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Evaluating the Impact of Climatic Factors on Tick-Borne Pathogen Prevalence: Insights from Ixodes scapularis and its Borrelia burgdorferi, Anaplasma phagocytophilum, and Babesia microti Pathogens

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Evaluating the Impact of Climatic Factors on Tick-Borne Pathogen Prevalence: Insights from *Ixodes scapularis* and its *Borrelia burgdorferi, Anaplasma phagocytophilum*, and *Babesia microti* Pathogens

A Thesis Presented for the Status of

Honors College Scholar

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April 17, 2024

AKNOWLEDGEMENTS

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ABSTRACT

Tick-borne zoonoses represent a significant and escalating public health threat, particularly in the United States, where ixodid (family Ixodidae) ticks serve as vectors for an array of tick-borne pathogens. With the release of climate change projections, understanding the complex interplay between environmental variables and disease dynamics becomes paramount. This study integrates epidemiological, molecular, and climatological data to investigate the influence of temperature and precipitation on the prevalence of *Borrelia burgdorferi*, *Babesia microti*, and *Anaplasma phagocytophilum* within adult *Ixodes scapularis* populations. Leveraging multiple linear regression models and climate projections, my analysis reveals associations between climatic factors and pathogen prevalence rates. While temperature emerges as a key determinant, precipitation exhibits a comparatively lower influence. By extrapolating current trends into future climate scenarios, our projections suggest a notable escalation in disease incidence by 2070. Despite inherent limitations in our model, including data constraints and potential overestimation of pathogen prevalence, our findings underscore the urgent need for continued research and proactive public health measures to mitigate the threat of tick-borne zoonoses amidst a changing climate. This comprehensive approach provides valuable insights into the dynamic nature of environmental factors, vector ecology, and disease transmission dynamics, informing targeted strategies for disease prevention and protection of the public health.

1 Introduction

1.1 Tick-Borne Zoonoses

The people are getting sicker—an evocative opening line for a dystopian narrative and an unequivocal truth regarding the state of public health within the United States (CDC, 2024). Among the myriad pathogens threatening human health, ticks, particularly ixodid ticks (family Ixodidae), emerge as formidable vectors of zoonotic diseases. These arthropods play a unique role in disease transmission, harboring and transmitting an array of pathogens including protozoa, bacteria, and viruses (Jongejan and Uilenberg 2004). Ticks are responsible for transmitting approximately 95% of locally acquired, nationally notifiable human vector-borne diseases reported annually to the Centers for Disease Control and Prevention (CDC)(Adams et al. 2016; Paddock et al. 2016). Over recent decades, the incidence of tick-borne diseases has surged, with expanding geographic distributions and the emergence of novel tick-borne pathogens compounding the public health burden (Paddock et al. 2016).

Of the numerous tick species inhabiting the United States, a select few from the families Ixodidae and Argasidae pose significant risks to human health due to their propensity to feed on humans (Merten and Durden 2000). These ticks, such as *Amblyomma americanum*, *Dermacentor variabilis*, and *Ixodes scapularis* (*I. scapularis*), are primary vectors of tick-borne illnesses, and orchestrate the transmission of pathogens to vertebrate hosts across their life stages. In light of the escalating threat posed by tick-borne zoonoses, a comprehensive understanding of tick ecology, pathogen dynamics, and vector-host interactions is imperative for devising effective strategies to mitigate disease transmission and safeguard public health.

The primary tick vector in the eastern United States, the blacklegged tick, *I. scapularis*, is associated with a diverse array of diseases. Associated with this vector are the zoonoses: Lyme

disease caused by *Borrelia burgdorferi* and *Borrelia mayonii*, relapsing fever caused by *Borrelia miyamotoi*, Anaplasmosis attributed to *Anaplasma phagocytophilum*, Ehrlichiosis induced by *Ehrlichia muris euclairensis*, and Babesiosis caused by *Babesia microti* (Dolan et al. 1997, 2016; Ebel 2010; Johnson et al. 2015; Karpathy et al. 2016; Krause et al. 2015; Piesman and Eisen 2008; Pritt et al. 2011, 2016a, 2016b, 2016c; Teglas and Foley 2006). Of these diseases, Lyme disease has garnered widespread attention for its ability to cause long-term complications if not promptly diagnosed and treated. Similarly, babesiosis and anaplasmosis have demonstrated an increasing incidence in recent years, underscoring the urgency of addressing their transmission dynamics across their shared vector.

Conventional public health strategies typically encompass diagnosis, testing, and treatment protocols - a perspective that perpetuates medicalization and urges ecologists and molecular geneticists to explore means to immunization. However, for diseases lacking a known vaccine, like Lyme disease, prevention strategies necessitate a nuanced understanding of local ecological dynamics. This proactive approach aims to mitigate potential cases by unraveling the intricate relationship between *I. scapularis* and its reservoir hosts. This relationship, classified as a highly complex ecological system, presents challenges that impede accurate and comprehensive research efforts (Mather & Ginsberg1994). Overcoming these obstacles is essential for advancing preventive strategies and empowering disease ecologists to assume a pivotal role in public health research.

1.2 Ixodid Distribution Modelling

Despite these challenges, considerable effort has been directed into understanding tick distribution models in an attempt to better understand the ecological forces that threaten human health. As of 2022, 107 peer-reviewed articles have been published detailing various distribution models for tick species in North America and Europe. Among them, the most frequently modeled species was *Ixodes ricinus* (n=23), followed closely by *I. scapularis* (n=16) (Kopsco et al., 2022). Recent publications have focused on population distributions of I. *scapularis* in newly suitable habitats, such as Florida (Glass et al., 2021; Kessler et al., 2019) and Ontario and Ottawa, Canada (Slatculescu et al., 2020; Soucy et al., 2018, respectively). Earlier studies examined regions more representative of New England, like New Brunswick (Lieske and Lloyd, 2018) and the United States as a whole (Hahn, 2016; Kopso, Smith, and Halsey, 2022).

