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Eastern Equine Encephalitis Amplification and Spillover in New England

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A Thesis submitted in partial fulfillment of the requirements for the degree of:
Master of Public Health in Epidemiology of Microbial Diseases
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Abstract

Introduction: Eastern Equine Encephalitis (EEE) is an alphavirus spread by the *Culiseta melanura* mosquitoe. While extremely rare, infection can lead to severe mental sequala and death, making it a public health concern for affected communities. Over the past two decades EEE outbreaks have become more frequent in occurrence and larger in size in the Northeast Region of the United States.

Objectives: The main objectives of this study are to 1) measure the association between mosquito abundance, infection rate, and disease incidence 2) characterize the relationship between seasonal climate variability in New England with *Cs. melanura* abundance, infection rates, and incidence of EEE in humans 3) Identify spatial patterns and distribution of vector species

Methods: Mosquito abundance, infection rate, and incidence of mammalian infection were compared using both simple linear regression techniques and the non-parametric Wilcox Rank Sum Test to determine the impact of *Cs. melanura* trends on EEE risk in humans. Association between infection rate and number humans/horses infected was measured using the Spearman correlation test. Statistically significant spatial clusters of mosquito abundance, infection rate, and human incidence were identified using a retrospective Poisson distribution model in SatScan v96.

Results: Mosquito abundance, infection rate, and incidence of mammalian infection were all highest in New London County, Connecticut. Abundance was higher in outbreak years compared to non-outbreak years but not significantly associated with human cases. Mean temperature during transmission season was associated with vector abundance while rainfall was not. Vector index was strongly associated with mammalian infection.

Conclusions: None of the risk factors studied contributed to EEE spillover significantly on their only. Likely, a combination of these factors and other environmental variables linked to climate change are what is causing the increased frequency of outbreaks. More mammalian data is needed to draw more concrete conclusions.

Acknowledgements

Nathan Grubaugh, Philip Armstrong, Joseph McMillan, Dasha & Robert Solomon, Jacob & Steven Hentoff, and Norma Solberg

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I. Introduction

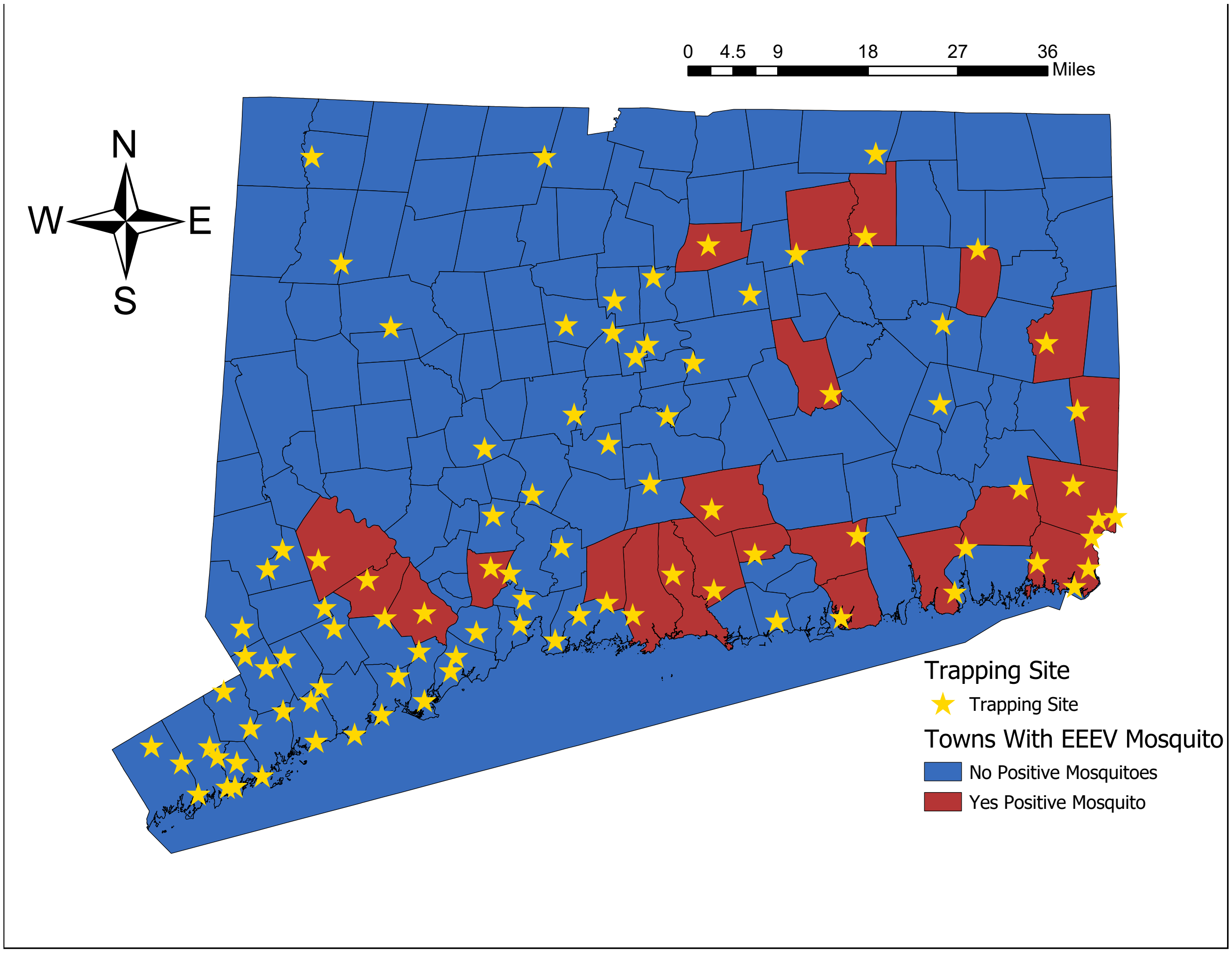
Eastern Equine Encephalitis Virus (EEEV) is a highly pathogenic mosquito-borne alphavirus that circulates in an enzootic cycle between *Culiseta melanura* mosquitoes and passerine birds in the freshwater hardwood swamps of the eastern and central United States (U.S.) and Canada.[i] Although rare, infection presents a significant health concern for both humans and horses, with the former having a mortality rate of 33% and the latter being 90%.[ii] Additionally, EEEV can lead to disabling and progressive mental and physical sequelae in humans, ranging from minimal brain dysfunction to severe intellectual impairment, personality disorders, seizures, paralysis, cranial nerve dysfunction, and death.[iii] Over the past 50 years, between five to ten human EEEV cases have been reported annually in the United States, however disease incidence has been steadily increasing over the past decade.[iv] Outbreaks of EEEV in humans and horses have historically been small in size and infrequent in occurrence, however this began to change at the beginning of the 2000's.[v] Between 2004 and 2019, EEEV transmission began expanding further north into the United States and Canada, with outbreaks occurring more frequently.[iv] Over the past 15 years, there have been 79 reported cases of EEEV in humans according to the Centers for Disease Control (CDC).[vi] In 2019, the CDC reported 36 human cases of neuro-invasive EEEV, 14 of which were fatal, across the Northeast, raising concerns about the future of EEEV transmission in New England.[iii] Currently, there is no vaccine or treatment available for EEEV. Increasing incidence and the high costs associated with mortality and after-care make EEEV a significant public health concern for the Northeast that warrants further research. It is unclear why EEEV outbreaks are occurring more frequently and across a larger geographic range. To better understand these new transmission patterns, I assessed the impact of several risk factor for EEEV between outbreak and non-outbreak years in the Northeastern region of the United States.

