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Soil Characteristics Associated with Horse Cases of Eastern Equine Encephalitis Virus in Florida

Fulya Guzelkucuk
University of South Florida

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Soil Characteristics Associated with Horse Cases of Eastern Equine Encephalitis Virus in Florida

by

Fulya Guzelkucuk

A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science in Environmental Science and Policy
Department of Environmental Science and Policy
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Major Professor: Joni Downs Firat, Ph.D.
Thomas Unnasch, Ph.D.
He Jin, Ph.D.

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ABSTRACT

Eastern Equine Encephalitis virus (EEEV) is a mosquito-borne virus found in Florida that affects humans and horses. Also, EEEV is an agricultural land management issue in Florida, as it causes mortality in large numbers of horses each year and sometimes humans. This study investigates the characteristics of soils associated with horse cases of EEEV in the State of Florida, focusing on differences in soil characteristics between summer and winter cases. This study analyzed a total of 676 EEEV cases during 2005-2018, including 611 summer cases and 65 winter cases. Soil characteristics that were examined include: soil texture, drainage class, hydrology group, and flooding frequency. First, horse cases were overlaid soils layers using a GIS, and soil characteristics at the case locations were summarized. Second, soil characteristics were summarized for 1.5-km buffers to understand soils immediately surrounding each case. For both the soil characteristics at the case locations and within the buffers, the observed values were compared with those expected at random. The results show that summer cases were positively associated with: sandy soils; medium drainage regimes; B, C, and D type hydrologic groups; and frequent flooding at the case locations but not within the surrounding landscape. Winter cases were positively associated with sandy soils; extreme drainage conditions; A type hydrologic group; and frequent flooding. This research improves our understanding about the relationships between soil characteristics and EEEV activity in Florida which may be useful for predicting hotspots of EEEV.
CHAPTER 1: INTRODUCTION

Eastern equine encephalitis virus (EEEV) is a mosquito-borne alphavirus that is endemic to the eastern United States. EEEV infects most species of birds without causing illness, but it is frequently fatal to horses, humans, and some other mammals. Even though EEEV transmission occurs frequently in the Northeastern and Central states of the USA, the largest number of human and horse cases have been seen in Florida (CDC, 2019). Throughout much of its range, EEEV activity occurs from late spring to early fall, but transmission occurs at low levels in Florida during even the winter months (Armstrong et al., 2008; White, 2016). There are many factors that influence EEEV transmission, such as vector and host availability, landcover, temperature, and precipitation, among others.

In Florida, EEEV transmission occurs through a complex cycle involving a number of mosquito vectors and animal hosts. EEEV is thought to be maintained during winter in small mammals, wetland birds, and reptiles, where the mosquito *Culiseta melanura* and similar species transmit the virus to songbirds. Then, throughout the year, bridge vectors such as mosquitoes of the genera *Aedes* and *Coquillettidia*, feed on both songbirds and mammals which transmit the virus more broadly to humans, horses, and other wildlife (CDC 2017). Horse cases of EEEV have been associated with two main land cover types: wetlands and coniferous forests (Vander Kelen et al. 2012). Wetlands support breeding of a variety of mosquito species, although flooded tree plantations and other forests support *Culiseta melanura* specifically, which lay their eggs on tree stumps (Carpenter and LaCasse 1955). Vander Kelen et al. (2014) developed a GIS-based
risk model to map potential locations of EEEV based on the distribution and abundance of wetlands and coniferous forests across Florida. Although the model has been shown effective at identifying hot spots of EEEV activity in horses (Downs et al. 2018), the model might be improved by including other variables that might be related to transmission, such as soils.

This study examines the characteristics of soils associated with EEEV horse cases in Florida, which have previously been unexplored. The goal of this research is to evaluate the relationship between EEEV horse cases and soil characteristics by investigating the soils data associated with the horse cases. The objective is to specifically assess the relationship between the soil characteristics and EEEV horse cases during the winter and summer seasons based on soil data for the State of Florida. If a relationship is found, then models predicting the occurrence of EEEV might be improved by adding soil variables to the model. Improving EEEV prediction models would be beneficial to horse and human health because surveillance and prevention efforts might be improved by a more accurate model.

