Spatial and Temporal Determinants Associated with Eastern Equine Encephalitis Virus Activity in Florida

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Spatial and Temporal Determinants Associated with Eastern Equine Encephalitis Virus Activity in Florida

by

Kristi M. Miley

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy with a concentration in Global Communicable Disease Department of Global and Planetary Health College of Public Health University of South Florida

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DEDICATION

First of all, I would like to thank my husband James, without his love and support none of this would have been possible. Thank you to my mom for always believing in me. Thank you to my children for their patience while allowing me to pursue my dreams. Thank you to the many people along life’s journey that pushed me in a direction I may not have chosen on my own, as it led me to exactly where I was meant to be. Thank you to the many mentors along my path for inspiring me to achieve my goals. Finally, special thanks to all those individuals who have passed from this earth, but who have shared in making me the person I am today. To my Dad, I miss you dearly, I hope that you are smiling down on me and that you are proud of the person I have become.
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ABSTRACT

Eastern Equine Encephalitis virus (EEEV) is considered the most pathogenic mosquito-borne illness in the United States. Human mortality has been estimated to range between 35 to 75%, with horses experiencing mortality rates greater than 90%. A large number of EEEV cases occur in Florida. Though we have come a long way since the first human infection was identified in the 1930s, there is still much to learn regarding the virus’s ability to maintain transmission year-round in Florida. Phylogenic studies support that Florida may serve as the geographic reservoir for EEEV. This research investigated spatiotemporal and ecological variables associated with risk for EEEV transmission during winter-spring seasons when vector abundance is low, yet EEEV activity is still present. A lag effect was demonstrated using the Southern Oscillation Index (SOI) that is predictive for EEEV equine outbreaks. Secondly this research sought to identify areas of high-risk for EEEV activity using GIS-based risk index modelling. Although mosquito abundance was low during winter field collections Culiseta melanura the major enzootic vector of EEEV was present in reasonable numbers at high risk sites in the winter and early spring months, while almost none were found at low-moderate risk sites. EEEV activity was also only detected in GIS-predicted high-risk sites. Combined these results offer models that can predict EEEV activity which could be used for optimization of surveillance and control strategies that protect against EEEV in Florida.
INTRODUCTION

Eastern Equine Encephalitis Virus

Eastern Equine Encephalitis virus (EEEV), an Alphavirus from family Togaviridae, is considered the most pathogenic mosquito-borne illness in the United States [1, 2]. Human mortality due to EEEV has been estimated to range between 35 to 75%, with horses experiencing mortality rates greater than 90% [3-6]. Human infections can cause either a systemic febrile illness similar to influenza, or neurologic disease where severe encephalic involvement causes neurologic deficits that often lead to lifelong sequelae in survivors [7-9]. Although neurological disease is thought to be rare, a large number of these cases occur in the southeastern United States with Florida reporting one to three cases annually [10, 11].

EEEV’s genome is a single-stranded positive-sense RNA 11.68 Kb in length [12-16]. The EEEV genome encodes three structural proteins (Envelope proteins E1 and E2, and one capsid) and four non-structural proteins (nsP1, nsP2, nsP3, and nsP4) that are responsible for binding, entry, and viral replication [16-18]. Other encephalic alphaviruses belonging to the family Togaviridae include Western Equine Encephalitis virus (WEEV) and Venezuelan Equine Encephalitis virus (VEEV) [12, 19]. Madariaga (MADV), Chikungunya virus, Sinbis virus, Ross River virus and Mayaro virus are also alphaviruses of the family Togaviridae, but rarely if ever cause severe neurological disease [19-21]. Although EEEV may have been the cause of many horse deaths dating back to the 1800’s, the first human cases were diagnosed in Massachusetts during an equine outbreak in 1938 [5, 6, 14, 22].

North American EEEV was previously thought to belong to four lineages (I, II, III, IV) [23-25]. However, recent research suggested that South American strains of the virus (previously known as EEEV isolates II, III and IV) differed considerably with regard to genetics and
pathogenesis compared to North American EEEV [20, 26, 27]. For many years comparisons between the North American and South American strains of EEEV have shown genetic differences in structural envelope proteins and non-structural proteins providing evidence that South American strains may have been a different virus all together [18, 28]. Further, research has indicated that *Culex (Melanoconion)* species mosquitoes were found to be the primary enzootic vectors for South American EEEV, as opposed to *Cs. melanura*, which is responsible for maintaining the enzootic cycle in North America [23, 28]. South American EEEV enzootic hosts include small mammals, primarily rodents, and occasionally birds and reptiles, with epizootics occurring in horses [20]. North American EEEV is immensely pathogenic to humans and horses with high mortality, whereas the South American strains have mild to no symptoms and rarely involve encephalic pathology [21, 23]. Unlike North American EEEV, South American EEEV is noted to be asymptomatic in most cases, but can cause febrile illness and malaise [20]. The major differences surrounding South American EEEV isolates (II, III and IV) led to their reclassification as a separate species, now referred to as Madariaga virus (MADV) [20, 21, 26]. With these classification changes, EEEV is currently only found in North America.

**Disease caused by infection with EEEV**

Although rare, human infections of EEEV can cause either systemic or encephalic illness. Systemic infection generally causes mild symptoms such as fever, weakness and myalgia, lasting up to two weeks [11]. Encephalic manifestations may cause similar symptoms as systemic infections in conjunction with headaches, vomiting, convulsions, coma and encephalitis, which may potentially lead to death [6, 7, 29]. It is thought that many infections of EEEV may show no symptoms; those individuals experiencing symptoms will usually exhibit illness within a few days of being bitten by an infectious vector mosquito [8, 12, 29]. Encephalic EEEV generally has a poor prognosis with a mortality rate approaching 75% and many survivors exhibit lifelong neurological deficits [1, 9, 15]. As there are no known treatments that
reverse the progression of illness, treatments rely solely on symptomatic palliative care [30, 31].

Once bitten by an infected mosquito, EEEV prefers invasion of multipotent stem cells with replication occurring in bone, tendons and muscle tissue (including cardiac muscle). Unlike other viruses, EEEV does not appear to replicate well in lymphoid tissues [32, 33]. Severe pathology occurs in progressive encephalic disease when EEEV enters the bloodstream and infects the neurons in various regions of the brain and brain stem [34]. Severity of disease, and detecting attributes of recovery, are diagnosed by repeat MRI imaging through T2-weighted fluid attenuated inversion recovery (T2-FLAIR) as this technique reveals lesions in the brain associated with encephalic diseases [8, 34, 35]. Case studies of neurological disease associated with EEEV infection show that the basil ganglia and thalamus are often affected with other areas of the brain having varying degrees of impact [8, 31, 36].

Diagnosis of EEE

Diagnostic tools are available to confirm whether an individual has contracted EEEV. In most cases, serological testing can be performed with an IgM capture enzyme-linked immunosorbent assay (ELISA) using cerebral spinal fluid or serum [12]. Confirmation can be obtained using a plaque reduction neutralization test (PRNT) that detects neutralizing antibodies specific to EEEV [12]. Unfortunately, EEEV symptomology can mimic other illnesses and EEEV infection may be misdiagnosed for quite some time during the process of differential diagnosis and ongoing care in hospitalized patients. The infrequent nature of EEEV infections may cause a lack of a definitive diagnosis in many cases. The most at risk for severe encephalic illness due to EEEV are children and the elderly, but no age barrier exists, as noted by recent cases in middle-aged individuals [11].

Though human cases of EEEV are rare, equine cases are more common and may provide a clear indication that risk for human transmission exists. Horses are more affected by EEEV than humans, especially in Florida, where upwards of 200 equine cases per year have
been documented [3, 37]. The first documented horse outbreak of EEEV in North America dates back to the early 1800s, but the first diagnosis in pathology from horse brain tissue was not reported until 1933 [15, 29, 38]. In severe equine cases of EEEV, onset of illness can be rapid, with a horse appearing physically healthy just hours prior to the appearance of severe neurological symptoms [39]. Severe encephalic involvement in horses includes recumbency, paralysis, thrashing and general unresponsiveness to outside stimuli [39]. EEEV targets neurons causing irreversible damage to these cells, leading to equine mortality greater than 90%, with most cases humanely euthanized to avoid undue pain and suffering [39].

Although no FDA approved immunizations are available for humans, vaccines are readily available and highly effective at protecting against EEEV infection in horses [40, 41]. EEEV vaccinations must be given every six months in order to be effective at protecting horses against infection [42]. Unfortunately, only about 63% of horses are vaccinated appropriately in compliance with the American Association of Equine Practitioners (AAEP) recommendations [40, 42]. Equine cases of EEEV can be prevented through adequate vaccination, and as a result most cases of EEEV have been found to occur in unvaccinated or inappropriately vaccinated animals [39, 43]. Although EEEV is found primarily in the eastern United States, the EEEV vaccine is considered one of the core vaccinations for all horses regardless of geographic location according to the AAEP, which includes immunizations that protect against both eastern and western equine encephalitis, West Nile virus, Tetanus, and Rabies [40, 42]. The occurrence of EEEV in horses is considerably higher on average in the state of Florida than any other state, lending weight to investigations into Florida as a potential geographic reservoir for the virus [3, 37, 44].