Earlier models utilized ground-observed environmental data to forecast the expansion of established *I. scapularis* populations. Autologistic analysis uncovered that maximum, minimum, and mean temperatures, in addition to vapor pressure, played significant roles in maintaining tick populations, achieving an impressive accuracy of 95% (Brownstein, 2003). Subsequent studies, exemplified by the work of Hahn et al. in 2016, employed response curves for climate, elevation, and land cover variables chosen by refined *I. scapularis* models to delineate suitable habitats where surveillance records had yet to designate counties as established tick habitats. Among these variables, maximum temperature and precipitation of the warmest quarter, and precipitation of the driest quarter emerged as the most influential climatic factors affecting county habitability, corroborated by multiple optimized models.

The more recent work by Lieske and Lloyd (2018) applied occupancy modeling through passive surveillance to determine if New Brunswick, Canada, an area sharing a northeast border with Maine, was suitable area for *I. scapularis* populations. The study found that milder winter conditions and rising annual precipitation were correlated with a positive predictor of tick occurrence. By this estimate, climate projections suggest that approximately a quarter of the region would be suitable for *Ixodes scapularis* populations by the 2080's.

Through the examination of climate-dependent dynamics throughout various stages of the tick life cycle, whether in nymphal or adult forms, these researchers employ a "bottom-up" methodology to dissect the zoonotic threats posed to human health. The "bottom-up" approach aims to elucidate the relationship of environmental factors on tick ecology across diverse landscapes, albeit with inherent assumptions that may introduce uncertainties into its broader application. Nonetheless, the collective findings consistently point towards a climate-mediated expansion of tick populations, underscoring the imperative for continued investigation. Given the demonstrable influence of climatic variables such as temperature and precipitation on tick population dynamics, the looming specter of climate change raises pertinent questions regarding its potential to exacerbate zoonotic disease transmission. The critical question then remains: to what extent will climate-induced alterations in environmental conditions foster an environment conducive to the proliferation of tick-borne diseases?

1.3 Climate and Ixodid-Host Dynamics

The literature suggests that the proliferation of tick populations is poised for continued expansion (Paddock et al., 2016). However, the intricate interplay between population dynamics and disease prevalence rates is multifaceted (Dobson, 2004). *I. scapularis* and its pathogens, *B.*

burgdorferi, *B. microti*, and *A. phagocytophilum*, operate within an enzootic transmission cycle (Prusinski, 2014). This would suggest that while tick population models anticipate a favorable trajectory for the habitat range of *I. scapularis*, these same environmental variables may exert differential impacts on the persistence and transmission rates of pathogens across diverse animal hosts.

1.3.1 Transmission Dynamics

The presence of pathogens within tick populations relies heavily upon the activity of *I. scapularis* and its reservoir hosts, a dynamic deeply intertwined with phenology (Couret, 2022). This relationship, defined by interspecies interactions, seasonality, spatiotemporal patterns, and climatic variance contribute to the high complexity of the tick-host system (Couret, 2022; VanAcker, 2022). While research has yet to fully illuminate the intricacies of the tick-host system, the partial insights gleaned from interdisciplinary efforts are invaluable in preventative measures to public health. Among these insights, the transmission dynamics of tick-borne pathogens, including *B. burgdorferi*, *B. microti*, and *A. phagocytophilum*, are particularly noteworthy, as they are intimately connected to the life cycle of their respective vectors.

The process begins with the hatching of uninfected larvae, which actively seek hosts, typically small mammals, or birds, to initiate the infection cycle. Since these pathogens are not transmitted transovarially, larvae acquire them during their first blood meal, potentially infecting the tick. As these larvae develop into nymphs, they may transmit the pathogen during subsequent feedings, often on small or medium-sized mammals (Arthur, 1962). As a result, if environmental factors affect the activity of ticks in their enzootic cycle, then differentiation between ixodid models and pathogen prevalence can be expected.

1.3.2 Biotic and Abiotic Factors Affect Activity

The activity of *I. scapularis* and the transmission cycle across its reservoir hosts are significantly influenced by climatic and environmental variables (Gray, 2009 Süss, 2008, Hartemink, 2019; Estrada-Peña, 2012, Hubálek, 2003). Ticks, like many other arthropods, are highly sensitive to climate conditions, which has been found to impact their development, survival, and questing behavior (Gray, 2009; Hubálek 2003). While warmer temperatures may favor tick survival and population growth, excessive heat and water stress can inhibit tick activity (Hartemink, 2019). Questing behavior, critical for finding hosts and transmitting pathogens, is influenced by temperature and humidity, affecting the intensity of tick infestations in reservoir hosts and the risks for humans (Süss, 2008).

1.4 Climate-Dependent Ixodid Zoonoses Prevalence Projections

The existing body of literature provides substantial evidence supporting a positive correlation between climatic variables, including temperature and precipitation, and various aspects of *I. scapularis ecology*, such as its survival, abundance, and population dynamics (Ogden et al., 2005; Schulze et al., 2009). Additionally, these climatic factors have been shown to alter the activity of the ixodid species, which would suggest differential results between ixodid projection models and prevalence rates. Given the ongoing phenomenon of climate change, which is projected to induce significant alterations in temperature and precipitation patterns across the continent, models forecasting the expansion of ixodid populations must also account for the potential implications for human health along the same dimensions.

Due to the intricate dynamics characterizing *I. scapularis* ecology, the myriad assumptions inherent in modeling render findings somewhat nuanced and occasionally discordant across

studies. Given the emerging phenomenon of co-infections, it becomes imperative for research endeavors to be transferable and applicable to broader contexts (Lou, 2017). Consequently, analyses that center on a specific pathogen may yield insights that are germane to understanding other related pathogens, fostering a more inclusive comprehension of the intricate interplay between vector ecology and disease transmission.