This thesis is organized as follows. The literature review in Chapter 2 provides general information about EEEV, including observed human and horse cases, transmission cycle, seasonal patterns, habitats, and risk models. The literature review also discusses soil characteristics like soil texture, drainage, hydrological group, moisture, and flooding frequency, including the relationship of soils to disease. Chapter 3 contains the goals and research objectives of this study. Section 4 describes the study area. Chapter 5 explains the methods and the data that were used in the research. Chapter 6 describes the results of the study. Finally, Chapter 7 provides discussion and conclusion of the findings.
CHAPTER 2: LITERATURE REVIEW

2.1 General Review of EEEV

Eastern Equine Encephalitis virus (EEEV) is an arthropod-borne virus (arbovirus) with a high degree of case fatality rate in both horses and humans. EEEV is transmitted by mosquitoes. It is endemic and distributed to South, Central, and North America and the Caribbean (Burkett-Cadena et al., 2015). The first EEEV disease outbreak in horses was reported during 1831 in Massachusetts, affecting approximately 75 horses (Hanson, 1957). The first human disease case was recognized in 1938 (Griffin, 2001). In Florida, the first documented human case occurred in 1952 (Bigler WJ et al., 1976). Currently, EEEV cases occur most commonly in the Atlantic and Gulf Coast states, with approximately 200 horse cases and six human cases per year in the US. (Bingham et al., 2014). Figure 1 illustrates the annual numbers of EEEV cases reported in the United States between 2005 and 2018. The highest number of equine cases of about 330 was seen in 2005, while the lowest number of 60 was observed in 2011. According to the Centers for Disease Control and Prevention (CDC) reports, Florida, Massachusetts, New York, North Carolina, and Georgia generally have the largest numbers of cases (Zacks & Paessler, 2010). Also, Florida is one of the most impacted states, with 25% of all stated human mortalities in the U.S. (Vander Kelen et al., 2012b; Estep et al., 2013).
Figure 1. The annual number of U.S. equine cases of EEE for 2005-2018 (Source: ArboNET, Centers for Disease Control and Prevention)

2.1.1 Diagnosis of EEEV

Symptoms of EEEV in horses involve a sleepy appearance, fever, staggering and weak gait, and muscle twitches (CDC, 2019). Like horses, humans have a high mortality rate. Most survivors have neurological complications, such as permanent brain damage (Deresiewicz et al., 1997; Silverman et al., 2013). Symptoms of EEEV in humans include fever, headache, chills, seizures, mental disorientation, and coma (CDC, 2019). There is no currently approved vaccine for humans, though experimental ones have been tested, nor is there an effective medical treatment (Maryam et al., 2019; Pandya et al., 2012). However, a vaccine is available to protect horses (Carossino et al., 2014).
2.1.2 Ecology of EEEV

The transmission cycle of EEEV is considered complicated due to the essential interactions between the disease vector and the vertebrate host. The arbovirus transmission cycle is through a vector (adult mosquito) feeding on an infected vertebrate host. Then, the mosquitoes carry the viral particles and, through blood-feeding, transmit the virus to other hosts (Vander Kelen., 2013). The primary vectors are enzootic (reservoir) and epizootic (bridge) vectors. EEEV remains enzootic in avian reservoir hosts and becomes epizootic in human and animal hosts by bridge vectors (Hassan et al., 2003). The transmission cycle includes bridge vectors which mosquitoes that feed on with mammals and birds. The bridge vectors transmit EEEV to horses, humans, and other mammals through epizootic outbreaks (Vander Kelen et al., 2014). These epizootic hosts, especially mammalian species, are known dead-end hosts for EEEV because they do not develop viral concentrations high enough to transmit mosquitoes (Hassan et al., 2003). Collectively, EEEV occurs in a transmission cycle including freshwater swamp mosquitoes, mammals, and avian reservoirs.

It is thought that birds are the primary reservoir host of EEEV. Humans, horses, and some birds are considered dead-end hosts for EEEV (Barba et al., 2019; Gruszynski, 2006). The primary enzootic vector of EEEV is Culiseta melanura, which is a freshwater hardwood swamp mosquito (Vander Kelen et al., 2012b; Armstrong & Andreadis, 2010; White et al., 2011). Also, Aedes, Culex, and Coquillettidia have been implicated as primary bridge vectors for EEEV transmission (Vander Kelen. 2013; Jacob et al., 2010; Unnasch et al., 2006). The bridge vectors of EEEV differ between habitats and regions. The virus is maintained in a cycle between Culiseta melanura and avian hosts generally found in freshwater hardwood swamps in the Great Lakes region and the Atlantic and Gulf Coast states (Weaver et al., 1999; Zacks & Paessler, 2010; Vander Kelen et al.,
The interaction between vectors and hosts is a significant factor in the transmission cycle. Also, many factors impact vectors such as geographic locations, ecological habitats, temperature, and population dynamics (Vander Kelen., 2013).

The transmission cycle of all mosquito-borne viruses is driven by four factors: the annual cycle of the mosquito vector; the annual cycle of the pathogen; the environmental factors that affect each biological cycle; the annual cycle of the reservoir, vertebrate hosts, and amplification that drive each biological cycle (Day & Shaman, 2011). Since these three biological cycles, vector, host, and pathogen, inform the transmission dynamics of arboviral disease, surveillance protocols have been developed to estimate the local risk each of these cycles and to monitor these cycles (Day & Shaman, 2011).

