocytophilum/B. miyamotoi*, and one nymph (1.54%) with *A. phagocytophilum*/Powassan virus. Only two nymphs (3.07%) demonstrated a triple infection of *B. burgdorferi/Ba. microti/A. phagocytophilum*

Pathogen presence and prevalence differed based on pathogen species. *B. burgdorferi* was the most spatially expansive pathogen, occurring at 36 sites. However, spatial distribution differed within sampling period. During round 1, 36 sites had confirmed *B. burgdorferi* presence decreasing to 24 sites during round 2 (Figure 3). Mean *B. burgdorferi* differed slightly between sampling rounds with a prevalence of 17.84%

and 15.91% for Round 1 and Round 2, respectively. Other pathogens demonstrated a similar decreasing trend concerning both distribution and prevalence for round 1 and round 2. *Borrelia miyamotoi* was found within nymphs at nine total sites. For round 1, *B. miyamotoi* had its maximum spatial extent of nine sites with a mean prevalence of 1.35% in nymphs (Figure 3). For round 2, distribution decreased to four sites with a lower prevalence of .77%. *Babesia microti* reached its maximum number of positive sites in round 1 with 32 sites with a mean infection rate of 7.40% (Figure 4). Only 16 sites with a mean infection rate of 6.02% were recorded in round 2 for *Ba. microti*. *Anaplasma phagocytophilum* was found infecting *I. scapularis* nymphs at a total of 21 sites. For round 1, 21 sites contained infected nymphs found. By round 2, *A. phagocytophilum* infected nymphs were found in only 12 sites. The mean infection rate for *A. phagocytophilum* was 6.52% and 6.95% for round 1 and round 2, respectively (Figure 4). Powassan virus was found to infect nymphs at three sites, one site in round 1 and 2 sites in round 2. All sites were located near one another, with a total area of ~8 sq miles (Figure 5). The prevalence of co-infected nymphs also changed within the sampling period. In round 1, we found co-infected nymphs at 17 sites with a mean co-infection rate of 4.24%. all pathogens. In July, 12 sites had coinfected nymphs with a mean co-infection rate of 4.02% (Figure 6).

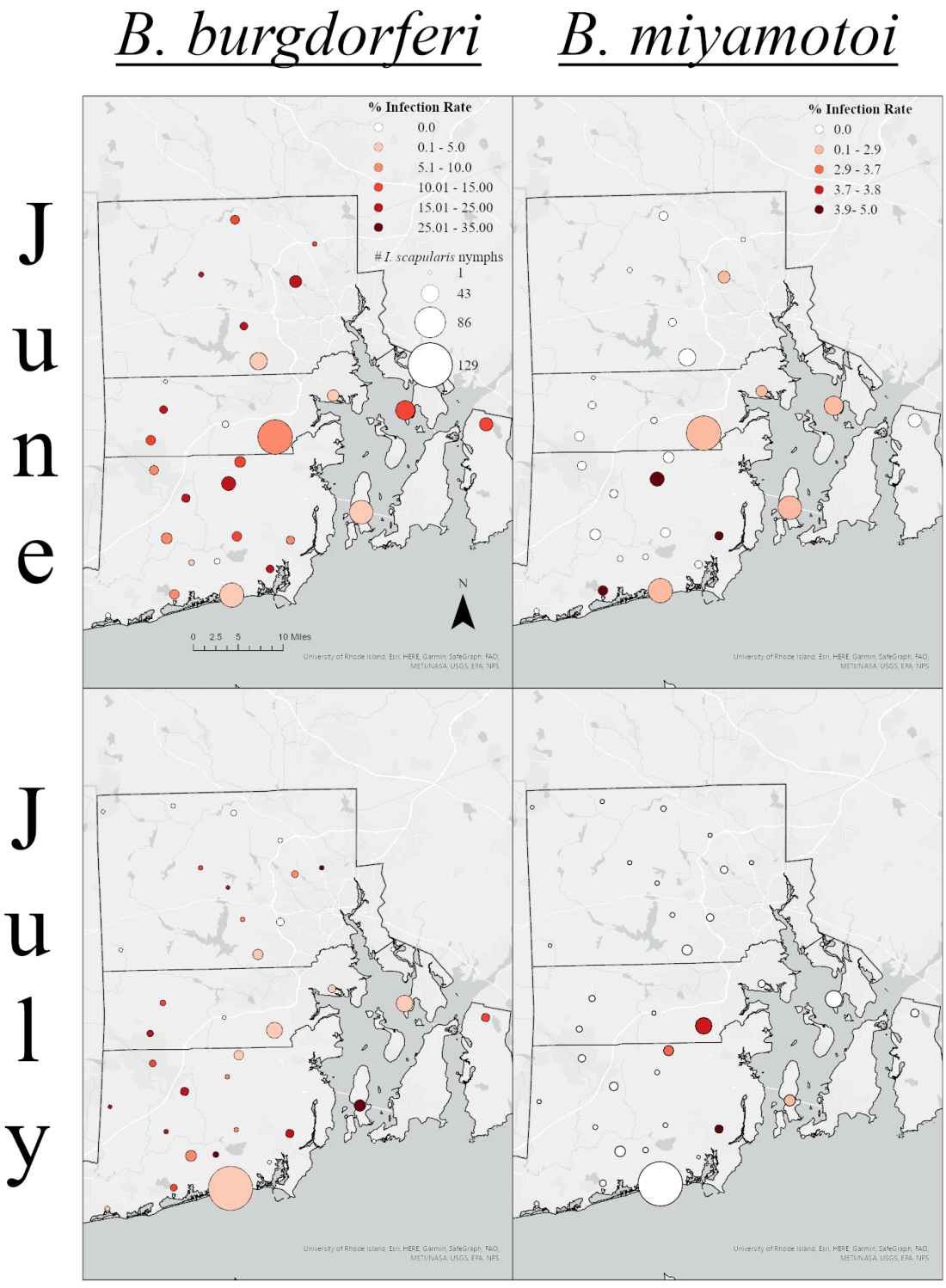


Figure 3. Site level of prevalence of *B. burgdorferi* and *B. miyamotoi* in *I. scapularis* nymphs across round 1 and round 2 in 2022. Dot color denotes prevalence of pathogen, with white meaning no infection to dark red representing high pathogen prevalence and dot size symbology denotes # of *I. scapularis* nymphs detected.