If climatic variables indeed wield significant influence over tick population dynamics and zoonotic pathogen prevalence, then it stands to reason that climate change will disrupt the observed incidence rates, deviating from what would be anticipated under a traditional exponential growth model. Moreover, by concurrently studying *Borrelia burgdorferi*, *Babesia microti*, and *Anaplasma phagocytophilum*, a multifaceted perspective emerges, complementing climate change projections with novel disease forecasts.

2 Methods

We merged four types of data to conduct the regression analysis in this study: meteorological data from the National Oceanic and Atmospheric Administration (NOAA), ground-level observed PCR results of adult *I. scapularis* samples, annual state-level epidemiological data on Lyme disease, babesiosis, and anaplasmosis cases from the CDC, and climate projections from CHELSA. The CDC, NOAA, and CHELSA databases are publicly available.

2.1 Meteorological Data

The Global Summaries dataset, referred to as GSOM for Monthly, provides monthly meteorological data spanning from 1763 to the present. It includes various parameters such as monthly mean maximum, mean minimum, and mean temperatures, total precipitation, snowfall, departure from normal temperature and precipitation, heating and cooling degree days, days with extreme temperatures and precipitation, foggy days, and thunderstorm occurrences. These data are sourced primarily from the Global Historical Climatology Network - Daily (GHCN-Daily) dataset (Lawrimore, 2016).

2.1.1 Forest Matching

New England state forests, in conjunction with established research sites affiliated with the UVM Wildlife Pathogens Laboratory, were aligned with NOAA weather stations by a spatial correspondence procedure. Leveraging the Haversine formula to account for the Earth's curvature, a cohort of 146 weather stations was successfully paired with their corresponding forest locations, exhibiting an average spatial separation of 10 kilometers. However, the spatial

disparity between forests and their matched weather stations exceeded one standard deviation for 77 forest locations, signifying a non-negligible level of spatial discrepancy.

2.1.2 Preprocessing

Among the matched weather stations, a total of 57 climatic variables were recorded; however, we found not all weather stations were uniform in their data collection capabilities. Remarkably, 42% of the 146 stations failed to measure climatic variables, excluding DP01 (number of days with >= 0.01 inch/0.254 millimeter in the month), DP10 (number of days with >= 0.1 inch/2.54 millimeters in the month), DP1X (number of days with >= 1.00 inch/25.4 millimeters in the month), DSND (number of days with snow depth >= 1 inch/25 millimeters), DSNW (number of days with snowfall >= 1 inch/25 millimeters), EMNT (extreme minimum temperature for month), EMXT (extreme maximum temperature for month.), EMXP (highest daily total of precipitation in the month), PRCP (total monthly precipitation), TAVG (average monthly temperature), TMIN (monthly minimum temperature), TMAX (monthly maximum temperature) and SNOW (total monthly snowfall), for the month of July 2022. Moreover, only 58% of stations provided measurements for average temperature. Forest sites paired with weather stations characterized by incomplete meteorological data were deemed inadequate for further analysis and thus excluded from subsequent investigations.

2.1.3 Site Selection

To identify representative forest sites for modeling New England climatic dynamics, two Principal Component Analyses (PCA's) were conducted. PCA[1], which incorporated all variables except TAVG, and PCA[2], which excluded PRCP, were employed. The variables TAVG and PRCP were plotted against their respective PCA axes. Through a rigorous selection

process, four forest sites were identified based on the following criteria: (1) representation of the four quartile ranges of average temperatures and precipitations in New England, (2) minimal summed residual across both regressions, and (3) accessibility and availability of scientific collection permits. The output of this selection process can be seen in Figure: 1. The sites deemed most representative for modeling New England climatic dynamics were: Mountain Laurel Estates, Goffstown, NH; Jericho Research Center, Jericho, VT; St. Mikes, Winooski, VT; and Ashburnham State Forest, Ashburnham, MA.
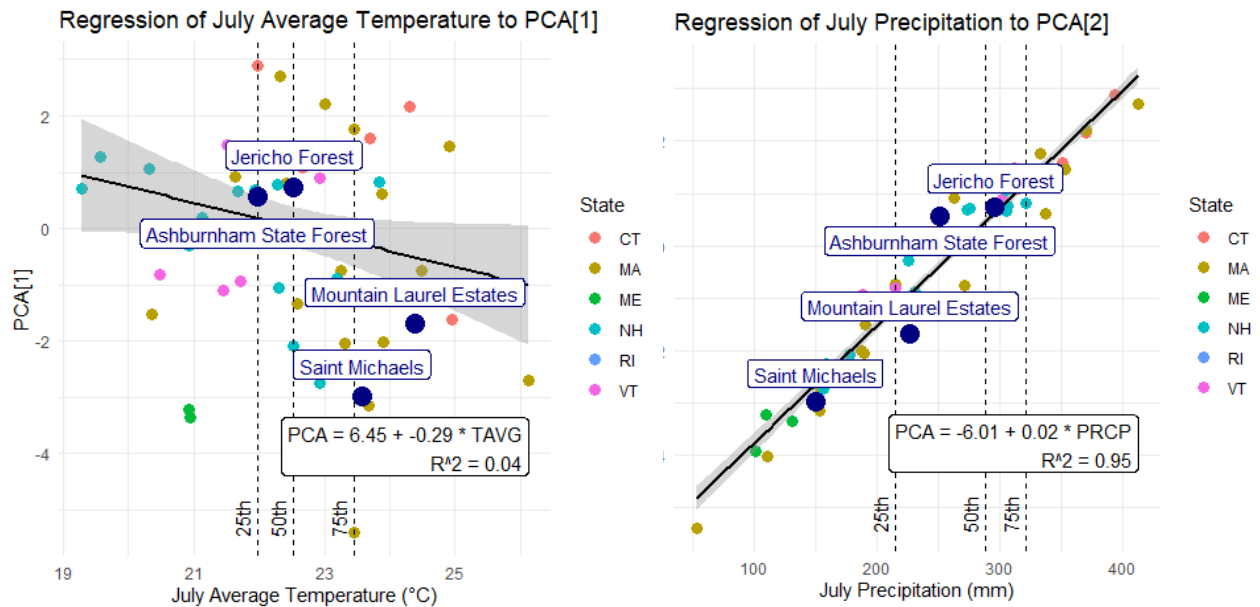
Figure 1. Relationship Between Average July Temperature, Precipitation, and PCA Scores. Scatter plots depicting the association between average July temperature, precipitation, and PCA scores. Black dotted vertical lines delineate quartile ranges, while a gray shadow around the regression line indicates the 95% confidence interval. The PCA equation and R² value for the regression are provided in the bottom right corner. Data points represent various state forests derived from preprocessed NOAA Monthly Summaries for July 2022. The identified forests, Ashburnham State Forest, MA; Jericho Forest, VT; Mountain Laurel Estates, NH; and Saint Michael's, VT were selected as the most representative sites for ixodid collection.

