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Rainfall, Precipitation, and Drought Patterns Associated with Wintertime Transmission of Eastern Equine Encephalitis Virus (EEEV) in Florida

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by

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A thesis submitted in partial fulfillment
of the requirements for the degree of
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DEDICATION

To My Parents and My Grandparents

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First of all, I would like to thank Dr. Joni for taking time for me and listening patiently. She helped me every time I went to her and did her best for my academic development. Also, she never withheld her smiling face and sincerity from me. I think that her great guidance will help me in my future academic and business life. I would also like to thank my dear professors, Dr. Thomas Unnasch and Dr. Ran Tao, who accepted my committee membership and shared their valuable ideas with me. I express my gratitude.

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ABBEREVIATIONS

EEEV	**:** Eastern Encephalitis Virus
hPa	**:** Hectopascal Pressure Unit
VPDmin	**:** Daily minimum vapor pressure deficit
VPDmax	**:** Daily maximum vapor pressure deficit
Mm	**:** millimeter
Tmax	**:** maximum temperature
Tmin	**:** minimum temperature
Ppt	**:** precipitation
Tmean	**:** Average Temperature
Tdmean	**:** Daily mean dew point temperature
D0	**:** Abnormally Dry
D1_D4	**:** Moderate to Exceptional Drought

ABSTRACT

Eastern Equine encephalitis virus (EEEV) is a highly pathogenic alphavirus that causes disease in humans and horses. EEEV cases are common in the eastern North America, especially in horses in the State of Florida. EEEV cases are most common in Florida during May to August but also occur year-round, unlike most other locations. According to the Florida Department of Health, 65 EEEV horse cases were documented in the winter months between 2005 and 2018. This study investigates the meteorological activities that affect the wintertime transmission of the EEEV virus to horses. In this, we examined meteorological data up to a year before the onset of the case. Specifically, we analyzed temperatures, precipitation, and drought. Fifty-six of 65 cases occurred when rain was observed in the previous 10 days. Sixty-one occurred when four or fewer days of freezing temperatures were observed in the previous 4 weeks. Forty-six cases occurred when drought or abnormally dry conditions occurred in the prior 16 weeks. Given that drought and freezing temperatures are relatively uncommon, these results suggest that cases are associated with mild temperatures occurring with abundant rainfall following previously dry conditions.

CHAPTER 1:

INTRODUCTION

1.1 Introduction

Eastern equine encephalitis virus (EEEV) is a highly pathogenic alphavirus occurring in different regions of the eastern United States (White, 2011; Molaei, 2015). In addition, EEEV is one of the most dangerous of arbovirus in the U.S. (Bingham, 2012; 2014). EEEV infections are fairly common in humans and horses during the summer months. In most of the northern states, horses and humans are diagnosed with EEEV between July and October (Liu, 2016; Shepard, 2016). However, EEEV outbreaks usually peak in Florida between May and August with transmission also continuing throughout the winter months (Liu, 2016). Many factors affect EEEV transmission, and most of them vary seasonally or year to year (Jacob, 2010). Due to its dependence on mosquito vectors, EEEV is highly seasonal and cycles with weather conditions, such as temperature. In this study, we will examine the relationships of temperature, rainfall, and drought to wintertime horse cases of EEEV in the State of Florida.

The literature review in Chapter 2 provides information on the general description of EEEV, its spread, and its relationship to climate. In addition, the literature review provides important information about the lethal effects of EEEV on humans and horses. Chapter 3 shows the research objectives for our study. Chapter 4 describes the study area, while Chapter 5 gives an overview of the data and methods used in the research. Chapter 6 describes the results of the study, while Chapter 7 provides a discussion of the findings.

CHAPTER 2:

LITERATURE REVIEW

2.1 General Review of Eastern Equine Encephalitis Virus (EEEV)

2.1.1 General Information About of EEEV

EEEV is an extremely pathogenic alphavirus occurring at different foci in the eastern US (Go, 2014; Burgueño, 2018; Barba, 2019). Among the arboviruses specific to the US, EEEV is especially dangerous and is a nationally reportable disease in the United States (Unnasch, 2006; Vander Kelen, 2012). In the southeastern U.S., EEEV infections often spread to humans and horses in the late summer, causing illness. In most states, cases of EEEV in horses and humans occur in the months between July and October (Liu, 2016; Shepard, 2016). However, EEEV outbreaks in Florida usually happen between May and August but often extend throughout the year (Liu, 2016; Vander Kelen, 2013). The spread of the EEEV virus usually occurs through mosquitoes, in a complex cylcle including multiple species, such as *Ae. vexans, Cq. perturbans and Cx. erraticus.* (Vaidyanathan, 1997; Cupp, 2003; Rochlin, 2014).

EEEV cases have been identified as an important public health problem as they cause high mortality in humans and horses. It is the most severe of the arbovirus-borne encephalitis diseases and has mortality rate of 30-70% (Sickness, 2008). Outbreaks can infect humans and other living things not only with birds but also reptiles. Some mosquitoes are the hosts in spreading the virus. In this case, viruses that cause disease in humans and other mammals have the ability to pass EEEV to humans and horses during epizootics, which are fed by link vectors such as mosquitoes, both birds and mammals.

2.1.2 History of EEEV

In the U.S., the first horse encephalomyelitis epizootics were seen in Massachusetts. In Massachusetts in 1831, about 75 horses died because of EEEV (Voakes, 2005). For the first time in the U.S., EEEV was isolated in 1933 in coastal cities in Maryland and Virginia in disease-infected horses (Weaver, 1999). EEEV studies in the 1930s also showed that mosquitoes of the genus *Aedes, Culex (Cx.)* and *Coquillettidia* can transfer EEEV from one vertebrate to another. In 1938, it was observed that this virus could also be transmitted to humans; 30 children from EEEV in the United States in 1930 (Liu, 2016). EEEV infection in mammalian hosts is highly virulent and causes about 70% of symptomatic cases in horses and humans. Most survivors struggle with residual neurological diseases in acute phase disease. About a few million dollars can be spent per person to combat these diseases. EEEV was isolated in 1949 in a mosquito in Georgia. At the same time, in the state of Louisiana, the EEEV virus was naturally isolated from a fly. In addition, scientists have found that many bird species are susceptible to EEEV and then found EEEV in birds such as pigeons, Peking ducks, and pheasants. According to US health data, approximately 270 human EEEV cases were identified and confirmed in the US between 1964 and 2010 (CDC, 2019). These cases in the United States are most common in states such as New Jersey and Florida (Go, 2014). The virus is also isolated from mosquitoes in other eastern and central US states and South America. For example, the EEEV has been continuously active in birds and mosquitoes in New York since 2003.

2.1.3 Transfer of EEEV

The life cycle of arboviruses is complex; that is because transmission of arboviruses arise from interactions between the vertebrate host and the vectors carrying the disease. The primary transmission cycle of arboviruses to other living things is through adult mosquitoes feeding on an infected host. First of all, mosquitoes, which are carriers, transmit the virus to their salivary glands where they store the virus. Then, the mosquitoes with the disease interact with other susceptible hosts for the new blood they need, thereby transmitting the virus to the other hosts by supplying the virus blood (Lim, 2018). Many factors contribute to host-vector interaction, and each increases or decreases the likelihood of virus infection to varying degrees. The host's reservoir competence is important for the successful spread of the virus. The reservoir competence is the ability of a given host to support multiplication of the virus to levels where it may be passed on to a mosquito taking a blood meal. Some environmental factors affect the transfer of the EEEV-carrying host to another virus, such as temperature. The interaction between competent vectors and the competent host is an important element in determining vector capacity (Cupp, 2005). Vector capacity varies according to some variables. They vary according to the intensity of the vector and the feeding of the vectors carrying the disease. Also, the incubation time required for a vertebrate host or mosquito to develop viral levels capable of be transmitted onward should be known. The EEEV consists of an enzootic bird-mosquito transfer cycle due to the frequent feeding of competent bird species by *Cs. melanura* mosquitoes. Mosquitoes feeding as opportunists can feed on viremic birds and then on mammalian hosts. Therefore, these mosquitoes act as bridge vectors and therefore transmit the virus to humans and horses. When we look at the analysis of *Cs. melanura*, which the author Molaei (2015) studied and analyzed in the northeast of America, it was found that 1-11% of the blood meals were mammalian derived including meals taken from horses and humans. As a result, it has been shown that this type of mosquito can transmit to humans and horses. When previous studies of EEEV transmission in the southeast of the United States were examined, some species of mosquitoes were identified in these regions. These were found to be mosquito vectors such as *Culiseta melanura* and *Culex erraticus.* (Burkett-Cadena, 2015). In addition, EEEV is more frequently found in the blood samples of birds that are frequently targeted by mosquitoes, especially younger ones which are generally more susceptible to bites and develop infectious viruses faster than adult birds.

2.1.4 Habitat of EEEV

One of the reasons for the rapid increase in EEEV is related to the areas of the wetland, because they provide a basic habitat for mosquito vectors and bird hosts carrying EEEV. In addition, wetlands are not homogeneous, and there are differences between types of wetlands, their sizes, and plant diversity. Wetlands may have different effects on EEEV activity, depending on the spatial scale assessed. According to Skaff (2007), the vegetation structure in wetlands significantly affects the abundance of *Cs. melanura.* For example, the abundance of *Cs. melanura* in deciduous forested wetlands is higher than in evergreen forests because these wetlands provide suitable underground habitats for the development of *Cs. melanura*. Finally, it can be said that wet hydrological conditions in the autumn/winter months prior to the transmission of the disease to another organism are associated with high *Cs. melanura* abundance and EEEV infection, and that wet conditions are suitable for EEEV transmission. EEEV distribution is generally in rural areas, and EEEV activity is generally observed in forests and wetlands. Humans, horses, small mammals, and domestic poultry are dead-end hosts of the

EEEV because, in general, viremia in these hosts is not high enough to infect mosquitoes. In South America, EEEV is mainly isolated from *Culex* mosquitoes, and in some areas, mosquito surveillance shows enzootic circulation and has been reported to have a high seroprevalence against EEEV. In South America, it is assumed that the insufficiency of human disease due to EEEV is a result of low pathogenicity of South American strains (Barba,2019). Also, EEEV has been increasing in the northeastern region of the US. However, the frequency, intensity, spatial, and temporal uncertainty of the increase in the number of virus cases are not well known. Therefore, the rate of virus infection cannot be estimated exactly. However, according to scientists, the abundance of *Cs. melanura* may be closely linked to soil cover characteristics and wetland cover. Therefore, when examining the increase in *Cs. abundance* and virus cases, it can help identify the key elements that increase potential outbreaks.

2.1.5 Diagnosis of EEEV

Since EEEV infections do not have specific clinical symptoms, laboratory tests should be performed to detect the disease. In addition, the detection of the EEEV virus should be kept separate from the Highland J Virus (HJV). Because of the geographical distribution of HJV, the distribution of EEEV can be confused. HJV is very similar to EEEV but is thought to confer much milder symptoms.

EEEV disease is observed in two forms. These are systemic and encephalitic diseases (Martin, 2019). Systemic disease occurs suddenly, and the symptoms are weakness, joint pain, and muscle pain. Muscles can also pain for several days, and the body temperature in the infected mammal can reach a maximum temperature of 40 ° C, which can have fatal consequences for humans (Liu, 2016). In animals infected with EEEV, a cough may last for about a few weeks. However, EEEV disease is more severe in living things in encephalitic form. In adults and older children, the incubation period is approximately eight days. This disease has some symptoms, such as fever, drowsiness, and headache (Liu, 2016). In addition to these symptoms, coma or contractions can be observed. In addition, patients deteriorate rapidly when neurological findings develop. Patients infected with EEEV often have cerebrospinal fluid abnormalities.