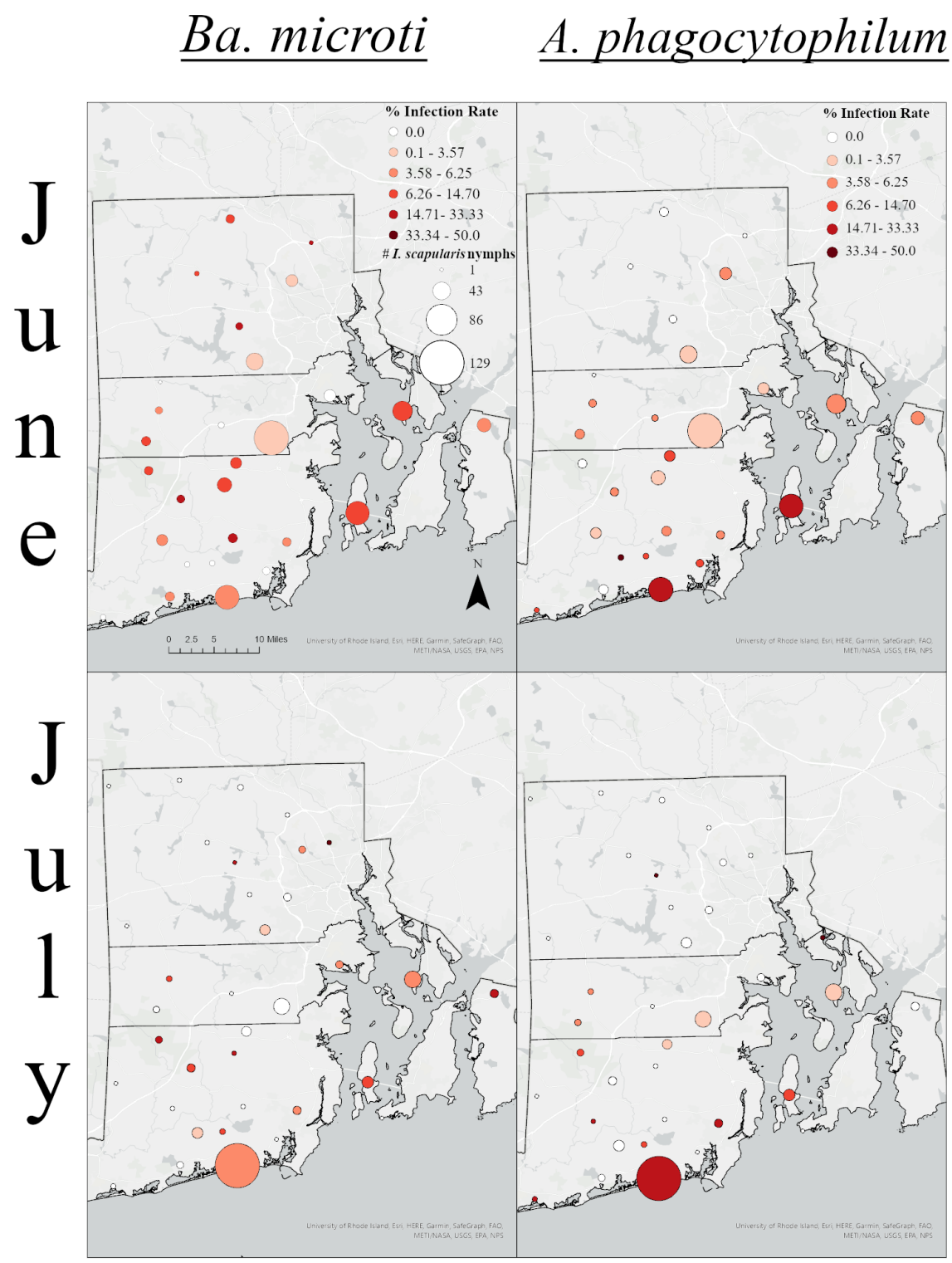


Figure 4. Site level of prevalence of *Ba. microti* and *A. phagocytophilum* in *I. scapularis* nymphs across round 1 and round 2 in 2022. Dot color denotes prevalence

of pathogen, with white meaning no infection to dark red representing high pathogen prevalence and dot size symbology denotes # of *I. scapularis* nymphs detected.

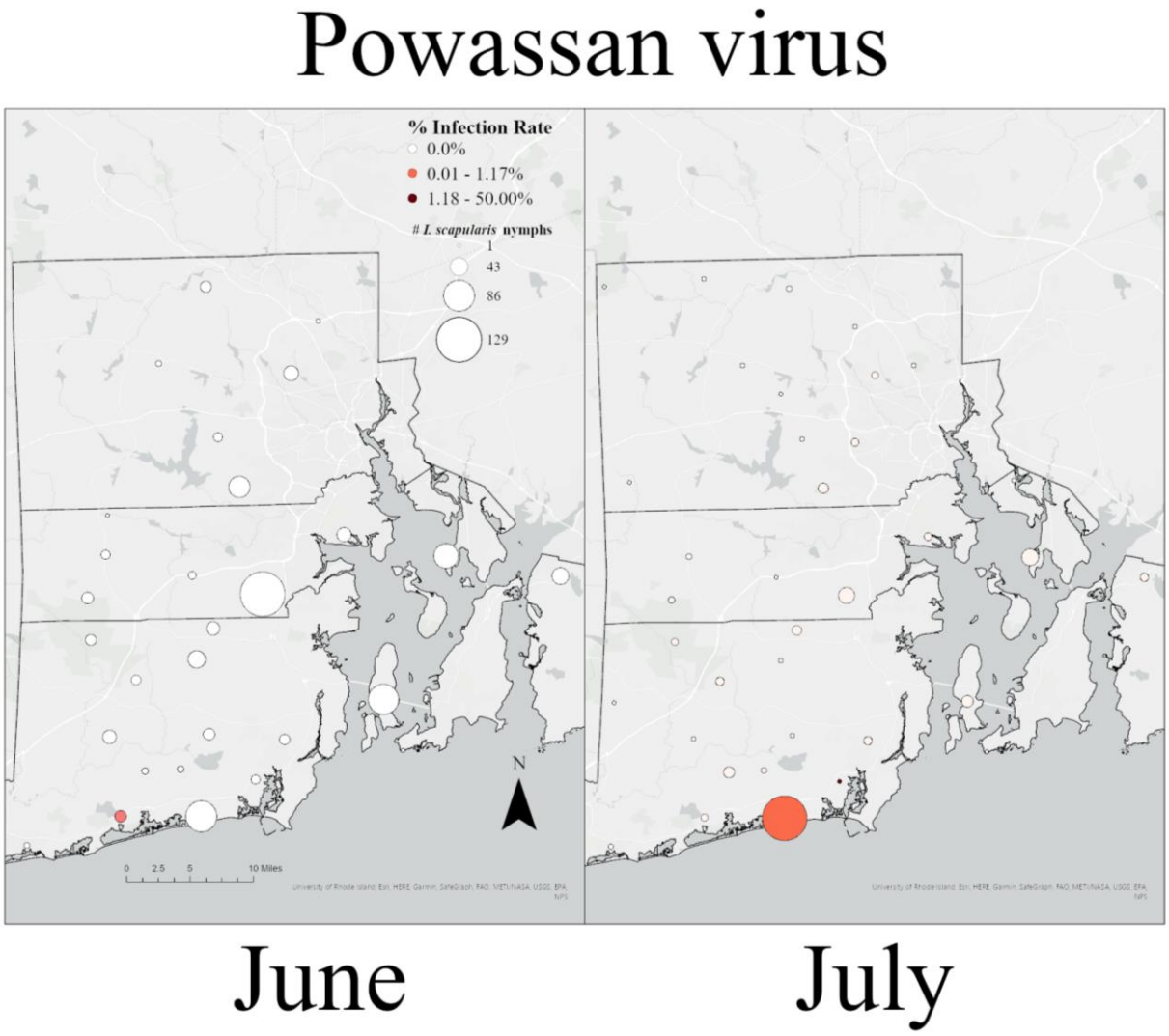


Figure 5. Prevalence and Distribution of Powassan Virus in Rhode Island in *I. scapularis* nymphs across round 1 and round 2 in 2022. Dot color denotes prevalence of pathogen, with white meaning no infection to dark red representing high pathogen prevalence and dot size symbology denotes # of *I. scapularis* nymphs detected.