Eastern Equine Encephalitis Virus transmission predominantly circulates between the bird feeding mosquito species *Culiseta melanura* and passerine birds including robins, chickadees, and wood thrushes.[i-ii] Studies suggest that *Cs. melanura* and *Cs. morsitans* are involved in enzootic cycling of EEEV among birds, whereas other mosquito species such as *Coquillettidia perturbans*, *Ochlerotatus canadensis*, *Aedes vexans*, and *Oc. sollicitans* were responsible for mammalian infection.[vi] The contribution of *Cs. Melanura*, to the epidemic transmission of infection to mammals remains unclear. *Culiseta melanura* reproduce in aquatic subterranean habitats that can be found among tree roots and under patches of peat moss in lowland freshwater swamps.[vii] These habitats provide a cool (<20°C), acidic and stable environment that allow for larval development and overwintering.[viii] Studies show that increased air and ground water temperature can hasten larval development of *Cs. melanura* and suggest that milder winter temperatures can produce larger and older than average mosquito populations during the summer, when most EEEV transmission occurs.[vi]. Current evidence suggests that inter-annual climate variability has a direct influence on the epidemiology of EEEV however this relationship is not yet well understood.[ix] Additionally, records show a trend toward milder winters and hotter summers, as well as increased extremes in both precipitation and drought in the northeastern United States.[vii]

Identifying the most significant factors that contributed to the 2019 EEEV epidemic, will assist in the future design and implementation of vector control strategies for a number of mosquitos spread diseases. In this study, mosquito surveillance data from the state of

Connecticut were analyzed using Graphpad Prism, Geographic Information Systems (GIS), and Spatial and Space-Time Scan (SatScan) statistics to identify risk factors for EEEV outbreaks. This study aims to 1) to measure the association between mosquito abundance, infection rate, and disease incidence 2) to characterize the relationship between seasonal climate variability in New England with *Cs. melanura* abundance, infection rates, and incidence of EEEV in humans 3) identify spatial patterns of vector distribution and disease transmission.

Figure 1: Map on Connecticut with mosquito trapping sites



OBJECTIVES:

1) Measure the association between mosquito abundance, infection rate, and disease incidence.

2) Characterize the relationship between seasonal climate variability in New England with *Cs. melanura* abundance, infection rates, and incidence of EEEV in humans.

3) Identify spatial patterns of vector distribution and disease transmission.

II. METHODS

ETHICS STATEMENT:

Ethical approval was not required for this review.

Study Site:

The study was conducted across the state of Connecticut. Connecticut has an area of 14,353 Km^2 and has 172,584 acres of wetlands. An estimated 9% of the total land cover in Connecticut is made up of wetlands, 88% of which are suitable habitats for *Cs. melanura* as well as many other mosquito species including *Coquillettidia perturbans, Ochlerotatus canadensis, Aedes vexans,* and *Oc. Sollicitans*.[vi] These freshwater wetlands also contain several species of birds including Green Heron, American Robin, Common Yellowthroat, and Black-capped Chickadee, all of which are known food sources for *Cs. melanura*.

Data Collection:

Vector Data

All Connecticut mosquito data was provided by the Connecticut Agricultural Experimentation Station.[x] Mosquitoes were collected in 90 CDC battery operated light traps dispersed across the state and were stored at the Connecticut Agricultural Experimentation Station for identification of species, sex, and infection status. Traps were checked for mosquitoes on a weekly basis between June and October. Weekly mosquito abundance was calculated using the mean number of mosquitoes collected per trap per night during a week. Virus isolates were counted by using a plaque assay of Vero cells follow by an immunofluorescent assay.[xi] The CDC's infection rate index was used to estimate the minimum infection rate per species (MIR) ([number of positive pools / total specimens tested] x 1000).[xii]

Host Data

National case counts of EEEV in humans, non-primate mammals, and passerine birds were provided by the Centers for Disease Control's ArboNet surveillance program.[xiii] Data included date and location of the diagnosis at the month and state level respectively.

