In summer 2022 (May, June & July) we sampled at 40 historical sites to determine the density of *Ixodes scapularis* nymphs across Rhode Island (RI). Additionally, we tested all nymphs for 6 tick-borne pathogens known or suspected to occur within RI. We analyzed our dataset to determine rates of infection and co-infection, environmental covariates that might mediate infection rates, and the spatial relationship of nymphal infection.

Overall, we collected 1566 *I. scapularis* across 38 sites (2 sites under testing) with no nymphs found to be infected for *Borrelia mayonii* or *Ehrlichia muris eauclairensis*. Infection rates, considered here as # of infected nymphs, were as follows: 266 for *B. burgdorferi (Bb), 17* for *B. miyamotoi (Bmy),* 107 for *Babesia microt (Bam)i*, 105 for *Anaplasmosis phagocytophilum (Ap)*, and 4 for Powassan Virus/Deer Tick Virus (DTV). Additionally, 65 nymphs were to be simultaneously infected with 2 or more pathogens simultaneously, hereafter referred to as co-infection. Among these co-infected nymphs, 2 nymphs were found to have a triple co-infection (simultaneous infection with Bb/Bam/Ap). The remaining 63 nymphs only had double co-infections of varying frequency between pathogen combinations (Fig. 1). Interestingly, despite there being roughly similar rates of individual infections across nymphs, co-infection rates are substantially different between *A. phagocytophilum* an *Ba. microti*. In co-infection with *B. burgdorferi*, *B. microti* had nearly 2.5 times as many infections than co-infection with *A. phagocytophilum*. All other co-infection between 2 pathogens were low, generally not any higher than 5 infected nymphs.

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Figure . Graph of frequency of double co-infections across all observed pathogen combinations.

Continuing down the line to analyze relationship between pathogens, we used parametric statistical tests to determine if there was a positive, negative, or random relationship for occurrence between pathogen specie pairs. We performed analysis in terms of both prevalence and presence/absence. We used a specific package called “coccur” in R, with built in tools for analysis. Relationship between pathogens were analyzed using the formula below (2). Here *N* = the number of sites where either species 1 or species 2 occurs. *J =* number of sites where both species 1 and species 2 occurs. *Pj* = the probability that 2 species co-occur at exactly *j* number of sites.

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Figure . Formula to determine relationship between pathogen into positive, negative, or random.

Based on this analysis we consider infection of terms of spatial sites were we sampled and as each individual nymph as a “site”. Since nymphs experience differing selection pressure due to host movements and interaction between pathogen, we think this a reasonable generalization for what constitutes. In terms of sites, we found that all possible double combinations for all pathogens except Deer Tick Virus we positively associated. This is unsurprising considering the number of collected nymphs and the closeness of sites. However, when we analyzed co-occurrence across individuals, we found that the only non-random positive association was between *B. burgdorferi* and *Ba. microti* (P-values .0001) (Figure 2.). All other pair combinations were found to be purely random. Additionally, we found that considering abundance of pathogen species at a site (# of infected nymphs) all associations between pathogen pairs were random.

Timeline

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Figure . Species Co-occurrence matrix for pathogen species within individuals I. scapularis nymphs.

We then fit a Generalized linear model (GLM) to understand if rates of non-*B. burgdorferi* influenced rates of *B. burgdorferi* infection in nymphs. This is distinct from the previous analysis since this only consider a corollary relationship among singular infection rates at the site level rather than co-infections. Using a gaussian distribution with a logit link function we used *A. phagocytophilum, Ba. microti,* and *B. miyamotoi* as predictors and *B. burgdorferi* as the response variable. All variables were square root transform to normalize the distribution. We found that while *B. miyamotoi* ( P-value .009) and *Ba. microti* (P-value < .0001) were significant predictors of *B. burgdorferi* singular infection rates, *A. phagocytophilum* was not (P value .759) (Table 1). Model Coefficients suggest that when *Ba. microti* and *B. miyamotoi* infection rates rise than *B. burgdorferi* infection rates rise in tandem. However, when we tested models *A. phagocytophilu*m was significant until *Ba. microti* was included as a predictor. *B. miyamotoi* Among all models tested AIC score was lowest for the currently discussed model, suggesting that the data is a good fit for this model. An issue of collinearity may arise due to the high correlation between *B. burgdorferi* and all other pathogens, but we consider the non-significance of *A. phagocytophilum* to be confirmation of non-collinearity.

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Figure . Summary table of B. burgdorferi infection rate as function of other pathogen infection rates.

We additionally tested models to understand if probability of a nymph becoming infected with a specific pathogen. As infection status is binary (infected/not infected) we assumed a binomial distribution with a logit link function in a GLM. We considered TAMEs (Tick adverse humidity events), % forest cover, and the interaction between them for use in predicting infection across all pathogens. We also attempted to use % forest cover across deciduous, coniferous, and mixed forest but found them to be highly correlated and collinear due to high coefficients we found in model testing We also fit a model with these covariates to determine if the same held true for co-infection probability. A separate GLM was fitted for each mode. The model of best fit for all pathogens and co-infections was found to be that included all covariates and the interaction between them. We found that models that included TAMEs and % forest cover and their interactions as the best fit for the data. We found that TAMEs, % forest cover, and interaction was not significant for *B. miyamotoi* and *A. phagocytophilum*. % forest cover was significant for *Ba. microti* and co-infection. TAMES, % forest cover, and interaction was only significant for *B. burgdorferi.*

Next, we analyzed spatial dependence for all pathogens. To do this considered all previous covariates but added a nugget effect to the model. In short, a low nugget effect suggests that a variable does not experience large scale variability compared to similar spatial points surrounding the measured point. A high nugget suggests the variability is so high that a spatial point, in our case a site, does not share similarity with nearby measured sites. Nugget must be compared to the range, which denotes distance between sites. We assumed a zero inflated distribution and a spherical spatial correlation function. We did not consider Deer Tick Virus due to low prevalence. We found there to be a moderate spatial association for A. phagocytophilum but not for any other pathogen, co-infections, or total infection.

Overall, while there are some associations between pathogens, we could not identify any strong spatial relationship for any pathogen or co-infection except for A. phagocytophilum.