Transmission of EEEV

While epizootic infections can occur in humans, horses and other mammals, EEEV is maintained in an enzootic cycle between the ornithophilic mosquito vector Culiseta melanura
(Coquillett, 1902 in the order Diptera of family Culicidae) and avian species [2, 3, 45-47]. EEEV epizootic events may have widely different mosquito vectors depending upon the region where transmission occurs [48]. Although many bridge vectors may be involved in the epizootic cycle, *Culex erraticus* (Coquillett) has been documented as potential vector for EEEV and has a relatively high abundance in north and central regions of Florida, making it a possible player in transmission to humans, horses and other mammals [46, 48]. EEEV enzootic hosts species are generally passerines or songbird species such as the Northern Cardinal (*Cardinalis cardinalis*), but many avian species have been documented as potential hosts including a variety of waterfowl and wading birds [3, 46, 49]. *Cs. melanura* has been identified as the primary vector for EEEV and is thought to rarely feed upon humans and other mammals [46, 50]. However, detection of human and other mammalian blood has been documented in *Cs. melanura* suggesting that some transmission could also occur through ornithophilic mosquito vectors that may feed opportunistically outside of their normal feeding behavior [51]. A variety of bridge vectors have been implicated as possible opportunistic spreaders for introduction of EEEV in the human and horse population [47]. Viral detection of EEEV has suggested the following mosquito species as potential bridge vectors for epizootic transmission; *Coquillettidia perturbans* (Walker), *Aedes vexans* (Meigen), *Aedes sollicitans* (Walker), *Aedes canadensis* (Theobald), *Anopheles punctipennis* (Say), *Anopheles quadrimaculatus* (Say), *Cx erraticus* and *Culex salinarius* (Coquillett) [38, 46, 47, 50, 52-54]. Although EEEV viral isolates have been detected in potential bridge vectors in various studies, their vector competency has often not been investigated. Therefore, whether a given species can effectively transmit the virus to humans and/or horses or other mammals remains an open question in many cases [53]. Though a zoonotic pattern of EEEV infection exists within the host avian populations and the primary vector, humans and horses are considered dead end hosts as they do not have the ability to further perpetuate the spread of the virus [29].
Biology of *Cs. melanura*

*Cs. melanura* is a medium-sized dark brown mosquito [55]. Though *Cs. melanura* may appear somewhat plain and resemble other moderately sized brown mosquitoes to the naked eye, it does have distinctive features that make it easy to recognize, such as a long curved proboscis and setae on the ventral base of the wing [55, 56]. *Cs. melanura* are typically found in freshwater hardwood swamps, as larvae tend to thrive in indentations or sinks formed in the land that hold water, which often happens when trees fall, leaving holes near root beds that collect water [46, 50, 55, 57]. *Cs. melanura* generally occupies low-lying land in the eastern United States, but has been noted in regions of higher elevation, even extending into parts of Canada [4, 55, 58-61]. *Cs. melanura* feeds primarily on avian species, usually passerines that live or frequent freshwater hardwood wetlands and may have a fondness for the Northern Cardinal in the state of Florida [3, 46, 49-51, 54, 57, 62]. Research has also shown seasonal shifts in *Cs. melanura* feeding patterns between various avian species including feeding on reptiles and occasionally mammals as a source of blood meals [45, 63]. The seasonal shifts and subtleties in *Cs. melanura*’s behavior, may lead to a better understanding of the complexities surrounding EEEV transmission in Florida and across the rest of the eastern United States.

EEEV has a geographic range primarily bordering the Atlantic and Gulf coasts of the United States, and more recently expanding into eastern parts of Canada [4, 53, 58, 59, 64]. Florida likely serves as a geographic reservoir for EEEV due to climatic factors which can create a suitable habitat for the primary vector *Cs. melanura*, leading to potential for year-round EEEV transmission [3, 45, 46, 65]. In support of this, recent research has linked most strains circulating outside of Florida to progenitor isolates in Florida [13, 66, 67]. The first case of EEEV in Florida was not documented until 1952, but evidence suggests that Florida may be a geographic reservoir for both the primary vector and EEEV activity during all months of the year [2]. This has led to many investigations into EEEV winter activity and the potential for the virus to overwinter in various species which may amplify transmission and northeasterly spread of the
virus during the peak summer months [3, 45, 46, 68]. Climate change may soon weigh heavy on the possibility for EEEV establishment in naïve regions that could provide a suitable habitat for the primary enzootic vector *Cs. melanura* to thrive as the climate warms [5].

**Climate change and infectious disease**

Investigating climate variables in relation to disease has been a hot topic for research which has allowed for concise evaluation regarding the effects climate may have on infectious diseases [69]. This is especially important for vector-borne illnesses where certain weather factors may become indicators to assist in determining if an imminent threat of an outbreak could to be expected [58, 70-74]. Weather anomalies may dictate redistribution patterns for a potential vector in the case of vector borne infections. This may be seen in regions where major rainfall turns arid land to wetland via flooding events. With increased rainfall and standing water, mosquito populations may have a greater abundance of progeny leading to increased risk for a variety of mosquito-borne illnesses [70-72]. There has also been speculation that warming trends may impact colder climates, as temperatures increase to a point that a suitable vector habitat may be established. Canada has experienced upsurges in many vector-borne diseases over the past few decades that illustrate the impact weather anomalies can have on the spread of disease [58].

Although it may be difficult to halt these global warming trends, there may be patterns that can assist in slowing certain infectious disease events by understanding how weather anomalies precede an outbreak. One such potential weather predictor that has been evaluated as a tool to protect against several global health threats is the El Niño Southern Oscillation (ENSO) and the calculations of the Southern Oscillation Index (SOI) [65, 72, 75-80]. Various illnesses have been studied through the lens of weather predictors and found to have attributable risk associated with certain weather conditions. One might expect this to be so with mosquito borne illnesses, as an uptick in mosquito abundance can be seen following times of
intense rainfall [71]. ENSO cycles may bring about weather conditions leading to heavy precipitation that leads to flooding and stagnant water providing breeding sites for mosquito vectors which increases the potential risk for contact with an infected mosquito, as noted in several dengue epidemics that followed strong La Niña events [71-73]. Evaluating the ENSO cycle in relation to EEEV brings an additional surveillance tool to the table enhancing our ability to protect against this rare but often fatal illness.

**EEEV in Florida**

EEEV surveillance and risk assessment in Florida can be challenging. Currently, EEEV risk is determined through the use of sentinel chicken surveillance and mosquito field collections to determine species abundance [2, 13, 46, 81]. GIS-based modeling of EEEV horse cases has been developed to aid in optimizing placement of sentinel chickens and mosquito collection devices to provide better information on EEEV risk potential for Florida [82-85]. Horse cases may indicate an increased threat to humans as EEEV may be present in greater proportions among potential bridge vectors that would be more likely to feed on mammalian hosts. The sentinel chicken surveillance program helps determine whether circulation of the virus is present in a given environment. This is achieved through weekly blood draws that are screened for the presence of EEEV [13, 81]. Chickens are generally very hardy and will establish active immune responses to EEEV whereby producing effective antibodies against the virus without incidence, which makes these birds essential surveillance tools [13]. Mosquito populations can also advise evidence of impending threat through evaluation of species abundance [86]. Mosquito field collections are usually performed through the use of capture devices such as the CO₂ baited CDC light trap or through passive collections such as resting shelters [3, 86-89]. Resting shelters can provide improved abundance information than collections from CO₂ baited CDC light traps and other attractant based collection devices, as passive collection of resting mosquitoes using an aspirator vacuum may provide a more precise characteristic sampling of a
given environment [88]. Although artificial resting shelters do not mimic every resting environment, they have proven to be a useful tool in collecting a variety of blood engorged mosquitoes including having an affinity for collecting the primary vector and some potential bridge vectors for EEEV [87, 88, 90, 91]. Further, collection of blood fed mosquitoes can provide insight regarding host interactions enabling greater understanding of the sylvatic habitat and potential for transmission of various mosquito-borne illnesses [48, 87, 88, 90, 92]. These collections can be sorted by species using dichotomous keys for identification, counted, and pooled for testing for the presence of EEEV viral RNA [3, 55, 56]. Although it can be difficult to detect EEEV viral RNA in mosquito pools, one could still utilize the abundance data from resting shelter collections to predict the risk potential for EEEV transmission in a given region [3, 46].

Epizootic transmission of EEEV generally occurs in the mid-summer months in the southeastern United States, with cases reported year-round in humans and horses in the state of Florida [2, 3, 36, 45, 46]. Though overwintering in the northern regions may occur in some instances, amplification and spread of EEEV to the northeastern United States may occur when birds return to these regions in the spring when infection is acquired in Florida prior to migration to the north [3, 13, 46]. The mechanisms at play regarding winter transmission of EEEV are quite perplexing, as research has shown gaps in understanding the ability to effectively overwinter EEEV in infected progeny of various mosquito species [23, 48]. *C. melanura* has shown its ability to feed upon reptiles through various field examinations of blood engorged mosquitoes [45, 51, 63, 93]. Given that ectotherms such as snakes maintain low levels of EEEV during experimental brumation and recovery post winter conditions, capabilities appear to exist in reptiles as a potential overwintering factor for EEEV transmission [45, 92-94]. Much of Florida rarely sees freezing temperatures, and winter conditions have been attributed to the ability for mosquitoes to change feeding behaviors as noted with studies on *Cx. erraticus* that could increase EEEV risk for humans, horses and other mammals [95]. Florida’s sub-tropical climate is just one of many reasons why Florida may be supporting year-round EEEV transmission. The
primary vector, *Cs. melanura*, is also a regularly detected resident mosquito species throughout the year in northern and central regions in Florida [46, 84]. Although *Cs. melanura* rarely feeds on humans and other mammals based on blood meal analysis, research shows that this mosquito species appears to have the highest detectable titers competent to transmit EEEV making this species viable for participation in both the enzootic cycle and epizootic events [3, 5, 47].

There are still many unanswered questions regarding wintertime transmission and impacts mild winters may have on EEEV peak season activity in Florida. Weather anomalies may grossly impact vector habitat suitability, potentially causing either cold drier conditions that minimize mosquito abundance, or warm wetter conditions that may increase abundance. This research aims at identifying alternative indicators associated with risk for EEEV in the state of Florida. The first aim of this research examines weather patterns that impact EEEV risk potential in Florida, looking at ENSO cycles in relation to retrospective horse case data. Horse cases indicate mammalian infection exists and can indicate risk to humans during an equine outbreak. Horse case data is useful when evaluating the potential for EEEV associated weather patterns that may also indicate risk. Secondly, this research seeks to improve upon present EEEV surveillance strategies by utilizing GIS-based risk index modeling to improve field-based evaluation of vector-host relationships during winter-spring seasons in Florida. Florida has an environment conducive for supporting a variety of mosquito species, therefore it is important to isolate areas that may provide an optimal habitat for the undertaking of field collection and surveillance. GIS-Based habitat suitability models can aid in pinpointing areas that could provide the ideal setting for risk based collection of *Cs. melanura* and other potential bridge vectors that may cohabitate in similar environments. This may also optimize placement of sentinel chicken coops to improve upon EEEV detection rates as opposed to random placement of surveillance tools in a given region. With the aforementioned possibility that Florida may serve as a geographic reservoir for EEEV, these studies may assist in detecting a link between wintertime
EEEV activity and peak transmission based on spatiotemporal analysis that could then be used to target areas of high risk across the state.