2.1.3 Risk Models for EEEV

To predict EEEV transmission risk to horses, Vander Kelen et al. (2014) developed a spatially explicit risk index (RI) model in Florida. The RI model was derived from analyses of the habitats based on EEEV transmission in Florida between 2005-2010 (Downs et al., 2018; Vander Kelen et al., 2012). The model includes five risk variables (RVs) to be related to EEEV transmission. RV1 measures EEEV transmission risk to horses associated with the local habitat type. RV2 measures EEEV transmission risk depending on wetland proximity. RV3 measures the EEEV transmission risk based on wetland composition. RV4 measures EEEV transmission risk associated with tree plantation-coniferous forest proximity. RV5 measures EEEV transmission risk to horses associated with tree plantation-coniferous forest composition. As this model does not incorporate soils, it potentially could be improved if soils are found to be associated with EEEV transmission.
2.1.4 Seasonal Patterns of EEEV

EEEV transmission is typically seasonal and occurs during the summer and early fall in temperate climates (Weaver et al., 1999; Armstrong et al., 2008). Epizootic outbreaks, including horses and humans, are the high level in August and September in the northeast and south-central states. However, in Florida, EEEV transmission occurs all the year-round, and most horse and human cases are seen in June and July (Armstrong et al., 2008; Vander Kelen et al., 2013; Burkett-Cadena et al., 2015). In another study, EEEV transmission was most intense during summer and early fall in the Northeast and Great Lakes region (CDC, 2019).

Outbreaks of EEEV have been correlated with warm and wet summers (Armstrong & Andreadis, 2013). Also, EEEV transmission is weather dependent because of its dependence on mosquito vectors. High precipitation, high summer and spring temperatures, mild winters, play an important role in vector abundance and spread (Armstrong & Andreadis, 2013). High levels of precipitation influence humidity and water table, which may promote mosquito abundance (Day & Shaman, 2008).

The physical environment factors play the most significant role in spreading EEEV. The environmental factors affect arbovirus transmission cycles directly and involve temperature, rainfall, humidity, surface water accumulation, and land-use practices (Cleckner et al., 2011; Day & Shaman, 2011). Additionally, these factors impact vertebrate amplification hosts, the survival of mosquito vectors transmission rates, and arboviral abundance (Day & Shaman, 2011; Gruszynski, 2006). According to Gage et al. (2008), environmental factors such as rainfall, temperature, and humidity are known to influence the development, reproduction, population dynamics, and behavior of mosquito vectors.
Some research shows that climate, particularly rainfall and high temperature affect mosquito population abundance (Shaman & Day, 2005). Also, climate influences mosquito-borne epidemics and diseases. Hydrologic conditions, including soil moisture and rainfall, are significant for increases in mosquito populations (Shaman et al., 2010 & Modelski, 2006). Previous studies demonstrate that warmer temperatures support mosquito activity, increase reproduction percentage, and thus support disease transmission (Modelski, 2006 & Shaman et al., 2010). Also, high rainfall conditions regenerate the population of vector and provide sufficient vector breeding sites (Tanser et al., 2003).

Rainfall is the most significant environmental factor influencing mosquitoes and is generally associated with water availability and also breeding site availability (Shaman et al., 2002 & Tanser et al., 2003). Therefore, many arbovirus transmissions are affected by hydrologic variability. Wet hydrological conditions are associated with a higher abundance of Culiseta melanura and EEEV infection in the transmission season, demonstrating that wet conditions are suitable for EEEV transmission (USDA, 2004). The variables of anthropogenic and hydrologic analyses illustrate that EEEV transmission is most severe in rural areas, and when hydrological conditions are especially wet during the transmission season (Skaff et al., 2017).

Rainfall has two important effects on the mosquito life cycle. First, the increased humidity related to rainfall promotes mosquito activity. Second, rainfall can change the abundance and type of habitat for mosquito (Shaman & Day, 2005). According to Shaman & Day (2005), rainfall increases part of surface soil wetness and can cause an expansion of some habitats. Consequently, habitats like swamps, which are preferred by many mosquito species, might increase in extent during wet years, which may result in increased mosquito breeding habitat.
2.2 Soil Characteristics

2.2.1 Soil Moisture

Rainfall also directly impacts soil moisture. Soil moisture is the main component of soil hydrology. Soil moisture affects climate and weather, plant productivity and growth, soil ecology, and hydrology (Pan et al., 2012). Soil moisture variability changes regionally in the United States. This variation is generally due to soil characteristics, precipitation, evapotranspiration demands, and vegetation differences (Modelski, 2006). Soil moisture is a significant factor in mosquito survival (Tanser et al., 2003). Several studies have conducted association soil moisture and mosquito abundance. For instance, Shaman et al. (2002) were observed the effect of hydrologic conditions on mosquito populations through water table depth simulation. They used a dynamic hydrology model to predict swamp and flood water mosquito abundance. Mosquito abundances were found to respond variously to changing wetness conditions. These differences were related to habitat preferences and breeding behavior. For example, mosquito abundance increased in wet conditions in flood and swamp water, whereas mosquitoes preferred to eutrophic habitats increased in dry conditions (Shaman et al., 2002).