2.1.6 The Result of the Disease

EEEV disease carries a more serious impacts for certain age groups, especially for children under the age of 15 and adults older than 56 years. During outbreaks, approximately 70% of infected people die within 2-10 days after the onset of the disease. The 35-80% of individuals who survive suffer severe mental and physical sequelae. Short-term death has also been associated with encephalitis in some cases of myocardial failure and lung involvement (Garlick, 2016).

2.1.7 RI Model for EEEV

A spatially open RI model is described by Vander Kelen et al. (2014), to estimate the risk of transferring EEEV to horses in Florida. The model is derived from the analysis of habitats related to EEEV transmission in Florida between 2005 and 2010 (Downs, 2018). The model uses land use/land cover data using a continuous scale ranging from 0.0 (no risk) to 1.0 (maximum risk) to assess risk at any location on the map. The model assesses risk based on five individual risk variables (RV1 - RV5) mathematically combined to generate an overall RI value (Downs, 2018). This risk model only utilizes land cover data, so meteorological data could be used to add a temporal component to risk mapping.

2.2. Climate

2.2.1 General Information of Climate over EEEV

Many factors affect EEEV transmission dynamics, and most of them vary seasonally or year by year. Examples include meteorological variables, climate factors, and vector diversity. Other external factors are horse vaccination rates and horse abundance (Skaff, 2017). Due to its dependence on mosquito vectors, the EEEV is highly seasonal and proliferates due to weather conditions. High rainfall levels affect water tables and humidity. Higher water tables can increase mosquito larvae carrying viruses and swamp areas where they are typically found. Therefore, both horses and humans are infected in warm rainy seasons, usually in mid-summer and even in autumn. The epizootic cycle usually appears in the northern states, such as Michigan, towards the end of August and September. EEEV cases in the southern states also appear in horses during an earlier or longer period of EEEV disease spell. This is usually June and July for Florida. The reason for EEEV's peak in these months was attributed to the increasing number of mosquitoes and birds. Recent studies also show that long-term trends in temperature and extreme precipitation in North America are increasing (White, 2016). This may have caused the second-largest increase in *Cs. melanura* abundance and the more positive EEEV after the warm winter in 1998 (White, 2016). In addition, it has shown that groundwater levels increase in the flood areas on average, especially in a temperate winter season, and will increase the spawning areas further.

2.2.2 The Effects of Climate Change Over Health

Climatic conditions are the determinant of many vector-borne, enteric, and waterborne diseases. The relationship between climate and infectious diseases varies from year to year.

Where climate change occurs and in less developed societies, climate-related diseases are much higher (Ludwig, 2019). The impact of global climate change on infectious diseases can be seen in El Niño. Excessive climate events are expected to become more common with climate change. The effects of extreme weather events (such as droughts, floods, storms, and related fires) on health are difficult to measure. Because there is no precise information about the consequences of extreme weather events on human health. What is certain is that the number of people affected by natural disasters caused by the events of El Niño is increasing. The impact of natural disasters is increasing. Moreover, the incidence of natural disasters is increasing. When historical data are analyzed and compared with the 1960s, we can see that the number of natural disasters has tripled in the last ten years. We can also say that the number of people affected by each disaster is more than in the past years (Bouma, 1997).

2.2.3 Diseases Transmitted by Vectors

Three main factors determine the infectiousness of diseases transmitted by vectors. These are the survival and reproduction conditions of the vector, the frequency of biting or insertion of vectors, and the rate of incubation of pathogenic agents in the vector. Each of these main factors increases the infectivity when suitable optimal climatic conditions are found. That is, climatic conditions are one of the main variables affecting the spread of diseases transmitted by vectors.

Various diseases are transmitted by vectors such as mosquitoes, lice, ticks, or rodents. Vectors are often affected by climate, especially humidity and temperature. Vectors have limited climate tolerance. Climate change will have an important influence on the geographical and seasonal activity of vectors. A change in vector distribution will also affect human health. However, it is difficult to determine the health effects of climate change in humans than to estimate vector distribution. A certain minimum temperature is required to complete the extrinsic incubation time. This is one of the factors limiting the spread of the disease (Kovats et al., 1999).

The increased mean environmental temperature is expected to affect the distribution rate, distribution, and multiplicity of vectors such as mosquitoes, resulting in faster proliferation and virulence of pathogens. For example, malaria is expected to become more prevalent in temperate climates and is expected to increase in tropical and subtropical high regions where it is still absent. This is expected to increase (Nairobi, Harare, Soweto, etc.), especially in high-rise cities and surrounding shanty towns in East Africa, resulting in an additional 20-30 million people at risk. Again, Indonesia and millions of other populations in South and Southeast Asia will face the risk of malaria. The prevalence and possibly mortality of other tropical and sub-tropical vector-borne diseases will also increase (Kovats et al., 1999).

2.2.4 El Nino

There are significant relationships between precipitation and disease-emitting vectors. One reason for this is that vectors can easily multiply and develop in stagnant waters after rainfall. Living and breeding places have been created for some mosquitoes that spread contagious diseases in regions where extreme rainfall occurs as a result of extreme climate events. At the same time, suitable environments for feeding and multiplying of mosquitoes can be formed in the river beds that dry as a result of drought. On the other hand, extreme rainfalls may not allow the survival of some vectors, especially mosquitoes, by flooding the feeding and reproduction sites of disease-emitting vectors. This may lead to a reduction in the predominant vector-borne diseases in the region (Rao, 2019).

Changes in temperature values also play an important role in the spread of vector-borne diseases caused by extreme precipitation. Indeed, increasing temperatures lead to a shortening of the proliferation time of the vector population. In addition, increasing temperature values reduce the development time of viruses and cause vectors to spread diseases more quickly. Time of precipitation and other climatic characteristics were the other factors affecting the survival and proliferation of disease-bearing vectors. Some mosquito species that have the chance to spread due to extreme rainfall during the period of el Nino cause vector-borne diseases such as malaria, dengue fever, Lyme and yellow fever in these regions where they are effective, and some rodent species increasing in polluted waters also cause infectious diseases such as hantavirus, leptospirosis, and plague can spread the spread (Caminade, 2017).

2.2.5 Possible Results of El Nino

Today, the effects of global warming and climate change are increasing, and it is expected that El Nino events that are repeated between 2 and 7 years will increase in frequency. It is obvious that these extreme climatic events, which will be experienced frequently, will, directly and indirectly, affect human health intensively. In countries where the risk of disease is high, inadequate infrastructure and socio-economic conditions will aggravate the situation. El Ninos, which will be seen more frequently in the coming years with the effect of global warming and climate change will trigger simple and complex extreme weather events, making infectious diseases more common in larger geographies (Fisman, 2016). The vectors and pathogens that threaten human health will increase the risk of infectious diseases in El Nino periods because they encounter appropriate humidity and temperature values for proliferation and spread after extreme climatic events. Extreme rainfall during El Nino periods will facilitate the transmission of water borne pathogens by increasing mixing of wastewater and domestic water. The number of diseases, such as cholera and diarrhea, will increase in South Asian countries due to contaminated domestic water. On the other hand, extreme precipitation or prolonged droughts in the Central and West African countries will lead to the expansion of the habitat of mosquitoes, ticks, fleas, and other rodent-type vectors. These vectors will have the opportunity to live in larger areas; malaria, dengue, plague, leptospirosis, hantavirus, and Lyme (Wu, 2016).

CHAPTER 3:

GOALS AND OBJECTIVES

The goal of this study is to analyze patterns of temperature, rainfall, vapor pressure, and drought associated with wintertime transmission of EEEV to horses. Documented horse cases from 2005 to 2018 will be used for the study. Specific research objectives include:

1. To identify patterns of temperature, rainfall, humidity, and drought associated with each individual horse case, and
2. To summarize overall trends associated with the wintertime cases as a collective.

This study will answer the following research questions: How do meteorological factors influence the transmission of EEEV during winter months? Which factors may be useful for predicting future outbreaks of EEEV during winter?

CHAPTER 4:

STUDY AREA

This research was conducted in the State of Florida (Figure 1). It covers an area of 140,098 km^2. The southern part of Florida is generally covered with plains, and the central and northern parts are hilly. The coast of Florida on the Atlantic Ocean extends over an area of 933 km, while the coast of the Gulf of Mexico is 1,239 km. Although the common climate is a humid subtropical climate, a tropical climate can be found in the extreme south. The climate in Florida is generally warm and temperate, with seasonally abundant rainfall and hot summertime temperatures. Freezing temperatures commonly occur in the panhandle but occur less frequently into the central and southern regions.

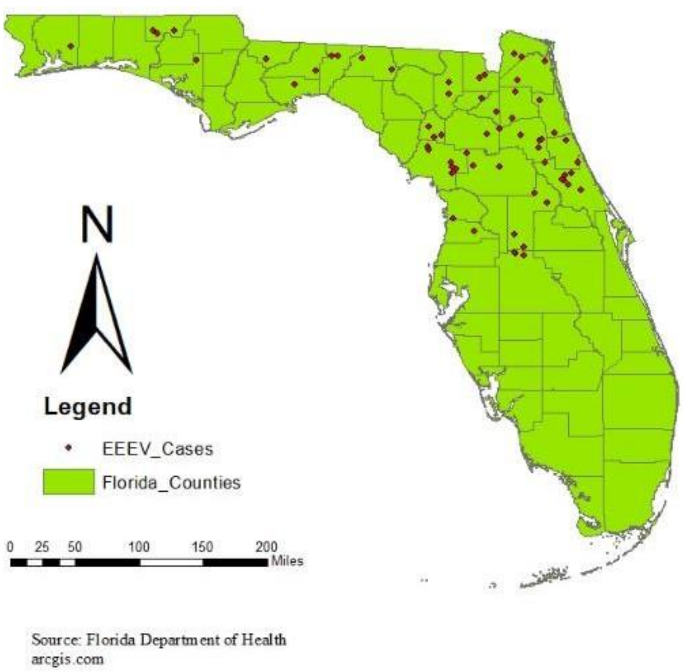

Figure 1. EEEV Case Map in County of Florida

CHAPTER 5:

METHODOLOGY

5.1 Data Collection

Documented wintertime horse cases from 2005 to 2018 were used for the study. The data were obtained from the Florida Department of Health and included both the date and spatial location associated with each case. There were 65 wintertime cases observed during this period (Figure 1). Most cases were observed in March. The total number of cases in March was 16. Then there were 14 cases of EEEV in February and 13 cases each in November and January. In December, 9 cases were observed. As can be seen from Figure 3, the greatest number of EEEV cases were observed in the 2008-2009 and 2009-2010 winter seasons. The fewest wintertime cases were observed in 2005-2007 and 2016-2017.