References


CHAPTER ONE

Note to Reader

This chapter has been previously published and has been used with publisher’s permission (Miley, K., et al., 2020. Impact of the Southern Oscillation Index, Temperature, and Precipitation on Eastern Equine Encephalitis Virus Activity in Florida., doi: 10.1093/jme/tjaa084)

Abstract

Eastern Equine Encephalitis virus (EEEV) is a highly pathogenic arbovirus affecting the eastern United States, especially Florida. Effects of the Southern Oscillation Index (SOI), precipitation, and cooling degree days on EEEV horse case data in Florida from 2004 to 2018 were modeled using distributed lag non-linear models (DLNMs). The analysis was conducted at statewide and regional scales. DLNMs were used to model potential delayed effects of the covariates on monthly counts of horse cases. Both models confirmed a seasonal trend in EEEV transmission and found that precipitation, cooling degree days, and the SOI were all predictors of monthly numbers of horse cases ($p < 0.05$). EEEV activity in horses was associated with higher amounts of rainfall during the month of transmission at the statewide scale, as well as the prior three months at the regional scale, fewer cooling degree days during the month of transmission and the preceding three months and high SOI values during the month and the previous two months, and SOI values in the prior 2 to 8 months. Horse cases were lower during El Niño winters but higher during the following summer, while La Niña winters were associated with higher numbers of cases and fewer during the following summer. At the regional scale,
extremely low levels of precipitation were associated with a suppression of EEEV cases for three months. Given the periodicity and potential predictability of ENSO cycles, precipitation, and temperature, these results may provide a method for predicting EEEV risk potential in Florida.

**Introduction**

Eastern Equine Encephalitis virus (EEEV) is the most pathogenic arbovirus in the eastern United States and has represented a significant public health concern for many years due to its high mortality from neuroinvasive infections in both humans and horses [1, 6, 11, 64]. Historically, Florida has been recognized as having one of the largest human caseloads in the southeast and is now believed to be the reservoir for outbreaks to the northeast [14, 45, 64]. Although neuroinvasive EEE is rare, the mortality rate is approximately 35% in humans and almost 100% in horses; those individuals that survive infection often have lifelong complications with an individual healthcare and economic burden in the millions [1, 9, 11]. With no FDA approved human vaccine available and treatment limited to palliative care, prevention relies on surveillance and protective vector control strategies [96]. EEEV in Florida has been extensively evaluated since the 1950s, but it still remains elusive with respect to providing an early warning system to predict and control outbreaks during periods of peak transmission [2, 11, 29].

Global climate anomalies may have a role in increasing outbreaks of vector borne infectious diseases due to heavy rainfall events and intense warming trends that may convert additional regions of the globe into suitable habitats for vectors of disease [70, 97, 98]. Increases in extreme weather conditions may bring about new challenges to interpreting the risk potential for many arboviral disease threats, which creates a need for evaluating weather anomalies that may affect the development of future outbreaks. Patterns in the El Niño Southern Oscillation (ENSO) have been associated with a variety of vector-borne diseases, including
malaria, Dengue, Chikungunya, Murray Valley encephalitis, Ross River virus and Rift Valley fever [72, 75-77, 80, 99-104]. ENSO has also been implicated in various other infectious diseases, including influenza, Varicella, Hantavirus, and Hand, Foot and Mouth disease (HFMD) [75, 79, 105-108]. The Southern Oscillation Index (SOI) is a global measure of large scale fluctuations in atmospheric pressure between the western and eastern tropical Pacific; strong negative SOI values coincide with warmer ocean temperatures and El Niño conditions, while strong positive values correspond to cooler ocean temperatures and La Niña conditions [69, 77, 109, 110]. In Florida, El Niño events appear to be associated with cooler, wetter winters, while La Niña events bring warmer, drier winter weather although the effects can vary spatially.

Currently, Florida’s EEEV surveillance relies heavily on sentinel chicken seroconversion rates and reported horse cases, with most counties utilizing EEEV seropositive sentinel chickens as indicators for increasing their mosquito control efforts and providing educational public service announcements to its residents [81, 111]. Unfortunately these surveillance methods may not provide enough warning to effectively reduce the risk to the human population as there may be limitations on the number of sentinel chickens representing an entire county, with some counties having no sentinel program. EEEV appears to be sustained in an enzootic cycle between birds and the mosquito vector Culiseta melanura, wherein risk of transmission to humans and other mammals is thought to occur through various bridge vectors, such as Coquillettididae pertubans, as well as certain Aedes and Culex species mosquitoes [3]. Although sentinel chicken surveillance is an integral part of prevention strategies and may indicate EEEV presence, this could also indicate transmission solely within the enzootic cycle Culiseta melanura rarely feeds upon mammals [47]. Human risk might be better predicted when sentinel surveillance is combined with the epizootic occurrence in horses, as this displays appearance of mammalian infection, suggesting it’s presence in bridge vectors that pose a more imminent risk of human exposure. Although a vaccine has been used in equines for many years, Eastern
Equine Encephalitis virus (EEEV) vaccine compliance in the United States has been reported at an average around 63% over the past 20 years, according to research on equine vaccination practices performed through the United States Department of Agriculture (USDA) and Animal and Plant Health Inspection Service (APHIS) [42]. Thus, the pool of unvaccinated animals is large. Indeed, it is rare to diagnose a case of EEEV in an appropriately vaccinated horse [44, 112].

A large portion of reported EEEV horse cases occur in north and central Florida (Figure 1). This is perhaps not surprising as this has a large population of horses. For example, the city of Ocala in Marion County has been called the “Horse Capital of the World” owing to its extensive involvement in the equine industry. Central Florida’s moderate climate provides ideal year-round conditions for the recreational equine and racehorse industry [113, 114]. Utilizing Florida for forecasting vector-borne disease risk through investigation of weather patterns and ENSO cycles related to EEEV horse case data may provide an additional mechanism to establish an early warning system for future planning and prevention strategies for EEEV in the state. In this study, we explored the effects of the SOI, precipitation, and temperature on monthly counts of EEEV horse cases in Florida during 2004 to 2018 using a distributed lag non-linear model (DLNMs). DLNMs were used in order to model potential delayed effects of the covariates on the counts of EEEV horse cases. This is ideal for uncovering relationships when exposure and response may involve a time delay or lag, such as climate changes that may affect vector breeding, survival or extrinsic incubation period [73, 115].

Materials and Methods

Distributed lag non-linear models (DLNMs) are a class of regression models that are useful for analyzing time series data where the lag dimension of an exposure-relationship is of interest but may be non-linear [116]. The lag response is modeled in terms of a cross-basis
function, which describes the shape of the exposure-relationship along both the space of the predictor and its lag dimension. DLNMs are frequently used to analyze epidemiological data summarized into regularly spaced time intervals, such as days, weeks or months, especially when delayed effects of weather or climate variables are expected [71, 78, 117, 118]. For the purpose of evaluating weather and climate factors associated with EEEV transmission in Florida, we used distributed lag non-linear models to investigate the effects of precipitation, temperature, and El Niño Southern Oscillation (ENSO) on monthly counts of EEEV horse cases from January 2004 to December 2018 for the entire state and for the central region where most cases are localized.

Horse case data were provided by the Florida Department of Health and summarized by calendar month and year. Yearly counts were combined from 2004 to 2018 and EEEV horse cases (n=730) were mapped by county. Mapping was conducted using ArcGIS Pro 2.4.3 and a county map derived from 2019 U.S Census Bureau. EEEV horse cases were plotted using proportional symbols to illustrate the variation of case counts within the given counties and determine areas of high case concentration within the state (Figure 1) [37, 119]. Along with a statewide analysis, 12 counties were selected in the central region of the state for fine scale examination. Together these represented 46.4% (n=339) of the horse cases in Florida during the study period. For the statewide analysis, mean monthly precipitation values for the state were obtained from the Florida Climate Center as derived from precipitation data archived by the National Climatic Data Center [120]. These values represent the total rainfall recorded each month averaged across all locations in the state. For the central analysis, mean monthly precipitation was averaged for five airport weather stations located within the region. Temperature was modelled as the statewide mean number of cooling degree days recorded each month using a base temperature of 0°C as obtained from the National Climatic Data Center [121]. A base temperature of 0°C was utilized, as this base temperature is relevant for
Florida, as freezing temperatures are relatively infrequent in the central and southern regions of the state. First, the numbers of cooling degree days for each month were obtained for 19 airport weather stations distributed across the state from the National Climatic Data Center. Then, a statewide mean was computed by averaging the number of cooling days for those 19 stations. Mean cooling degree days were calculated similarly for the central region using five airport weather stations. ENSO conditions were measured using Southern Oscillation Index (SOI) values archived by NOAA [122]. The complete datasets included 180 monthly records of horse cases, mean precipitation, mean number of cooling degree days, and SOI data (Figure 2).

We used DLNMs to explore the impacts of weather and climate on counts of EEEV horse cases in Florida with five covariates at both the state-wide and regional scales. First, sine and cosine functions of month were used as co-variates for both models to account for the seasonal trend in EEEV cases, as per Fisman et al. (2016). This was done because transmission generally follows a regular annual pattern with relatively fewer cases occurring during winter months when compared to summer, with lows generally occurring during mid-winter and peaks during mid-summer [45, 78]. For the state-wide analysis, mean statewide precipitation was included as an unlagged covariate, since preliminary exploration of the data did not find any evidence suggesting it had a delayed effect on EEEV transmission at the monthly time scale. For the regional analysis, we specified the lagged effect of mean monthly precipitation as a first-degree polynomial function for up to 3 months of lag. For both models, we specified the lagged effect of cooling degree days as a first-degree polynomial function for up to 6 months of lag. We specified the lagged effect of SOI as a second-degree polynomial with a maximum lag of 12 months. Though the choices of shape and length of lag for DLNMs can be somewhat arbitrary, these choices were based on guidance both from previous studies and fitting the data in hand, as per [116]. The state-wide Poisson-family GLM can be written in simplified terms as:
monthly count of EEEV horse cases ~ sine(month) + cosine(month) + mean statewide monthly precipitation + cross-basis (mean cooling degree days) + cross-basis (SOI)

while the regional model can be simplified as:

monthly count of EEEV horse cases ~ sine(month) + cosine(month) + cross-basis(mean statewide monthly precipitation) + cross-basis(mean cooling degree days) + cross-basis (SOI)

This DLNM was applied using R statistical software using package dlnm [123]. Fit of the model was assessed using standard methods, utilizing Cook’s distance to identify any potential outliers that may have been over- or under-predicted by the model [124].

Results

The total number of horse cases observed in Florida during 2004 to 2018 was 730; the total count in the central region was 339. State-wide monthly counts ranged from 0 to 52 cases, with an average of 4.1 reported horse cases per month. Counts in the central region ranged from 0 to 31, with an average of 1.7. State-wide mean monthly precipitation values ranged from 6 to 334.3 mm, with an average of 114.3 mm, while regional values ranged from 7.5 to 334.1 mm and averaged 99.6 mm. Mean state-wide cooling degree days ranged from 0 to 10.1 per month, with an average of 0.78. For the central region, these values ranged from 0 to 7.25 per month with an average of 0.43. During this time frame, SOI values fluctuated between -3.1 to 2.9, with an average of 0.24.