Environmental Data

Environmental variables were selected according to previously reported physiological and behavioral characteristics of *Cs. melanura* in New England.[xiv] Meteorological data for the state of Connecticut was provided by the PRISM Climate Group and included precipitation, minimum temperature, maximum temperature, mean dew point, minimum vapor pressure deficit, and maximum vapor pressure deficit. Data was aggregated by both weekly and monthly averages.[xv] Seasonal designations were also made for accumulated precipitation and temperature. Spring was defined as April and May, Winter was defined as the previous year's December through current year's February ,and Fall was defined as the previous year's September through November. Wetland raster data was provided by U.S. Fish and Wildlife Service National Wetlands Inventory (NWI) and was categorized by type using the Cowardin et al. (1979) method. *Cs. melanura* habitat was defined as freshwater emergent wetlands, freshwater forested shrub/wetlands, and freshwater ponds.

Temporal Analysis:

Mosquito abundance, MIR, incidence of mammalian infection, and human population size were compared using both simple linear regression techniques and the non-parametric Wilcox Rank Sum Test to determine the impact of *Cs. melanura* trends on EEEV risk in humans. Association between MIR and humans/horses infected was measured using the Spearman Correlation test. The test was repeated several times with the date of infection kept stationary and MIR calculated for each week preceding a new case. The same method was used to measure the association between mosquito abundance and human infection. Mosquito indices and weather variability were compared between years with reported mammalian EEEV cases and those without using the Wilcoxon Rank Sum Test. Statistical significance between groups was determined with two-tailed t tests, and values of $P < 0.05$ were considered significant. A Poisson model was used to identify temporal clusters of mosquito abundance, MIR, and human infection. Temporal cluster analysis was done in SatScan and all other temporal anylases was done in GraphPad Prism.

Spatial Analysis:

Statistically significant spatial clusters of mosquito abundance, MIR, and human incidence were identified using a retrospective Poisson distribution model in SatScan v96. The Monte Carlo method was used to assess statistical significance of findings. The wetland raster file from U.S. Fish and Wildlife Service National Wetlands Inventory (NWI) was uploaded into GIS and trimmed to the state boundary of Connecticut. Only freshwater emergent wetlands, freshwater forested shrub/wetlands, and freshwater ponds were included in this analysis. The data was rasterized. The density of wetlands was measured by assigning all wetland pixels a value of one and all non-wetland pixels a value of zero. The number of wetland pixels was divided by the area of the town that each pixel fell in in order to calculate wetland density per town. This process was repeated to create several density maps including kernel density maps of mosquito, human, and horse incidence as well as high abundance traps (defined as traps that had a larger mosquito count per night then the statewide average). All maps were layered together using the raster calculator to create a risk index for EEEV infection.

III. RESULTS

Over the past two decades, the frequency, intensity, and geographic range of Eastern Equine Encephalitis virus has dramatically increased. The CDC reported the largest outbreak of neuro-invasive EEEV in recorded history between June and October of 2019 with 36 human cases.[vi] In this study I used case data, vector distribution, and climate records to identify spatial and temporal risk factors for EEEV in New England.

Culiseta melanura

I tested how *Cs. melanura* population and dispersion changed before during and after the EEEV transmission season. Mosquitoes were collected annually between June and October across the 90 light traps dispersed within Connecticut. Characteristics of captured *Cs. melanura* including mosquito per trap night, infection rate, and vector index are described in Table 1. During the study period, a total of 3,213,824 mosquitoes were collected. Other species that have highly been implicated in the epizootic transmission off EEEV, including, Coquillettidia *perturbans* (590,885), Ochlerotatus canadensis (482,593), and *Aedes vexans* (326,759), were the most abundant species collected. In order to better understand the distribution of *Cs. melanura* across the state, I used a Poisson spatial cluster analysis and found 11 significant spatial clusters (figure 2). These clusters of high abundance encapsulated regions that have observed mammalian transmission of EEEV. On average abundance peaked in July (19.47 caught per trap night) and was lowest in October (5.84 caught per rap night) . In order to test the hypothesis that higher *Cs. melanura* abundance during transmission season would lead to a larger number of both insect and mammalian cases, I compared *Cs. melanura* abundance during years without EEEV outbreaks in Connecticut to

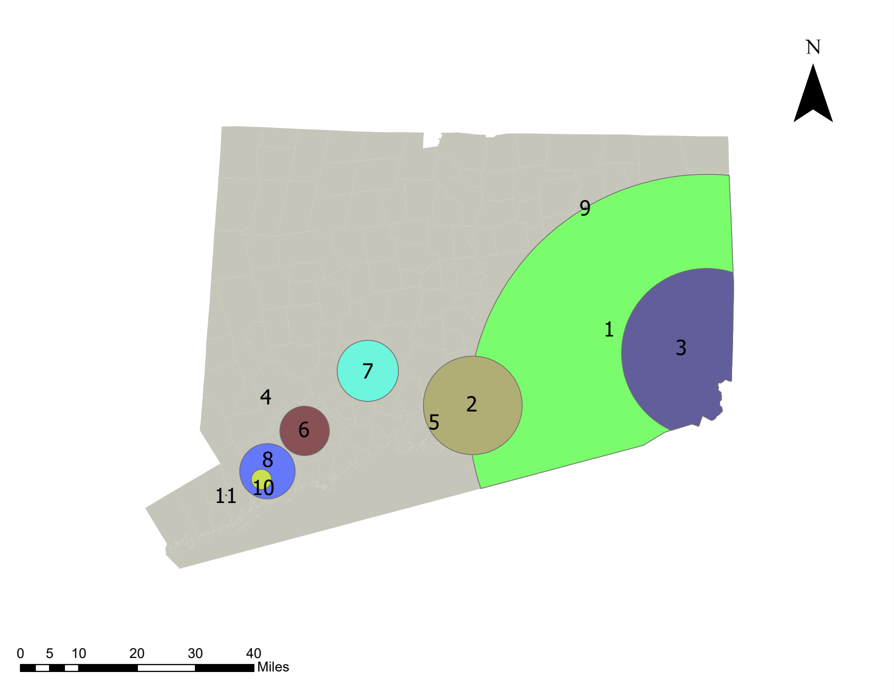
Table 1: Mean *Cs. melanura* caught per trap night, MIR, and VI in Connecticut 2001-2019