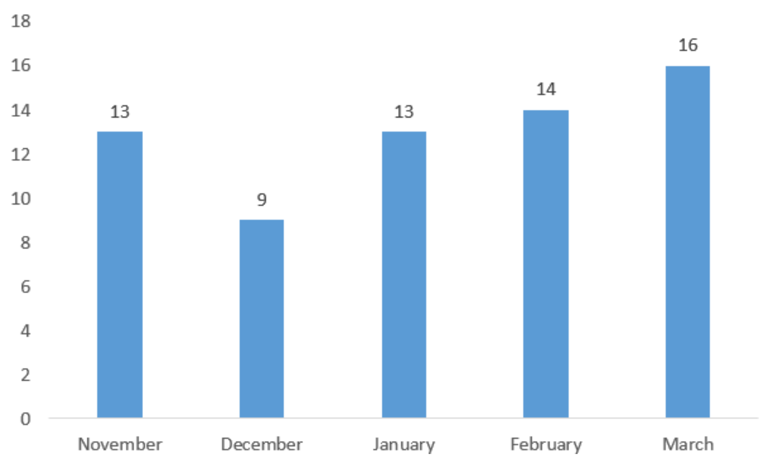

Figure 2. Count of Wintertime EEEV Cases Month**ly**

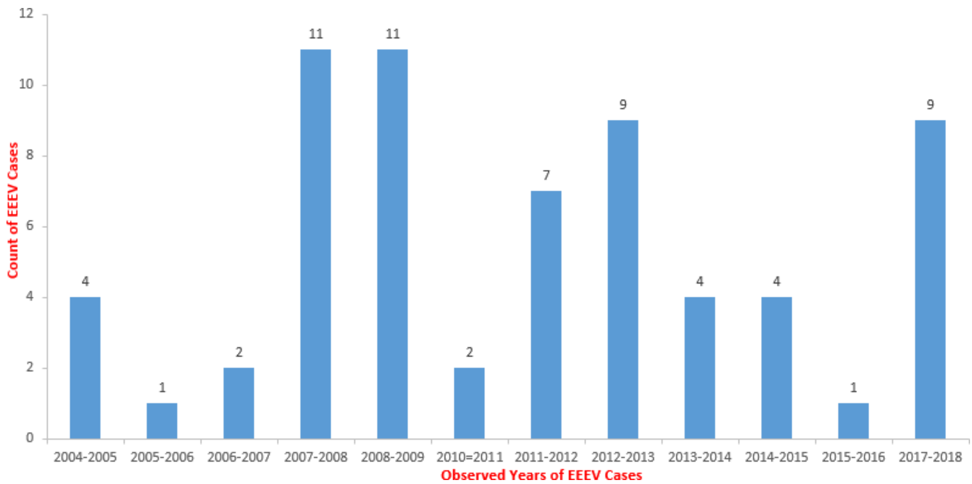

Figure 3. Number of Cases of EEEV by Wintertime Season

For each case, we downloaded data on precipitation and temperature from http://prism.oregonstate.edu/. We downloaded daily precipitation, maximum temperature, minimum temperature, average temperature, average dew point, maximum vapor pressure deficit, and minimum vapor pressure deficit data for a one-year period prior to each case onset for analysis. Values for each of these variables were downloaded for each case based on the recorded spatial locations. Drought data were downloaded from the Drought Monitor at https://droughtmonitor.unl.edu/Data/DataTables.aspx/. We recorded if abnormally dry (D0) or moderate to exceptional drought (D1-D4) conditions were present at each case. D0 is used for areas that show dryness, but are still in a drought, or drought recovery areas. D1-D4 includes the combination of droughts D1 (moderate), D2 (severe), D3 (extreme), and D4 (exceptional). Specifically, we determined the most recent week in which the county containing the case was classified as abnormally dry or under moderate to exceptional drought conditions. If the value in the table was 100 percent, the case definitely experienced dry conditions as the entire county was affected. If it was 0 percent, then the case was recorded as unaffected that week. If there was a value between 0 and 100, we downloaded the values for that week as a shapefile from the website. Shapefiles allowed us to map the spatial distribution of drought to overlay the associated case point to determine if it occurred during dry conditions or not. The final data table recorded the number of weeks since the most recent drying or drought event.

5.2 Data Analysis

Temperature, precipitation, vapor pressure, and drought data were analyzed in several ways in order to explore their relationships with wintertime horse cases of EEEV. First, the weather data was summarized for the date of the case onset. The minimum temperature, maximum temperature, mean temperature, precipitation, mean dewpoint temperature, minimum vapor pressure deficit (VPD), and maximum VPD, were recorded for the date of onset. Then, summary statistics (minimum, maximum, mean, standard deviation) were calculated across all cases. This analysis revealed the range of and average conditions associated with wintertime case onset.

Second, prior weather events were analyzed for the wintertime cases. Days since the most recent rainfall event, days since the most recent cooling event (below 0°C, below 7.2°C), and weeks since the most recent period of dryness or drought were a record for each case. Then, summary statistics (minimum, maximum, mean, standard deviation) were to be calculated across all cases. This analysis revealed the range and average of prior cold weather events associated with wintertime cases.

Third, weekly weather patterns were explored over one year prior to the case onset. The minimum temperature, maximum temperature, mean temperature, precipitation, mean dewpoint temperature, minimum VPD, and maximum VPD were summarized over the year preceding each case. Standard errors were calculated and plotted by week to explore temporal patterns in weather associated with case onset. The examination of those patterns was used to categorize the data for cross-tabulation analysis. Three-way cross-tabulation analysis was used to analyze the relationship between weather variables and wintertime case onset. The weekly summaries were used to divide the most important weather data into classes. The classes were determined using some histogram classification scheme appropriate to the data (e.g., Natural Breaks). The counts of cases in each class were used for cross-tabulation between variables and analyzed in R for associations.

CHAPTER 6:

RESULTS

Table 1. Weather Variables Associated with EEEV Wintertime Case Onset During 2005 to 2018

	ppt (mm)	tmin (°)	tmean (°C)	tmax (°C)	tdmean (°C)	VPDmin (hPa)	vpdmax (hPa)
Minimum	0.00	-5.4	2.5	8.0	-10.5	0.04	1.9
Maximum	15.2	19.9	23.7	29.7	20.1	4.7	25.8
Mean	0.7	9.1	15.7	22.3	9.8	0.7	14.5
Standard Deviation	2.3	5.1	4.6	5.1	6.0	0.8	5.3

6.1 Case Onset

We examined weather variables associated with the onset of EEEV horse cases between 2005 and 2018 in the State of Florida (Table 1). Sixty-seven percent of the cases occurred in abnormally dry or drought conditions. At the beginning of EEEV cases, minimum precipitation (ppt) was 0 mm, maximum precipitation was 15.24 mm, and average precipitation was 0.70 mm. The average temperature at the beginning of the 65 EEEV cases in these counties ranged between 2.5 ^{o}C and 23.70 ^{o}C, and the average temperature for these lcoations was 15.71 ^{o}C, with the lowest temperature being -5.4 ^{o}C and the highest temperature being 29.7 C. The average dew point (tdmean) value between these dates ranged from -10.5 ^{o}C to 20.1 C, and the average was

9.8 C. Furthermore, the maximum vapor pressure gap (vpdmax) values ranged from 1.9 hPa to 25.8 hPa, and the minimum vapor pressure gap (VPDmin) values ranged from 0.04 hPa to 4.7 hPa.

6.2 Metrological Conditions Prior to Case Onset

We examined the weekly total precipitation data of 65 wintertime EEEV cases between 2005 and 2018 from the week of occurrence to one year earlier (Figure 4). Across all weeks, total precipitation ranged from 0 to 379.69 mm. In general, precipitation totals were highest 16 to 42 weeks prior to case onset, coinciding with the rainy, summer months in Florida. The maximum weekly precipitation total occurred 18 weeks before the case onset for a case in Putnam County. Weekly totals for the first twelve weeks prior to onset averaged between 10 and 23 mm (Figure 5). The number of days since the last rainfall event averaged 4.7 days, with a minimum of zero and a maximum of 18 days. About 77% of cases experienced rain during the week prior to onset, while 94% of cases experienced rain during the previous two weeks. Total rainfall for the four-week period ranged from 2.1 to 194.5 mm, with an average of 59.6 mm (sd = 46.5). According to figure 5, total rainfall for the 12-week period ranged from 41.6 to 475.9 mm, with an average of 199.1 mm (sd = 102.4).

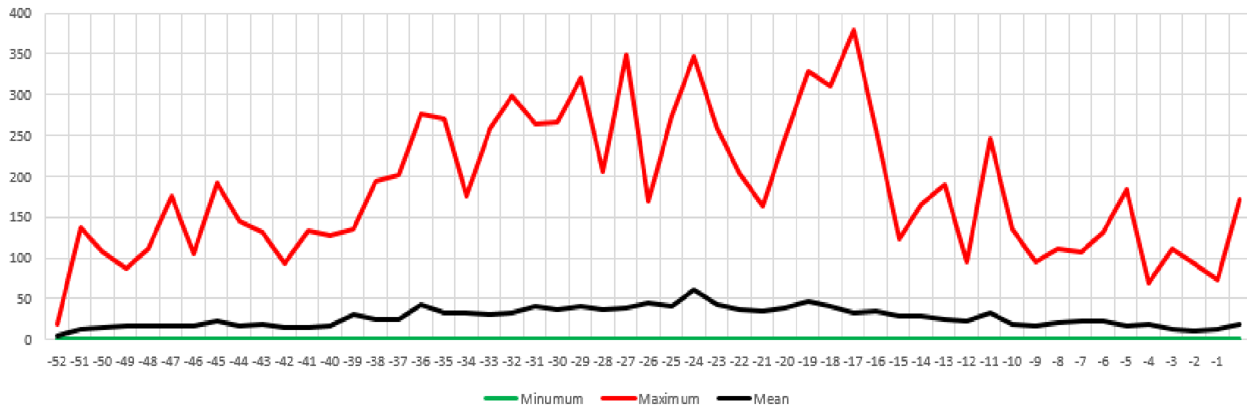

Figure 4. Weekly Minimum, Maximum, and Average Precipitation for One Year Prior to the Onset of EEEV Horse Cases in Florida During 2005-2018

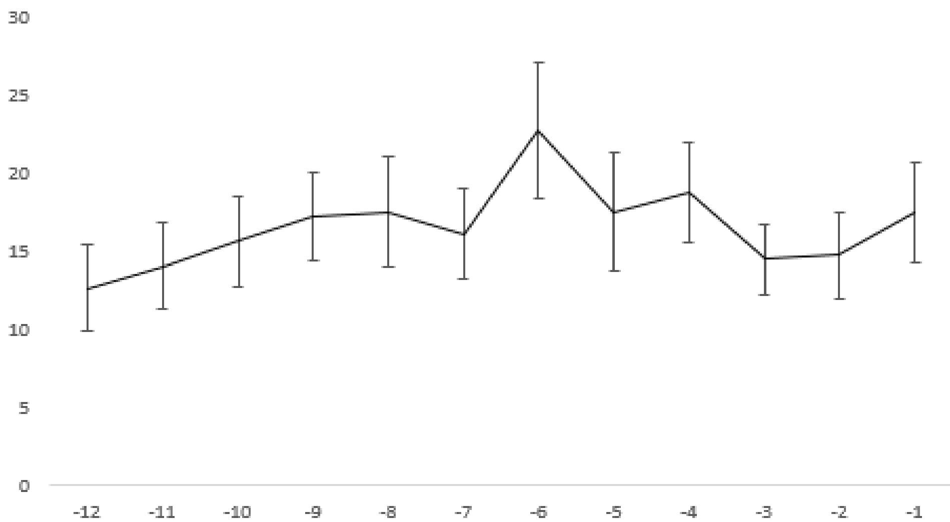

Figure 5. Average total precipitation (with standard error bars) in the first 12 weeks' period

When we looked at the minimum number of rainfall days for the 65 cases, rain was not observed in all weeks (Figure 6). The most precipitation was recorded in the 28th week prior to case onset. The number of rainy days per week varied between 0 and 7 days. The number of rainy days varied between 1 and 3 days during the 12 weeks prior to case onset (Figure 7). The number of rainy days varied between 1.82 and 4.45 days during the 4 weeks prior to case onset. The average was 2.90 days (sd = 1.86) during the 12 weeks prior to case onset. The average value varied between 1.85 and 4.12 days during the 4 weeks prior to case onset, and the average was 2.99 days (sd = 1.90) (Figure 7).