The state-wide DLNM confirmed the seasonal trend in EEEV transmission and found that precipitation, cooling degree days, and SOI were all predictors of monthly numbers of horse cases (p < 0.05). Cook’s distance identified one potential outlier with a value ≥1. This was June 2005, where the model predicted above average counts but not as high as those actually
observed (n=28 vs n=52 cases respectively). All other months were produced residuals with Cook’s distances <1. Mean monthly rainfall produced a weak but significant effect on the response (Table 1). Relative risk measures the degree of the positive or negative impacts on the response variable and is the standard utilized in Poisson regression models such as those used in this study. An overall depiction of the effect of cooling degree days on case incidence is shown in Figure 3a, while two-dimensional cross-sections with 95% confidence intervals of the relative risks (RR) are shown in Figure 3b-d for 0 to 6 mean cooling days per month. These results suggested that a mean of 0 cooling degree days is associated with a slight increase in risk during the month and up to about 1.5 months later, while a mean of 1 degree-day had no impact on the response. An average of 2-3 cooling degree days per month produced minor reductions in monthly cases for a period up to 3 months. Once the average number of cooling degree days reaches 4 or more, the impact on relative risk is substantial and is associated with a reduction EEEV transmission for a period of 3 months.

The overall effects of the SOI on the counts of EEEV horse cases are illustrated in Figure 4. The relationship is more complex than that observed for temperature. The cross-sections depicted in the figure represent three benchmark SOI values: 3 (La Niña), 0 (ENSO neutral), and -3 (El Niño). The resulting graphs suggest that strong La Niña conditions, which mostly occur during winter months but may extend into summer, produce larger numbers of cases for the winter season (Figure 4b). This La Niña effect lasts up to 2 months, with the relative risk declining for a period of eight months before it increases again. On the other hand, El Niño conditions, which also are more likely to occur during winter but can extend into summer, are associated with relatively lower numbers of cases for winter (Figure 4d). The El Niño effect is observed for approximately two months and is followed by an eight-month period of elevated risk. The ENSO neutral conditions have no impact on the response (Figure 4c).
At the regional scale, the DLNM confirmed the state wide seasonal trend in EEEV transmission and found that precipitation, cooling degree days, and SOI were all predictors of monthly numbers of horse cases \((p < 0.05)\) (Table 1). Cook’s distance identified one potential outlier with a value \(\geq 1\). This was May 2005, where the model predicted above average counts but not as high as observed \((n=6 \text{ vs } n=21 \text{ cases respectively})\). All other months produced residuals with Cook’s distances \(<1\). Unlike the state-wide model, the regional model found that monthly precipitation had a significant lagged effect on monthly cases (Figure 5). Extremely low monthly amounts of rainfall were associated with a suppression in the number of cases for a period of 3 months (Figure 5b), while average values had a null effect (Figure 5c). Above average amounts were associated with an increased number of cases for a period of 3 months (Figure 5d). Lagged effects of cooling degree days (Figure 6) and SOI (Figure 7) were similar to those found for the state-wide model, except stronger impacts were observed with larger numbers of cooling days for the region.

**Discussion**

The results of the DLNMs explain the variation in monthly numbers of observed horse cases of EEEV at both the state-wide and regional scales, which vary considerably both within and between years. The main advantage of the DLNM approach was that it allowed non-linear weather and climate relationships to be modelled over time. Notwithstanding the seasonal trend of more cases in summer than winter, increased state-wide EEEV activity in horses was associated with: (1) higher amounts of rainfall during the month of transmission, (2) fewer cooling degree days during the month of transmission and the preceding three months, and (3) high SOI values during the month and the previous two months, as well as low SOI values in the prior 2 to 8 months. Similar observations were made at the regional scale, although: (1) a lagged effect of precipitation found, with unusually low amounts of precipitation suppressing
EEEV activity for three months and above average amounts associated with increased numbers of cases; and (2) large numbers of cooling degree days were associated with much stronger reductions in cases for a period of three months. These results suggest that the statewide model has utility in predicting EEEV transmission in horses over a broad geographic area, but the regional model was able to detect stronger associations, likely because it better captures local conditions that may vary considerably across the state.

These associations explain both winter and summer peak counts. For example, the fewest numbers of summer counts in Florida were observed during 2011 and 2017 when only 4 and 7 cases were observed respectively. Summer 2011 experienced below average precipitation early in the season; it was preceded by extreme numbers of cooling degree days in the preceding December and January, with freezing extending into March; and was preceded by a strong La Niña winter event. Summer 2017 experienced a drought, with far below average rainfall, followed La Niña winter conditions, and had moderate numbers of cooling degree days in the preceding 3 months, explaining the small numbers of cases. At the opposite end of the scale, the greatest numbers of summer counts were observed during May-July 2005 and July-August 2010, months that were associated with above average precipitation, preceded by three months of mild winter temperatures, and which followed strong winter El Niño events. Although the models did not predict the extreme values observed June 2005, it did predict well above average numbers of cases for that year and also predicted those observed in 2010, explaining the large numbers of observations. Less extreme monthly counts for both seasons were generally associated with less extreme amounts of rainfall, moderate numbers of cooling degree days, and more neutral ENSO conditions for both models.

The DLNMs explained the variation in historical monthly numbers of horse cases of EEEV, both across the State of Florida and for its most affected region, showing potential for forecasting when peak EEEV activity is expected based on current and previous weather and
climatic conditions. This may allow mosquito control districts to implement enhanced surveillance, prevention and control methods before transmission begins to intensify, potentially reducing future observed numbers of cases. A spatial risk map for EEEV in horses has been developed and validated for Florida showing locations across the state where transmission to horses is most likely to occur based on habitat conditions [84, 85]. Combining the spatial predictions of EEEV transmission from the risk map with temporal predictions from the DLNM could provide a spatiotemporal prediction method to improve EEEV surveillance and control across the state.

The models developed here are based on long term fluctuations, and thus validation of the model will require several years when low, medium, and high numbers of cases are observed. However, according to the SOI data for 2019, the winter of 2018 into 2019 favored ENSO neutral conditions with a slight potential for El Niño illustrated in early spring, while much of the year conditions remained neutral. Annual precipitation for the state was above average, while winter temperatures in the central region were relatively mild. This suggests that the numbers would be expected to be average or below average for the year. From 2004-2018, the average number of horse cases was 49 per year, while in 2019 just 28 horse cases were recorded statewide. Similarly, for the central region, 6 cases were observed compared to an average of 19. Future work should explore validating the model using longer term data.

Although it appears that horse cases may be lessening over time (as depicted in Figure 2), this could actually be reflective of the overall decrease in the equine population. According to the USDA National Agricultural Statistics Service (NASS) equine census, the equine population in the United States, the number of documented horses has dropped from approximately 4 million in 2007 to around 2.8 million in 2017, a decrease of roughly 30% [125]. This decline in the population may be in part responsible for the observed decline in horse cases.
Although the DLNMs explain much of the variation in monthly EEEV horse cases based on weather and climatic variables, it does have some limitations. These are related to the scales of analysis. First, the analysis is constrained to the monthly time scale. Predictions could possibly be improved with finer scales, including using daily or weekly temperature or rainfall data, although SOI values are only available monthly. Second, the regional analysis was better able to pinpoint the lagged effects of precipitation and temperature than the state-wide analysis. This is likely because local trends are masked at such a broad scale. Although it may be desirable to conduct a similar analysis at finer scales or for other regions, the low numbers of case counts are likely to limit the use of DLMNs at that scale. Additionally, although SOI is a global variable, particular ENSO conditions can impact a specific region differently, e.g. producing wetter conditions in some areas while creating drier conditions in others. However, despite these limitations, the DLNM results may be effective for forecasting general patterns of EEEV transmission in the state of Florida. ENSO predictors of EEEV could be utilized with previously validated EEEV risk mapping, alongside current mosquito abundance and chicken serological surveillance to aid in targeted prevention strategies that reduce EEEV transmission potential. Future work should investigate ENSO effects at fine local scales. This exploration of ENSO in relation to EEEV risk potential is in no way meant to create an inclusive predictive model; rather its proposed as a potentially valuable mechanism to utilize in conjunction with the current surveillance and control activities which may reduce future EEEV outbreaks in Florida and their potential spread to the northeastern United States.
## Tables and Figures

**Table 1.1** Effect of statewide monthly temperature, precipitation, and Southern Oscillation Index (SOI) on counts of EEE horse cases in Florida.

<table>
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<th>Statewide Analysis</th>
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<th>Standard Error</th>
<th>$p$</th>
</tr>
</thead>
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<tr>
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<td>0.018</td>
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<td>Cooling Degree Days (Lag 2)</td>
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<td>SOI (Lag 1)</td>
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<td>0.001</td>
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Figure 1.1 Florida Map of statewide EEEV horse cases from 2004 to 2018, Including highlighted counties selected for Regional fine-scale analysis.
Figure 1.2  Mean monthly precipitation (a), mean monthly number of degree cooling days (b), and SOI values (c) plotted over monthly counts of EEEV horse cases in Florida.
Figure 1.3 Overall effect of mean monthly numbers of degree cooling days (a) on monthly counts of EEEV horse cases in Florida over 6 month of lag, with 95% confidence intervals calculated using standard methods in DLMN package plotted for means of 6 (b), 3 (c), and 0 (d), days per month.
Figure 1.4 Overall effect of monthly SOI values on monthly counts of EEEV horse cases in Florida over 12 month of lag (a), with 95% confidence intervals calculated using standard methods in DLMN package plotted for SOI values of 3 (b), 0 (c), and −3 (d).
Figure 1.5 Regional model of monthly precipitation effect on monthly EEEV horse cases in Florida over 3 month lag (a), with 95% confidence intervals calculated using standard methods in DLMN package for plotted means of 50 mm (b), 100 mm (c), and 250 mm (d) of precipitation per month.
**Figure 1.6** Regional effect of mean monthly numbers of degree cooling days (a) on monthly counts of EEEV horse cases in Florida over 6 month of lag, with 95% confidence intervals calculated using standard methods in DLMN package plotted for means of 6 (b), 3 (c), and 0 (d) days per month.
Figure 1.7  Regional effect of monthly SOI values on monthly counts of EEEV horse cases in Florida over 12 month of lag (a), with 95% confidence intervals calculated using standard methods in DLMN package plotted for SOI values of 3 (b), 0 (c), and −3 (d).
References


CHAPTER TWO

Note to Reader

This chapter has been submitted for publication, but is not yet published (Miley, K. et al. 2020. Field Analysis of Biological Factors associated with Sites at High and Low to Moderate Risk for Eastern Equine Encephalitis Virus Winter Activity in Florida)

Abstract

Eastern Equine Encephalitis virus (EEEV) is the most pathogenic arbovirus endemic to the United States. Studies have demonstrated Florida’s role as a regional reservoir for the virus and its ability to support year-round transmission. Previous research has developed risk index models for mapping locations most at risk for EEEV transmission. We compared vector abundance, vector feeding behavior, potential host species, and fauna presence at high versus low-moderate risk sites during the winter and spring. Predicted high-risk sites had a significantly greater abundance of mosquitoes overall, including Culiseta melanura (Coquillett), the primary enzootic vector of EEEV. Twenty host species were identified from Cs. melanura bloodmeals, with the majority taken from avian species. Culiseta melanura largely fed upon the Northern Cardinal (Cardinalis cardinalis), which accounted for 20% to 24.4% of the bloodmeals obtained from this species in years one and two respectively. One EEEV positive mosquito pool (Cs. melanura) and nine EEEV seropositive sentinel chickens were confirmed during winter-spring collections from high-risk sites; no seropositive chickens nor mosquito pools were found at the low-moderate risk sites. These results suggest that high-risk sites for EEEV activity are characterized by habitats that support populations of Cs. melanura and which may also provide ample opportunities to feed upon Northern Cardinals. The overall low level of mosquito
populations during the winter also suggests that control of *Cs. melanura* populations in winter at high-risk sites may prove effective in reducing EEEV transmission during the peak summer season.