Year	Mosquito per Trap per Night	MIR	VI
2001	9.40	4.21	15.79
2002	7.97	0.00	0.00
2003	20.06	4.29	51.66
2004	11.44	1.93	21.68
2005	8.59	0.00	0.00
2006	12.56	1.67	8.54
2007	9.30	2.54	9.50
2008	11.20	0.00	0.00
2009	26.97	4.35	82.15
2010	9.57	0.27	1.87
2011	17.61	0.35	4.46
2012	11.24	1.14	10.01
2013	17.17	6.03	85.13
2014	9.78	0.00	0.00
2015	12.25	0.00	0.00
2016	8.73	0.24	1.68
2017	17.42	1.17	17.25
2018	23.75	0.63	8.06
2019	26.60	4.50	89.15

those that did (2003,2009, 2013, and 2019) using the Wilcox Rank Sum Test. The mean number of *Cs. melanura* per trap night was significantly higher for outbreak years than it was for non-outbreak years (P=0.0310, t=3.262).

Mosquito Infection Rate

Figure 2: 11 significant spatial clusters of EEEV positive Mosquitoes



Minimum Infection Rate (MIR), which is an estimate of the prevalence of a given arbovirus in a population of vectors, is common surveillance indicator, and is used for a variety of mosquito and tick-spread infections. A Spearman correlation test revealed a weak and non-significant association between MIR and abundance (r = -0.01775, P = 0.8652). The same analysis was repeated to compare MIR during years with and without EEEV outbreaks using a Wilcox Rank Sum Test. The annual average MIR has significantly higher during outbreak years compared to non-outbreak years (P=0.0002, t=7.380). In order to test the hypothesis that higher MIR's lead to more human cases of EEEV, I compared monthly mean MIR for months with human cases in counties with reported human to the MIR in all other counties during the same period. In 2019 there were four human cases of EEEV, three in New London County and one in New Haven County. All cases were reported in September. There were no significant differences in MIR between states for the period that cases were reported (P=0.8644, t=0.1783). I repeated this test comparing monthly

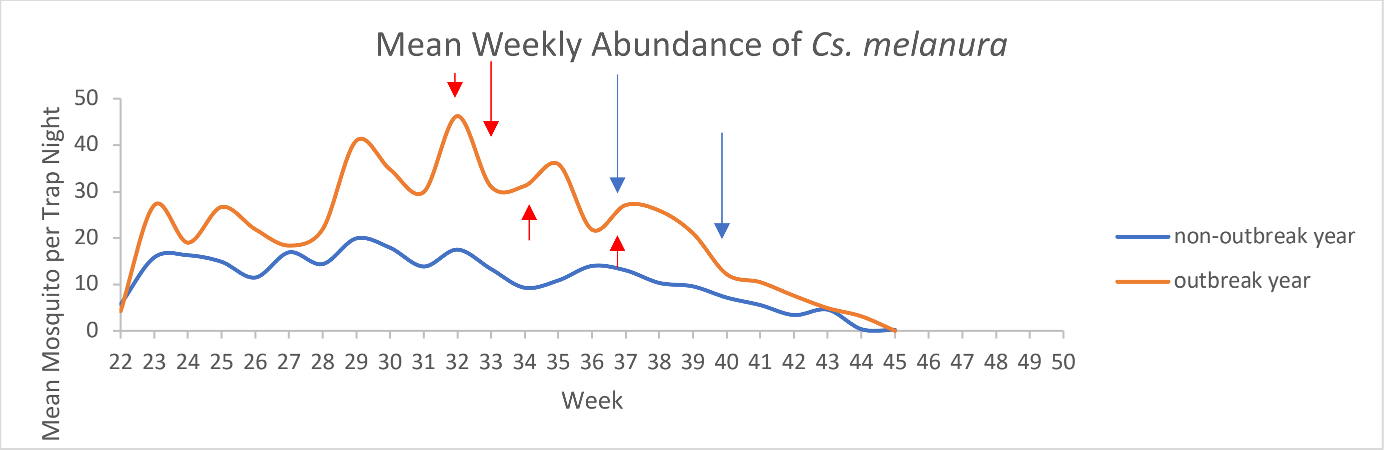
MIR in New London preceding the date of reported cases to monthly MIR in all other counties during the same period but found no statistically significant difference between means (P=0.4203, t=0.8874).

Vector Index

Vector Index (VI) has been used in many studies to analyze trends in mosquito infection rate by adjusting for vector species composition and vector population density. On average, VI is lowest at the beginning of the transmission season and peaks in September. To estimate the association between vector index and human EEEV incidence, as well as the strength of this associate compared to other entomological factors, I plotted monthly average VI, Infection rate, and abundance for years with and without outbreaks between 2001 through 2019 (Figure 4). Vector Index was significantly higher in years that had outbreaks compared to those that did not have outbreaks. The average difference in mean VI between outbreak and non-outbreak years is 74.3± 8.77 (P=0.0029, t=8.029)

Figure 3: a) Mean Weekly Abundance of *Cs. melanura* b) Mean Weekly MIR of *Cs. melanura* c) Mean Weekly Vector Index of *Cs. melanura*. Red arrows signify horse cases and blue around signify human cases.

