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EQUINE WEST NILE VIRUS IN KENTUCKY: CHARACTERISTICS OF HISTORICAL TESTING AND ANALYSIS OF CAUSAL FACTORS

Kaelyn Short
University of Kentucky, kaelyn.short@yahoo.com

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Kaelyn Short, Student

Dr. Wayne T. Sanderson, Committee Chair

Dr. Sarah Wackerbarth, Director of Graduate Studies

EQUINE WEST NILE VIRUS IN KENTUCKY: CHARACTERISTICS OF HISTORICAL TESTING AND ANALYSIS OF CAUSAL FACTORS

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By

Kaelyn Short

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Chair
Wayne T. Sanderson, Ph.D., CIH

Committee Member
Craig Nash Carter DVM, MS, PhD

Committee Member
Kimberly I. Tumlin, PhD, MS, MPH

ABSTRACT

EQUINE WEST NILE VIRUS IN KENTUCKY: CHARACTERISTICS OF HISTORICAL TESTING AND ANALYSIS OF CAUSAL FACTORS

As the arbovirus with the highest incidence in both humans and horses in the United States, West Nile Virus (WNV) presents a significant risk to the equine population of Kentucky. Widespread infection has the potential to cause a significant economic impact to the state and long-term health complications for the horses. To better understand the burden of equine WNV, historical diagnostic testing archives the University of Kentucky Veterinary Diagnostic Lab were analyzed along with environmental and census data collected from additional sources. A total of 2146 test results were analyzed utilizing descriptive statistics, chi-square tests of independence, and binary logistic regression. Results indicated that WNV infections in Kentucky were seasonal with the majority of cases occurring in the fall months. Age and sex were statistically significant factors in overall testing characteristics. Breed testing and positivity results tended to correlate with population census data. Both testing and positive cases were found to be widespread across the state. Environmental factors of surface area of land and water by county and total horse population by county were found to have a statistically significant relationship with positive test results. As land and water surface area increased, the positivity rate increased. As total horse population by county increased, positivity rates decreased. Total rainfall by county was not found to have a statistically significant relationship with positive test results. Future research should examine equine WNV vaccine protocols in Kentucky and the purpose for testing, whether surveillance of asymptomatic horses or diagnosis of symptomatic horses.

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By
Kaelyn Short

Wayne T. Sanderson, Ph.D., CIH
Director of Thesis

Sarah Wackerbarth, Ph.D.
Director of Graduate Studies

April 19, 2021
Date

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Dedication

To my mom and dad, Mike and Nancy Short, for their constant and unwavering love and encouragement throughout every stage of my life. I appreciate all you have done for me.

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Introduction

West Nile Virus (WNV) is a flavivirus with documented infections across the world in Europe, Africa, the Middle East, parts of Asia, Australia, and North and South America (Hayes et al., 2005). In 1999, the first outbreak of WNV in the United States occurred in Queens, New York where a cluster of human cases was detected. Since the first outbreak, WNV has become the arbovirus with the highest incidence in the United States with cases reported in all 48 contiguous states, many of which now consider WNV endemic (Petersen, 2019).

WNV is transmitted by the bite of an infected mosquito with *Culex* mosquitos serving as primary vectors (Molaei et al., 2006). *Culex pipiens* and *Culex restuans* take the majority of their blood meals from birds including American robins and house sparrows and *Culex salinarius* take mixed blood meals from both birds and mammals. Birds act as an amplifying host for WNV and *Culex salinarius* serve as a bridge vector for WNV between birds and mammals. Grey squirrels, white tailed deer, horses, and humans are the preferred mammals for *Culex* mosquito blood meals.

As one of the preferred mammalian hosts for *Culex* mosquitos, horses are at high risk for WNV infection. Fever and neurological symptoms including ataxia, paralysis of the limbs, muscle tremors and rigidity, and changes in behavior are the most common indicators of WNV in horses (Castillo-Olivares & Wood, 2004). Approximately ten percent of horses infected with WNV will present with neurological disorders (Castillo-Olivares & Wood, 2004). The mortality rate for horses infected with WNV that exhibit clinic signs of infection is 33 percent (AAEP, 2020). Approximately 40 percent of horses that survive initial infection will suffer from residual effects over a period of six months after their initial diagnosis (AAEP, 2020). There is no treatment for equine WNV and supportive care is recommended for symptomatic horses. A vaccination for WNV for horses has been available since 2001 (Seino et al., 2007). The American Association of Equine Practitioners recommends yearly vaccination in the spring for adult horses to provide protection before the insect vector season (AAEP, 2020).