**Introduction**

Eastern Equine Encephalitis virus (EEEV) (*Togaviridae: Alphavirus*) is the most pathogenic arbovirus endemic to in the United States. It is estimated that about 35% of individuals infected with EEEV will succumb to the illness, and many survivors will develop long-term neurological deficits [3, 6, 9, 11, 94]. There are approximately 7 human cases of EEEV in the U.S. annually, with about half of those occurring in Florida [6, 10]. Florida also reports the highest number of horse cases of EEEV on average in the U.S. [3, 10]. This may be in large part due to the presence of both an abundant vector habitat [46] and a climate conducive to year-round transmission of EEEV [65]. Recent phylogenetic studies have demonstrated Florida’s role as a geographic reservoir for the virus for the rest of the country [13, 94]. These studies suggest that the northern foci of EEEV may persist for several successive seasons, eventually becoming extinct and being replaced by reintroductions from Florida [2, 45, 66, 126]. Thus, the wintertime transmission of EEEV in Florida plays a key role in the maintenance and dissemination of EEEV throughout the USA.

Spatial epidemiological studies have shown that certain habitats in Florida appear to have greater association with EEEV transmission than others [114]. EEEV risk models utilizing habitat association and horse case data have indicated that areas of high risk are equally predictive regardless of season [83]. Although these risk maps predict wintertime and peak season EEEV activity, they do not explain the ecological and biological underpinnings that make a particular habitat high or low risk. Therefore, it is important to validate geological risk mapping through field analysis in order to further explain why certain habitats are more conducive to EEEV transmission.
For this study, we focused on the biological and ecological factors relevant to EEEV transmission at sites predicted to be at high or low-moderate risk for EEEV activity. The goal was to gain a better understanding of the biological drivers of winter-spring EEEV activity that may allow for optimized surveillance and treatment strategies during these periods of low mosquito abundance.

Materials and Methods

Risk maps were created for three counties in Florida (Walton, Hernando, and Citrus Counties) using a risk index (RI) model, as previously described [83, 84]. The RI model was originally developed to predict EEEV transmission risk based on habitat configurations of known horse cases [83, 84]. Here, we applied a more generic RI model that assesses risk for a broader array of species, such as humans and birds [127]. This model estimates transmission risk on a continuous scale of 0.0 to 1.0, where 0 indicates no risk and 1 indicates maximum risk. Risk is evaluated based on five habitat variables that are combined to calculate a final RI for each raster cell in a land cover map: local habitat, wetland proximity, wetland composition, coniferous forest-tree plantation proximity, and coniferous forest-tree plantation composition (Table 1). Areas receive maximal RI values when they are in close proximity to an abundance of both wetlands and coniferous forests/tree plantations. For specific details on the geographic information system (GIS) application of the RI models, see [83, 84].

The risk maps were then used to identify surveillance study sites for mosquito collection and placement of resting shelters and sentinel chicken coops at sites classified as high-risk (RI Values >0.8) or low-moderate risk (RI Values < 0.7) (Figure 1). A total of 20 sites (16 high risk and four low-moderate risk) were chosen for inclusion in this study (Table 2). Resting shelters were deployed at all sites; sentinel chicken coops were also placed at all but two of the sites to monitor EEEV activity [87, 89]. A total of 60 resting shelters were deployed at all sites (three shelters at each site) for use as a passive collection method for Cs. melanura, the primary
enzootic vector responsible for wintertime transmission in Florida [3]. Resting shelters have been effectively utilized for collection of a variety of mosquitoes, especially when attempting to retrieve blood fed mosquitoes, as they mimic the seclusion and darkness of tree holes and other sheltered locations that a blood fed female mosquito may seek for egg maturation [3, 63, 128]. Mosquito collections were carried out twice per week at all sites from October to May for each collection year (2017-2018, 2018-2019). This represented a total of 5,184 trap nights over the course of the study.

Mosquitoes were retrieved using a modified vacuum aspirator with a mesh bottom collection cup as previously described [3, 88, 128]. Collections were placed in -20°C and transferred to 50mL tubes labelled by site and date of collection. Mosquito collections were identified utilizing region specific dichotomous keys [55, 56] and separated by species using a chill table. The sorted mosquitoes were combined into pools of 50 or less by species, collection site, and collection date. They were placed in 2.0mL microcentrifuge tubes and stored at -80°C. All specimens were catalogued, and species were combined into two-week intervals to increase pool size, due to low wintertime collection rates. All blood fed mosquitoes were separated into individual specimens, placed in 1.7mL microcentrifuge tubes for PCR-based bloodmeal analysis. Overall mosquito abundance was evaluated to determine differences between collections at high-risk versus low-moderate risk sites. A t-test was used to determine significance between the means of high and low-moderate risk groups. A Chi-square test was used to determine if differences existed between Cs. melanura counts compared to other mosquito species at high versus low-moderate risk sites.

Bloodmeal analysis was carried out as described by West et. al [63]. DNA extracts were first queried using primers specific for the vertebrate cytochrome b gene. Samples found to be negative in this PCR assay were further tested using a variety of other primers in a stratified approach to detect other potential host classes [63, 129-131]. PCR products were subjected to
gel electrophoresis (1% agarose, SYBR Safe gel stain, O'GeneRuler 100bp DNA ladder; Thermo Scientific®) and samples displaying a distinct band were sent to a commercial facility to determine the amplicon’s DNA sequence [63]. The resulting sequences were evaluated and compared to known sequences using BLASTn (NCBI). Bloodmeals were classified as having been derived from a given species when the sequence from the PCR amplicon exhibited a ≥ 95% match to a sequence in the GenBank sequence databank [63].

Mosquito pools (≤ 50 mosquitoes/pool) were homogenized in Biological Field Diluent (90% Minimum Essential Medium with Hank’s Salts, 10% Fetal Bovine Serum, with antibiotics 200U/mL Penicillin, 200ug/mL Streptomycin, 2.5ug/mL Amphotericin B) using Qiagen® TissueLyser II at 25Hz for 4 minutes, as previously described [3]. RNA extraction of homogenized samples was performed from 140uL of homogenized supernatant using QIAamp® Viral RNA Mini Kits with automated processing performed using a QIAcube® (Qiagen). Eluted RNA was then used as a template in a RT-PCR assay employing the iTaq universal probes one-step kit (BioRad®) and the EEEV Primer/Probe A set [132]. Specimens with a Ct value of 37 or less in this assay were classified as putative positive and confirmatory testing carried out using a EEEV Primer/Probe B set [132]. Samples exhibiting Ct values of <37 in both assays were confirmed positive.

Wildlife cameras were placed at all sites in Citrus County to monitor local wildlife around the mosquito resting shelters. This data was analysed by visit count (allocated for a given species as 1 independent visit during a minimum 10 minute period, longer if determined that the same animal was apparent in consecutive photos) similar to previous studies [133, 134]. Wintertime trail camera photos for each winter season were examined, identified by species, and catalogued to compare species visit counts with mosquito host use and vector abundance data collected at each site. Feeding indices were calculated for wildlife visit counts using published methods [135, 136].
Results

Winter-spring collections (October to May) from resting shelters for 2017-2019 yielded a total of 4,925 mosquitoes collected across all sites combined, representing 38 different species. The study encompassed a total 5,184 trap nights of collections representing a collection rate of 0.95 mosquitoes per trap night. An average of 282 mosquitoes were collected from each of the high-risk sites (n=16, 48 shelters), while the low-moderate risk sites (n=4, 12 shelters) yielded a mean of 103 mosquitoes per site indicating a marginally significant difference in total mosquito populations between high and low-moderate risk sites ($t = 1.75$, $p = 0.047$). All 38 different mosquito species collected in the study were found at one or more of the high-risk sites, while 21 species were collected from the low-moderate risk sites. The top five species collected at the high-risk sites were *Culex quinquefasciatus* (Say), *Cs. melanura*, *Anopheles crucians* (Wiedemann), *Anopheles perplexens* (Ludlow), and *Culex erraticus* (Dyar and Knab), making up 25.7%, 25.0%, 13.6%, 8.3% and 7.7% of the collections, respectively (Figure 2A). Various other mosquito species (n=33) made up the remaining 19.7% of the collections at the high-risk sites (Table 3). The top five species collected at the low-moderate risk sites included *Cx. erraticus*, *Culex salinarius* (Coquillett), *Cx. quinquefasciatus*, *Culex nigripalpus* (Theobald), and *An. crucians*, making up 34.4%, 15.7%, 14.5%, 7.3% and 6.0% of the collections, respectively (Figure 2B), with other species (n=16) making up the remaining 22.8% of the collections (Table 3). *Culiseta melanura* made up 25.0% of the total collection from the high-risk sites and just 3.4% of the collections from the low-moderate risk sites ($X^2$ with Yates correction = 97.0, $p < 0.0001$).