a



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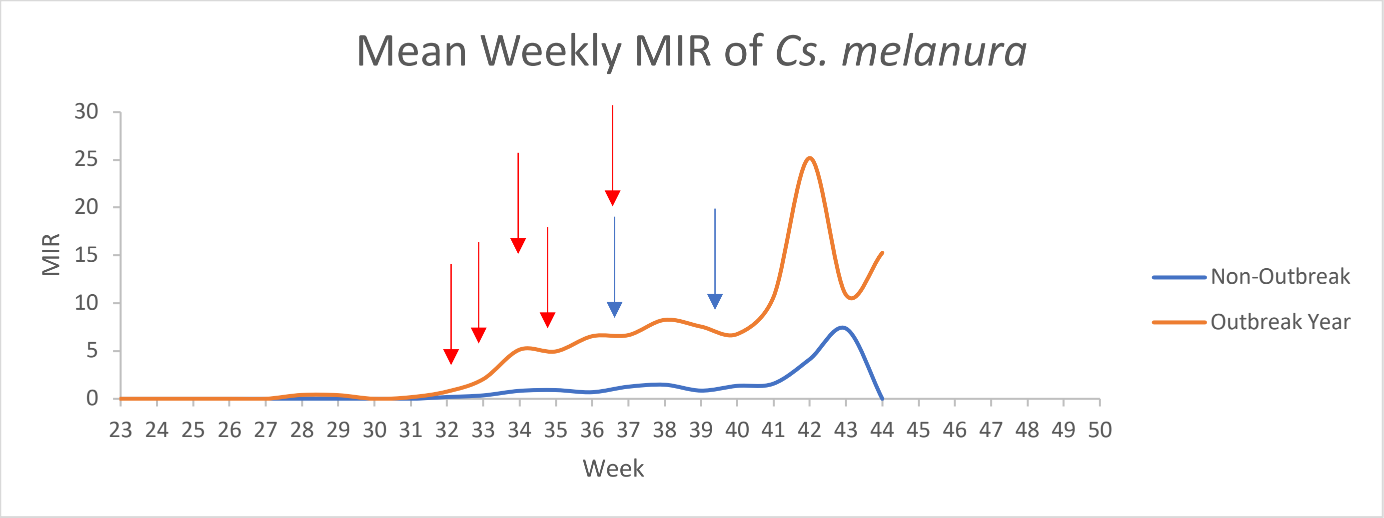

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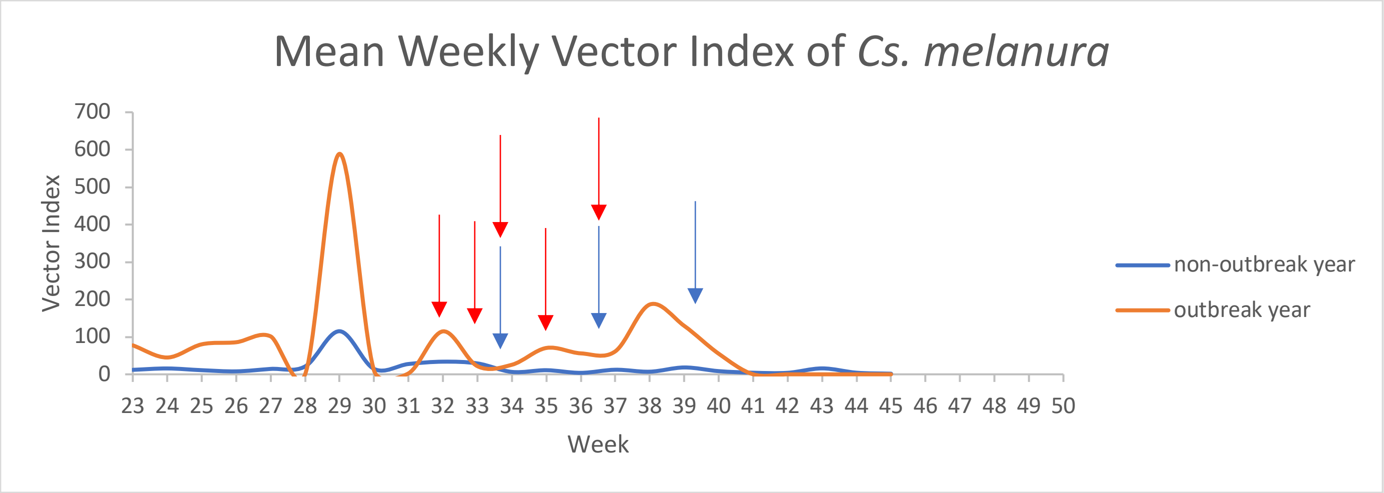


Climate Analysis

Current evidence suggests that both seasonal and annual climate variability influence mosquito abundance, vector capacity, viral fitness, and EEEV transmission dynamics.[vii] Using historic climate data, I sought to identify annual and seasonal weather patterns associated with entomological indices and EEEV transmission. Association was measured between each climate variable and each vector indices before and during transmission season. Results are shown below in Table 2. The strongest association was between mosquito abundance and mean temperature during the transmission season (r=0.5285, P<0.0001). There were no significant associations between vector indices and accumulated rainfall, average min/max tempurature during the previous Winter or Fall season. Mean temperature was not significantly different between outbreak and non-outbreak transmission seasons (P=0.2105).