Soil moisture is one of the significant stimuli included in choosing of oviposition sites. Thus, it follows that it might be learned about the dispersion of eggs by searching the soil moisture distribution (Strickman, 1980). The soil moisture distribution and its influences on oviposition by *Aedes vexans* contribute to a significant part of this species. After located in the around of an oviposition site, female mosquitoes look for moist soil for the accumulation of eggs and also avoid dry soil (Strickman, 1980).
2.2.2 Drainage

Soil drainage conditions also affected the mosquito life cycle and abundance. Various soil properties impact the drainage of soils because of the amount of air and water that find among soil particles (Soil Survey Manual, 1993). Soil drainage is classified into five classes: well-drained, moderately well-drained, poorly drained, somewhat poorly drained and very poorly drained. Poorly drained soils are high clay content, little space between particles, and very small particle sizes. Also, these soils tend to limit downward water flow and cause accumulate on the surface when rain is frequent and water percolation into downward is slow. In addition, soils with restrictive layers like fragipans and rock result in slow percolation (Hart, 2010). Well-drained soils have a high sand content and large space among particles. Thus, the soils tend to drain effectually because of large space among particles and quick percolation. Soils with poorly drainage accumulate surface water more readily, facilitating the persistence of water. Also, these soils are more convenient for mosquito breeding after rain and would allow for adequate time for the cycle of mosquito life to be completed (Hart, 2010). As a result, poorly drained soils may be more optimum mosquito breeding areas than well-drained soils.

Keating et al. (2004) reported the relationship between mosquito abundance and soil drainage anomaly. In this study, they collected more mosquito larvae in well-drained areas than in poorly drained soils. Well-drained areas were in a tendency in more suitable breeding habitats compared to poorly drained areas (Keating, 2004). They were associated with this finding of increased breeding habitats to socioeconomics and human activity, such as infrastructure and community-level development. Thus, higher densities of mosquito were just indirectly related to the ecology of mosquito.
2.2.3 Soil Texture

Soil texture can also affect oviposition by mosquitoes. For example, according to Russo (1977), substrate texture is an oviposition stimulus for Aedes mosquitoes. Also, oviposition sites may be identified based on the substrate texture. Some mosquitoes can select smooth or rough oviposition surfaces. In this study, Russo (1977) was observed that *Aedes vexans* has a significant oviposition range among different particle sizes. In comparison of different types of texture substrates, medium size texture was significantly preferred more than coarse or fine texture.

2.2.4 Hydrologic Soil Group

Soils are classified into four Hydrologic Soil Groups by the Natural Resource Conservation Service. These soil groups are based on the runoff potential of soil. The four Hydrologic Soils Groups are A, B, C, and D. Also, three dual classes are A/D, B/D, and C/D (Engineering Staff, 1993). The groups are described as follows. Group A has low runoff potential and a high rate infiltration. These soil groups have a high rate water transmission. Group B has a moderate infiltration rate and moderately low runoff potential. Also, these soil groups have moderate rate water transmission. Group C has slow infiltration rate and moderately high runoff potential. These soil groups have slow rate of water transmission. Group D has high runoff potential and very slow infiltration rate. These soil groups have a very slow even restricted infiltration rate. In addition, dual groups (A/D, B/D, and C/D) are used for particular wet soils that can be enough drained (Engineering Staff, 1993). The first leading letter describes the drained condition and the second letter describes the un-drained condition.
CHAPTER 3: GOALS AND OBJECTIVES

The goals of this study are to identify the characteristics of soils associated with EEEV horse cases in the State of Florida and to compare soil characteristics between summer and winter cases. The study will be involved in documented horse cases between 2005 and 2018. The objectives include:

1. To summarize soil characteristics for the locations of the cases (both at the case locations and within 1.5 km buffers)

2. To compare those values to those for random points in the state to identify if the soils at the cases are significantly different than expected at random.
CHAPTER 4: STUDY AREA