The collections from 2017-2019 yielded 1,458 mosquito pools that were tested for presence of EEEV via RT-PCR. One mosquito pool (*Cs. melanura* pool n=4 collected on March 1, 2019 from site Hern 1 in Hernando County, a high-risk site) was confirmed RT-PCR positive for EEEV. One sentinel chicken (blood collected on 03/04/19) was also found to be seropositive for EEEV at this site. A total of nine EEEV positive sentinel chickens were also confirmed during
winter-spring collections from 2017-2019, with three sites in Walton County, one in Citrus and one in Hernando (Walton County n=6, Citrus County n=2, Hernando County n=1). This represented an infection rate of 0.51 per 1000 coop days of potential exposure to chickens by vector mosquitoes at high-risk sites. All seropositive chickens were located at high-risk sites (Table 2).

Bloodmeal analysis was performed on blood engorged female mosquitoes of all species collected from wintertime resting shelters during the entire collection period (2017-2019). The results of the first winter collection season (October 2017 – May 2018) are shown in Figure 3. In the potential bridge vectors (i.e. other mosquito species collected not including the primary vector), a total of 70.5% of the blood meals were derived from avian hosts, 26.5% from mammalian hosts and 3.0% from reptile hosts (Figure 3A). In contrast, 82.0% of the bloodmeals found in Cs. melanura, the primary vector for EEEV, were derived from avian hosts and 18.0% were from reptile hosts (Figure 3B). In all species other than Cs. melanura, the chicken was the most common host, representing 64.7% of the bloodmeals (likely due to resting shelter placement near sentinel coops), with the White-tailed deer (Odocoileus virginianus) the second most commonly fed upon host at 11.8% (Figure 3C). For Cs. melanura, the Northern Cardinal (Cardinalis cardinalis) was the most commonly utilized host at 24.4% while chickens were second at 15.6% (Figure 3D). In the second winter collection season (October 2018 – May 2019), 67.0% of the bloodmeals derived from mosquito species other than Cs. melanura were from avian hosts, 25.5% from mammalian hosts, while 7.5% were reptile derived (Figure 4A). During the second winter collection, 86.7% of the Cs. melanura bloodmeals were avian-derived, while 8.9% were mammalian derived, and 4.4% were reptile-derived (Figure 4B). In the second winter season, 53.4% of the bloodmeals of mosquito species other than Cs. melanura were derived from chickens, with humans being the second most utilized host at 10.5% and the White-tailed deer third at 6.0% (Figure 4C). For Cs. melanura in 2018-2019, the chicken was the
most utilized host at 37.8%, followed by the Northern Cardinal at 20.0%, with 8.9% of the meals in *Cs. melanura* being derived from humans (Figure 4D). Excluding bloodmeals from the sentinel chickens, the Northern Cardinal was the most commonly targeted species by *Cs. melanura*.

The results of the number of visits of different potential host species captured by the wildlife cameras are shown in Figure 5. The top four species observed in the camera imagery at the high-risk sites included the Northern raccoon (*Procyon lotor*), followed by White-tailed deer (*Odocoileus virginianus*), Nine-banded armadillo (*Dasypus novemcinctus*) and the Eastern grey squirrel (*Sciurus carolinensis*) (Figure 5A). These made up 57.5%, 12%, 8.2%, and 7.5% of the collective visits in the high-risk areas, respectively (Figure 5B). The top four species observed at low-moderate risk sites included the wild turkey (*Meleagris gallopavo*) followed by Eastern cottontail (*Sylvilagus floridanus*), Nine-banded armadillo and the Eastern grey squirrel (Figure 5A). These made up 34.3%, 17.1%, 14.3%, and 14.3% of the collective visits in low-moderate risk areas, respectively (Figure 5C). Other wildlife documented across the camera deployment sites included Virginia opossum (*Didelphis virginiana*), feral pig (*Sus scrofa*), bobcat (*Lynx rufus*), house sparrow (*Passer domesticus*) and the Cotton mouse (*Peromyscus gossypinus*) (Figure 5A). The deer appeared to be the most targeted mammalian host by mosquito species other than *Cs. melanura*, with a feeding index of 5.9. Raccoons were the most commonly observed visitors, but they were rarely fed upon by the mosquito population (feeding index of < 0.1; Table 4). Although not detected on camera imaging, humans were the only mammal noted to be fed upon by *Cs. melanura*.

**Discussion**

Through phylogenetic studies and vector habitat association, Florida has been documented as a key contributor to EEEV transmission and dissemination across the eastern
United States [2, 13, 114]. Although wintertime transmission of EEEV in Florida is rare, evidence of EEEV persists in high risk foci year round. Most EEEV activity occurs in the north and central regions of Florida [84]. While much of Florida offers a potential habitat for vector mosquito populations, certain locations across the state have increased risk of EEEV transmission; these provided the setting for field research and validation of RI mapping [3, 45, 46, 85, 94].

The high-risk sites had a greater mosquito abundance overall than that observed at the low-moderate risk locations. Importantly, collections of *Cs. melanura* were 28-fold greater at the high-risk than at the low-moderate risk sites. *Culiseta melanura* were rarely found at the low-moderate risk sites, being present in low numbers at only one of the low-moderate risk sites. Together these data indicate that the high-risk sites harboured greater numbers of mosquitoes overall than did the low-moderate risk sites, and most importantly harboured significantly greater populations of *Cs. melanura*, the major enzootic vector of EEEV. These results suggest that a major biological underpinning of the difference between high-risk and low-moderate risk sites is likely to be the presence of a habitat suitable for *Cs. melanura*. This finding provides an ecological reason for the difference between high-risk and low-moderate risk sites, suggesting that GIS-based risk modelling and the applied risk index can predict suitable habitat locations for EEEV activity. Such predictions may prove useful for optimizing both surveillance and control strategies [45-47, 127, 137].

Similar to previous studies, this work documented a large proportion of bloodmeals in *Cs. melanura* were derived from avian hosts [45, 46, 62]. During the winter-spring seasons of 2017-2019, 20 host species were identified from *Cs. melanura* bloodmeals, with the majority of feedings taken from avian species. The Northern Cardinal was the primary species fed upon by *Cs. melanura* in 2017-2018 collections, and it was the second most fed upon species in 2018-2019 collections (representing 20%-24% of the *Cs. melanura* derived blood meals) which may
further indicate that this species has potential as an amplifier for EEEV activity [46, 49]. Although chicken blood meals were detected in high numbers during these collections, this was probably due to the presence of the chicken coops near mosquito resting shelters. Previous studies have suggested that the Northern Cardinal may be a super spreader for EEEV and other mosquito-borne viruses [49, 138]. Taken together, these results suggest that high-risk sites for winter-spring EEEV activity are characterized by habitats that support populations of *Cs. melanura* and which also provide ample opportunities for this species to feed upon Northern Cardinals. These findings are further supported by the enzootic vector habitat which generally consists of freshwater hardwood swamps where Northern Cardinals may frequent the hardwood borders and vegetation [49, 57]. The presence of both the major enzootic vector and an important enzootic reservoir for the virus at high-risk sites may explain why these represent ideal habitats for EEEV activity.

*Cs. melanura* was found at only one low-moderate risk site (Citrus 5) and four of the the blood fed individual *Cs. melanura* collected from this site were found to have fed upon Northern Cardinals. This site thus shared some biological characteristics with the high risk sites, though the number of *Cs. melanura* collected at this site was very low (n=14 over two years). It is possible that Citrus 5 was misclassified as low-moderate risk, as the RI model was found in validation studies to be more accurate at predicting high-risk sites in Northern Florida and the Panhandle than in the Central and Southern parts of the state [84, 139]. Alternatively, it is possible that Citrus 5 may have included a small habitat suitable for *Cs. melanura* which was below the resolution used to classify habitat in the RI model (30m x 30m) [84, 139].

While the number of mosquitoes collected at the high-risk sites were significantly greater than the low-moderate risk sites, the numbers of mosquitoes collected were quite low overall. As documented in previous studies of mosquito abundance during winter periods, these low collection rates may be due to climatic variables during both winter collection seasons that could
have reduced the resident mosquito population [45, 46]. These climatic patterns may have led to a lower overall mosquito abundance that might be present in years with more suitable climatic conditions [65]. Although there was a small number of mosquitoes collected one positive mosquito pool was found to be positive for EEEV at a high risk site, and several seropositive chickens were detected at the high risk sites, representing an infection rate of 0.51 per 1000 coop days. Taken together with historical data collected by the Florida Department of Health [140] and previous studies [3, 45, 46], our results suggest that EEEV, though active year-round in Florida, may be inefficiently transmitted during the winter and spring months. These results also illustrate that the EEEV cycle may be particularly vulnerable to interruption through targeted vector control during winter and spring months. If one could disrupt EEEV transmission during this vulnerable period, it may be possible to prevent the peak in EEEV activity that generally occurs in Florida during the early summer months. Furthermore, it may be possible to prevent the dissemination of EEEV from Florida to the north-eastern states, thereby protecting this region from EEEV outbreaks.
Tables and Figures

Table 2.1 Calculation of an Eastern Equine Encephalitis Virus risk index (RI) model using five risk variables (RV).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Input</th>
<th>Equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>RV1</td>
<td>Local Habitat</td>
<td>Cover type of focal cell</td>
</tr>
</tbody>
</table>
| RV2                    | Wetland Proximity                      | Meters to nearest wetland, $d_w$                                       | $\begin{cases} 
1 & \text{if } d_w \leq 400 \\
1 - \left(\frac{d_w - 400}{1500 - 400}\right) & \text{if } 400 < d_w < 1500 \\
0 & \text{if } d_w \geq 1500 
\end{cases}$ |
| RV3                    | Wetland Composition                    | Proportion of wetlands within 1500 meters, $p_w$                      | $\begin{cases} 
1 & \text{if } p_w \geq 0.18 \\
p_w & \text{if } p_w < 0.18 \\
0.18 & \text{if } p_w < 0.18 
\end{cases}$ |
| RV4                    | Tree Plantation-Coniferous Forest Proximity | Meters to nearest tree plantation or coniferous forest, $d_c$         | $\begin{cases} 
1 & \text{if } d_c \leq 250 \\
1 - \left(\frac{d_c - 250}{1500 - 250}\right) & \text{if } 250 < d_c < 1500 \\
0 & \text{if } d_c \geq 1500 
\end{cases}$ |
| RV5                    | Tree Plantation-Coniferous Forest Composition | Proportion of tree plantations and coniferous forest within 1500 meters, $p_c$ | $\begin{cases} 
1 & \text{if } p_c \geq 0.20 \\
p_c & \text{if } p_c < 0.20 \\
0.20 & \text{if } p_c < 0.20 
\end{cases}$ |
| RI                     | Risk Index                             | $= RV1 \times \left[\frac{RV2 + RV3 + RV4 + RV5}{4}\right]$          |
Table 2.2  Characteristics of Study Sites; GIS-Based Mosquito Collection Sites (n=20) including EEEV Risk Index values and presence or absence of Sentinel Chicken Surveillance. (present at 90% of sites)