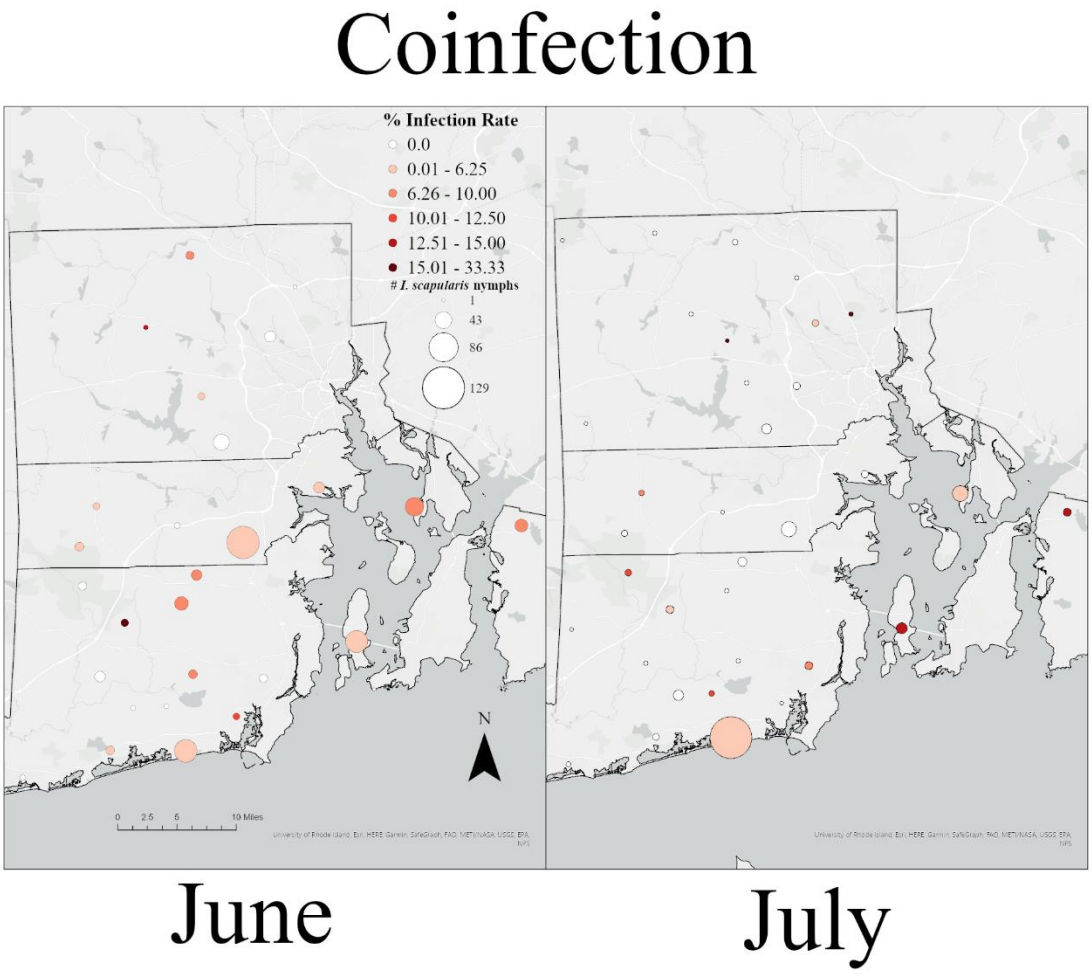


Figure 6. Site level of prevalence of co-infection in *I. scapularis* nymphs across round 1 and round 2 in 2022. Dot color denotes prevalence of pathogen, with white meaning no infection to dark red representing high pathogen prevalence and dot size symbology denotes # of *I. scapularis* nymphs detected.

There were some significant differences detected between round 1 and round 2 for *I. scapularis* nymph infection rate among pathogens. Significant difference in mean infection rate between round 1 and round 2 included *B. burgdorferi* (P-value = .007), *B. miyamotoi* (P-value = .032), *Ba. microti* (P-value = .022), and co-infections (P-value = .046). No significance was found for *A. phagocytophilum* (P-values = .184). Powassan virus was excluded from analysis due to low prevalence in nymphal *I. scapularis.*

For the co-occurrence of pathogens analysis, we only considered *B. burgdorferi, B. miyamotoi, Ba. microti,* and *A. phagocytophilum* for the probabilistic model. Powassan virus was excluded from analysis due to the low prevalence of infected nymphs. We found that *B. miyamotoi* did not have a significant co-occurrence association between either of its observed co-infecting pathogens, *B. burgdorferi* (P-value = .320) or *A. phagocytophilum* (P-value = .692). We also found no significant co-infection interaction between *A. phagocytophilum* and *B. burgdorferi* (P-value = .622) or *Ba. microti* (P-value = .938) in *I. scapularis* nymphs. The only significant interaction found was a positive association between the number of co-infected nymphs for *B. burgdoferi/Ba. microti* co-infection (P-value < .0001) (Table 5)

Table 5. Summary of probabilistic model for observed co-infections of *I. scapularis* nymphs in Rhode Island in 2022.

Pathogen Co-infections	Observed Co-occurrence	Expected Co-occurrence	P-value
B. burgdorferi + *B. miyamotoi*	4.0	2.9	.320
B. burgdorferi + *Ba. microti*	42.0	18.1	<.0001
B. burgdorferi + *A. phagocytophilum*	17	17.7	.622
B. miyamotoi + *A. phagocytophilum*	1	1.1	.692
Ba. microti + *A. phagocytophilum*	4	7.1	.938

Historical data of *I. scapularis* nymphs sampled annually from 1994 to 2014 shows that our recorded abundance in 2022 falls within the range of previous annual nymphal

abundance (Figure 7). However, 2023 was the highest abundance year for *I. scapularis* nymphs recorded in Rhode Island.

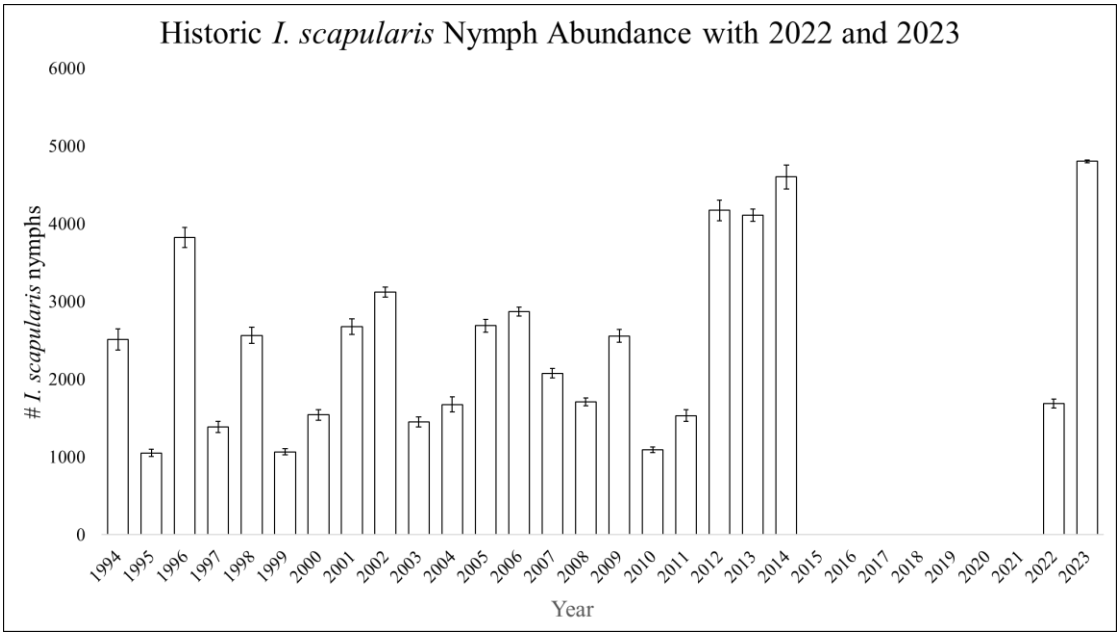


Figure 7. Historical data of annual *I. scapularis* nymphal abundance from 1994 to 2014. Includes abundance data we generated in 2022 and 2023. Only sites we sampled in 2022 and 2023 were used for comparison.

Analysis of NOAA RH data for Rhode Island shows that that median RH differed between years. For 2022, overall median RH was 72.99% while round 1 median RH was 76.82% and round 2 median RH was 75.39%. For 2023, overall median RH was 85.03% while round 1 median RH was 76.83% and round 2 median RH was 75.39%. Overall, 2022 was a much drier sampling period compared to 2023.

CHAPTER 4

CONCLUSIONS

During our surveillance of hard ticks during 2022 and 2023 we recorded annual varying abundances of *I. scapularis*, novel abundance and distribution data of *A. americanum* in Rhode Island and expanding presence of *H. longicornis*. Additionally, we determined the prevalence of several emerging TBPs and confirmed absence of several suspected TBPs in *I. scapularis* nymphs in 2022. We also identified that differences in prevalences between round 1 and round 2 were significant for *B. burgdorferi*, *B. miyamotoi*, *Ba. microti* and for co-infections. Among co-infections of TBPs in *I. scapularis* nymphs we identified a significant association between *B. burgdorferi/Ba. microti*.