Table 2: a) correlation between climate variables and *Culiseta melanura* abundance & infection rate December – February b) correlation between climate variables and *Culiseta melanura* abundance & infection rate September - November c) correlation between climate variables and *Culiseta melanura* abundance & infection rate March - May

		PPT (INCHES)	TMIN (DEGREES F)	TMEAN (DEGREES F)	TMAX (DEGREES F)
ABUNDANCE WINTER	r	0.2351	-0.2405	-0.1887	-0.2817
	95% confidence interval	-0.2589 to 0.6316	-0.6350 to 0.2536	-0.6015 to 0.3036	-0.6607 to 0.2117
	p-value	0.3326	0.3214	0.4392	0.2426
INFECTION RATE	r	0.3044	-0.1346	-0.1071	-0.1186
	95% confidence interval	-0.1878 to 0.6745	-0.5648 to 0.3532	-0.5455 to 0.3773	-0.5537 to 0.3673
	p-value	0.2051	0.5828	0.6625	0.6286
ABUNDANCE FALL	r	-0.03509	0.1633	0.2501	0.3817
	95% confidence interval	-0.4927 to 0.4377	-0.3272 to 0.5845	-0.2439 to 0.6411	-0.1020 to 0.7195
	p-value	0.8866	0.5041	0.3017	0.1068
INFECTION RATE	r	-0.2655	-0.264	0.08411	0.3834
	95% confidence interval	-0.6507 to 0.2284	-0.6497 to 0.2299	-0.3971 to 0.5290	-0.1001 to 0.7204
	p-value	0.272	0.2748	0.7321	0.1052
ABUNDANCE SPRING	r	0.09123	-0.101	-0.107	-0.03949
	95% confidence interval	-0.3910 to 0.5342	-0.5412 to 0.3827	-0.5455 to 0.3774	-0.4960 to 0.4341
	p-value	0.7103	0.6809	0.6628	0.8725
INFECTION RATE	r	-0.1735	-0.124	-0.05487	0.01771
	95% confidence interval	-0.5913 to 0.3178	-0.5574 to 0.3626	-0.5075 to 0.4215	-0.4516 to 0.4794
	p-value	0.4776	0.613	0.8235	0.9426

Spatial Analysis

For this study I used ArcGIS Pro and SaTScan v9.6 to create a risk map of human Eastern Equine Encephalitis transmission in Connecticut (figure 4). All mammalian cases were reported in New London or New Haven County. Figure 5 shows that New London had the highest amount of EEEV positive mosquitoes as well as the largest number of mammalian cases. According to the spatial clustering analysis, the largest clusters of infected mosquitoes fell almost entirely in the New London county with a few smaller ones in New Haven County. New London also had the highest density of freshwater wetlands compared to all other counties in Connecticut. Results from the finished risk map align with the observations made from all previous mapping of mosquito and mammal distribution and incidence of EEEV.

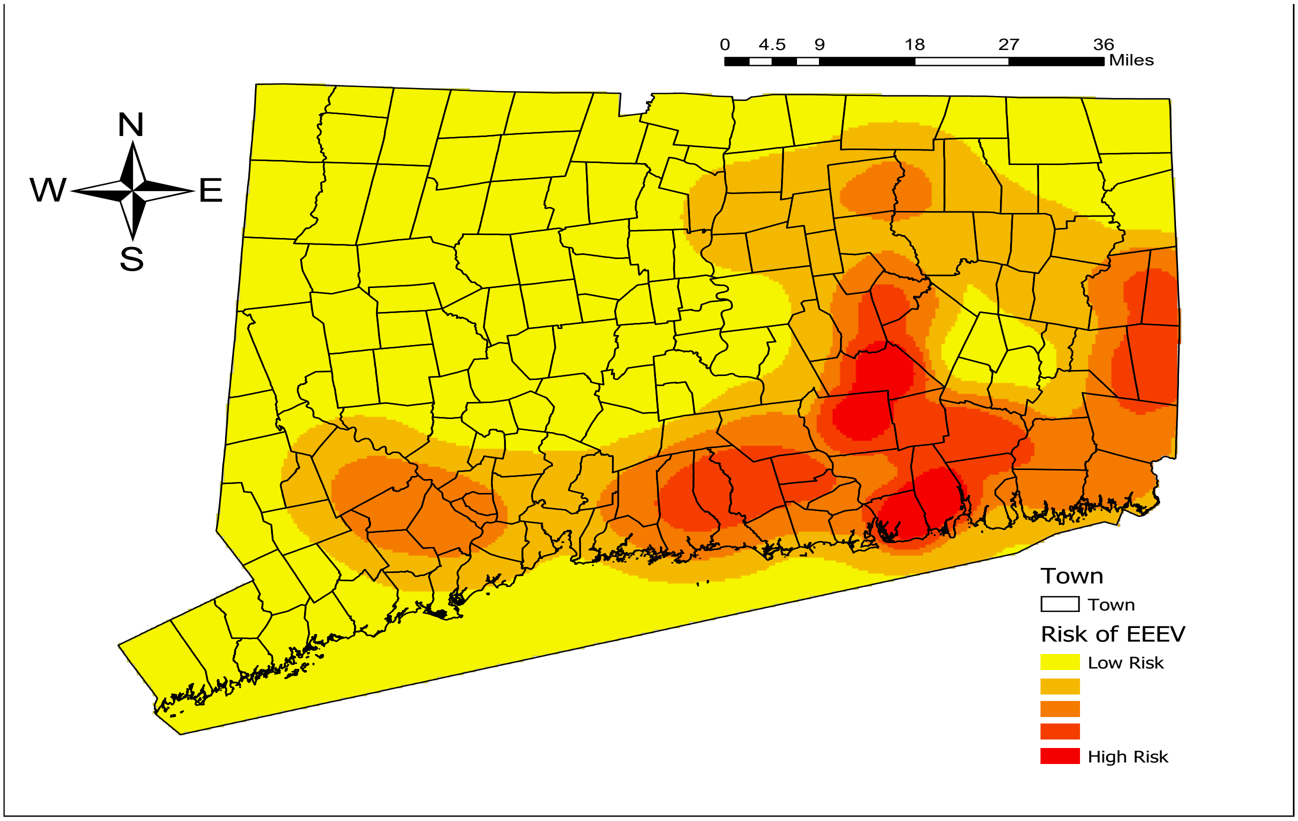
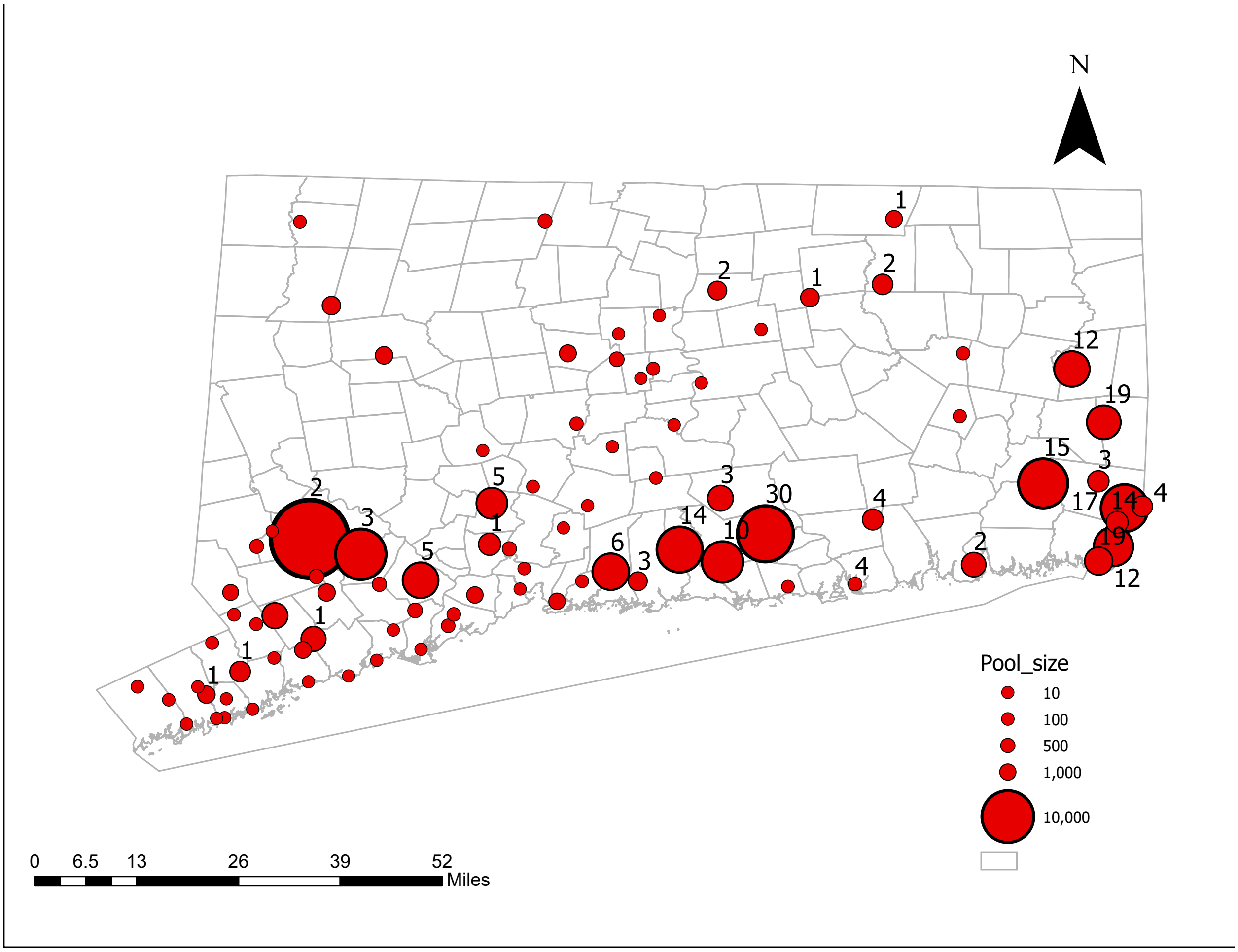