The equine industry in Kentucky is a significant part of the state's economy, with a total economic impact of $3 billion in 2012 and the creation of forty thousand jobs across the state (Wiemers, 2013). In light of this, it is important to understand the potential health threats to these horses, including WNV. Previous research has focused primarily on transmission to humans or general characteristics of mosquitos. This investigation is focused on the relationship between WNV and horses in Kentucky. This research will seek to understand the characteristics of historical equine WNV testing and positive cases in Kentucky and the potential causal effects of environmental factors on infection.

Literature Review

Equine WNV

Although Kentucky has not been the focus of past WNV research, studies examining WNV outbreaks in horses have been conducted in other regions around the world. These studies primarily focused on cases of equine encephalitis that occurred as a result of WNV infection. An outbreak of equine WNV occurred in southern France in 1962 and again in 2000 (Murge et al., 2001). In 1962, most cases were reported in the summer months with the peak number occurring between August 15 and September 15. Across all farms studied, the fatality rate among infected horses that developed neurologic disorders as a result of infection was between 25 and 30 percent. In 2000, another equine WNV outbreak was identified with a total of 58 cases reported from August to November. 82.9 percent of positive cases were reported in September and the last case was reported on November 3, 2000. Infected horses ranged in age from 3 years to 30 years with a median age of 10 years and all but 3 positive cases were within a 15km radius. A serologic survey conducted on all horses within a 10km radius to detect antibodies and identify the number of asymptomatic infections determined that 13.1 percent of horses sampled had either IgG or IgM antibodies. The area where the outbreak occurred included, “wetlands, rice fields, garriguea (a geographical dry area, typical of the Mediterranean basin), and fauna.” This study establishes the seasonal nature of the virus and known characteristics of symptomatic infection. It also identifies geographic areas where infection may be more likely.

In the late summer of 1998, a retrospective study in the Tuscany region of Italy identified an epidemic of equine WNV encephalitis (Autorino et al., 2002). While 14 cases had neurologic symptoms, a serologic survey conducted over a 700 square kilometer area showed a prevalence of 38 percent positive for WNV-specific antibodies, confirming widespread asymptomatic infection in the region. There were no differences in age detected among the horses infected and those that died. The 43 percent fatality rate was similar to the rates observed in other outbreaks including France and the United States.

Between August 20, 2002 and October 15, 2002, the Ontario Veterinary College Veterinary Teaching Hospital identified 28 cases of equine WNV encephalomyelitis (Weese et al., 2003). While the age of infected horses ranged from 5 months to 20 years, there was no age predisposition in horses to developing WNV infection. It was recommended that all horses with neurological symptoms in summer, fall, or early winter be tested for WNV. The study also noted the sporadic nature of equine WNV outbreaks in Canada and hypothesized that, “large numbers of horses have been exposed to WNV and have not developed clinical signs, while developing protective immunity.” The study suggested that this natural immunity, combined with immunization of horses, should decrease susceptibility over time.

Three years after WNV was first detected in the United States, Texas experienced the largest epidemic to date of equine WNV (Ward et al., 2006). The 2002 epidemic saw a total of 1698 confirmed cases occurring from June 27, 2002 to December 17, 2002. The epidemic occurred in three phases and peaked on October 5, 2002 with fifty percent of cases reported from September 3, 2002 to October 17, 2002. Analysis of case characteristics found that 90.2 percent of cases lived in rural areas and the risk of death as a result of infection was associated with age, gender, travel outside of home county, vaccination status, and severity of symptoms. One hypothesis to explain how WNV was introduced into Texas was by infected migratory birds that coincided with the mosquito breeding season. Another hypothesis proposed that infected mosquitoes were already present in the state but weather conditions including temperature and rainfall allowed an increase in the infected mosquito population, leading to high rates of infection.