Historical data compared to our collection data, shows that 2023 was the year with the highest number of collected *I. scapularis* nymphs. However, the difference between the 2 highest abundance years was only 206 nymphs (Figure 7). While this might signify a general increase in nymphal populations a gap in historical surveillance data for 2014 -2021 limits our ability to identify trends over time. Distribution of *I. scapularis* differed between sampling years. In 2022, high encounter rate sites were in the southern, coastal portion of Rhode Island. Inland sites had much lower abundance and encounter rates. In 2023, distribution was much more even between coastal and inland sites. This is consistent with previous estimates of abundance. This variation may in part be explained by differences in annually climatic condition. As discussed earlier, Berger et al. (2014) demonstrated that the number of TAMEs is directly linked with annual abundance of *I. scapularis* nymphs. 2022 was marked a period of severe

drought from May to July which resulted in high median temperatures and low relative humidity (McCarthy et al. 2023). This is consistent with previous estimates that low annual humidity is linked with low abundances of *I. scapularis* nymphs. High humidity in 2023 may have been responsible for the high abundances we reported. Given the differences of monthly relative humidity in June between 2022 and 2023, we see that Berger's hypothesis of TAMEs being responsible for annual differences in *I. scapularis* nymphal abundance still holds. While landscape measures, such as urban and rural landcover, were not measured within this study we noticed certain trends in *I. scapularis* abundances in urban areas. In a Bristol County site (Colt State Park), a highly developed area surrounded by suburbs, encounter rate for *I. scapularis* was similar the high encounter sites located in the southern half of Rhode Island across both 2022 and 2023 (Fig. 1). Another area in northern Providence surrounded by highways and only occurring in a small .25 x .25 sq mi forest patch also exhibited high nymphal encounter rates comparable to the southern half of the state (Fig. 1). Landscape factors were not formally quantified in our study, limiting conclusions we can draw from habitat factors modifying nymphal densities. Piedmonte et al. (2018) found *I. scapularis* nymphal densities were higher in edge habitats compared to interior woodland areas, but this effect was not found to impact the pathogen prevalence of *B. burgdorferi* in ticks. An analysis of landscape predictors in Canada showed that the proportion of forested areas was the only significant predictor for *I. scapularis* nymph densities. While landscape factors affecting *I. scapularis* nymphs are present in their entire geographic range, these factors relative contribution could

vary across the geographic range. Future work needs to build on this by analyzing how measures of human development could impact *I. scapularis* nymphal populations.

While *A. americanum* has been known to occur in Rhode since at least 1990, this is the first known attempt to quantify their abundance and distribution. Between 2022 and 2023, *A. americanum* population increased throughout the state following a similar trend to *I. scapularis* abundance. Low humidity is a strong source of mortality for *A. americanum,* so a low overall humidity may have resulted in lower *A. americanum* abundances compared to 2023 (Shulze et al. 2001). The spatial extent of *A. americanum* was limited, with populations only being encountered near coastal areas, with the highest abundances detected on Conanicut Island, and at sites in Washington and Bristol Counties. This may be due to higher RH in these sites due to placement near the ocean compared the lower RH inland areas. All sites where *A. americanum* was encountered in high abundances were situated ≤three mile from the coast. Springer et al. (2014) defined *A. americanum* as established if ≥six ticks or ≥two life stages were found within a county. They found through their review of historical data and the literature that only Newport County had established populations while Washington County only had reported detections. We found multiple life stages and >six ticks across every county each year demonstrating that *A. americanum* is now fully established in Rhode Island. Several models have suggested that Rhode Island is within the predicted range of the *A. americanum* range expansion, but such models are limited, in part, by lack of survey data (Sagurova et al. 2019) (Raghavan et al. 2019a) (Raghavan et al. 2019b). In Connecticut, a passive surveillance system identified an upward trend of submitted *A. americanum* specimens from 1996 -2017 but the overall

abundance suggested from submissions was much lower than what we identified from active sampling (Stafford III et al. 2018). They identified a total of 1,261 submissions across 21 years of surveillance while we collected half that many specimens throughout two years of active sampling. It may be that passive surveillance does not provide an accurate estimate of abundance within localities. Nearby states such as Massachusetts have reported populations of *A. americanum*, but no active surveillance has been attempted to measure overall abundance throughout the state of Rhode Island. Given that multiple sites with confirmed *A. americanum* populations border Massachusetts future efforts should focus on determining abundance within mainland Massachusetts as well.

Distribution of *A. americanum* life stages differed between the two years of this study. In 2022, *A. americanum* nymphs were found to be present in inland sites while in 2023 only adult *A. americanum* were found at inland sites. This difference may be explained by the differences in host preference of across hard ticks. All life stages of *A. americanum* prefer *O. virginianus* as their principal host. Compared to *P. leucopus*, the primary host of larval and nymphal *I. scapularis, O. virginianus* has a much larger habitat and dispersal range (Gaughan and DeStefano, 2005, McShea and Madison, 1992). It may be that while *A. americanum* populations are predominantly found in coastal areas, hosts with large ranges and the ability to cover large distances are allowing them to travel inland. Questing behavior mediated by environmental conditions may offer an explanation. Experimental evidence suggests that drier environmental conditions cause *A. americanum* to quest at higher heights, increasing their likelihood of finding a host (Nielebeck et al. 2022). In 2022, Rhode Island

experienced an intense, short hydrological drought period, causing lower atmospheric RH which may explain both the lower populations and a larger geographic distribution of *A. americanum* nymphs (McCarthy et al. 2023). Complementary to this was that in 2023 the nymphal period was marked by a higher overall median RH compared to 2022, where we found a more restricted geographic distribution of *A. americanum* nymph. As for adults, it might be that the higher humidity increased the overall population, allowing a higher proportion of adults to successfully attach to a host increasing their distribution compared to nymphs. While we did not test our *A. americanum* specimens for pathogens, previous studies have found pathogens to be present in Rhode Island *A. americanum* populations along with nearby states such as Connecticut confirming pathogen presence (Stafford III et al. 2018, Ijdo et al. 2000). Nymphal seasons of lower humidity may put the public at greater risk for *Amblyomma*-borne pathogens present in Rhode Island, including ehrlichiosis and alpha-gal syndrome, compared to higher humidity nymphal seasons. However, in higher mediant humidity years risk may be higher but more localized due to higher populations occurring a smaller area.

We found very few *H. longicornis* individuals compared to other species of hard tick vectors. However, all *H. longicornis* were collected in 2023 during a period of high abundance for all *I. scapularis* and *A. americanum* populations, suggesting that *H. longicornis* may experience annual increases in abundance mimicking other hard tick species. However, *H. longicornis* in Rhode Island has not reached abundances compared to other endemic areas of the United States. Field studies in other states, such as Pennsylvania, Virginia, and New York, have confirmed this but noted seasonal

phenology and period of peak populations are consistent annually (Piedmonte et al. 2021, Thompson et al. 2021). Price et al. (2021) found that *H. longicornis* nymph and adult densities were higher compared to *I. scapularis* nymphal densities within the same area and habitat, demonstrating similar environmental preferences. However, comparison between our study and other field studies is limited by differences in sampling methodology. Other studies use distance-based line transects compared to our time-based transects and sampled sites weekly compared to our monthly sampling events (Price et al. 2021). However, our sampling revealed a single *H. longicornis* specimen collected in Providence County. To our knowledge, this is currently the most northeastern distribution of *H. longicornis* in the United States. With low overall abundance across multiple years, it seems that *H. longicornis* has yet to establish in the same degree compared to its invasive range in the mid-Atlantic United States.

Powassan virus Lineage II (DTV) was restricted to a small area of ~8 sq miles. We found DTV infected *I. scapularis* nymphs in roughly the same area as previous studies (Goethert et al. 2021). Within that study, they only sampled 2 sites within Rhode Island while we sampled roughly the entire state. Our estimated infection rates were slightly lower than what Goethert et al. estimated with a lower infection rate of 1.17% for a southern coastal site (Trustom Wildlife Refuge) and over 4% for a single site located on private land. Regardless, the distribution of infected nymphs is similar suggesting that DTV is highly localized in Rhode Island, only occurring in the southern half of the state. This may be due to low competence of DTV in wildlife hosts. Other studies have noted reservoir hosts, such as *P. leucopus*, which are abundant and widely distributed throughout the Northeast are not competent hosts for

Powassan virus (Mlera et al. 2017). Goethert et al. found that among Powassan-infected nymphs in Rhode Island most fed on shrews (*Blarina brevicauda)* with 1 shrew tested being confirmed for Powassan virus infection. However, the population and distribution of shrews is poorly understood in Rhode Island and future efforts should focus on quantify their contribution to enzootic maintenance of Powassan virus. Other studies have demonstrated the restricted host profile of Powassan virus showing that competent hosts for Powassan virus do not overlap significantly with other tick-borne pathogens (Dupuis II et al. 2012). Future efforts should better quantify Powassan virus prevalence though hosts found in Rhode Island.