Figure 4: Risk map for mammalian transmission of Eastern Equine Encephalitis based of proximity to high abundance trap, proximity to rap with positive EEEV mosquito, and proximity to mammalian case

Figure 5: Approximate abundance of *Culiseta melanura* at each trap site. Number above traps indicate the number of EEEV positive *Culiseta melanura* at that site



IV. DISCUSSION

This study aims to 1) to measure the association between mosquito abundance, infection rate, and disease incidence 2) to characterize the relationship between seasonal climate variability in New England with *Cs. melanura* abundance, infection rates, and incidence of EEEV in humans 3) identify spatial patterns of vector distribution and disease transmission. Given the rarity of the disease and the small amount of publicly available data, I was not able to address all of these aims to completion but have completed a surface level analyses of risk factors EEEV in Connecticut that can be used to create and implement targeted and effective vector control strategies for Connecticut as well as the other surrounding states affected by EEEV. Broadly speaking, the study has five major findings 1) *Cs. melanura* are more abundant during years with reported mammalian cases 2) High abundance traps were close in proximity to reported mammalian cases, both of which were concentrated in New London County 3) Infection rate was not correlated with mosquito abundance, however years with outbreaks did tend to have higher infection rates than years that did not observe outbreaks. 4) Vector index appears to be a better predictor of mammalian risk compared to mosquito abundance and infection rate. 5) mean temperature during the transmission season was most strongly associated with outbreaks compared to all other climatic factors.

Cs. Melanura abundance in Connecticut between 2001 and 2019 was highly variable but peaked during years with high infection rates. Temporal patterns of *Cs. melanura* did not change between outbreak and non-outbreak years, peaking in mid-July and ending by mid-October across all years analyzed. It is difficult to tease apart exactly what these findings could mean for vector control because *Cs. melanura are* known to have a multiple clutch within a year and the timing of larval development varies greatly depending on climate and other environmental factors. The finding that outbreak years have higher mosquito abundance overall confirms previous studies on vector distribution and makes sense biologically. If there are more mosquitoes present then there are more opportunities for them to bite a susceptible mammal whether that be human or horse. Additionally, the abundance of *Cs. melanura* has remained stable between 2001 and 2019 which may indicate that pathogen, or environmental characteristics may play a larger role in EEEV transmission during outbreak years than vector abundance alone. This could indicate that a different vector has a larger impact on likelihood of outbreak in mammals, and that *Cs. melanura* is exclusively responsible for disease amplification within its sylvatic cycle of transmission. Further studies on the vector capacity of suspected bridge species are needed to fill in the gaps in the current transmission cycle. It's hard to draw these conclusions however, given the small number of human cases that have occurred in the state.