The State of Florida has 67 counties (Figure 2). Florida is located to the north adjacent to Georgia, to the south by the Straits of Florida, to the northwest by Alabama, and to the west by the Gulf of Mexico. Florida is known as the flattest state in the U.S. Florida is covered hundreds of streams in the north and northwest and the central region thousands of lakes. Also, there are many drainage basins. The climate of South Florida is a tropical climate, while the north and central parts of Florida, the climate is humid subtropical. Summers are long, warm, and especially humid, whereas winters are very short and dry. The hottest month is July. The wet season extends from May to October, with the dry season between November and April. Also, Florida has the highest rainfall rates of the United States. Generally, the soil of Florida consists of clay, sand, loam, and muck.
Figure 2. Study area map showing observed horse cases of Eastern Equine Encephalitis Virus in Florida during 2005-2018.
CHAPTER 5: METHODS

5.1 Data Collection

5.1.1 EEE Horse Data

Horse case data were obtained from the Florida Department of Health. The case data included the date of onset and the spatial locations (latitude and longitude) of the cases. There were 676 EEEV cases during 2005-2018 (Figure 3), including 611 summer cases (Figure 4) and 65 winter cases (Figure 5). Summer cases were more prevalent than winter. Also, these cases were observed in 60 different counties of the state of Florida between 2005 and 2018.

![Bar chart showing number of EEE horse cases in Florida from 2005 to 2018](image)

**Figure 3.** Number horse cases of EEE in Florida during 2005-2018
Figure 4. Observed summer horse cases of EEE in Florida during 2005-2018

Figure 5. Observed winter horse cases of EEE in Florida during 2005-2018
5.1.2 Soils Data

For the 676 EEE cases, we downloaded soil survey data, including soil texture, drainage class, and hydrology group from FGDL, which is Florida Geographic Data Library Documentation. These data were collected from the FGDL from (https://www.fgdl.org/metadataexplorer/explorer.jsp). The data set is a digital soil survey. Also, this data is the most detailed soil geographic data made by the National Cooperative Soil Survey. The information was created by digitizing maps, digitizing and compiling information into a correct planimetric base, or revising digitized maps using remote sensing and other information. Variables of interest include; texture (soil texture class), hydric rating (indicator if the soil is hydric), drainage class (a measure of the frequency and duration of wet periods), and flooding frequency (annual probability of flood event).

5.1.3 Data Analysis

Horse case locations were overlaid the soil layers using a geographic information system (GIS), specifically ArcGIS v 10.5 (ESRI, Inc.). First, the basic soil parameters were identified at each case location. Soil texture categories included: sand, loam, muck, and submerged soils. Drainage classes included: excessively drained, somewhat excessively drained, well drained, moderately well drained, somewhat poorly drained, poorly drained, very poorly drained, and submerged. Hydrological group classes included: Class A, A/D, B, B/D, C, C/D, D, and submerged. The percentage of cases falling within in each category was summarized by season.

Second, the categories for each variable were summarized within 1.5 km of each case location. This buffer is meant to represent a broader area of transmission, as both vectors and
hosts move around in space and cases may be as associated with surrounding habitat conditions as those at the recorded case location. This distance was selected as the typical distance flown by mosquitoes associated with EEEV transmission (Vander Kelen et al., 2014). The percentages of each soil category were computed for each case buffer and then summarized over seasons. The results of this analysis were shown which soil characteristics are associated with horse cases. The analysis was conducted for cases in each season independently.

While the previous analysis shows which soil characteristics are associated with case locations, it does not indicate if those associations are of any significance. To determine if the soils characteristics of the case locations are significant, they were compared to the same values for points distributed at random. If the characteristics are different than expected at random, then an association would be found. To accomplish this, random points were generated in equal numbers to the cases. Then, the soil characteristics both at the points and within buffers of those points were summarized. This process was repeated 100 times in order to generate a confidence interval for the values that were compared to the observed case data. If the observed values fall outside the confidence interval, then we can say they are statistically different than expected at random and conclude there is an association.
CHAPTER 6: RESULTS

6.1 Soil Texture

About 92.0% of summer cases were located in soils with a texture of sand (Figure 6a), slightly more than expected at random (95% CI 91.3-91.4%). Summer cases were located in 4.1% loam more than expected at random (95% CIs 2.9-3.0%). Summer cases were located in muck 2.0% and submerged soils 2.0%, less than expected at random (95% confidence interval 2.5-2.6% and 3.1-3.2%, respectively). About 98.5% of winter cases were located in soils with a texture of sand (Figure 6b), more than expected at random (95%CI 89.0%-91.0%). One case (1.5%) was located within muck, while none were located within loam or submerged soils. These percentages were all lower than expected (95% CIs 2.2-3.3%, 2.4-3.5%, and 3.8-4.8%, respectively).
Figure 6: Soil texture classes associated with horse cases of EEEV in Florida during summer (a) and winter (b) for 2005-2018