Our analysis of co-infections showed that *B. burgdorferi* and *Ba. microti* were the only significant pathogen co-infection pair in Rhode Island. Other studies have confirmed a similar relationship in Connecticut, New York, and Pennsylvania while another study in New Jersey did not (Narvaez et al. 2023). It has been suggested this pathogen interaction is driven by the immune system of *P. leucopus* where *B. burgdorferi* lowers the immune response of a mouse and allows for easier colonization by *Ba. microti* (Djokic et al. 2019). In habitats where *P. leucopus* populations are low compared to other pathogen host species, this interaction may not occur. Other studies have noticed a link between *A. phagocytophilum* and *B. burgdorferi* co-infections which they suggest is due to the immune system suppression effects of *A. phagocytophilum* (Dumler, 2012). Narvaez et al. (2023) reported that in New Jersey where *Ba. mcroti* prevalence was lower in local *I. scapularis* populations there was no association for *B. burgdorferi*. However, they did notice co-infections for *A. phagocytophilum/B. burgdorferi* were much higher than expected from individual preferences. Strain

pathogenicity may be important for this interaction as Edwards et al. (2019) found that only *A. phagocytophilum* strain Ap-ha was significantly associated with *B. burgdorferi* co-infections. Our study was limited in our ability to decipher strain level associations, as we tested for *A. phagocytophilum* broadly with no concern for strain phenotype. This may explain, despite similar singular infection rates for both *Ba. microti* and *A. phagocytophilum*, only *B. microti* was significantly associated with *B. burgdorferi* co-infection. Both Ap-ha and Ap-v1 are broadly distributed across the northeastern US, generally occurring within the same geographic area. However, given our undifferentiated testing, we are unable to identify if the spatial distribution of these pathogens is different or if there is a significant interaction between *B. burgdorferi* and Ap-ha. In an anaplasmosis endemic area in New York, Ap-ha populations were shown to cluster separately from Ap-v1 populations in a broad geographic area (Prusinski et al. 2023). However, it is unknown if this is also the case in Rhode Island. To identify if Ap-ha is the primary strain circulating in Rhode Island, and identify a possible link to co-infections, future studies should differentiate between strains during molecular testing. Additionally, as strain Ap-v1 is entirely incapable of expressing as a disease in humans, strain differentiation would more accurately represent disease risk to local human populations (Massung et al. 2005)

This study revealed the number of *B. miyamotoi*-infected nymphs occurring in Rhode Island is lower in both infection rate and distribution compared to *B. burgdorferi* infected nymphs. *Borrelia miyamotoi* wasn't found in most sites (eight out of 40 sites). For sites where *B. miyamotoi*-infected nymphs where found, pathogen infection rate reached up to 5% suggesting that *B. miyamotoi* is incapable of reaching the prevalence

and distribution demonstrated by *B. burgdorferi,* such that *B. miyamotoi* will only ever occur were *B. burgdorferi* is present. Other studies in the Northeastern US have noticed a lower infection rate and spatial distribution of *B. miyamotoi* compared to *B. burgdorferi* (Keesing et al.2021). Reservoir host competence of both *B. burgdorferi* and *B. miyamotoi* are similar. However, most studies report, a consistently lower prevalence of *B. miyamotoi* in reservoir hosts and vectors (Cleveland et al. 2023). The key difference between the two Borrelia species is that *B. miyamotoi* is capable of transovarial transmission in *I. scapularis* while *B, burgdorferi* is not (Bruener et al. 2018). Experimental studies have shown *B. miyamotoi* is more efficient at transmitting vertically to larval offspring than horizontally to wildlife hosts (Lynn et al. 2022). It may be that *B. miyamotoi* is incapable of reaching high infection rates during the nymphal season as *I. scapularis* larvae would be unlikely to acquire it from a host due to poor horizontal transmission efficiency. However, efficient transovarial transmission would potentially lead to larval *I. scapularis* exhibiting high infection rates in local populations. This may mean that the peak risk for *B. miyamotoi* infection would be during the larval season rather than the nymphal season. However, Future field studies need to quantify the infection prevalence of *B. miyamotoi* in larval *I. scapularis* populations and compare them to nymphal prevalence of the same cohort.

When comparing how prevalence of pathogens varies within the nymphal season in 2022, we reported that the overall infection and individual pathogen species, except *A. phagocytophilum*, prevalence decrease from round 1 to round 2 was significant. This means that infected *I. scapularis* nymphs are either dying in a higher proportion compared to uninfected nymphs or that infected nymphs are questing and taking a

bloodmeal more than uninfected nymphs. For infected nymphs to be more efficient than uninfected nymphs at questing, pathogen infection status would need to be the primary reason for this change in questing behavior. Lefcort and Durden (1996) reported that *B. burgdorferi* infected *I. scapularis* nymphs were more attracted to light and vertical surfaces compared to uninfected nymphs. However, we are not aware of any studies that directly link *B. miyamotoi*, *Ba. microti,* or Powassan virus to manipulation of questing behavior in *I. scapularis*. Additionally, given that differences between prevalences were so small it would suggest that if a benefit towards questing is conferred by pathogen infection it is minor. Future efforts that collect pathogen prevalence data must focus on comparing intra-season variation in prevalence to determine if this a consistent annual relationship or how it could vary in higher prevalences years.

During our study, we estimated infection rates for various *Ixodes*-borne pathogens, co-infection rates between those pathogens, and the abundance and distribution of hard tick vector species in Rhode Island. However, conclusions on broad-scale temporal trends for infection rates of pathogens and abundance of non-*I. scapularis* is limited by a lack of historical data. Ideally, a multiple-year active surveillance system would be created in Rhode Island that monitors TBP infection rates and provide an index of tick infection rates to answer the current unknowns and provide guidance for an appropriate public health response. Future studies should also focus on identifying the environment and landscape drivers for the abundance of *A. americanum*. Coupled with this needs to be an analysis of *Amblyomma*-borne health risk, including both pathogen prevalence and alpha-gal syndrome in local *A. americanum* populations to determine

if infection rate varies alongside abundance and distribution. While testing for *Amblyomma*-associated pathogen has been done for *A. americanum* populations in RI before, this involved sampling from a high abundance site which did not capture the full distribution of *A. americanum* demonstrated by this study (Ijdo et al. 2000). For *Ixodes*-borne pathogens, more annual data should be generated to determine how infection rates vary across seasons and with densities of *I. scapularis* nymphs. As both tick vectors and other newly invading species continue to emerge as threats to public health, monitoring studies such as this project provide new insights into how these tick vector species expand. Additionally, emphasis must be placed on monitoring for TBP's and strains capable of causing human disease for all tick vector species.

BIBLIOGRAPHY

Barbour, A.G., Bunikis, J., Travinsky, B., Hoen, A.G., Diuk-Wasser, M.A., Fish, D. and Tsao, J.I. 2009. Niche partitioning of *Borrelia burgdorferi* and *Borrelia miyamotoi* in the same tick vector and mammalian reservoir species. *The American journal of tropical medicine and hygiene*. 81(6): 1120.

Belongia, E.A. 2002. Epidemiology and impact of co-infections acquired from *Ixodes* ticks. *Vector-borne and zoonotic diseases*. 2(4): 265-273.

Berger, K.A., Ginsberg, H.S., Dugas, K.D., Hamel, L.H. and Mather, T.N. 2014. Adverse moisture events predict seasonal abundance of Lyme disease vector ticks (Ixodes scapularis). *Parasites & vectors*. 7(1): 1-8.

Bratton, R. L., & Corey, G. R. (2005). Tick-borne disease. *American family physician*. 71(12): 2323-2330.