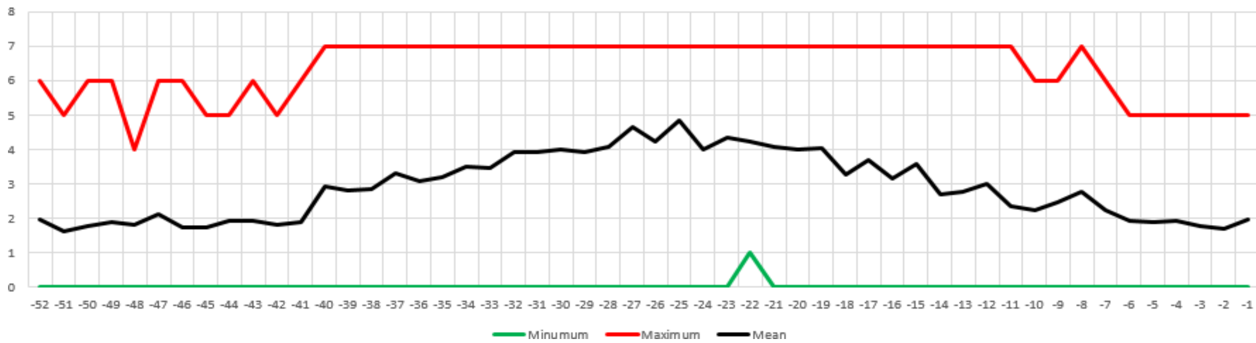

Figure 6. Weekly Minimum, Maximum, and Average Days with Precipitation for One Year Prior to the Onset of EEEV Horse Cases in Florida During 2005-2018

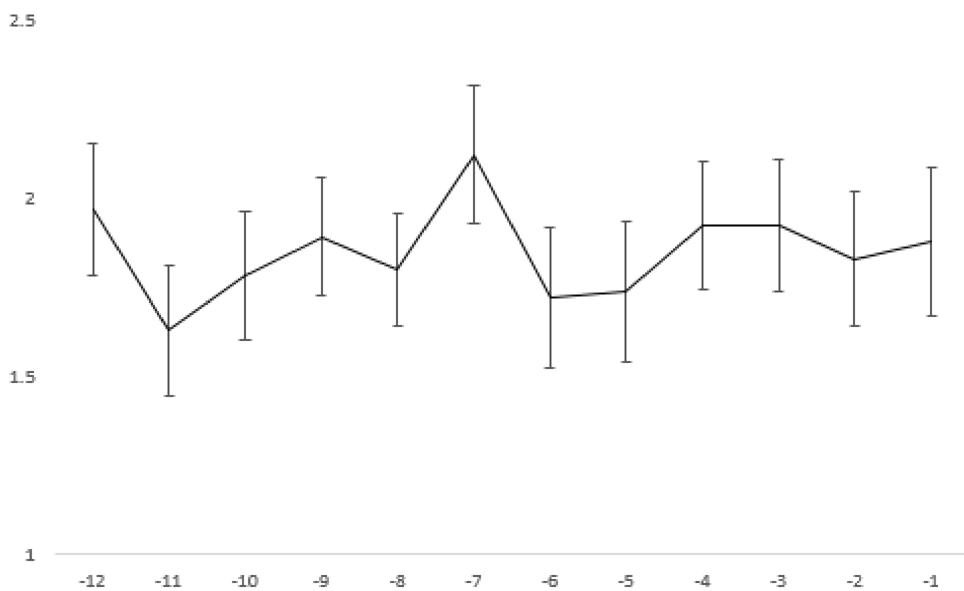

Figure 7. Average Days with Precipitation (with standard error bars) in the first 12 weeks' period

Minimum temperature values varied between -8.20 °C and 25 °C (Figure 8). Lower temperatures in the 10 weeks prior to case onset reflected changes in season. In addition, minimum average temperature values of the first 12 weeks values ranged between 2 °C and 9 C (Figure 9). Four-week minimum temperature values ranged from 3.24 °C to 19.32 °C with an average of 11.44 (sd = 5.94). The average minimum temperature values of 12 weeks ranged from 5.28 °C to 18.56 C, and the average was 11.97 (sd = 6.01) (Figure 9).

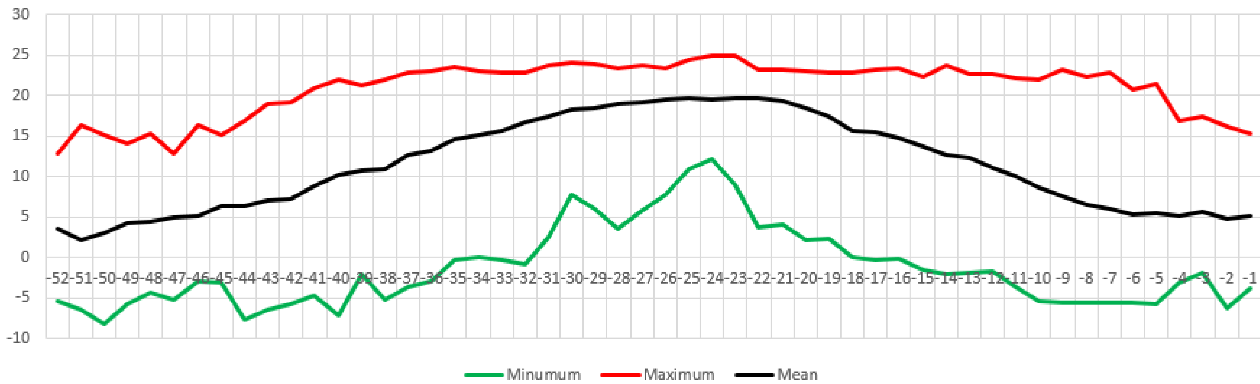

Figure 8. Weekly Minimum, Maximum, and Average tmin for One Year Prior to the Onset of EEEV Horse Cases in Florida During 2005-2018

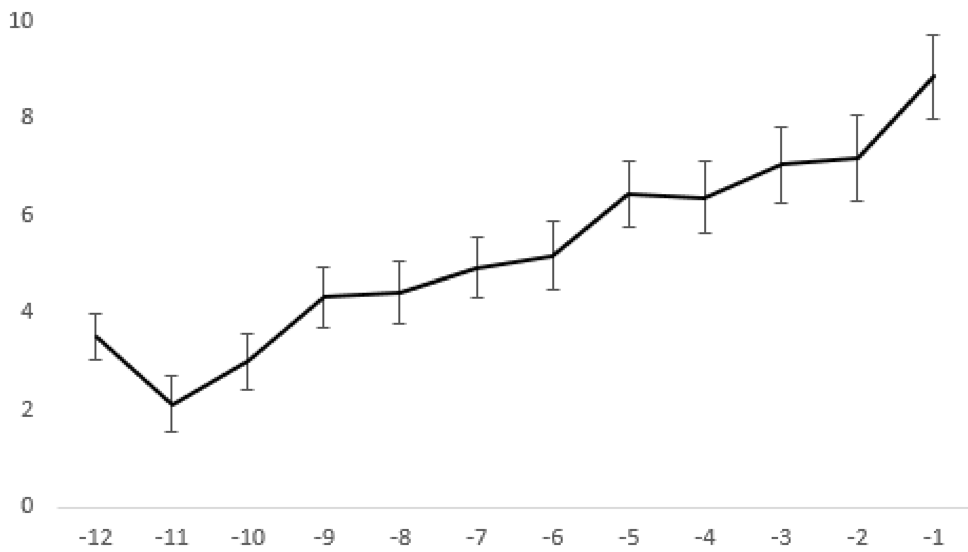

Figure 9. Average of Temperature Minimum (with standard error bars) the first 12 weeks' period

Weekly average temperature data ranged from 3.17 °C to 30.04 °C (Figure 10). The average temperature data of the first 12 weeks varied between 13 °C and 20 °C (Figure 11). The 4-week data of the average temperature varied between 15.20 °C and 26.44 °C, and the average was 20.98 (sd = 4.18). Average temperature data varied between 16.88 °C and 25.71 °C during the previous 12 weeks, and the average was 21.34 °C (sd = 4.21).

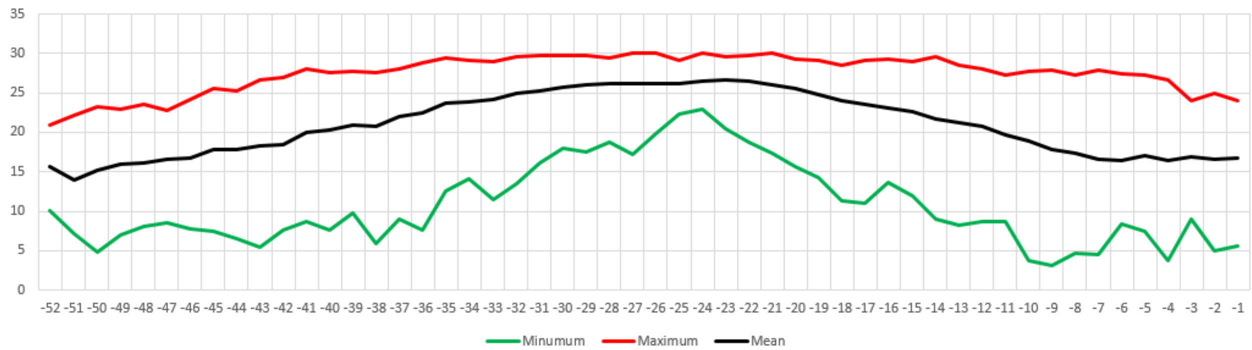

Figure 10. Weekly Minimum, Maximum, and Average Temperature mean for One Year Prior to the Onset of EEEV Horse Cases in Florida During 2005-2018

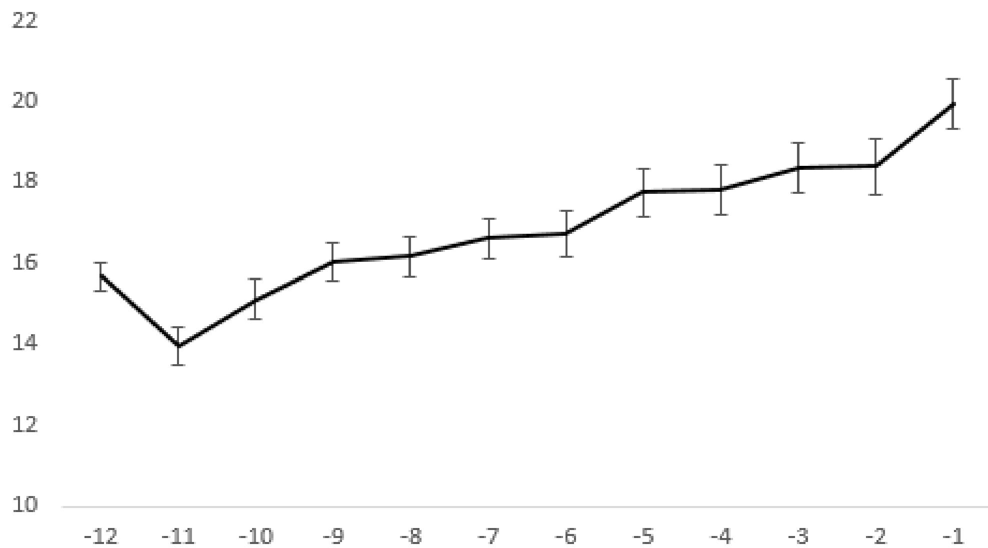

Figure 11. Average of Temperature (with standard error bars) the first 12 weeks' period

Maximum temperature values ranged from 14.80 °C to 37.60 °C per week (Figure 12). Maximum temperature data started to decrease 15 weeks before the virus was seen. When we examined the first 12 weeks of maximum temperature data, the average maximum data was between 25 °C and 30 °C (Figure 13). The 4-week maximum temperature varied between 26.42 °C and 33.34 °C (Figure 12). The average of the 4-week maximum temperature data was 29.98 °C (sd = 3.03). The 12-week maximum temperature data was between 27.66 °C and 32.88 °C, and the average was 30.24 °C (sd = 3.05) (Figure 13).