On average, summer cases were surrounded by 92.0% sand and 3.0% muck within 1500-m buffers (Figure 7a), amounts more than expected at random (95% CI 89.2-89.4% and 2.02-2.11%, respectively). Summer cases were surrounded by 4.0% loam and 1.0% submerged soils, less than expected at random (95% CIs 5.3-5.5% and 3.25-3.34%, respectively). On average, winter cases were surrounded by 87.0% sand (Figure 7b), amounts more than expected at random (95% 82.3-84.6%). Winter cases were surrounded by 2.0% loam and 9.0% muck, less than expected at random (95% CIs 2.3-3.1% and 11.3-13.1%, respectively), while 2.0% submerged soils equal with expected at random (95% CI 1.2-2.2%)
Figure 7: Mean percentage of soil texture classes within 1500-m buffers surrounding summer (a) and winter (b) horse cases of EEEV in Florida during 2005-2018

6.2 Drainage

About 16.20% of summer cases were located in soils with an excessively drained (Figure 8a), equal with expected at random (95% CI 16.07-16.22%). Summer cases were located in 13.42% moderately well drained and 32.24% poorly drained, less than expected at random (95% CIs 13.84-13.89% and 34.56-34.72% respectively). Summer cases were located in 1.64% somewhat excessively drained, 9.66% somewhat poorly drained, 6.38% very poorly drained, 18.49% well drained and 1.96% submerged class, more than expected at random (95% CIs 1.51-1.53%, 9.58-9.64%, 5.64-5.68%, 16.92-17.03%, and 1.58-1.62%, respectively). Winter cases were located in 13.8% moderately well drained, 1.5% somewhat excessively drained, and 10.8% somewhat poorly drained (Figure 8b), equal with expected at random (95% CIs 13.2-15.5%, 1.4-2.2%, and 9.3-11.3%, respectively). Winter cases were located in 26.2% poorly drained, 9.2% well drained, and 0% submerged class, less than expected at random (95% CIs 30.5-33.6%, 5.5-7.1%, and 1.6-2.5%, respectively). Also, excessively drained with 29.2% and very poorly...
drained with 9.2% were located in winter cases, more than expected at random (95% CIs 15.0-20.4% and 5.5-7.1%, respectively).

**Figure 8**: Drainage classes associated with horse cases of EEEV in Florida during summer (a) and winter (b) for 2005-2018

On average, summer cases were surrounded by 16.00% excessively drained, 3.0% somewhat excessively drained, 20.0% well drained, and 2.0% submerged class within 1500-m buffers (Figure 9a), amounts more than expected at random (95% CIs 15.1-15.3%, 1.7-1.8%, 17.6-18.0%, and 1.7-1.8%, respectively). Summer cases were surrounded by 13.0% moderately well drained, 32.0% poorly drained, 9.0% somewhat poorly drained, and 6.0% very poorly drained, less than expected at random (95% CIs 13.64-13.82%, 32.53-32.98%, 10.6-10.8%, and 6.4-6.6%, respectively). On average, winter cases were surrounded by 13.0% moderately well drained and 9.0% well drained within 1500-m buffers (Figure 9b), amounts equal with expected at random (95% CIs 12.9-15.2% and 8.5-9.9%, respectively). On average, winter cases were surrounded by 20.0% excessively drained, 31.0% poorly drained, and 2.0% submerged class, amounts were more than expected at random (95%CI 10.8-12.2%, 24.8-27.4%, and 0.5-1.1%, respectively). Winter cases were surrounded by 1.0% somewhat excessively drained, 9.0%
somewhat poorly drained, and 16.0% very poorly drained, amounts were less than expected at random (95% CI 1.4-2.2%, 11.2-13.1%, and 23.1-25.7%, respectively).

Figure 9: Mean percentage of drainage classes within 1500-m buffers surrounding summer (a) and winter (b) horse cases of EEEV in Florida during 2005-2018

6.3 Hydrological Soil Group

About 42.72% of summer cases were located in soils with group A (Figure 10a), less than expected at random (95% CI 46.53-46.63%). Summer cases were located in 4.26% group B, 6.55% group C, 1.31% group D, 23.08% group A/D, 13.58% group B/D, 6.55% C/D, and 1.96% submerged group, more than expected at random (95% CIs 4.02-4.07%, 5.92-5.95%, 0.92-0.94%, 21.46-21.51%, 13.26-13.34%, 6.16-6.18%, and 1.53-1.58%, respectively). Winter cases were located in 55.4% group A, 6.2% group B, 1.5% group D, and 7.7% group C/D (Figure 10b), more than expected at random (95% CIs 42.1-45.2%, 3.0-4.3%, 0.7-1.3%, and 4.8-6.2%, respectively). Winter cases were located in 0.0% group C, 13.8% group A/D, and 0.0% submerged group, less than expected at random (95% CIs 6.0-7.3%, 22.9-24.6%, and 1.8-2.6%, respectively).
Figure 10: Hydrologic soil groups associated with horse cases of EEEV in Florida during summer (a) and winter (b) for 2005-2018