2.2 Molecular Data

2.2.1 Field Sampling

Adult *I. scapularis* specimens were collected from designated sites between May and September of 2023, following landowner consent. Collection was conducted using a 1x1 meter white drag cloth. Each collected specimen was preserved in 250 mL plastic tubes containing 100 mL of 100% ethanol. To maintain and store specimens, each tube accommodated a maximum of 10 samples and was stored at room temperature until transferred to a freezer set at -17.8°C until future molecular analysis. Given that the sampling period coincided with a period of dormancy for adult *I. scapularis*, site revisits were conducted weekly until an approximate total of 50 samples per site were obtained.

2.2.2 Molecular Methods

To detect the presence *of B. burgdorferi*, *B. microti*, and *A. phagocytophilum*, all specimens were screened by PCR methods. DNA was first extracted from full body samples using the Qiagen DNeasy Blood and Tissue Kit following manufacturer's instructions. Following the procedure for Multiplex PCR in Diagnostic Virology, we used the forward primers RecR, 5BM, and 5AP and the reverse primers RecF, 3BM, and 3AP (Elnifro et al., 2000). In the thermocycler, the samples were run in cycles of 94°C for 30 seconds, 56°C for 90 seconds, and 72°C for 90 seconds. A total of 40 cycles were completed, with a 15-minute denaturation at 95°C and a final extension of 72°C for 10 minutes.

The PCR products were subjected to gel electrophoresis using 2% agarose gels to facilitate size differentiation. This technique enabled the identification of distinct bands corresponding to the amplified DNA fragments. Specifically, a band at approximately 550 base pairs signified a

positive infection of *B. burgdorferi*, while a band at approximately 310 base pairs indicated a positive infection of *B. microti*. Additionally, the presence of a band at approximately 1200 base pairs was indicative of a positive infection of *A. phagocytophilum*. The recorded infections were documented, and the samples were properly disposed of in accordance with established laboratory protocols.

2.3 Empirical Strategy

Using an incidence model derived from an exponential growth model and climatic dependent multiple linear regression, the regression model to examine the impact of the change in climatic variables C_i - in our case, the change in average temperature and total precipitation in July—on an outcome of interest Y_t - in our case, the prevalence of pathogens - takes the form:

$$Y_t = Y_0(1+\rho)^t + \sum_{i=1}^{n} \frac{\partial Y}{\partial C_i}(\beta_i C_i + \epsilon_i) \qquad (1)$$

where i indexes different climatic variables, t indexes time, and ρ indexes the growth rate of Y independent of the change in climatic variables defined by C_i. The error process ϵ_i is modeled using standard errors, allowing for arbitrary correlation over time and space in the covariance matrix clustered at the state level.

Recognizing the random variations in climatic variability, akin to weather changes that draw from the broader state climate distribution, C_i considers the change in such variables with the assumption that variability will be reduced across dimensions. The future projection for C is collected from v2.1 CHELSA monthly projections across the models SSP126, SSP370, and SSP575 (Karger, 2017). Consequently, *Ci* pinpoints the causal impact of climatic variations on

disease incidence. The inclusion of the observed growth rate, denoted as ρ, effectively absorbs unobservable spatial characteristics.

The selection of temperature and precipitation as primary climatic variables in our study is rooted in their profound impact on the activity and abundance of *I. scapularis* (Ogden et al., 2005; Schulze et al., 2009; Burtis et al., 2016). Temperature regulates the mobility of these ticks (Burtis et al., 2016; Benelli, 2020), influencing their ability to seek hosts, while humidity governs their questing behavior (Knülle, 1982). While relative humidity is an optimal measure, its limited availability necessitated the use of precipitation, which also significantly affects tick behavior (Burtis et al., 2016). Thus, comprehending the intricate interplay between temperature, precipitation, and tick activity is paramount for understanding disease dynamics and devising effective preventive strategies.

A fundamental issue with Equation 1 is regarding the functional form of *C*. Based off the noted trends in collected data, it was determined to consider the variables across a continuous spectrum, as opposed to bins for temperature and precipitation. As a result, the output is considered across a continuous spectrum. In this context, the functional form of *C* is determined by the nature of the climate data and how it varies continuously over time or space, that adequately captures the continuous variation in temperature or precipitation over the relevant time period or geographic area. The choice of employing β values for each variable, represents an effort to allow the data, rather than polynomial assumptions, to determine the incidence-variable relationship. This degree of flexibility and freedom from assumption arises from the careful selection of sites that are intended to be highly representative of New England ecology.