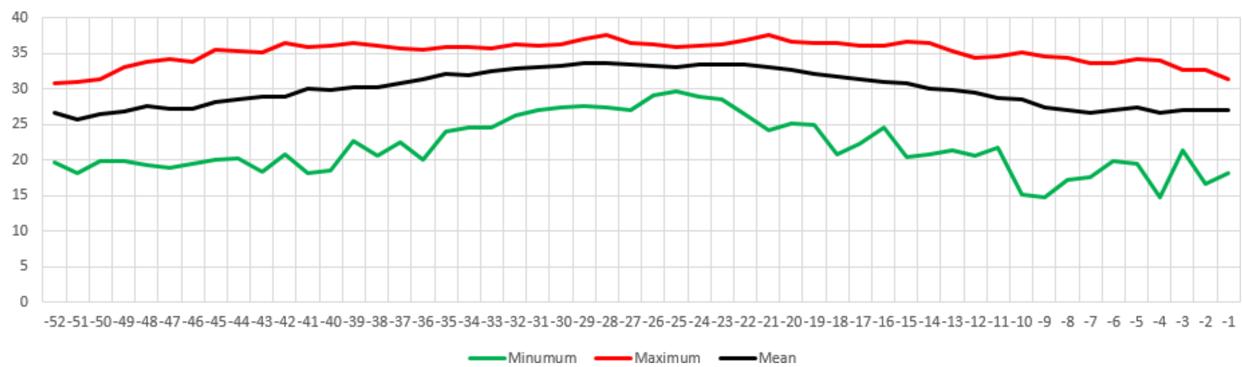

Figure 12. Weekly Minimum, Maximum, and Average Temperature max for One Year Prior to the Onset of EEEV Horse Cases in Florida During 2005-2018

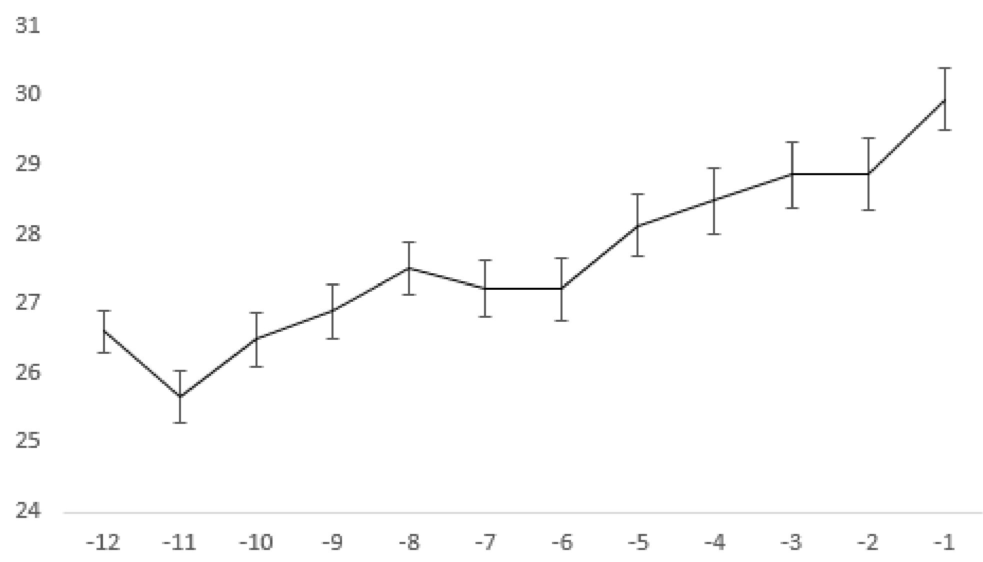

Figure 13. Average Maximum of Temperature (with standard error bars) the first 12 weeks' period

Minimum VPDmin ranged between 0 hPa and 3.41 hPa. The average of minimum one-year VPDmin was 0.41 hPa (sd = 0.36) (Figure 14). The average minimum VPDmin value ranged from 0.25 hPa to 0.40 hPa for the first 12 weeks (Figure 15). The 4-week minimum mean VPDmin values varied between 0.26 hPa and 0.58 hPa (Figure 14). The 12-week minimum mean VPDmin varied between 0.32 hPa and 0.54 hPa (sd = 0.37) (Figure 15).

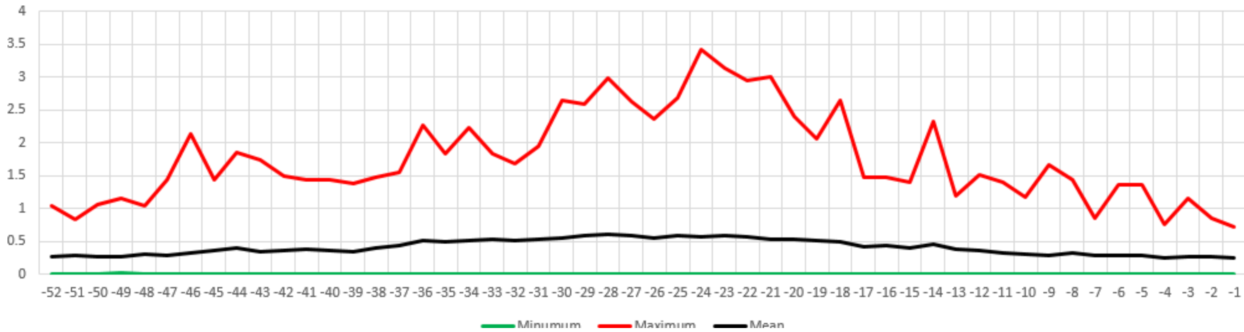

Figure 14. Weekly Minimum, Maximum, and Average Minimum VPDmin for One Year Prior to the Onset of EEEV Horse Cases in Florida During 2005-2018

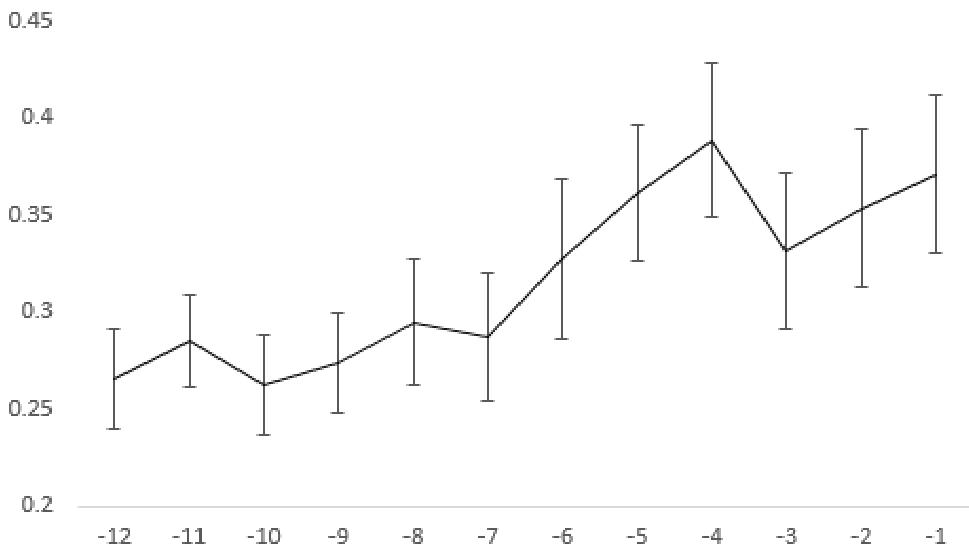

Figure 15. Average of Minimum VPDmin (with standard error bars) graph in the first 12 weeks' period

When we looked at the average VPDmin values, we saw that the values varied between 0 hPa and 4.59 hPa weekly, and the annual average VPDmin was 0.79 hPa (sd = 0.51) (Figure 16). The first 12-week average VPDmin ranged from 0.55 hPa to 0.90 hPa, as in Figure 17. The average 4-week VPDmin values varied between 0.59 hPa and 0.97 hPa (Figure 16). Average VPDmin valuesranged between 0.67 hPa and 0.94 hPa in the preceding 12-week period (Figure 17).

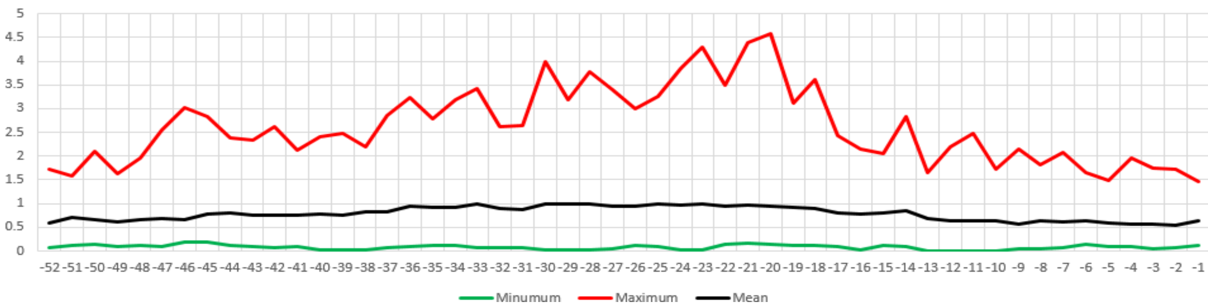

Figure 16. Weekly Minimum, Maximum, and Average Mean of VPDmin for One Year Prior to the Onset of EEEV Horse Cases in Florida During 2005-2018

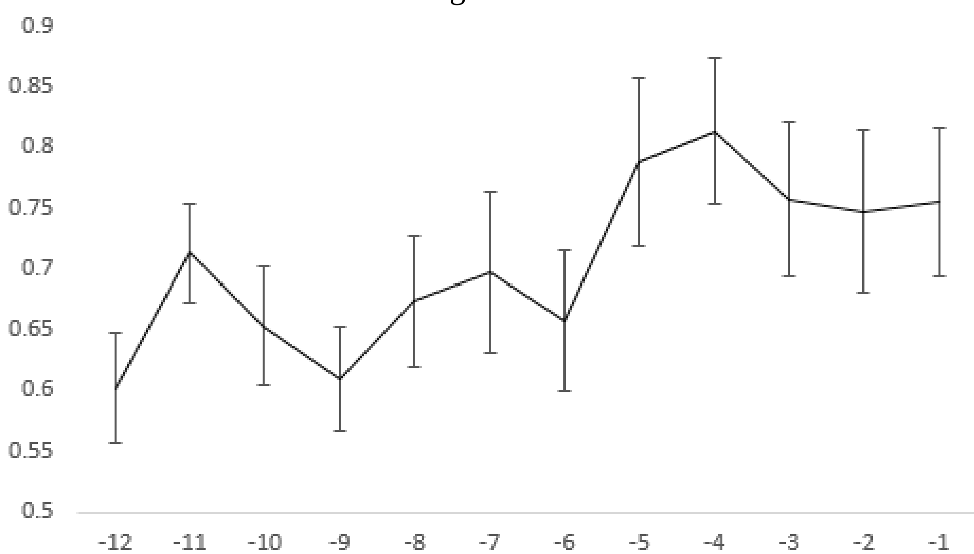

Figure 17. Average of Average VPDmin (with standard error bars) graph in the first 12 weeks' period

Maximum VPDmin valuesvaried between 0 hPa and 8.24 hPa (Figure 18). The one-year average maximum VPDmin value was 1.4 hPa (sd = 0.9) (Figure 18). The maximum VPDmin value varied between 1 hPa and 1.6 hPa during the prior 12 weeks, as in Figure 19. The average maximum VPDmin values for four weeksranged between 1.18 hPa and 1.62 hPa. In the 12-week periods, the average maximum VPDmin was between 1.21 hPa and 1.51 hPa (Figure 19).