There is limited data describing the number of equine WNV cases in Kentucky. The Kentucky Department of Agriculture reported a total of 744 confirmed positive equine WNV cases from 2001 to 2017 (KDA, 2020). Of those cases, 95.7% were not adequately vaccinated, defined as not vaccinated during the preceding 12-month period or having no booster vaccination. 223 (29.97%) of these cases were fatal.

Equine Value and Potential Economic Impact

The economic value of a horse is dependent on several factors including: genetics, training and behavior, conformation (impacting athletic ability), age, health conditions, and breed popularity (Freeman, 2016). Horses with unwanted or unusable traits will have a near zero value and horses with superior traits will have the highest value. Young horses intended for uses like racing or show will be valued higher than older horses. Middle-aged horses with training will be valued higher than older horses with similar characteristics due to longevity and ability to resell. While the economic value of a horse is what impacts the horse industry of a state and subsequently the economy, other factors including emotional attachment to a horse must also be considered when assessing equine value. In situations where the horse is owned as a hobby or for purely recreational purposes, the horse is treated more as a pet. This leads to an emotional attachment between the horse and its owner, and the emotional value of the horse would grow as the horse aged.

The 2012 Kentucky Equine Survey found that around 33 percent of Kentucky’s equine population was primarily used for trail riding or pleasure riding (UK CAFE et al., 2013). 16 percent were used as broodmares, 14 percent were idle or not working, 10 percent were used for competition or show, 9 percent as yearlings, weanlings, or foals, and 6 percent for racing. While the majority of Kentucky’s horse population is used for recreational purposes, around 62 percent of employment from the state’s horse industry comes from breeding-related activities, racing, and competition, while only 1 percent comes from recreation.

The horse industry is a significant part of Kentucky's economy. In 2012, the value of all horses, ponies, mules, and donkeys in the state was $6.3 billion (UK CAFE et al., 2013). Total value was highest for thoroughbreds at $5.5 billion, followed by American Saddlebreds at $173 million, Quarter Horses at $146 million, Standardbreds at $119 million, and Walking Horses at $71 million. In 2011, the total estimated equine-related income for equine operations was $1.1 billion. In addition to employment, operating expenditures, and taxes that contribute to the state's economy, Kentucky's equine industry also supports tourism through national and international equine events. The economic impact from just one racing venue, Keeneland, was estimated to be $15 million in 2012.

As the equine industry is such a significant part of Kentucky's economy, it is important to understand the potential economic impact of equine WNV. A 2002 study examined North Dakota's horse population of ~43,415, describing the state's equine industry as small but with "activities such as racing, showing, rodeo, ranch work, and other forms of recreation" (Ndiva et al., 2007). In 2002, North Dakota reported 569 cases of equine WNV, with an incidence rate of 13 cases per 1000 horses. The cost to horse owners as a result of WNV was estimated to be over $1.5 million including both treatment and vaccination of horses, however this figure is thought to be underestimated as the actual number of cases of WNV and vaccination for the disease was likely underreported. While the economic value of the horse can have negative implications for the state's equine industry in the event of a disease outbreak, there are additional costs of equine WNV. These costs included general emotional stress for impacted horse owners and loss of enjoyment as a result of being unable to ride the horse. For owners where their horses are treated more as companions or pets, infection and illness results in stress and worry over the wellbeing of the horse. Including the cost of vaccination, treatment, and euthanasia attributable to WNV, the total financial cost to horse owners in the US was $7.4 million in 2003 causing substantial economic impact. While the emotional impact of the virus on owners is not able to be measured, it should still be considered as a significant burden associated with WNV. The study suggested potential solutions of increased vector control programming as well as education of horse owners and further research into the disease.