On average, summer cases were surrounded by 43.0% group A and 6.0% group C/D within 1500-m buffers (Figure 11a), amounts less than expected at random (95%CI 46.7-46.8% and 6.10-6.15%, respectively). Summer cases were surrounded by 5.0% group B, 7.0% group C, 2.0% group D, 23.0% group A/D, 14.0% group B/D, and 2.0% submerged group, amounts more than expected at random (95% CIs 3.8-4.0%, 5.99-6.05%, 0.9-1.0%, 21.6-21.7%, 13.1-13.2%, and 1.5-1.6% respectively). On average, winter cases were surrounded by 6.2% group B within 1500-m buffers (Figure 11b), amounts equal with expected at random (95%CI 5.8-7.3%). Winter cases were surrounded by 40.0% group A, 13.0% group B/D, and 2.0% submerged group, amounts more than expected at random (95% CIs 31.3-34.0%, 8.8-10.0%, and 0.8-1.6% respectively), while group B with 5.0%, group A/D with 29.0, and group C/D with 8.0% were surrounded less than expected at random in winter cases (95% CIs 5.8-7.3, 37.1-39.5%, and 8.3-9.9%, respectively). Also, winter cases were surrounded by 2.0% group C and 1.0% group D, amounts equal with expected at random (95% CIs 1.5-2.2% and 0.7-1.2%, respectively).
Figure 11: Mean percentage of hydrologic soil groups within 1500-m buffers summer (a) and winter (b) horse cases of EEEV in Florida during 2005-2018

6.4 Flooding Frequency

About 97.22% of summer cases were located in soils with none (no flooding) (Figure 12a), slightly less than expected at random (95% CI 97.44-97.47). Summer cases were located in 1.64% soils with frequent flooding and 1.15% soils with unclassified, more than expected at random (95% CI 1.46-1.48% and 1.06-1.09% respectively). About 98.5% of winter cases were located in soils with none (no flooding) (Figure 12b), more than expected at random (95% CI 96.6-97.5%). Winter cases were located 1.5% in soils with frequent flooding, equally expected at random (1.4-2.1%), while 0.0% soils with unclassified less than expected at random (95% CI 0.8-1.4%).
Figure 12. Flooding frequency associated with horse cases of EEEV in Florida during summer (a) and winter (b) for 2005-2018

On average, summer cases were surrounded by 98.0% none (no flooding) within 1500-m buffers (Figure 13a), more than expected at random (95% CIs 97.11-97.2%). Summer cases were surrounded by 1.0% frequency and 1.0% unclassified, less than expected at random (95% CI 1.57-1.61% and 1.2-1.3%, respectively). On average, winter cases were surrounded by 93.0% none (no flooding) (Figure 13b), less than expected at random (95% CI 93.9-95.4%). Winter cases were surrounded by 6.0% frequency and 1.0% unclassified, more than expected at random (95% CIs 3.9-5.3% and 0.46-0.97%, respectively).
Figure 13: Mean percentage of flooding frequency within 1500-m buffers surrounding summer (a) and winter (b) horse cases of EEEV in Florida during 2005-2018
CHAPTER 7: DISCUSSION

This research was examined the characteristics of soils associated with EEEV horse cases in Florida, which have previously been unexplored. This research assessed the relationship between EEEV horse cases and soil characteristics by investigating the soil data compared to the horse cases. Texture, drainage, hydrological class, and flooding frequency were all explored.

The most predominant soil texture in Florida is sand. The other soil types are found loam, muck, and clay. Despite the predominance of sand in the landscape, the results of this study show that the texture of sand had a higher percentage than expected when compared to the random points, both for the case locations and the 1500-m buffers. In contrast, loam, muck, and submerged soils had a lower rate than expected random points in both summer and winter cases. These results suggest that there is an association between sandy soil and horse cases of EEEV. This may be because horses are most commonly kept in agricultural and rural residential areas, which may be associated with sand. Alternatively, there might be some association with mosquito activity and sandy soil, perhaps related to flooding or other factors.

Drainage explains the duration and frequency of soil saturation during wet periods. There were differences in association with drainage and EEEV horse cases between seasons. Summer cases were positively associated with medium levels of drainage (somewhat excessively drained, somewhat poorly drained). Winter cases were positively associated with extremes at both ends of the drainage scale, being located more in often in excessively drained and very poorly drained soils. This suggests that there is some seasonal difference in the relationship between EEEV
activity and soil drainage in Florida. It seems that summer activity is enhanced by more moderate levels of drainage, while winter activity occurs both in soils that retain water during dry seasons and those that drain well during very wet periods. These drainage patterns might best provide habitat for breeding mosquitoes in the respective seasons.