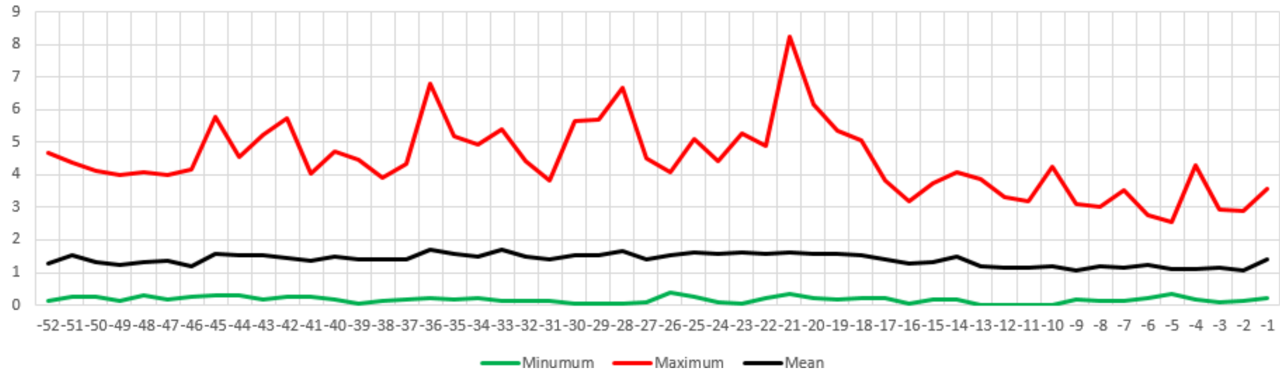

Figure 18. Weekly Minimum, Maximum, and Average Max of VPDmin for One Year Prior to the Onset of EEEV Horse Cases in Florida During 2005-2018

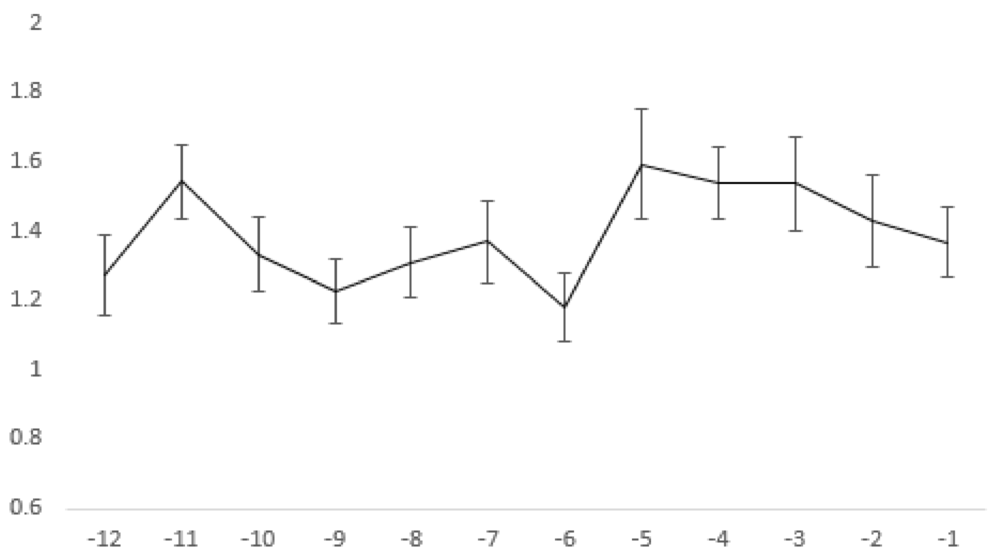

Figure 19. Average of Maximum VPDmin (with standard error bars) graph in the first 12 weeks' period

The minimum weekly

VPDmax value ranged from 0 hPa to 34.14 hPa (Figure 20). Average minimum VPDmax varied between 8 hPa and 13 hPa during the 12-week period before the occurrence of cases (Figure 21). The minimum VPDmax range for the 4-week period was between 8.14 hPa and 15.44 hPa (Figure 20), with an average of 11.88 hPa (sd = 5.92). When we examined the minimum VPDmax for 12-week periods, the minimum VPDmax value was between 9.81 hPa and 14.23 hPa, and the average of 12 weeks is 12.19 hPa (sd = 6.06) (Figure 21).

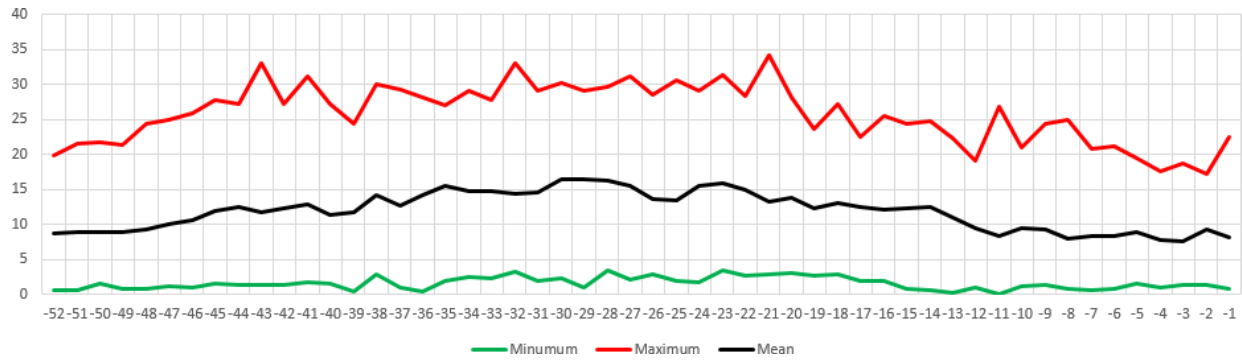

Figure 20. Weekly Minimum, Maximum, and Average Min of VPDmax for One Year Prior to the Onset of EEEV Horse Cases in Florida During 2005-2018

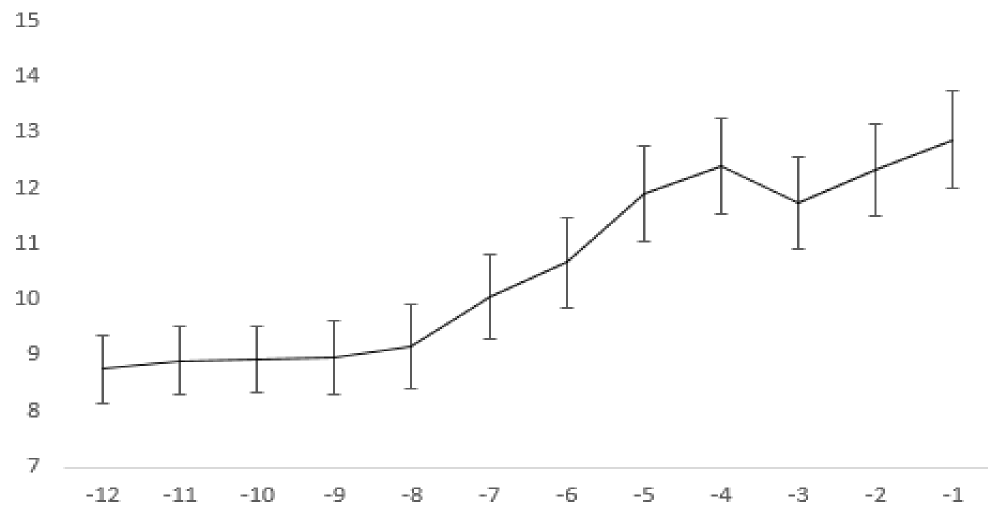

Figure 21. Average of Minimum VPDmax (with standard error bars) graph in the first 12 weeks' period

Mean VPDmax values ranged from 3.9 hPa to 37.33 hPa (Figure 22). The week with the lowest mean VPDmax was seen nine weeks prior to the incident. The highest mean VPDmax was observed for a case in Lake County in the 12th week prior to onset. When we looked at an average of 12 weeks of VPDmax, we saw that the values varied between 14 hPa and 21 hPa (Figure 23). When we look at the 4-week average VPDmax periods, the values varied between 14.29 hPa and 23.28 hPa. The 4-week average of VPDmax is 18.74 hPa (sd = 5.18) (Figure 22). In the 4-week periods, the lowest values appear in the last two months. When we look at the 12-week average VPDmax values, we see that the values varied between 16 hPa and 21.86 hPa, while the average was 19.10 hPa (SD = 5.31) (Figure 23).

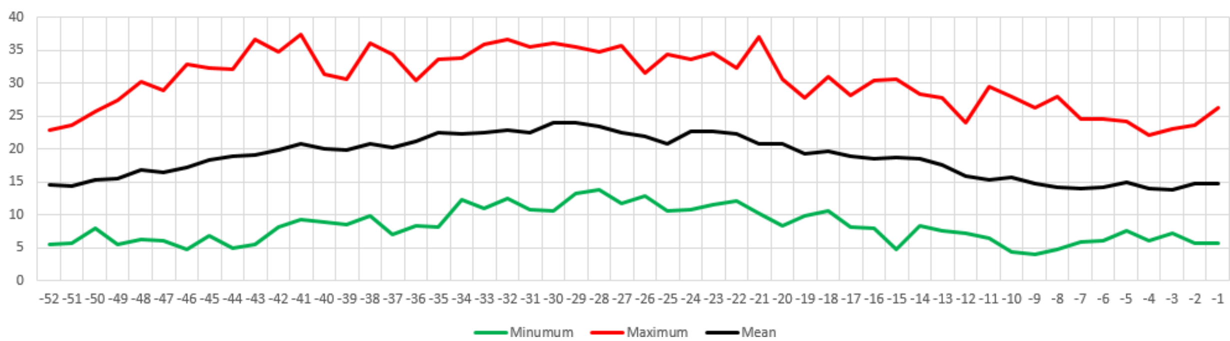

Figure 22. Weekly Minimum, Maximum, and Average Mean of VPDmax for One Year Prior to the Onset of EEEV Horse Cases in Florida During 2005-2018

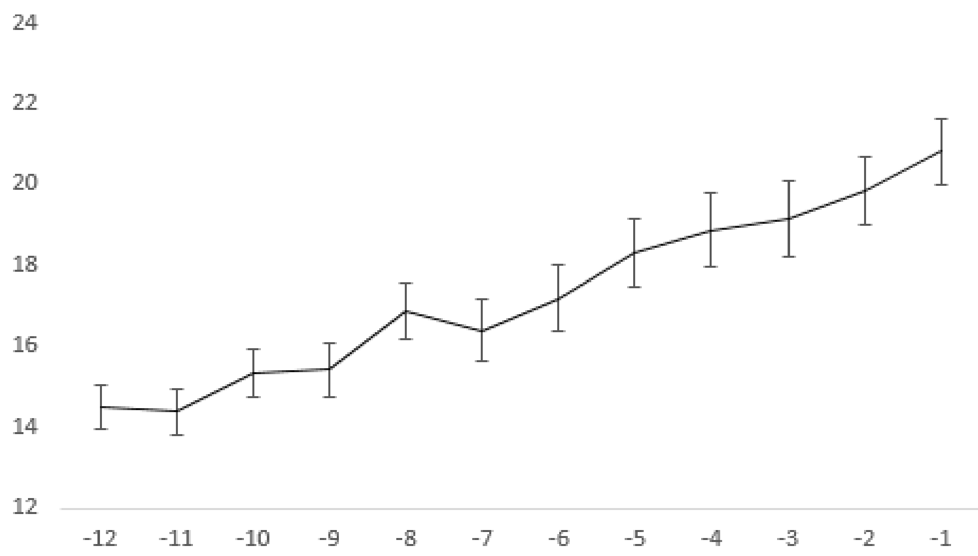

Figure 23. Average of Average VPDmax (with standard error bars) graph in the first 12 weeks' period

Maximum VPDmax values ranged between 7.59 hPa and 45.07 hPa (Figure 24). In the first 12 weeks, maximum VPDmax values were between 19 hPa and 28 hPa (Figure 25). In the most recent 4-week period, the maximum VPDmax values were between 19.31 hPa and 29.88 hPa, and the average was 24.58 hPa (5.56) (Figure 24). In the 12-week period, the maximum VPDmax range varied between 21.32 hPa and 28.42 hPa. The average 12-week VPDmax value was 25.01 hPa (5.69) (Figure 25).