<table>
<thead>
<tr>
<th>Site ID</th>
<th>Latitude</th>
<th>Longitude</th>
<th>Risk Index</th>
<th>Risk Class</th>
<th>Sentinel Chickens Present</th>
<th>EEEV Positive Chickens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citrus 1</td>
<td>28.704</td>
<td>-82.5641</td>
<td>1.00</td>
<td>High</td>
<td>Yes</td>
<td>2</td>
</tr>
<tr>
<td>Citrus 2</td>
<td>28.832784</td>
<td>-82.566586</td>
<td>0.93</td>
<td>High</td>
<td>No</td>
<td>-</td>
</tr>
<tr>
<td>Citrus 3</td>
<td>28.88845335</td>
<td>-82.5719984</td>
<td>0.95</td>
<td>High</td>
<td>Yes</td>
<td>0</td>
</tr>
<tr>
<td>Citrus 4</td>
<td>28.919264</td>
<td>-82.636198</td>
<td>0.88</td>
<td>High</td>
<td>No</td>
<td>-</td>
</tr>
<tr>
<td>Citrus 5</td>
<td>29.010104</td>
<td>-82.54341657</td>
<td>0.62</td>
<td>Low-Moderate</td>
<td>Yes</td>
<td>0</td>
</tr>
<tr>
<td>Hern 1</td>
<td>28.481659</td>
<td>-82.616481</td>
<td>0.94</td>
<td>High</td>
<td>Yes</td>
<td>1</td>
</tr>
<tr>
<td>Hern 2</td>
<td>28.461048</td>
<td>-82.630529</td>
<td>0.97</td>
<td>High</td>
<td>Yes</td>
<td>0</td>
</tr>
<tr>
<td>Hern 3</td>
<td>28.573118</td>
<td>-82.576955</td>
<td>1.00</td>
<td>High</td>
<td>Yes</td>
<td>0</td>
</tr>
<tr>
<td>Hern 4</td>
<td>28.484582</td>
<td>-82.43194</td>
<td>0.63</td>
<td>Low-Moderate</td>
<td>Yes</td>
<td>0</td>
</tr>
<tr>
<td>Hern 5</td>
<td>28.5331692</td>
<td>-82.4703581</td>
<td>0.62</td>
<td>Low-Moderate</td>
<td>Yes</td>
<td>0</td>
</tr>
<tr>
<td>Hern 6</td>
<td>28.47741469</td>
<td>-82.2858239</td>
<td>0.67</td>
<td>Low-Moderate</td>
<td>Yes</td>
<td>0</td>
</tr>
<tr>
<td>NW 1</td>
<td>30.58928</td>
<td>-86.02623</td>
<td>1.00</td>
<td>High</td>
<td>Yes</td>
<td>0</td>
</tr>
<tr>
<td>NW 2</td>
<td>30.513137</td>
<td>-86.108255</td>
<td>1.00</td>
<td>High</td>
<td>Yes</td>
<td>0</td>
</tr>
<tr>
<td>NW 3</td>
<td>30.954142</td>
<td>-86.180023</td>
<td>1.00</td>
<td>High</td>
<td>Yes</td>
<td>0</td>
</tr>
<tr>
<td>NW 4</td>
<td>30.807207</td>
<td>-86.08923</td>
<td>0.81</td>
<td>High</td>
<td>Yes</td>
<td>0</td>
</tr>
<tr>
<td>SW 1</td>
<td>30.374583</td>
<td>-86.185333</td>
<td>1.00</td>
<td>High</td>
<td>Yes</td>
<td>0</td>
</tr>
<tr>
<td>SW 2</td>
<td>30.361167</td>
<td>-86.095667</td>
<td>1.00</td>
<td>High</td>
<td>Yes</td>
<td>2</td>
</tr>
<tr>
<td>SW 3</td>
<td>30.313892</td>
<td>-86.087986</td>
<td>1.00</td>
<td>High</td>
<td>Yes</td>
<td>1</td>
</tr>
<tr>
<td>SW 4</td>
<td>30.355084</td>
<td>-86.240399</td>
<td>0.90</td>
<td>High</td>
<td>Yes</td>
<td>0</td>
</tr>
<tr>
<td>SW 5</td>
<td>30.365</td>
<td>-86.22605</td>
<td>0.91</td>
<td>High</td>
<td>Yes</td>
<td>3</td>
</tr>
</tbody>
</table>
### Table 2.3 Mosquito Species Collected at High and Low-Moderate Risk Sites from 2017-2019:

<table>
<thead>
<tr>
<th>Mosquito Species</th>
<th>High Risk Sites</th>
<th>Low-Moderate Risk Sites</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(N) Site Mean</td>
<td>%</td>
</tr>
<tr>
<td>Aedes albopictus</td>
<td>3 0.19</td>
<td>0.07%</td>
</tr>
<tr>
<td>Aedes atlanticus/tormentor</td>
<td>53 3.31</td>
<td>1.17%</td>
</tr>
<tr>
<td>Aedes canadensis canadensis</td>
<td>6 0.38</td>
<td>0.13%</td>
</tr>
<tr>
<td>Aedes dupreei</td>
<td>2 0.13</td>
<td>0.04%</td>
</tr>
<tr>
<td>Aedes infirmatus</td>
<td>161 10.06</td>
<td>3.57%</td>
</tr>
<tr>
<td>Aedes japonicus</td>
<td>1 0.06</td>
<td>0.02%</td>
</tr>
<tr>
<td>Aedes vexans</td>
<td>2 0.13</td>
<td>0.04%</td>
</tr>
<tr>
<td>Anopheles atropos</td>
<td>2 0.13</td>
<td>0.04%</td>
</tr>
<tr>
<td>Anopheles barberi</td>
<td>2 0.13</td>
<td>0.04%</td>
</tr>
<tr>
<td>Anopheles crucians</td>
<td>613 38.31</td>
<td>13.59%</td>
</tr>
<tr>
<td>Anopheles perplexens</td>
<td>374 23.38</td>
<td>8.29%</td>
</tr>
<tr>
<td>Anopheles punctipennis</td>
<td>15 0.94</td>
<td>0.33%</td>
</tr>
<tr>
<td>Anopheles quadrimaculatus</td>
<td>64 4</td>
<td>1.42%</td>
</tr>
<tr>
<td>Anopheles quadrimaculatus group</td>
<td>50 3.13</td>
<td>1.12%</td>
</tr>
<tr>
<td>Coquillettidia perturbans</td>
<td>3 0.19</td>
<td>0.07%</td>
</tr>
<tr>
<td>Culex territans</td>
<td>30 1.88</td>
<td>0.66%</td>
</tr>
<tr>
<td>Culex coronator</td>
<td>2 0.13</td>
<td>0.04%</td>
</tr>
<tr>
<td>Culex erraticus</td>
<td>345 21.56</td>
<td>7.65%</td>
</tr>
<tr>
<td>Culex interrogator</td>
<td>10 0.63</td>
<td>0.22%</td>
</tr>
<tr>
<td>Culexiolambdis</td>
<td>8 0.5</td>
<td>0.18%</td>
</tr>
<tr>
<td>Culex nigripalpus</td>
<td>146 9.13</td>
<td>3.24%</td>
</tr>
<tr>
<td>Culex peccator</td>
<td>5 0.31</td>
<td>0.11%</td>
</tr>
<tr>
<td>Culex pilosus</td>
<td>2 0.13</td>
<td>0.04%</td>
</tr>
<tr>
<td>Culex quinquefasciatus</td>
<td>1159 72.44</td>
<td>25.69%</td>
</tr>
<tr>
<td>Culex restuans</td>
<td>10 0.63</td>
<td>0.22%</td>
</tr>
<tr>
<td>Culex salinarius</td>
<td>153 9.56</td>
<td>3.39%</td>
</tr>
<tr>
<td>Culex territans</td>
<td>122 7.63</td>
<td>2.70%</td>
</tr>
<tr>
<td>Culiseta inornata</td>
<td>2 0.13</td>
<td>0.04%</td>
</tr>
<tr>
<td>Culiseta melanura</td>
<td>1121 70.06</td>
<td>24.84%</td>
</tr>
<tr>
<td>Mansonia dyari</td>
<td>1 0.06</td>
<td>0.02%</td>
</tr>
<tr>
<td>Psorophora ferox</td>
<td>28 1.75</td>
<td>0.62%</td>
</tr>
<tr>
<td>Uranotaenia lowii</td>
<td>4 0.25</td>
<td>0.09%</td>
</tr>
<tr>
<td>Uranotaenia sapphirina</td>
<td>4 0.25</td>
<td>0.09%</td>
</tr>
<tr>
<td>Wyeomyia vanduzeei</td>
<td>1 0.06</td>
<td>0.02%</td>
</tr>
</tbody>
</table>
Table 2.3 (Cont.)

<table>
<thead>
<tr>
<th></th>
<th>High Risk Sites</th>
<th></th>
<th>Low-Moderate Risk Sites</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(N)</td>
<td>Site Mean</td>
<td>%</td>
<td>(N)</td>
</tr>
<tr>
<td>Total Collected</td>
<td>4512</td>
<td>282</td>
<td>100</td>
<td>413</td>
</tr>
</tbody>
</table>
Table 2.4  Feeding Indices for Wildlife Observed at Study Sites:

<table>
<thead>
<tr>
<th>Species</th>
<th>Count (High Risk)</th>
<th>Count (Low-Moderate Risk)</th>
<th>Blood Meals (High Risk)</th>
<th>Blood Meals (Low-Moderate Risk)</th>
<th>Feeding Index (High Risk)*</th>
<th>Feeding Index (Low-Moderate Risk)*</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Odocoileus virginianus</em> (Deer)</td>
<td>17</td>
<td>2</td>
<td>15</td>
<td>0</td>
<td>5.86</td>
<td>&lt; 0.08</td>
</tr>
<tr>
<td><em>Didelphis virginiana</em> (Opossum)</td>
<td>6</td>
<td>3</td>
<td>3</td>
<td>0</td>
<td>3.32</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td><em>Sus scrofa</em> (Feral pig)</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>4.42</td>
<td>&lt; 0.02</td>
</tr>
<tr>
<td><em>Procyon lotor</em> (Raccoon)</td>
<td>84</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0.08</td>
<td>&lt; 0.02</td>
</tr>
<tr>
<td><em>Meleagris gallopavo</em> (Turkey)</td>
<td>6</td>
<td>12</td>
<td>1</td>
<td>0</td>
<td>1.11</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td><em>Dasypus novemcinctus</em> (Armadillo)</td>
<td>12</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>&lt; 0.06</td>
<td>&lt; 0.03</td>
</tr>
<tr>
<td><em>Sciurus carolinensis</em> (Squirrel)</td>
<td>11</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>&lt; 0.06</td>
<td>&lt; 0.03</td>
</tr>
<tr>
<td><em>Lynx rufus</em> (Bobcat)</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>&lt; 0.22</td>
<td>-</td>
</tr>
<tr>
<td><em>Passer domesticus</em> (House Sparrow)</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>&lt; 0.33</td>
<td>-</td>
</tr>
<tr>
<td><em>Peromyscus gossypinus</em> (Cotton Mouse)</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>&lt; 0.33</td>
<td>-</td>
</tr>
<tr>
<td><em>Sylvilagus floridanus</em> (Rabbit)</td>
<td>0</td>
<td>6</td>
<td>0</td>
<td>0</td>
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<td>&lt; 0.03</td>
</tr>
</tbody>
</table>