I calculated Minimum Infection Rate (MIR) in order to estimate of the prevalence of EEEV in Cs. melanura in Connecticut. A Spearman correlation revealed a week non-significant correlation between infection rate and *Cs. melanura* abundance. Unexpectedly, MIR was lowest at the beginning of the transmission season (June) and increased linearly until October, even as *Cs. melanura* abundance was declining. The same phenomenon was observed in Alabama and could be caused by blood meal preferences, host availability, and host–vector interaction changes in late summer.[xvi] [xvii] [xviii] My results contrast that of other studies which suggest high infection rates early in June may be correlated with mammalian transmission in August-October. The high MIR rate observed August-September is counteracted by the lower population size and biting rate of the *Cs. melanura. Cs. melanura* most often feed upon fledgling birds in the early Spring because these new born chicks have less mobility and no feathers to protect themselves from biting insects. By August, most fledging birds have grown their feathers and are able to fly away from their nests and therefor are able to better protect themselves from biting insects. All four reported human cases of EEEV in Connecticut happened in August, despite the fact that MIR continued to rise in September. High infection rate in the late season (September & October) may more so contribute to the following year's transmission as *Cs. melanura* are known to overwinter.

Vector Index (VI) has been used in many studies to analyze trends in mosquito infection rate by adjusting for vector species composition and vector population density. I found that vector index has a much higher associated with mammalian transmission compared to MIR or vector abundance alone. Given the derivation of vector index, these results indicate the MIR and vector abundance together contribute to EEEV transmission but not as a separate risk factor. There must be both a high abundance of *Cs. melanura* and a simultaneously high MIR for a transmission event to occur. The highest VI was found in New London county. Although New Haven county had a similarly high mosquito abundance to New London, the MIR was markedly lower in New Haven than it was in New London. This combination of high abundance and high MIR may explain why all mammalian cases of EEEV were reported in New London county. The average difference in mean VI between outbreak and non-outbreak years is 74.3± 8.77 (P=0.0029, t=8.029). These results suggest vector control efforts may be more successful if they focus exclusively on preventing amplification of EEEV early in the season, or keep mosquito abundance low in the later season (primarily August).

Current evidence suggests that both seasonal and annual climate variability influence mosquito's abundance, vector capacity, viral fitness, and EEEV transmission dynamics.[xix] Using historic climate data, I sought to identify annual and seasonal weather patterns associated with entomological indices and EEEV transmission. Mean daily temperature showed a moderate association with mosquito abundance during the transmission season (r=0.5285, P<0.0001). A study from the Journal of Medical Entomology conducted laboratory experiments to assess the effects of temperature on *Cs. melanura* development and adult biting rate. [xvi] Researchers found that warmer temperature led to more prolific mosquito reproduction and biting activity. Water and air temperatures in freshwater wetlands also may regulate the northern limit for virus amplification each year. [xvi] Fall, Winter, and Spring temperatures almost all had non-significant correlations with *Cs. melanura* abundance as seen in table 2. These findings are possibly a result of sampling bias given the rarity of infection especially in mammals as well as the short study period (18 Years). These results may change if climate and mosquito abundance was assessed over a much longer time period.

For this study I used ArcGIS Pro and SaTScan v9.6 to create a risk map of human Eastern Equine Encephalitis transmission in Connecticut. Results show high mosquito abundance in both New Haven and New London County. Compared to New Haven County, New London county has a much higher density of freshwater wetland habitat as well as a larger number of EEEV infected mosquitoes reported. The high abundance of *Cs. melanura* may be a result of sampling bias given that these traps were likely checked most frequently and properly maintained given their close proximity to the Agricultural Experimentation Station. New Haven county had no reported mammalian cases in 2019 while New London had six horse cases and four human cases reported. While there is similar *Cs. melanura* abundance in both counties, New London has a higher density of mosquito habitat as well as a high infection rate among *Cs. melanura.* These results that suggest that neither abundance nor MIR on their own contribute to EEEV transmission but rather that both must be high in order for a transmission event to occur. These results also emphasize the importance of wetland habitat in EEEV amplification. There are more susceptible passerine birds in New London than in New Haven, suggesting that

EEEV is most prolific in areas that have both high mosquito abundance and high abundance of fledging birds.

The Connecticut State Health Department currently recommends using personal insect repellent to prevent EEEV transmission. Results from this study suggest that largescale insecticide spray in the county of New London, especially in freshwater wetlands, may combat the incidence of EEEV in humans. Spraying before mosquito abundance and MIR reach their peak (around august) could prevent mammalian transmission of EEEV in the state of Connecticut.

This study has some limitations including lack of data, scale of analysis, and distribution of mosquito traps. Some of the temporal findings lack sufficient statistical significance because the number of mammalian cases has been so low in the state of Connecticut. Future studies that look at distribution of *Cs. melanura,* freshwater wetlands, and passerine birds in bordering states would reveal why Connecticut seems to be an outlier for total mammalian cases reported. A second limitation is the dispersion of mosquito traps throughout the state of Connecticut. Traps are concentrated in high risk areas, meaning those that have both a high density of wetlands as well as a large nearby susceptible human population. The traps in the northwest region of the state are sparse and may bias results from the spatial analysis in particular. Inclusion of data on wild bird populations, population level immunity in both birds and mammals, and virus strain may allow for a more robust assessment of human risk in the state of Connecticut. Ultimately, this study was able to conclude that the occurrence of a transmission event is dependent upon the culmination of several different environmental, entomological, and host factors working simultaneously.

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