The second aspect to consider regarding Equation 1 pertains to the necessity of an accurate and precise ρ. The observed rate of growth is defined as the annual change in the prevalence of the

disease-causing pathogens, accommodating unobserved variations across sites. The underlying assumption posits that ρ represents the average rate of change over each time step, t, and remains independent of the climatic variables, C. As time (t) progresses, the significance of ρ increases exponentially. Furthermore, the assumption is made that pathogen prevalence follows an exponential growth model. This assumption finds partial support from CDC observations of annual incidence cases and the absence of evidence suggesting a carrying capacity beyond the presence of ticks and reservoir species. Consequently, this equation is applicable solely in scenarios where ixodid ticks are anticipated to be present. The methodology for computing ρ is elaborated upon in the subsequent section.

2.4 Epidemiological Data

In order to calculate ρ, we collected CDC incidence reports for Lyme disease, Babesiosis, and Anaplasmosis, and applied a rigorous function that seeks to fit an exponential model to the dataset. The function begins by initializing parameters for time, where each data point is indexed sequentially. Subsequently, it defines an objective function, which computes the sum of squared differences between the observed data and the predicted values based on the exponential growth model. The function then utilizes optimization techniques to minimize this objective function, effectively estimating the parameters of the exponential model, including the R value. This method employs the Nelder-Mead method for nonlinear optimization.

An inherent limitation of the current methodology lies in its temporal scope. Despite the redefinition of Lyme Disease in 2008, available data from the CDC spans only the past 16 years, providing an annual record of its incidence across states. Conversely, babesiosis and anaplasmosis are diseases of emerging concern, with data on Babesiosis incidence by state

spanning from 2014 to 2020 and Anaplasmosis incidence recorded from 2017 to 2021. Consequently, the estimation of respective ρ values suffers from a degree of statistical underpowering, thereby compromising the precision of our projections.

The last assumption inherent in this model pertains to the equivalence between disease incidence and the prevalence of corresponding pathogens within tick populations. This assumption implies a consistent frequency of human-tick interactions, and consequently, tick bites on humans, over time therefore, any alteration in pathogen prevalence is presumed to yield a proportional change in disease incidence. It is assumed that state-level incidence data can serve as a surrogate measure for pathogen prevalence within the respective state. In that respect, ρ can be derived from incidence data.

3 Results and Discussion
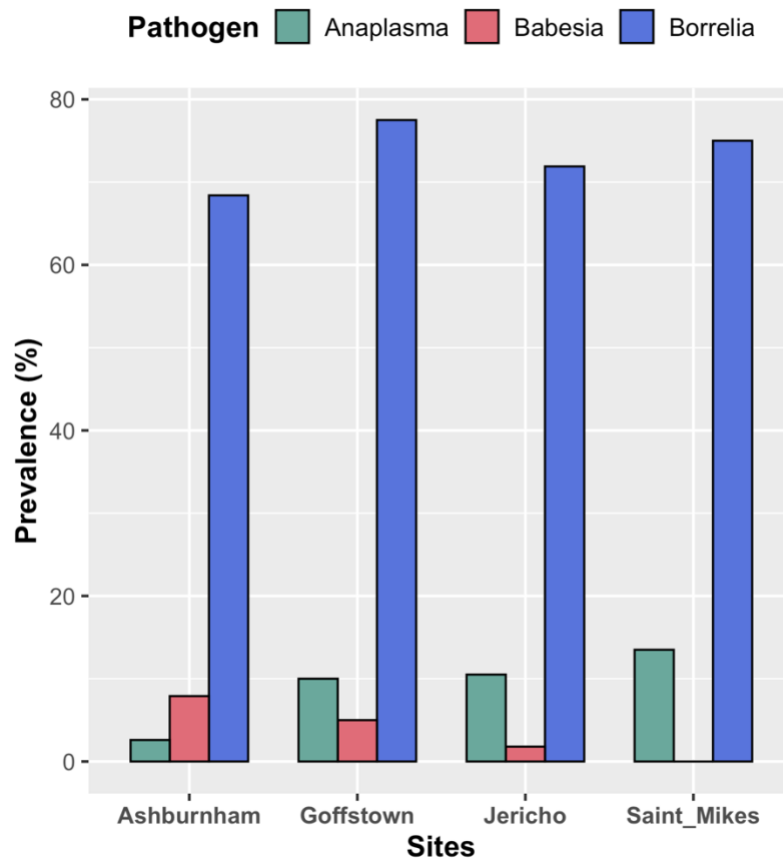
Figure 2. Prevalence of *Borrelia burgdorferi*, *Babesia microti*, and *Anaplasma phagocytophilum* Across Four Sites. The figure depicts the prevalence of *Borrelia burgdorferi* (blue), *Babesia microti* (red), and *Anaplasma phagocytophilum* (green) across four sites: Ashburnham (n=38), Goffstown (n=40), Jericho (n=57), and Saint Mikes (n=52). Pathogen prevalence is expressed as a percentage of the total sample collected at each site. The results were obtained through multiplex PCR analysis of ticks collected at the representative sites, with sample sizes indicated for each location. (ANOVA: p = 2.433E-9).
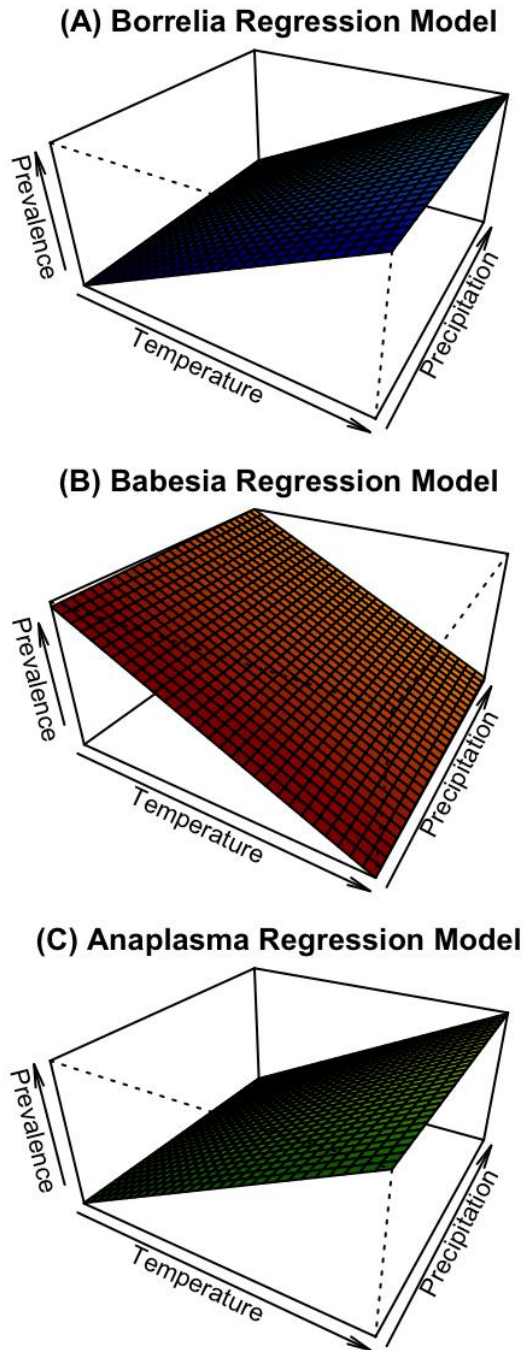
Figure 3. Multiple Linear Regression Models for *Borrelia*, *Babesia*, and *Anaplasma*.
(A) *Borrelia* Regression Model, (B) *Babesia* Regression Model, and (C) *Anaplasma* Regression
Model illustrates the relationship between temperature (x-axis), precipitation (z-axis), and
pathogen prevalence (y-axis). The coefficient of determination (R^2) for each model is provided:
0.98 for *Borrelia*, 0.18 for *Babesia*, and 0.45 for *Anaplasma*. Temperature was found to exert a
greater influence on pathogen prevalence across all sites, while precipitation exhibited a lesser
significance. Statistical analysis indicated no significant p-values ($p > 0.05$) for all variables
across all factors.