Environmental Factors

West Nile Virus infections are dependent on a wide variety of environmental factors including the climate, season, mosquito and host animal population, landscape factors, and socioeconomic conditions of the area (Liu & Weng, 2008). An ecologic study focusing on eight northeastern US states attempted to understand the risk factors for WNV infection in humans (Brown et al., 2008). Examining WNV cases in these states from 1999 to 2006, the study found urbanization was a risk factor for WNV infection. Areas with less forested areas and more urban areas were found to have higher likelihood of WNV infection. In a study of positive WNV reports for mosquitos and hosts from 2001 to 2006 in Cook County, Illinois, urban and grassy areas were associated with the spread of WNV (Liu & Weng, 2008). Higher temperatures were also associated with more cases of WNV with the higher temperatures attributed to pollution and high vehicle use in the county. The study suggested that a potential control measure for WNV could be decreasing the sizes of existing waterways such as ponds and lakes as well as education on elimination of areas with small amounts of water accumulation in order to decrease the mosquito population. A similar study examined the relationship between environmental factors and WNV risk in the greater Chicago, IL region from 2004 to 2008 (Ruiz et al., 2010). This study focused on role of temperature and precipitation in WNV infection finding that 80 percent of the variation in infection could be explained by weather conditions. Higher air temperatures led to increased infection rates in mosquitos. Lower rates of precipitation were associated with lower rates of infection in mosquitos and lower rates of infection in mosquitoes was correlated to lower rates of human infection.

The activity patterns of mosquitos impact the transmission of WNV. *Culex* mosquitos lay their eggs on the surface of water including both natural and man-made bodies of water and polluted or clear water (ECDC, 2020). This variance allows the mosquitos to lay their eggs and populate highly varied environments. Past research on mosquito activity in Italy showed seasonal patterns in mosquito activity with *Culex pipiens* being active at night from July to October (Veronesi et al., 2012). In Cook County, Illinois, the pre-adult stages of the mosquito's life were found to be impacted by weather conditions 8 to 20 days prior to capture and adult mosquitos were found to be impacted by weather conditions 20 to 28 days prior to capture (Lebl, Brugger, & Rubel, 2013). The abundance of mosquitos was correlated with daytime length over the previous four to five weeks and temperature during the previous two weeks. Longer daytime length and higher temperatures were associated with the highest mosquito abundance. High wind speed was associated with lower capture rates of mosquitos likely due to the inability of the mosquito to be effectively attracted to the baited trap, while high humidity was found to increase mosquito capture rates. The study found rainfall to contribute to higher adult mosquito populations, contradicting previous research that suggested a potential negative impact on mosquito larvae as a result of high levels of rainfall. These results show that weather changes cause generational impacts as changes in the mosquito population were found to occur as a result of weather conditions during the previous generation.

Impacts of Vaccination and Disease Control

The United States Department of Agriculture has approved four WNV vaccines for use in horses with the first vaccine made available in 2001 (Seino et al., 2007). The schedule for each vaccine varies, but all require two primary doses followed by a booster dose every twelve months (AAEP, 2021). Each vaccination provides one year of immunity. For horses that have previously received the vaccination, the American Association of Equine Practitioners recommends annual vaccinations in the spring prior to the insect vector season. For horses that have not previously received the vaccination, two doses four to six weeks apart are recommended. Pregnant mares are recommended to receive vaccination four to six weeks before foaling. A study on the efficacy of each vaccine showed all vaccines were protective against clinical disease and resulted in a 100 percent survivorship against severe WNV encephalomyelitis (Seino et al., 2007). The study strongly supported the use of vaccination as a preventative against equine WNV infection. However, an Animal and Plant Health Inspection Service (APHIS) study from 1998 to 2015 showed low rates of all equine vaccinations in the United States (APHIS, 2018). Data was collected from a representative sample in 28 states. In 2005, 63.8 percent of respondents reported vaccinating any of their horses against WNV in the past 12 months, but in 2015, only 56.3 percent of respondents reported vaccinating against WNV. Reasons for not vaccinating included expense of the vaccinations, potential adverse effects, “likelihood and consequence of disease,” availability of a veterinarian to schedule a time for vaccination, and owner perception of risk for their animals being infected.

In 2002, the same year as the Texas epidemic of equine WNV, misleading information regarding the safety of vaccination in pregnant mares was published by local news outlets (Connell, 2003). These reports alleged that the equine WNV vaccination produced by Fort Dodge Animal Health was linked to abortion and deformed foals in pregnant mares following vaccination. This allegation was proven false and the USDA and AAEP still recommend vaccination in pregnant mares. These allegations did cause fear and hesitancy in horse owners to proceed with vaccination around 2002 which caused a potential decrease in vaccination rates for pregnant mares.