* In species with observed visit counts where bloodmeals were not detected (zero), bloodmeal numbers were set to 0.1 for calculation purposes.
Figure 2.1 GIS-Based risk maps; Panel a: Citrus County, Panel b: Hernando County, Panel c: Walton County.
Figure 2.2 Mosquito abundance at high and low-moderate risk sites. Panel A: Percentage of different mosquito species captured in resting shelters during winter months in high-risk sites. Panel B: Percentage of different mosquito species captured in resting shelters during winter months in low-moderate risk sites.
Figure 2.3  Mosquito host use 2017-2018. Panel A: Percentage of identified bloodmeals from different host classes identified in mosquito species other than the primary vector *Cs. melanura*. Panel B: Percentage of bloodmeals from different host classes identified in *Cs. melanura*. Panel C: Percentage of bloodmeals from different host species detected in mosquito species other than the primary vector *Cs. melanura*. Panel D: Percentage of bloodmeals from different host species detected in *Cs. melanura*. 
Figure 2.4 Mosquito Host Use 2018-2019. Panel A: Percentage of identified bloodmeals from different host classes identified in mosquito species other than the primary vector *Cs. melanura*. Panel B: Percentage of bloodmeals from different host classes identified in *Cs. melanura*. Panel C: Percentage of bloodmeals from different host species detected in mosquito species other than the primary vector *Cs. melanura*. Panel D: Percentage of bloodmeals from different host species detected in *Cs. melanura*.
Figure 2.5 Wildlife camera collection data from high and low-moderate risk sites during the winter collections 2017-2019. Panel A: All species detected at high-risk and low-moderate risk sites. Panel B: Percentage representation of the four most commonly observed species at the high-risk sites. Panel C: Percentage representation of the four most commonly observed species at the low-moderate risk sites.
References


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CONCLUSION

Although EEEV infection in humans is a rare occurrence, the threat is real with the virus circulating throughout much of Florida year-round. Phylogenetic, biological, spatiotemporal and environmental studies have demonstrated that Florida may be a geographic reservoir for EEEV with vector-host activity present at some level year round in the state [1-5]. While there is a great deal of research that has been done regarding vectors for EEEV transmission in Florida, there are still knowledge gaps related the elusiveness of EEEV activity during winter-spring seasons [2-4, 6, 7]. Phylogenetic studies show Florida’s relationship to EEEV isolates across northern regions which demonstrates that the state may hold the key to EEEV activity and risk for transmission nationwide [1, 8-11]. Examination regarding specific weather patterns that could affect EEEV activity has not been fully described. While all the mechanisms at play may not be completely understood, information regarding predictable weather patterns could inform enhanced treatment and control strategies that protect against EEEV infection in Florida, which may further interrupt the spread to the northern regions of the United States. The goal when researching mosquito borne illness like EEEV is that the knowledge gained will be applied towards reducing or eliminating risk of transmission. Extreme weather patterns have been linked to events of increased risk for dengue virus infections, but no known study has examined these weather anomalies through ENSO cycles compared to risk for transmission of EEEV [12-16]. This study demonstrated that El Niño winter conditions, which produce mild winters and increased precipitation in Florida, correlate with the number of observed horse cases during the following peak season (summer). Because there is a lag between the SOI predictor variable in the ENSO cycle and the breeding maturation process following appropriate weather conditions,
this information may assist in predicting when an equine outbreak of EEEV is threatening in the future. Further these weather predictors would also illustrate risk for transmission of EEEV to humans, whereby this information can advise mosquito control towards appropriate preventative treatment and control measures to reduce the potential risk.

The second portion of this research also seeks to provide tools that improve EEEV surveillance strategies that could optimize control efforts, especially where resources may be limited. GIS risk mapping is a novel advent that grown over the years allowing a means for precise tracking for many illnesses of public health concern [5, 17-21]. Research utilizing GIS-based risk index modelling of EEEV has determined this tool shows the ability to optimize surveillance of EEEV risk, notably during winter-spring months when mosquito abundance is low. It can be difficult to detect EEEV through these surveillance methods during the winter-spring months, as mosquito populations are generally quite small compared to what are present during peak season collections. However, this study provided proof that winter circulation can be detected through risk-based trap placement and surveillance, as a pool of four Cs. melanura tested positive for EEEV RNA and one sentinel chicken was confirmed positive for antibodies at the same site of collection during the same timeframe. These GIS-based high-risk site collection results occurred in early March and therefore demonstrated winter-spring EEEV activity and risk for human and equine transmission in Florida. This evidence taken together further supports that trap placement and surveillance can be optimized through the use of GIS-based risk index modelling.

Additional research is necessary to explore why EEEV continues to be active in the winter-spring season when the overall mosquito abundance is low. One thought is that birds maintaining the enzootic cycle may be amplifying the virus through other modes of transmission during periods of low vector abundance. Examining passerine families for potential fecal-oral transmission or possibly feather-picking and/or grooming transmission of EEEV may provide
further insight into overwintering conditions that keep the virus viable all year in Florida. If we look at how EEEV fatally affects emus, a non-native bird species, with rampant disease occurring through an entire flock from EEEV infected feces (as many emus will develop hemorrhagic diarrhea that quickly spreads the illness), then we could hypothesize that the virus may be transmitted horizontally among a passerine colony during times of low mosquito abundance [22]. Northern Cardinals would be an ideal species to investigate in this regard, as they have been implicated as possible super spreaders for EEEV [23]. Research has also demonstrated relatively high EEEV antibody seroprevalence in Northern Cardinals [4, 24-26]. Though other passerines have also been suspected as enzootic hosts for EEEV, research suggests that the Northern Cardinal may play a large role in maintaining the virus, especially during winter-spring seasons in Florida as this bird species thrives as a permanent resident in the state year-round [23, 26, 27]. Notably, studies, including this one, have demonstrated that the Northern Cardinal appears to be fed upon quite frequently by the primary vector for EEEV, *Cs. melanura* [26]. Further, *Cx. erraticus*, a potential bridge vector for EEEV, has been documented as feeding upon Northern Cardinals which may increase risk for transmission to humans and horses as described through seasonal shifts associated with this vector [4, 7].

Although *Cs. melanura* is rarely documented feeding on mammals, the same cannot be said for *Cx. erraticus* which may amplify EEEV risk potential upon feeding on infected songbirds [4, 6, 7, 28]. *Cx erraticus* is also a highly abundant mosquito species found in marsh lands across the United States and is likely to serve as both an enzootic and epizootic vector for EEEV [2, 7, 28, 29]. Future studies should seek to further evaluate the vector-host and spatiotemporal involvement of *Cx. erraticus* in the EEEV transmission cycle in Florida in greater detail.

Protecting communities from arboviral threats presents its own set of problems outside of the ability to provide accurate knowledge regarding various vector-host relationships and habitable risks associated with a given region. Many mosquito vectors reside in marshland
habitats, making Florida a prime habitat for mosquito borne illnesses [2-4, 30-32]. One of many challenges with arboviral surveillance and control is that much of the marshland in Florida is uninhabited but may border susceptible communities [33]. Furthermore, these marshlands may be considered conservation protected lands which are generally not exposed to treatment measures against mosquito vectors. Therefore, those charged with mosquito prevention and control strategies are continually seeking new perspectives to handling this age old problem and prevent the spread of arboviruses [33]. This research attempts to improve upon current mosquito control applications by enabling concise surveillance and treatment sites that could essentially reduce the financial burden when protecting against EEEV.

It is possible that the methods used here, i.e. both ENSO prediction and the GIS-based risk index analysis could be applied in the case of other arboviruses, such as West Nile Virus (WNV) which is another important threat to Florida’s human and equine population. Although it is not likely that the ENSO and SOI weather predictors, when applied to WNV, would depict the same pattern described for EEEV. This analysis may still provide a distinctive prediction that supports early warning of possible WNV outbreaks. Additionally, the current GIS-based risk index model approach could be applied to WNV. In this way it may be possible to identify points of overlap in high-risk locations for EEEV, which could represent ideal surveillance locations for both viruses. EEEV and WNV are thought to share in potential bridge vectors, primarily of Aedes and Culex species mosquitoes, which could facilitate optimization of surveillance and control strategies that reduce financial expenditures in areas demonstrated as high risk for both viruses [34-37]. EEEV and WNV also share similar host relationships between the vector mosquito and the avian host, primarily passerines wherein the Northern Cardinal appears to play an important role in the enzootic cycle for WNV [35, 38, 39]. This information taken together demonstrates how this research could be applied to improve optimization of surveillance and prevention methods to a variety of mosquito borne illnesses present in Florida.
These studies provide clear explanations for weather related frequency of historical horse cases that can be utilized for development of a EEEV equine outbreak early warning system through the use of ENSO cycles and SOI predictors [40]. This research endeavor also demonstrated that mosquito control can benefit from GIS-based risk mapping as an additional component in surveillance. This may make the mosquito control program surveillance activities more effective, eliminating needless expenses and allowing mosquito control districts to participate in year-round surveillance. This could provide a comprehensive picture of EEEV risk as opposed to current surveillance practices that do not afford wintertime treatment and control. Another benefit to the aforementioned GIS-based Risk Index research is that future studies could attempt wintertime mosquito treatments at high-risk areas and then evaluate vector reduction during peak season and during the subsequent winter. Wintertime treatment strategies at sites depicted as high-risk could be an important step towards diminishing EEEV transmission in Florida, hitting the mosquito vectors during low abundance when they would likely be most vulnerable. This strategy might effectively reduce EEEV risk potential in Florida during winter months and perhaps interrupt subsequent spread of the virus to the northern states. With EEEV there is no known cure, the best solution is prevention.

References


APPENDIX A: Supplemental Material

Appendix for Chapter One Permissions

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