Breuner, N.E., Ford, S.L., Hojgaard, A., Osikowicz, L.M., Parise, C.M., Rizzo, M.F.R., Bai, Y., Levin, M.L., Eisen, R.J. and Eisen, L. 2020. Failure of the Asian longhorned tick, *Haemaphysalis longicornis*, to serve as an experimental vector of the Lyme disease spirochete, *Borrelia burgdorferi* sensu stricto. *Ticks and tick-borne diseases*. 11(1). 101311.

Breuner, N.E., Hojgaard, A., Replogle, A.J., Boegler, K.A. and Eisen, L. 2018. Transmission of the relapsing fever spirochete, *Borrelia miyamotoi*, by single transovarially-infected larval *Ixodes scapularis* ticks. *Ticks and tick-borne diseases*. 9(6): 1464-1467.

Cleveland, D.W., Anderson, C.C. and Brissette, C.A. 2023. *Borrelia miyamotoi*: A Comprehensive Review. *Pathogens*. 12(2): 267.

Couret. J., and Garrett, J. 2021. potential Effects of Climate Change on tick-borne diseases in Rhode Island. *Rhode Island Medical Journal*. 104(9): 29-33.

Cutler, S.J., Vayssier-Taussat, M., Estrada-Peña, A., Potkonjak, A., Mihalca, A.D. and Zeller, H. 2021. Tick-borne diseases and co-infection: Current considerations. *Ticks and tick-borne diseases*. 12(1): 101607.

Dergousoff, S.J., Galloway, T.D., Lindsay, L.R., Curry, P.S. and Chilton, N.B. 2013. Range expansion of *Dermacentor variabilis* and *Dermacentor andersoni* (Acari: Ixodidae) near their northern distributional limits. *Journal of Medical Entomology*. 50(3): 510-520.

Diuk-Wasser, M.A., VanAcker, M.C. and Fernandez, M.P. 2021. Impact of land use changes and habitat fragmentation on the eco-epidemiology of tick-borne diseases. *Journal of Medical Entomology*. 58(4): 1546-1564.

Diuk-Wasser, M.A., Vannier, E. and Krause, P.J. 2016. Co-infection by *Ixodes* tick-borne pathogens: ecological, epidemiological, and clinical consequences. *Trends in parasitology*. 32(1): 30-42.

Djokic, V., Akoolo, L., Primus, S., Schlachter, S., Kelly, K., Bhanot, P. and Parveen, N., 2019. Protozoan parasite *Babesia microti* subverts adaptive immunity and enhances Lyme disease severity. *Frontiers in microbiology*. 10: 1596.

Dolan, M.C., Hojgaard, A., Hoxmeier, J.C., Replogle, A.J., Respicio-Kingry, L.B., Sexton, C., Williams, M.A., Pritt, B.S., Schriefer, M.E. and Eisen, L. 2016. Vector competence of the blacklegged tick, *Ixodes scapularis*, for the recently recognized Lyme borreliosis spirochete Candidatus Borrelia mayonii. *Ticks and tick-borne diseases*. 7(5): 665-669.

Dumler, J.S., 2012. The biological basis of severe outcomes in *Anaplasma phagocytophilum* infection. *FEMS Immunology & Medical Microbiology*. 64(1): 13-20.

Dunn, J.M., Krause, P.J., Davis, S., Vannier, E.G., Fitzpatrick, M.C., Rollend, L., Belperron, A.A., States, S.L., Stacey, A., Bockenstedt, L.K. and Fish, D. 2014. *Borrelia burgdorferi* promotes the establishment of *Babesia microti* in the northeastern United States. *PLoS One*. 9(12): 115494.

Durden, L.A. and Keirans, J.E. 1996. Nymphs of the genus Ixodes (Acari: Ixodidae) of the United States: Taxonomy, identification key, distribution, hosts, and medical/veterinary importance. *Thomas Say Publications in Entomology: Mongraphs, Entomological Society of America*, Lanham, MD, 95p.

Edwards, M.J., Russell, J.C., Davidson, E.N., Yanushefski, T.J., Fleischman, B.L., Heist, R.O., Leep-Lazar, J.G., Stuppi, S.L., Esposito, R.A. and Suppan, L.M. 2019. A 4-yr survey of the range of ticks and tick-borne pathogens in the Lehigh Valley region of eastern Pennsylvania. *Journal of medical entomology*. 56(4): 1122-1134.

Egizi, A., Bulaga-Seraphin, L., Alt, E., Bajwa, W.I., Bernick, J., Bickerton, M., Campbell, S.R., Connally, N., Doi, K., Falco, R.C., Gaines, D.N., Telleasha, L.G., Harper, V.L., Heath, A.C.G., Jiang, J., Klein, T.A., Maestas, L., Mather, T.N., Occi, J.L., Oskam, C.L., Pendleton, J., Teator, M., Thompson, A.T., Tufts, D., Umemiya-Shirafiju, R., Vanacker, M.C., Yabsley, M.J., Fonseca, D.M. 2020. First glimpse into the origin and spread of the Asian longhorned tick, *Haemaphysalis longicornis*, in the United States. *Zoonoses and public health*. 67(6): 637-650.

Fiorito, T.M., Reece, R., Flanigan, T.P. and Silverblatt, F.J. 2017. *Borrelia miyamotoi* polymerase chain reaction positivity on a tick-borne disease panel in an endemic region of Rhode Island: a case series. *Infectious Diseases in Clinical Practice*. 25(5): 250-254.

Fish D. 1993. Population ecology of *Ixodes dammini*. In Ginsberg H. S. [ed.], Ecology and environmental management of Lyme disease. Rutgers University Press, New Brunswick, NJ

Gaughan, C.R. and DeStefano, S. 2005. Movement patterns of rural and suburban white-tailed deer in Massachusetts. *Urban Ecosystems*. 8(2): 191-202.

Ginsberg, H.S. and Ewing, C.P., 1989. Comparison of flagging, walking, trapping, and collecting from hosts as sampling methods for northern deer ticks, *Ixodes dammini*, and lone-star ticks, *Amblyomma americanum (Acari: Ixodidae). Experimental & applied acarology.* 7: 313-322.

Ginsberg, H.S. and Zhioua, E., 1996. Nymphal survival and habitat distribution of *Ixodes scapularis* and *Amblyomma americanum* ticks (Acari: Ixodidae) on Fire Island, New York, USA. *Experimental & Applied Acarology*. 20: 533-544.

Ginsberg, H.S. and Zhioua, E., 1996. Nymphal survival and habitat distribution of Ixodes scapularis and Amblyomma americanum ticks (Acari: Ixodidae) on Fire Island, New York, USA. *Experimental & Applied Acarology*. 20:533-544.

Ginsberg, H.S., E.L. Rulison, J.L. Miller, G. Pang, I.M. Arsnoe, G.J. Hickling, N.H. Ogden, R.A. Lebrun, and J.L. Tsao. 2020. Local abundance of *Ixodes scapularis* in forests: effects of environmental moisture, vegetation characteristics, and host abundance. *Ticks and Tick-borne diseases*. 11(1): 101271

Goddard, J. and Varela-Stokes, A.S., 2009. Role of the lone star tick, *Amblyomma americanum* (L.), in human and animal diseases. *Veterinary parasitology*. 160(1-2): 1-12.

Goethert, H.K., Mather, T.N., Johnson, R.W. and Telford III, S.R., 2021. Incrimination of shrews as a reservoir for Powassan virus. *Communications Biology*. 4(1): 1319.

Hermance, M.E. and Thangamani, S. 2017. Powassan virus: an emerging arbovirus of public health concern in North America. *Vector-Borne and Zoonotic Diseases*. 17(7): 453-462.

Hersh, M.H., Ostfeld, R.S., McHenry, D.J., Tibbetts, M., Brunner, J.L., Killilea, M.E., LoGiudice, K., Schmidt, K.A. and Keesing, F. 2014. Co-infection of blacklegged ticks with *Babesia microti* and *Borrelia burgdorferi* is higher than expected and acquired from small mammal hosts. *PloS one*. 9(6): 99348.

Ijdo JW, Wu C, Magnarelli LA, Stafford KC 3rd, Anderson JF, Fikrig E. 2000. Detection of Ehrlichia chaffeensis DNA in *Amblyomma americanum* ticks in Connecticut and Rhode Island. *Journal of Clinical Microbiology*. 38(12): 4655-4656.