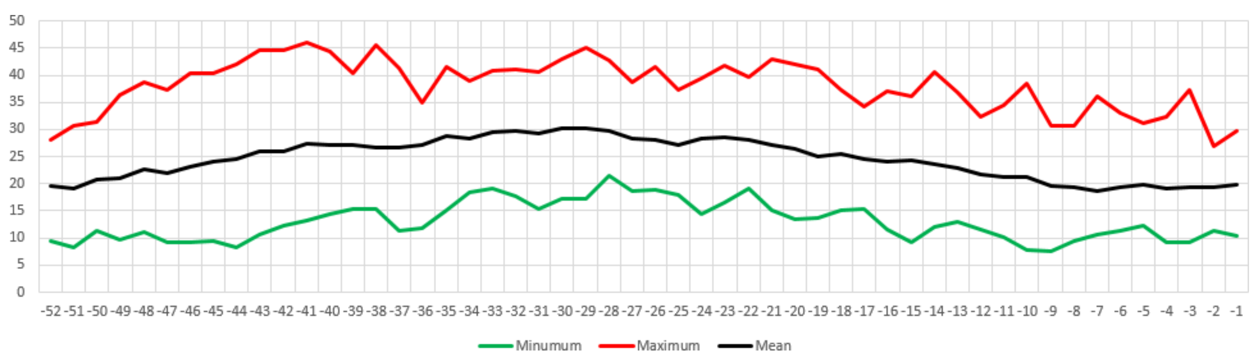

Figure 24. Weekly Minimum, Maximum, and Average Max of VPDmax for One Year Prior to the Onset of EEEV Horse Cases in Florida During 2005-2018

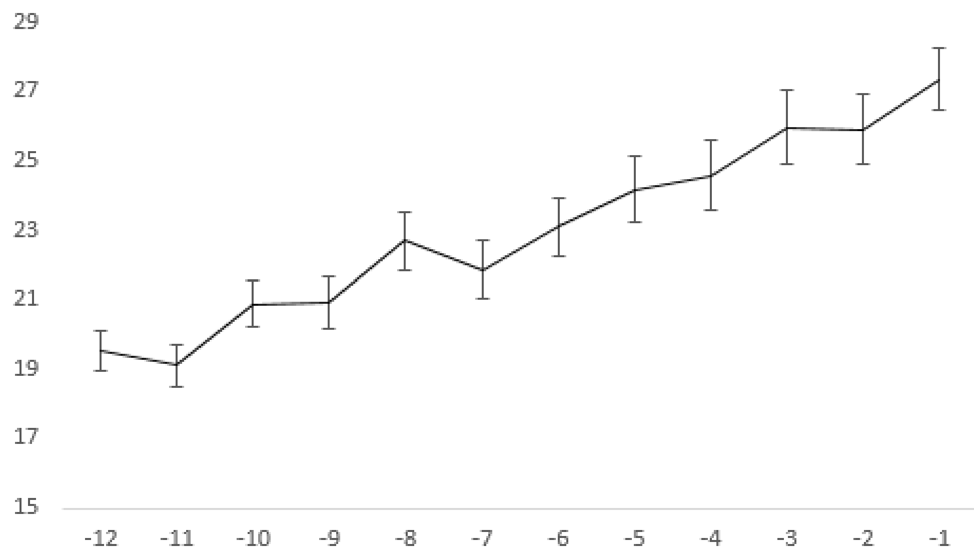

Figure 25. Average of Maximum VPDmax (with standard error bars) graph in the first 12 weeks' period

We tabulated the cooling days of 65 virus cases in Florida as <0 °C and <7.2 °C per week. The number of days below <0 °C changed between 0 and 6 days weekly. For the first 12 weeks prior to case onset, the number of days below 0 °C varied between 0.20 and 0.90 (Figure 26). When we looked at the 4-week periods, the number of days below <0 °C varied between 0 and 0.52. The average was 0.21 (sd = 0.54) (Figure 26). During the 12-week period, the number of days below 0 °C was between 0 and 0.43, on average, 0.20 (sd = 0.51) (Figure 27). Over the course of the year, the number of days below 0 °C gradually increased up to the 17th week. The number of days since the last <0 °C cooling varies between 1 and 366 days, with an average of 101.42 (135.04) (Figure 26). Four cases did not fall below 0 °C during the year when the cases were seen, and 55 of them did not fall below 0 °C for the first 300 days. In 3 of the cases, the first day was seen 52 weeks before the virus occurred. Fifty-five of the cases also fell below 0 °C in the week prior to case onset.

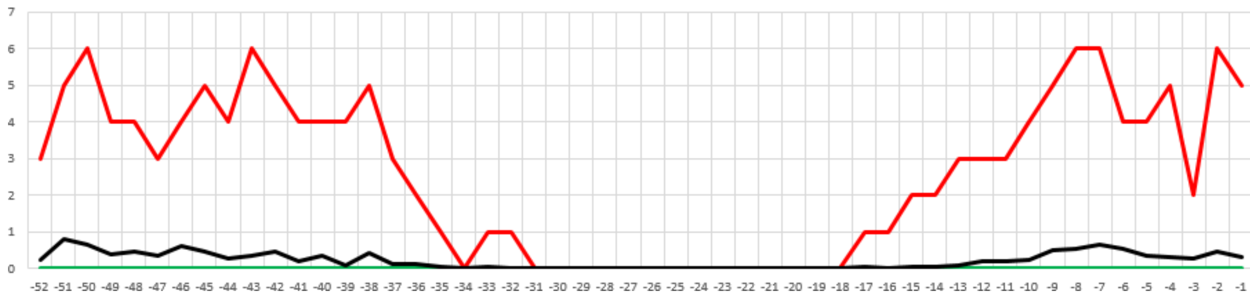

Figure 26. Weekly Minimum, Maximum, and Average days <0°C for One Year Prior to the Onset of EEEV Horse Cases in Florida During 2005-2018

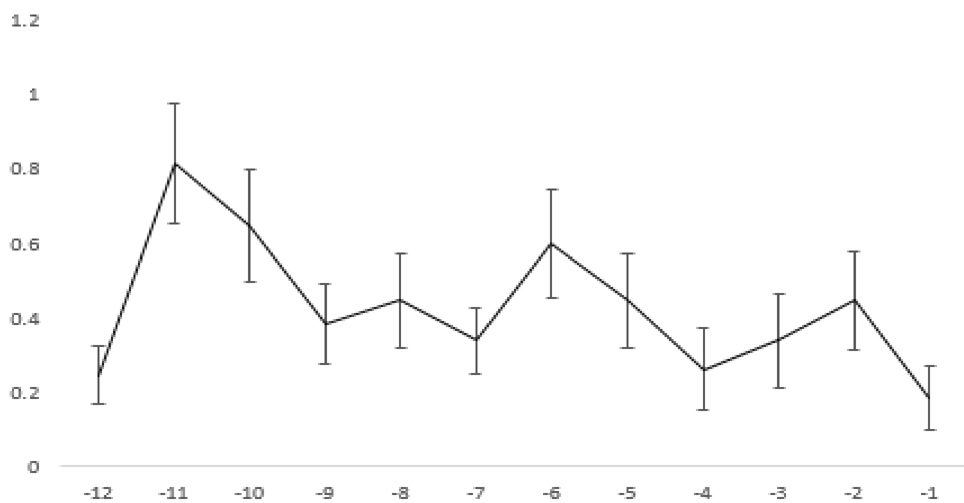

Figure 27. Average number of days <0°C (with standard error bars) graph in the first 12 weeks' period

The number of days of cooling below 7.2 °C days ranged between 1 and 20 days. The average was 4.52 days (sd = 4.53) (Figure 28). The number of days below 7.2 °C per week varied between 0 and 7 days. When we examined the first 12 weeks, the number of days below 7.2 °C varied between 1 and 4 days (Figure 29). When we examined the 4-week periods, the average number of days below 7.2 °C was between 0.02 and 2.93 with an average of 1.21 (sd = 1.48) (Figure 28). When we looked at the 12-week periods, the average number of days was between

0.15 and 2.26, while the average was 1.13 (sd = 1.43) (Figure 29). In the 23rd and 26th weeks, the temperature in all cases did not drop below 7.2 °C. The air temperature dropped below 7.2 °C every day for 14 weeks before the virus cases began to appear.

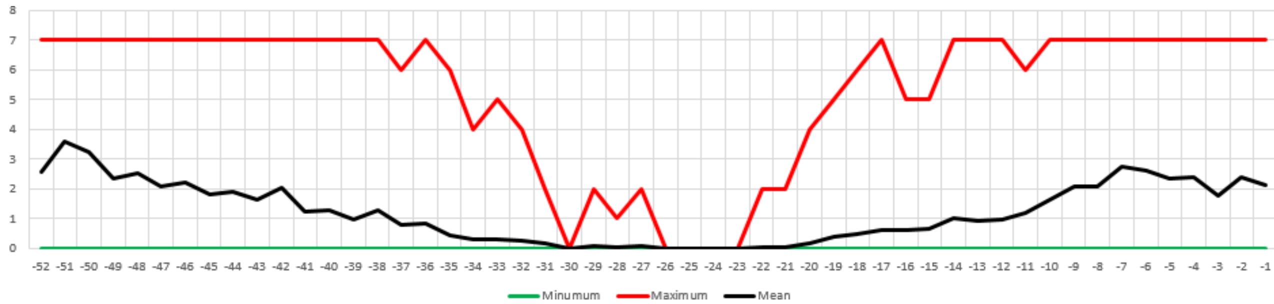

Figure 28. Weekly Minimum, Maximum, and Average days 7.2<°C for One Year Prior to the Onset of EEEV Horse Cases in Florida During 2005-2018

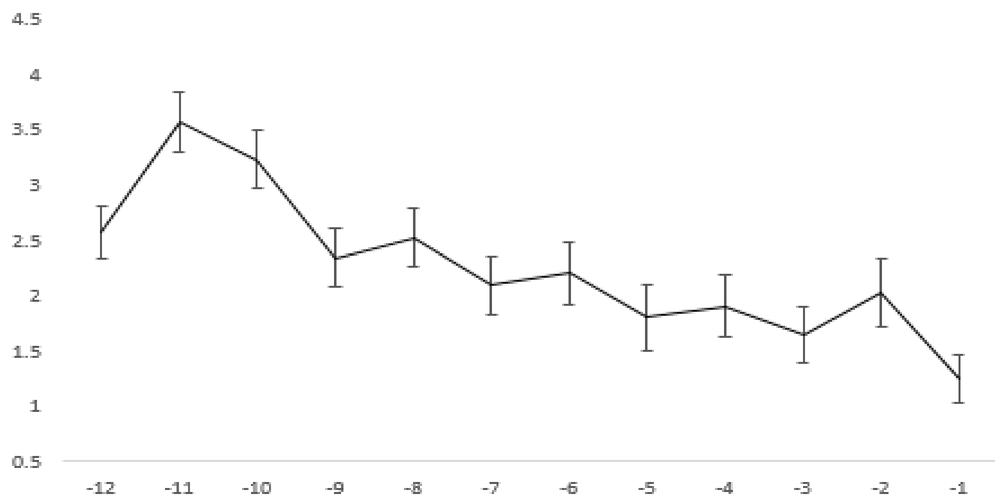

Figure 29. Average number of days <7.2°C (with standard error bars) graph in the first 12 weeks' period

We examined the times since the last abnormally dry period or drought for each case. We divided the extreme drought and normal drought into 4 groups as 0-8, 9-23, 24-42, and 43 and more as in the Table 3. When we examined the periods of extreme drought of the cases, the time since the extreme drought ranged between 1 and 52+ weeks, with an average of 17.03 (SD = 17.35) (Table 2). In 7 cases, drought did not appear in the preceding year, but in 30 cases, moderate to excessive drought was observed between 0 and 8 weeks. For abnormally dry conditions, the times since the last drought ranged from 1 to 52 weeks, with an average of 30.28 (sd = 15.92) (Table 3).