Table 1. Summary of Regression Models. This table presents the statistical analysis derived from the multiple linear regression analyses conducted for the *Borrelia*, *Babesia*, and *Anaplasma* pathogens. The reported values include Pathogen Model, R^2 (coefficient of determination), β_0 (intercept), Variable (predictor variable), β (regression coefficient), SE(β) (standard error of the coefficient), t-statistic, p-value, 2.50% (lower bound of the 95% confidence interval), and 97.5% (upper bound of the 95% confidence interval).

Pathogen Model	R^2	β_0	Variable	β	SE(β)	t	p	2.50%	97.50%
Borrelia	0.98	-13.6	Temperature	3.72	0.662	5.61	0.11	-4.69	12.1
			Precipitation	0.003	0.012	0.3	0.81	-0.144	0.151
Babesia	0.18	12.3	Temperature	-0.551	3.46	-0.159	0.89	-44.6	43.5
			Precipitation	0.0176	0.0606	0.291	0.82	-0.752	0.788
Anaplasma	0.45	-43.8	Temperature	2.42	3.76	0.648	0.64	-45.3	50.2
			Precipitation	-0.0133	0.0657	-0.203	0.87	-0.849	0.821
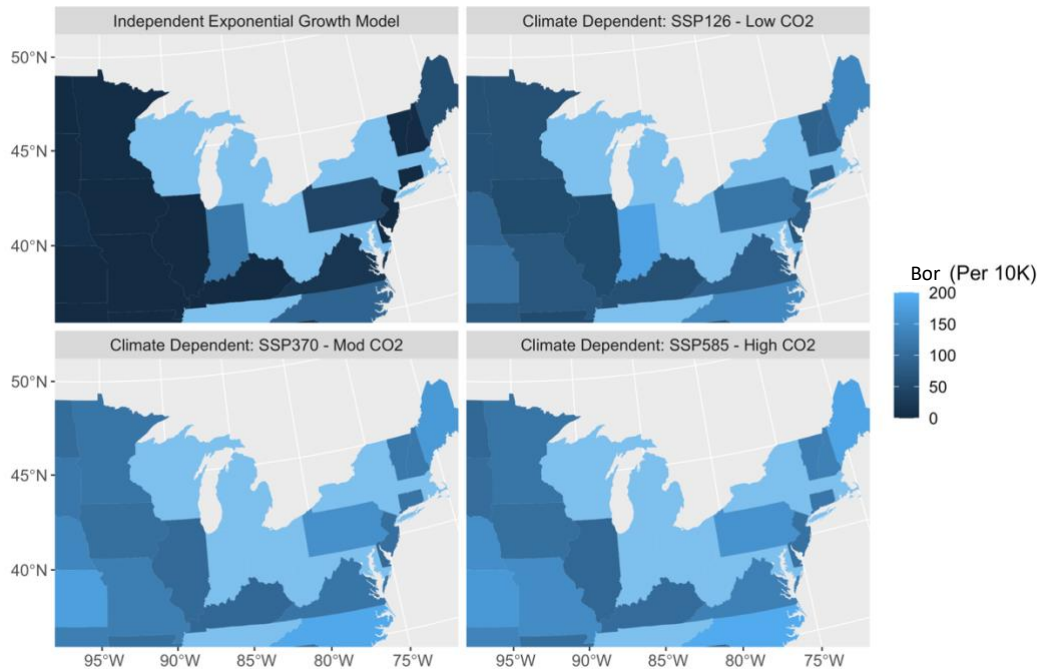
Figure 4. Projected Prevalence of *Borrelia burgdorferi* in the Northeastern United States for the Year 2070. The maps depict varying shades of blue, indicating the prevalence ranging from dark blue (low prevalence) to light blue (high prevalence). From the upper left to the bottom right, the maps represent the growth rate assuming an exponential model, as well as three scenarios based on different SSP: SSP126 (Low CO2 emissions), SSP370 (Moderate CO2 emissions), and SSP585 (High CO2 emissions).The figure suggests a positive correlation between CO2 emissions, temperature, precipitation, and pathogen prevalence on the prevalence of *Borrelia burgdorferi* in populations of *Ixodes scapularis*.
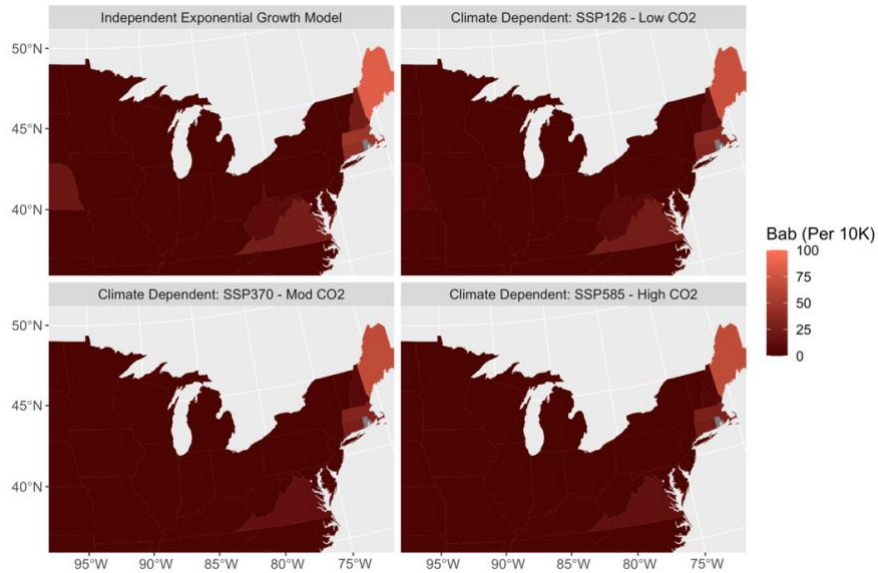
Figure 5. Projected Prevalence of *Babesia microti* in the Northeastern United States for the Year 2070. The maps depict varying shades of red, indicating the prevalence ranging from dark red (low prevalence) to light red (high prevalence). From the upper left to the bottom right, the maps represent the growth rate assuming an exponential model, as well as three scenarios based on different SSP: SSP126, SSP370, and SSP585. The figure suggests a low correlation between CO_2 emissions, temperature, precipitation, and *B. microti* prevalence in *I. scapularis*.
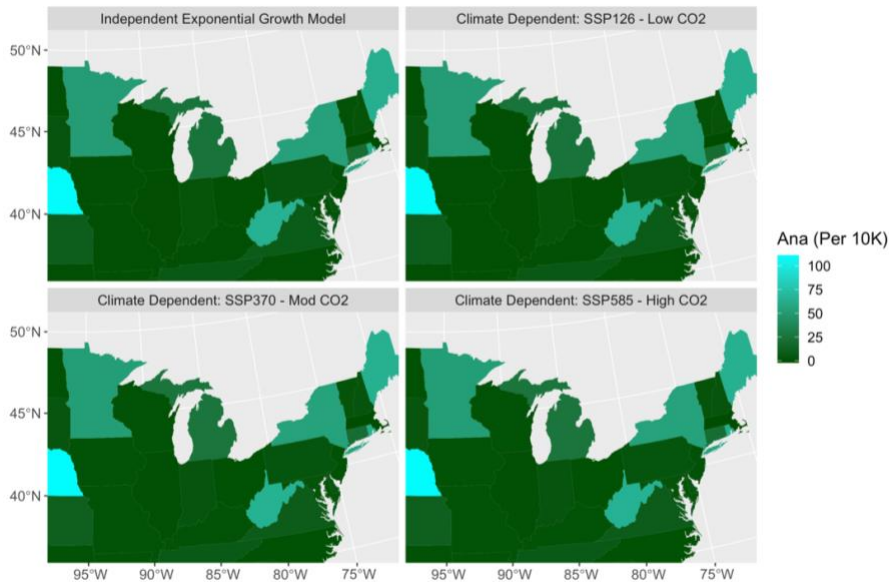