Jordan, R.A. and Egizi, A. 2019. The growing importance of lone star ticks in a Lyme disease endemic county: passive tick surveillance in Monmouth County, NJ, 2006–2016. *PLoS One*. 14(2): 0211778.

Keesing, F., McHenry, D.J., Hersh, M.H. and Ostfeld, R.S. 2021. Spatial and temporal patterns of the emerging tick-borne pathogen *Borrelia miyamotoi* in blacklegged ticks (*Ixodes scapularis*) in New York. *Parasites & vectors*. 14: 1-7.

Keirans, J.E. and Clifford, C.M. 1978. The genus Ixodes in the United States: A scanning electron microscope study and key to the adults. *Journal of Medical. Entomology.* Suppl. 2: 1-149.

Kilpatrick, H. J., Labonte, A. M., & Stafford III, K. C. 2014. The relationship between deer density, tick abundance, and human cases of Lyme disease in a residential community. *Journal of Medical Entomology*. 51(4): 777-784.

Knapp, K.L. and Rice, N.A. 2015. Human co-infection with *Borrelia burgdorferi* and *Babesia microti* in the United States. *Journal of parasitology research.* 2015*:* 1-11.

Kollars Jr, T.M., Oliver Jr, J.H., Durden, L.A. and Kollars, P.G. 2000. Host associations and seasonal activity of Amblyomma americanum (Acari: Ixodidae) in Missouri. *Journal of Parasitology*. 86(5): 156-1159.

Krause, P.J. 2019. Human babesiosis. *International journal for parasitology*. 49(2): 165-174.

Krause, P.J., Narasimhan, S., Wormser, G.P., Barbour, A.G., Platonov, A.E., Brancato, J., Lepore, T., Dardick, K., Mamula, M., Rollend, L. and Steeves, T.K. 2014. *Borrelia miyamotoi* sensu lato seroreactivity and seroprevalence in the northeastern United States. *Emerging infectious diseases*. 20(7): 1183.

Lefcort, H. and Durden, L.A. 1996. The effect of infection with Lyme disease spirochetes (*Borrelia burgdorferi*) on the phototaxis, activity, and questing height of the tick vector *Ixodes scapularis*. *Parasitology*. 113: 97–10

Levin, M.L., Stanley, H.M., Hartzer, K. and Snellgrove, A.N. 2021. Incompetence of the Asian longhorned tick (Acari: Ixodidae) in transmitting the agent of human granulocytic anaplasmosis in the United States. *Journal of medical entomology*. 58(3): 1419-1423.

Lindsay, L.R., S.W. Mathison, I.K. Barker, S.A. McEwen, T.J. Gillespie, and G.A. Surgeoner. Microclimate and habitat in relation to *Ixodes scapularis* (Acari: Ixodidae)

populations on Long Point, Ontario, Canada. 1999. *Journal of Medical Entomology*. 36(3): 255-262

Lubelczyk, C.B., S.P. Elias, P.W. Rand, M.S. Holman, E.H. Lacombe, and R.P. Smith. 2004. Habitat associations of *Ixodes scapularis* (Acari: Ixodidae) in Maine. *Enviromental Entomology.* 33(4): 900-906

Luo, L.M., Zhao, L., Wen, H.L., Zhang, Z.T., Liu, J.W., Fang, L.Z., Xue, Z.F., Ma, D.Q., Zhang, X.S., Ding, S.J. and Lei, X.Y. 2015. *Haemaphysalis longicornis* ticks as reservoir and vector of severe fever with thrombocytopenia syndrome virus in China. *Emerging infectious diseases*. 21(10): 1770.

Lynn, G.E., Breuner, N.E., Hojgaard, A., Oliver, J., Eisen, L. and Eisen, R.J. 2022. A comparison of horizontal and transovarial transmission efficiency of *Borrelia miyamotoi* by *Ixodes scapularis*. *Ticks and Tick-borne Diseases*. 13(5): 102003.

Madison-Antenucci, S., Kramer, L.D., Gebhardt, L.L. and Kauffman, E. 2020. Emerging tick-borne diseases. *Clinical microbiology reviews*. 33(2): 10-1128.

Massung, R.F. Mauel, M.J., Owens, J.H., Allan, N., Courtney. J.W., and Stafford, K.C. III. 2002. Genetic variants of *Ehrlichia phagocytophila,* Rhode Island and Connecticut. *Emerging Infectious Diseases*. 8: 467–472

Mather, T. N., Nicholson, M. C., Donnelly, E. F., & Matyas, B. T. 1996. Entomologic index for human risk of Lyme disease. *American Journal of Epidemiology*. 144(11): 1066-1069.

Mather, T.N. and Mather, M.E. 1990. Intrinsic competence of three ixodid ticks (Acari) as vectors of the Lyme disease spirochete. *Journal of medical entomology*. 27(4): 646-650.

Mather, T.N., Nicholson, M.C., Donnelly, E.F. and Matyas, B.T., 1996. Entomologic index for human risk of Lyme disease. *American Journal of Epidemiology*. 144(11): 1066-1069.

McCarthy, D.E., LeNoir, J.M., and Lombard, P.J., 2023. 2022 drought in New England: U.S. Geological Survey Scientific Investigations Report 2023–5016. 34. https://doi.org/10.3133/sir20235016.

McShea, W. J., and D.M. Madison. 1992. Alternative approaches to the study of small mammal dispersal: insights from radiotelemetry. *dispersal: small mammals as a model.* 319-332

Minigan, J.N., Hager, H.A., Peregrine, A.S. and Newman, J.A. 2018. Current and potential future distribution of the American dog tick (*Dermacentor variabilis*, Say) in North America. *Ticks and Tick-borne Diseases*. 9(2): 354-362.

Murphy, D.S., Lee, X., Larson, S.R., Johnson, D.K.H., Loo, T. and Paskewitz, S.M. 2017. Prevalence and distribution of human and tick infections with the Ehrlichia muris-like agent and *Anaplasma phagocytophilum* in Wisconsin, 2009–2015. *Vector-Borne and Zoonotic Diseases*. 17(4): 229-236.

Narvaez, Z.E., Rainey, T., Puelle, R., Khan, A., Jordan, R.A., Egizi, A.M. and Price, D.C., 2023. Detection of multiple tick-borne pathogens in *Ixodes scapularis* from Hunterdon County, NJ, USA. *Current Research in Parasitology & Vector-Borne Diseases*. 4: 100140.

Nicholson, M.C. and Mather, T.N., 2014. Methods for evaluating Lyme disease risks using geographic information systems and geospatial analysis. *Journal of medical entomology*. 33(5): 711-720.

Nielebeck, C., Kim, S.H., Dedmon, L., Pangilinan, M., Quan, J., Ota, W. and Monzón, J.D., 2022. A novel laboratory method to simulate climatic stress with successful application to experiments with medically relevant ticks. *Plos one.* 17(9): 0275314.

Ogden, N.H., & L.R. Lindsay. 2016. Effects of climate and climate change on vectors and vector-borne diseases: Ticks are different. *Trends in parasitology*. 32(8): 646-656

Ogden, N.H., Ben Beard, C., Ginsberg, H.S. and Tsao, J.I., 2021. Possible effects of climate change on ixodid ticks and the pathogens they transmit: Predictions and observations. *Journal of Medical Entomology*. 58(4): 1536-1545.

Ogden, N.H., Mechai, S. and Margos, G. 2013. Changing geographic ranges of ticks and tick-borne pathogens: drivers, mechanisms and consequences for pathogen diversity. *Frontiers in cellular and infection microbiology*. 3: 46.

Parveen, N. and Bhanot, P. 2019. *Babesia microti—Borrelia burgdorferi* co-infection. *Pathogens*. (3): 117.

Piedmonte, N.P., Shaw, S.B., Prusinski, M.A. and Fierke, M.K. 2018. Landscape features associated with blacklegged tick (Acari: Ixodidae) density and tick-borne pathogen prevalence at multiple spatial scales in central New York state. *Journal of medical entomology*, 55(6): 1496-1508.