Table 2. D0 and D1-D4 Droughts Data for 65 cases

Case	D0	D1-D4	Case	D0	D1-D4
1	42	43	34	1	27
2	>52	>52	35	1	27
3	>52	>52	36	23	23
4	>52	>52	37	15	15
5	17	>52	38	21	23
6	1	1	39	1	27
7	1	1	40	30	30
8	17	20	41	1	35
9	1	22	42	1	1
10	4	25	43	1	1
11	5	38	44	1	1
12	5	26	45	21	39
13	1	1	46	1	31
14	2	29	47	21	39
15	1	29	48	29	30
16	5	30	49	11	>52
17	1	33	50	19	48
18	20	21	51	>52	>52
19	28	>52	52	>52	>52
20	27	>52	53	>52	>52
21	22	22	54	8	8
22	1	25	55	>52	>52
23	1	26	56	14	14
24	1	1	57	1	6
25	1	1	58	32	33
26	26	27	59	35	36
27	1	27	60	13	39
28	13	>>52	61	37	38
29	1	29	62	40	40
30	1	>>52	63	14	40
31	22	36	64	7	40
32	36	37	65	39	40
33	1	22	66	1	41

Table 3. D0 and D1_D4 Droughts Table

	0-8	9-23	24-42	43 or more
D0	30 (46.15%)	16 (24.62%)	12 (18.46%)	7 (10.77%)
D1_D4	10 (15.38%)	9 (13.85%)	32 (49.23%)	14 (21.54%)

6.3 Association of Weather Variables

From our examination of weather variables preceding case onset, we categorized the most apparent variables into classes to best describe weather patterns associated with the horse cases. Fifty-six of 65 cases occurred when rain was observed in the previous 10 days. Sixty-one occurred when four or fewer days of freezing temperatures were observed in the previous 4 weeks. Forty-six cases occurred when drought or abnormally dry conditions occurred in the prior 16 weeks. The combinations of these variables are shown in Table 4. The cross-tabulation test revealed no significant association between the variables Drought/Abnormally Dry, Rainfall, Number of days in last 4 weeks that were below 0 ^{o}C, but independently cases are characterized by recent rainfall that following dry periods that occur during mild wintertime temperatures.

Table 4. Meteorological Unexpected Situation Table

Drought/ Abnormally Dry	Rainfall	Number of days in last 4 weeks that were below 0 ^oC	
		1-4	5 or more
0-16 weeks	Rain in last 10 days	38	3
	No rain in last 10 days	5	0
17 weeks or more	Rain in last 10 days	14	1
	No rain in last 10 days	3	1

CHAPTER 7:

DISCUSSION

7.1 Discussion

This study explored the weather associations of variables—temperature, precipitation, vapor pressure deficit, and drought—with the occurrence of wintertime horse cases of EEEV in Florida. First, the findings suggest that cases are associated with recent rainfall. Generally, winter months are drier than summer months in Florida. Although precipitation varies spatially, the statewide average winter precipitation averaged 71.8 mm per month from 2005 to 2018 (Florida Climate Center, 2020). Given that rainfall within the four weeks prior to case onset averaged 59.6 mm, this suggests cases were associated with recent rainfall of less than to average amounts. Precipitation is important for EEEV transmission, because mosquito vectors need water for breeding. Mosquitoes, such as *Cs. melanura*, require a water surface to develop their larvae. According to Andreadis et al. (2012), the time spent as larvae in the warm winter periods will be shorter than the normal time. According to White (2016), the increase in spring rain is thought to cause subsequent accumulation of moisture and surface water and supports the second and third generation mosquitoes by increasing the spawning zones later in the year. Precipitation in winter months may not be very effective in spreading EEEV. In fact, according to White (2016), they said that the abundance of mosquitoes in the long term is not associated with increased precipitation and that only short-term examination has good predictability for disease. However, Day and Shaman (2014) reported the opposite and said that wet winter months would increase the spawning and laying area and increase transmission. This study suggests precipitation is needed for wintertime transmission to horses but not necessarily above average amounts.

Second, observed horse cases occurred when frequent freezes were absent. Number of freezing days varies widely across the state, being more common in the Panhandle and North (about 15 days per year), less frequent in the Central region (1-5 days per year), and extremely rare in the south (average <1 day per year) (Runkle et. al, 2017). Because wintertime horse cases—which were located in the Panhandle, North, and Central regions—were almost always associated with four or fewer freezing days in the preceding 4 weeks, this suggests that they are associated with milder winter temperatures. Temperature is relevant for EEEV transmission, since it affects mosquito breeding. Mosquitoes develop from their eggs at temperatures of 12 ^{o}C to 32 ^{o}C, and adult mosquitoes emerge, and one mosquito can lay 75 to 400 eggs at a time (Seymen, 2018). Suitable temperatures will be able to complete and develop within 10-15 days. In addition, temperature data below 0 ^{o}C and 7.2 ^{o}C suppress or halt mosquito reproduction (Seymen, 2018). Also, when we examine the average temperature of 65 EEEV cases, we see that the average temperature is 15.29 ^{o}C. This average temperature provides a suitable temperature environment for the development of mosquito larvae.

Third, our study looked at vapor pressure deficits. Although we did not discern any clear associations of cases with VPD, it can be important for EEEV transmission, particularly in spring. VPD is found by measuring humidity in a particular area, and the VPD determines the difference between the amount of air humidity and the amount of air that the air can hold. Therefore, it is important to know the vapor pressure deficit for the plants to grow. Because the vegetation provides a suitable habitat for mosquitoes and birds carrying EEEV. As the VPD grows, the water requirement of the plants' increases, and they dry more and thus may result in fewer hosts and vectors.

Fourth, we examined the relationships between drought and EEEV horse cases. Although extreme drought during winter likely suppresses winter transmission, as evidenced by the very few cases during 2010-2011, dry conditions during the previous summer months were associated with horse cases. A possible explanation for this is that drought concentrates vectors and hosts in the scarce open water sources, which facilities the amplification of the virus in the hosts. Then, the virus spreads widely as vectors and hosts disperse when rains finally return (Caminade, 2017).

Finally, we tested for associations between the meteorological variables. Although rainfall, temperature, and drought seem to be important in EEEV transmission independently from one another, no associations were found between them. However, the absence of a relationship between the variables in our analysis results does not mean that our variables are not important; it reveals there is no association between them. An example of an association that we did not find would be that cases were high both when temperatures were cold/rain was abundant and when temperatures were hot/rain was scarce.

The implications of these findings are as follows. First, this study improves our understanding of wintertime transmission to horses. We identified that near average precipitation amounts, mild temperatures, and recent droughts were associated with transmission to horses during winter. Second, when these conditions are present during winter in Florida, we might predict high numbers of cases and increase EEEV surveillance and control efforts in high risk areas. High risk areas might be identified using previously mentioned risk mapping approaches. Third, knowledge of factors associated with elevated numbers of horse cases can also protect human health, as both horses and humans are dead end hosts in the EEEV transmission cycle.

7.2 Limitations

In our study, we only examined the meteorological events in the places where EEEV cases were observed and investigated the connection of these events with the EEEV case seen in the winter months. According to some studies, many factors, such as seasonal weather changes, socioeconomic conditions, vector control programs, environmental changes, insecticide resistance, climate changes, affect the epidemiology of vector creatures and vector-borne diseases (White, 2016). It is assumed that these factors increase the host and bring out epidemics in the short term and gradually different diseases in the long term. However, when we did our research and analysis, we did not analyze the habitats of the hosts carrying the virus. Many climatic and environmental conditions are important for the reproduction of mosquitoes carrying EEEV. We did not analyze the abundance suitable habitat environments for mosquitoes, since that has been reported in other studies (Vander Kelen et al., 2014). In addition, there was no data indicating the connection between the EEEV case observed in the wintertime in these regions regarding the habitat structure or soil moisture. Therefore, this study does not address the ecological or interspecific relationships of hosts carrying EEEV that may affect the abundance and reproductive biology. Rainfall in Florida contributes to groundwater level measurements, but its effects on mosquito abundance differ significantly. Considering low to freezing temperatures, it is more likely to increase development time in the larval stage. Adding this categorical data will provide more information about the abundance levels of the hosts. All of these can be addressed in future research.

7.3 Future Work

Today, many scientists state that climate-related outbreaks are a more dangerous threat than biological attacks (Nikiforuk 2008). While many organisms cannot adapt to new conditions caused by climate change, they are negatively affected, while viruses are among the rare organisms that adapt best to these conditions. This suggests that climate changes will significantly affect the number and impact of viral outbreaks. Although methods to combat emerging viral outbreaks have been developed, viruses may have more dangerous consequences than previous viral epidemics with their ability to adapt to new conditions. In addition, it should not be ignored that outbreaks occurring as a result of climate change in one region affect other regions. It should not be forgotten that in our globalizing world, viruses can find new endemic fields with increasing animal trade, new routes of migratory birds, numerical increases in vectors, and tourism. In conclusion, the studies and their results that we have examined within the scope of this review reveal that viral outbreaks likely to be shaped by climate change are of international importance. Further studies might examine connections of EEEV to climatic changes or climate variables, such as the El Nino Southern Oscillation.

In order to obtain clearer results, it is necessary to know the environmental conditions and habitat characteristics of EEEV events. Knowing environmental data may be useful for future studies. Future studies should consider preserving more workarounds of the original data to examine climate-related intra-year trends. In addition, for the vector abundance response, consecutive days of extreme events should also be examined as biologically relevant to the vector's life cycle. For example, the consecutive days of average measurement for successive temperature or precipitation in winter may be more useful than using average temperatures, minimum or maximum temperatures, or simple precipitation. It is also important to capture variables or create a factor that represents the aquatic habitat for *Cs. melanura*. A mosquito-borne viral disease requires the presence and a sufficient number of mosquitoes. If we effectively measure aquatic habitats in high resolution, we can work towards accurate and timely vector disease system modeling to alleviate outbreaks and improve ongoing surveillance efforts.

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