Figure 6. Projected Prevalence of *Anaplasma phagocytophilum* in the Northeastern United States for the Year 2070. The maps depict varying shades of green, indicating the prevalence ranging from dark green (low prevalence) to light green (high prevalence). From the upper left to the bottom right, the maps represent the growth rate assuming an exponential model, as well as three scenarios based on different SSP: SSP126, SSP370, and SSP585. The figure suggests a low correlation between CO_2 emissions, temperature, precipitation, and *A. phagocytophilum* prevalence in *I. scapularis*.

Tick-borne illnesses pose a significant public health risk, with their prevalence on the rise both in the United States and globally. The interactions between human activity and climate change are playing a role in the emergence of new diseases and the spread of existing ones into previously unaffected regions (Caminade, 2019). Factors like temperature and humidity have a direct impact on the abundance of ticks, availability of hosts, their survival rates, and the transmission of diseases. Studies, both empirical and simulation-based, have highlighted the positive impact of a warming climate on tick population expansion through increased survival rates and better access to hosts for feeding. Our study seeks to investigate how temperature and precipitation influence Lyme disease, babesiosis, and anaplasmosis incidence across U.S. states. Our hypothesis was that an increase in temperature and precipitation would lead to an increase in pathogen prevalence across all zoonoses because an increase in temperature and precipitation would contribute to an environment that is conducive to heightened activity and questing (Ogden et al., 2005; Schulze et al., 2009), which would increase the rate of pathogen transmission across ticks and reservoir species.

The results of the multiplex PCR analysis of ticks collected across the four representative sites, Asburnham (n= 38), Goffstown (n= 40), Jericho (n=57), and Saint Mikes (n=52), can be seen in Figure 2. Gel electrophoresis revealed an average *Borrelia burgdorferi* prevalence of 73.2%, an average *Babesia microti* prevalence of 3.7%, and an average *Anaplasma phagocytophilum* prevalence of 9.2% across all sites. The p-value across all sites considering pathogen prevalence for all three pathogens was significant (p = 2.433E-9).