Piedmonte, N.P., Vinci, V.C., Daniels, T.J., Backenson, B.P. and Falco, R.C. 2021. Seasonal activity of *Haemaphysalis longicornis* (Acari: Ixodidae) in southern New York state. *Journal of Medical Entomology,* 58(2): 676-681.

Price, K.J., Witmier, B.J., Eckert, R.A., Boyer, C.N., Helwig, M.W. and Kyle, A.D. 2021. Distribution and density of *Haemaphysalis longicornis* (Acari: Ixodidae) on public lands in Pennsylvania, United States. *Journal of Medical Entomology*. 58(3): 1433-1438.

Pritt, B.S., Respicio-Kingry, L.B., Sloan, L.M., Schriefer, M.E., Replogle, A.J., Bjork, J., Liu, G., Kingry, L.C., Mead, P.S., Neitzel, D.F. and Schiffman, E. 2016. *Borrelia mayonii* sp. nov., a member of the *Borrelia burgdorferi* sensu lato complex, detected in patients and ticks in the upper midwestern United States. *International journal of systematic and evolutionary microbiology*. 66(11): 4878-4880.

Prusinski, M., O'Connor, C., Russell, A., Sommer, J., White, J., Rose, L., Falco, R., Kokas, J., Vinci, V., Gall, W. and Tober, K. 2023. Associations of *Anaplasma phagocytophilum* bacteria variants in *Ixodes scapularis* ticks and humans, New York, USA. *Emerging Infectious Diseases*. 29(3): 540.

R Team Core. 2018. R: a language and environment for statistical computing. https://www.gbif.org/tool/81287/r-a-language-and-environment-for-statistical-computing

Raghavan, R.K., Barker, S.C., Cobos, M.E., Barker, D., Teo, E.J.M., Foley, D.H., Nakao, R., Lawrence, K., Heath, A.C.G. and Peterson, A.T. 2019A. Potential spatial distribution of the newly introduced long-horned tick, *Haemaphysalis longicornis*, in North America. *Scientific reports*. 9(1): 498.

Raghavan, R.K., Peterson, A.T., Cobos, M.E., Ganta, R. and Foley, D., 2019B. Current and future distribution of the lone star tick, *Amblyomma americanum* (L.)(Acari: Ixodidae) in North America. *PloS one*. 14(1): e0209082.

Rocha, S.C., Velasquez, C.V., Aquib, A., Al-Nazal, A., and Parveen, N. 2022. Transmission Cycle of Tick-Borne Infections and Co-Infections, Animal Models and Diseases. *Pathogens*. 11(11): 1309

Rochlin, I., Egizi, A. and Lindström, A. 2022. The original scientific description of the lone star tick (*Amblyomma americanum*, Acari: Ixodidae) and implications for the species' past and future geographic distributions. *Journal of Medical Entomology*. 59(2): 412-420.

Rodino, K.G., Theel, E.S. and Pritt, B.S. 2020. Tick-borne diseases in the United States. *Clinical chemistry*. 66(4): 537-548.

Sack, A., Naumova, E.N., Price, L.L., Xu, G. and Rich, S.M., 2023. Passive Surveillance of Human-Biting *Ixodes scapularis* Ticks in Massachusetts from 2015–2019. *International Journal of Environmental Research and Public Health*. 20(5): 4306.

Sagurova, I., Ludwig, A., Ogden, N.H., Pelcat, Y., Dueymes, G. and Gachon, P. 2019. Predicted northward expansion of the geographic range of the tick vector Amblyomma americanum in North America under future climate conditions. *Environmental Health Perspectives*. 127(10): 107014.

Schulze, T.L., Jordan, R.A. and Hung, R.W. 2001. Effects of selected meteorological factors on diurnal questing of *Ixodes scapularis* and *Amblyomma americanum* (Acari: Ixodidae). *Journal of Medical Entomology*. 38(2): 318-324.

Schwartz, A.M., Hinckley, A.F., Mead, P.S., Hook, S.A. and Kugeler, K.J., 2017. Surveillance for lyme disease—United States, 2008–2015. *MMWR Surveillance Summaries*. 66(22): 1.

Schwartz, A.M., Kugeler, K.J., Nelson, C.A., Marx, G.E. and Hinckley, A.F. 2021. Use of commercial claims data for evaluating trends in Lyme disease diagnoses, United States, 2010–2018. *Emerging infectious diseases*. 27(2):499.

Schwartz, S., Calvente, E., Rollinson, E., Sample Koon Koon, D. and Chinnici, N. 2022. Tick-Borne Pathogens in Questing Blacklegged Ticks (Acari: Ixodidae) From Pike County, Pennsylvania. *Journal of Medical Entomology*. 59(5): 1793-1804.

Smalley IV, R., Zafar, H., Land, J., Samour, A., Hance, D. and Brennan, R.E. 2022. Detection of *Borrelia miyamotoi* and Powassan virus lineage II (deer tick virus) from *Odocoileus virginianus* harvested *Ixodes scapularis* in Oklahoma. *Vector-Borne and Zoonotic Diseases*. 22(4): 209-216.

Springer, Y. P., L.Eisen, L.Beati, A. M.James, and R. J. Eisen. 2014. Spatial distribution of counties in the continental united states with records of occurrence of *Amblyomma americanum* (Ixodida: Ixodidae). *Journal of Medical Entomology.* 51: 342–351.

Stafford III, K.C., Molaei, G., Little, E.A., Paddock, C.D., Karpathy, S.E. and Labonte, A.M., 2018. Distribution and establishment of the lone star tick in Connecticut and implications for range expansion and public health. *Journal of medical entomology*. 55(6): 1561-1568.

Stafford K. C. Cartter M.L. Magnarelli L. A. Ertel S. H. Mshar P. A. 1998. Temporal correlations between tick abundance and prevalence of ticks infected with Borrelia burgdorferi and increasing incidence of Lyme disease. *Journal of Clinical Microbiology*. 36: 1240–1244.

Swanson, S.J., Neitzel, D., Reed, K.D. and Belongia, E.A. 2006. Co-infections acquired from Ixodes ticks. *Clinical microbiology reviews*. 19(4): 708-727.

Thapa, S., Zhang, Y. and Allen, M.S. 2019. Bacterial microbiomes of *Ixodes scapularis* ticks collected from Massachusetts and Texas, USA. *BMC microbiology*. 19: 1-12.

Thompson, A.T., White, S.A., Shaw, D., Garrett, K.B., Wyckoff, S.T., Doub, E.E., Ruder, M.G. and Yabsley, M.J. 2021. A multi-seasonal study investigating the

phenology, host and habitat associations, and pathogens of *Haemaphysalis longicornis* in Virginia, USA. *Ticks and Tick-borne Diseases*. 12(5):m101773.

Thompson, J.M., 2023. Geographic Distribution of Suspected Alpha-gal Syndrome Cases—United States, January 2017–December 2022. *MMWR. Morbidity and Mortality Weekly Report*. 72(30): 815 - 820

Tsao, J.I., Hamer, S.A., Han, S., Sidge, J.L. and Hickling, G.J. 2021. The contribution of wildlife hosts to the rise of ticks and tick-borne diseases in North America. *Journal of medical entomology*. 58(4): 1565-1587.

Tufts, D.M. and Diuk-Wasser, M.A., 2021. First hemispheric report of invasive tick species *Haemaphysalis punctata*, first state report of *Haemaphysalis longicornis*, and range expansion of native tick species in Rhode Island, USA. *Parasites & Vectors*. 14(1): 1-6.

Wilson, M.L., Adler, G.H. and Spielman, A., 1985. Correlation between abundance of deer and that of the deer tick, Ixodes *dammini* (Acari: Ixodidae). *Annals of the Entomological Society of America*. 78(2):172-176.

Xu, G., Foster, E., Ribbe, F., Hojgaard, A., Eisen, R.J., Paull, S. and Rich, S.M., 2023. Detection of *Ehrlichia muris eauclairensis* in Blacklegged Ticks (*Ixodes scapularis*) and White-Footed Mice (*Peromyscus leucopus*) in Massachusetts. *Vector-Borne and Zoonotic Diseases*. 23(6): 311 – 315

Xu, G., Mather, T.N., Hollingsworth, C.S. and 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.