The hydrologic group describes the infiltration rates of the soils and their potential of drainage. The results of this analysis showed a clear pattern between the hydrological group between summer and winter cases for both the case locations and the buffers. Summer cases were negatively associated with class A soils and positively associated with B, C, and D type soils. On the other hand, winter cases showed a positive association with class A soils, as well as B type. Class A soils have a low runoff potential (Engineering staff, 1993) and may not provide ideal breeding environments for mosquitoes during summer, while class D soils are thought to be more favorable for mosquitoes (Elwell, 2006). However, Class A soils may better support breeding during winter.

Flood frequency also showed disparities between winter and summer cases. Summer cases were associated with frequent flooding at the case location but no flooding within the surrounding buffers. This suggests summer cases were associated with soils that frequently flood were the horses in habitat but not necessarily nearby. Winter cases, however, were associated with a lack of flooding at the case location but frequent flooding in the surrounding area. This suggests that winter cases might be enhanced by localized flooding in the immediate area of the horses.
7.1 Limitations

Literature has not yet seen a contribution to the relationship between soil characteristics and EEEV. Therefore, this study presents a preliminary exploration of the associations between soil and horse cases of EEEV. The results obtained in this study only show the analysis of the soil properties in that region and independently of one another. This study did not explore the possible relationships between soil variables associated with the cases. For example, winter cases were associated with sandy soil, extreme drainage regimes (either excessively well drained or very poorly drained), Class A hydrology, and frequent flooding in the nearby area. However, we did not explore the relationships between these variables. It might be found that there is a relationship between drainage and flooding; for example, very poorly drained soils might be associated with no flooding, while excessively drained soils might be associated with frequent flooding. Furthermore, there are many characteristics of soils that were not explored here that might be important. Other important soil characteristics might include: soil color, nutrient levels, pH, soil moisture, soil structure, and electrical conductivity.

Additionally, we did not analyze habitat environments for mosquitoes, though this was reported in other studies (Vander Kelen et al., 2014). In addition, we did not take into account environmental variables like temperature and precipitation, since they were examined in other studies (White, 2016). Other factors that might be important could be those related to topography, such as elevation, slope, and aspect. Also, there was no data observing the relationship between the EEEV case indicate in the summer and winter regarding the habitat structure. However, many environmental conditions have significantly affected the reproduction of mosquitoes carrying EEEV. This research does not refer to the relationships of vectors EEEV that might influence the abundance and breeding sites.
Finally, in terms of the analysis, we did not consider how spatial autocorrelation might have impacted the results. Spatial autocorrelation describes how things located closer to one another are likely to have similar values. The presence of spatial autocorrelation can cause issues with some statistical techniques. The spatial distribution of horse cases in Florida, especially during summer, does show some clustering. In some cases, there was overlap between neighboring buffers, although this analysis did not factor any potential effects of spatial autocorrelation.

7.2 Implications

This research applied analysis to evaluate the relationship between soil properties and EEEV cases during summer and winter in Florida. Understanding the relationship between the characteristics is a significant guide in predicting future locations of EEEV and potential habitat-soil areas for them to survive. This research is essential because EEEV is an emerging infectious disease in the United States. There are no studies that focus on factors that the relationship between EEEV horse cases and soil characteristics. To provide this information can be a contribution to disease surveillance, detection, and prevention in communities. This study might help communities to find areas of concern during the time of the outbreak season. The communities might set up better-monitoring systems and occur the required precautions to protect the people. Also, once the relationship soil characteristics and EEEV is predicted, the results might be used in current risk models (RI) to predict the risk of outbreak transmission from these mosquito species. In this case, although there were significant differences between soils associated with cases and those at random, the magnitudes of those differences were quite small for texture in particular. This suggests that soil texture may not greatly improve a risk model.
However, hydrological class showed important differences between season, so that might be a useful variable to include.

### 7.3 Future Work

First, future work should explore further relationships between EEEV and soil. In particular, relationships among the soil variables examined in this paper should be analyzed. Understanding the interactions between variables might be important for understanding the relationships with EEEV cases. Second, other soil characteristics should be explored, such as soil moisture. Moisture in the soil affects the survival of mosquito larvae, so it should be explored in future studies. Soil moisture was not included in this study, due to difficulties in finding data at a suitable spatial and temporal resolution for the time frame of the study. However, it is expected that there would be a relationship between soil drainage or flooding and soil moisture. Along with soil factors, other environmental features should be more analyzed to determine how they affect the distribution of the virus. These might include variables related to topography, such as elevation, slope, and curvature, or those related to the distribution of land cover, such as habitat fragmentation, which remain unexplored.
REFERENCES