The statistical weight of temperature and precipitation on pathogen prevalence across *Borrelia, Babesia*, and *Anaplasma* was assessed by plotting the results in a three-dimensional space. This analysis, depicted in Figure 3, provides insights into the influence of these environmental

variables. Table 1 presents summary statistics for these models. Notably, the models reveal a significant impact of temperature on the prevalence of Borrelia, Babesia, and Anaplasma, as evidenced by the respective coefficients (β) of 3.72 -0.551, and 2.42, while precipitation exhibits comparatively lower effects β= 0.003, 0.0176, -0.0133, respectively). The Borrelia model demonstrates a high level of fit (R-squared = 0.98), indicating strong agreement between observed and predicted values. However, the models for Babesia and Anaplasma exhibit lower levels of fit (R-squared = 0.18 and 0.45, respectively). Notably, all p-values for the variables in these models exceed 0.05, suggesting non-significance in the observed relationships. All models had a VIF less than 5.0 which suggests a low level of collinearity.

The exploration of non-significant results in the regression models was coupled with the integration of v2.1 CHELSA climate projections for the year 2070. Leveraging the socio-economic pathways (SSPs) of SSP126, SSP370, and SSP385, these models facilitated the generation of novel disease forecasts that deviate from traditional climate-independent growth models. By extrapolating current trends into future climate scenarios, our study unveils potential trajectories of disease prevalence.

CHELSA projections using the Borrelia model for 2070 suggest a high level of variation between climate independent exponential growth projections, and climate dependent models (Figure 4). The independent model suggests a future incidence of around 50-100 cases per 10,000 people across most states, with notable exceptions including New York, Massachusetts, Rhode Island, Maryland, West Virginia, Ohio, Michigan, and Wisconsin - which have projected rates exceeding 200 cases per 10,000 people. This is attributed to a particular high ρ, which is derived from notable increase in the rate of incidence in the last 10 years. The SSP models, however, project

heighted incidence, with the SSP585 model projecting upwards of 150 cases per 10,000 people across much of the northeast of the United States.

CHELSA projections using the Babesia model for 2070 suggest a low level of variation between climate independent exponential growth projections, and climate dependent models (Figure 5). The independent model suggests a future incidence of around 0-25 cases per 10,000 people across most states, with notable exceptions including Maine - which have projected rates exceeding 100 cases per 10,000 people. This is attributed to a particular high ρ, and a relatively low change in projected temperature and precipitation. The SSP models project decreased incidence across all states in comparison to the independent exponential growth model.

CHELSA projections using the Anaplasmosis model for 2070 suggest a low level of variation between climate independent exponential growth projections, and climate dependent models (Figure 6). The independent model suggests a future incidence of around 0-75 cases per 10,000 people across most states, with notable exceptions including Nebraska - which have projected rates exceeding 100 cases per 10,000 people. This is attributed to a particular high ρ, which is in part explained by a limited record of incidence on an annual basis. The SSP models revealed little differentiation between climate projections and the independent exponential growth models.

Our findings indicate that climate change is poised to exert a significant influence on the prevalence of Borrelia, Babesia, and Anaplasma within Ixodes scapularis populations in the foreseeable future. Employing a model that accounts for the effects of temperature and precipitation on the prevalence of tick-borne pathogens, our projections suggest an approximate escalation to 150 cases per 10,000 individuals by the year 2070, translating to an augmentation of around 3 Lyme disease cases per county per year. Additionally, our model predicts 25 cases of Babesiosis and 50 cases of Anaplasmosis per 10,000 individuals. These projections were derived

by juxtaposing observed prevalence rates in New England forests with anticipated climate scenarios in the northeastern United States. However, it is important to acknowledge the inherent limitations of our model, as indicated by a p-value exceeding 0.05. Consequently, our projections may tend to overestimate the prevalence of Borrelia and Anaplasma while underestimating that of Babesia. Given the restricted scope of our dataset, our model likely inflates ρ leading to an exaggerated annual rise in pathogen prevalence.

This data partially supports our hypothesis that climate change would have an effect on the prevalence of tick-borne pathogens. However, the lack of significant evidence leaves our investigation unresolved and further research must continue to answer this question. While the methodology may provide a reproducible approach that enhances our understanding of the complex interplay between climate change and tick-borne pathogen prevalence, its limitations underscore the need for continued investigation. Further research efforts should focus on expanding the scope and diversity of the dataset used for site selection, as well as incorporating a more comprehensive and representative dataset for tick-borne pathogen prevalence across various temporal scales.

By augmenting the dataset in this manner, the pathogen models derived from the multiple linear regression analyses can be refined, thereby improving their accuracy and reliability for future projections. Additionally, a more robust dataset will provide a stronger foundation for illuminating the intricate climate-dependent associations governing tick-host ecological systems. Unlike "bottom-up" models that rely on a plethora of assumptions regarding ecological dynamics, our methodology is grounded in the application of readily available datasets, thus minimizing the risk of bias introduced by speculative assumptions. However, the power of our

projections and the scientific validity of our findings are inherently constrained by the quality and comprehensiveness of the incorporated data.

In conclusion, while our study provides valuable insights into the potential impacts of climate change on tick-borne pathogen prevalence, it also highlights the necessity for ongoing research efforts to address the inherent limitations and uncertainties. By refining our methodologies and expanding our datasets, we can advance our understanding of these complex ecological systems and better inform public health interventions aimed at mitigating the risks posed by emerging infectious diseases.

Data Availability

The data used to support the findings of this study are publicly available through NOAA, CHELSA, and the CDC.

Conflict of Interest

The authors declare that they have no conflicts of interest.

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