Vaccination is the most effective intervention for preventing equine infection. Beyond vaccination, vector control is the strongest method to reduce infection (Giedt, 2018). As horses do not serve as reservoir for WNV and cannot spread the virus to other horses, quarantine of horses is not necessary to control the spread of disease. Reducing exposure of horses to mosquitoes through removal of standing water, application of insect repellant, keeping horses in barns at night with insect screens, and eliminating bird roosting areas can also minimize exposure.

Methodology

Data Collection

This study uses secondary data analysis of equine WNV diagnostic testing data generated by the University of Kentucky Veterinary Diagnostic Laboratory (UKVDL). It is comprised of testing data for all horses tested for WNV at the UKVDL from November 17, 2000 to September 16, 2020. Tests were comprised of the following testing procedures: IgM capture ELISA testing, Real-time PCR testing, or WNV screening. In order to be included in the analysis, the test must have met the following criteria: must receive one of the three available diagnostic tests for WNV at the UKVDL during the specified time period, species must be equine, and must be from Kentucky.

Results were divided into geographic regions determined by county (Figure 1). These regions were defined using a regional map published by the Kentucky National Digital Newspaper Program (KY-NDNP, n.d.). The number of horses per county data was collected from the United States Department of Agriculture National Agriculture Statistics Service from the 2017 Census of Agriculture- Chapter 2, Table 18- Equine- Inventory and Sales; the commodity "Equine, Horses & Ponies – Inventory" was used (NASS, 2017). Rainfall data per county was collected from the National Oceanic and Atmospheric Administration (NOAA) National Centers for Environmental Information (NOAA, 2021). Daily summaries of precipitation were collected for each county. Surface area of land and water per county was collected from the 2019 American Community Survey conducted by the U.S. Census Bureau (U.S. Census Bureau, 2010). Data was collected from TIGERWeb and analyzed using QGIS software. Surface area of land was defined as the "area measurement providing the size, in square meters, of the land portions of geographic entities for which the Census Bureau tabulates and disseminates data (U.S. Census Bureau, 2010). Surface area of water was defined as “water area figures include inland, coastal, Great Lakes, and territorial sea water. Inland water consists of any lake, reservoir, pond, or similar body of water that is recorded in the Census Bureau geographic database. It also includes any river, creek, canal, stream, or similar feature (U.S. Census Bureau, 2010).

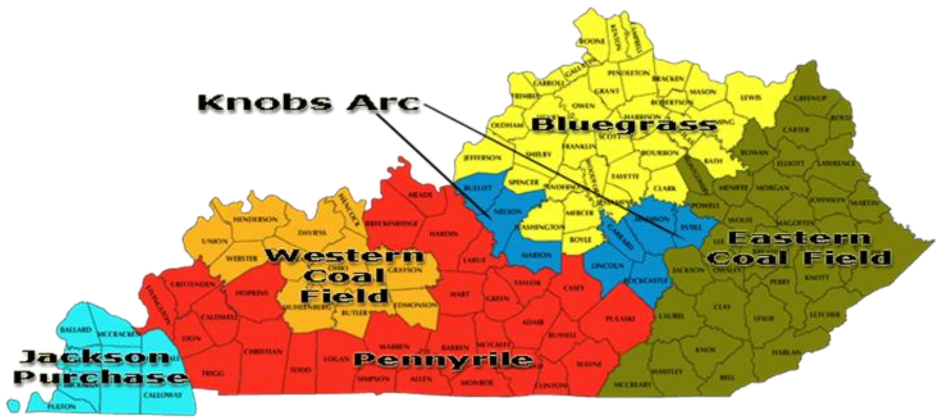

Figure 1. Geographic regions of Kentucky (KY-NDNP, n.d.)

Coding of Variables

Variables were recoded for analysis. Results were grouped by a “negative” or “not detected” result and a “positive” or “numeric” (signifying the virus was detected) result. Breeds were recoded into an “other” category if there were fewer than 10 horses of that breed in the dataset. The age of the horse was recoded into the following categories: Fetus, Juvenile (under 5 years), Adult (5 to 14 years), and Senior (15 years or older). Total precipitation per county was calculated from the total rainfall from 10 to 24 days before the date of the diagnostic test. This accounts for the life cycle of the mosquito and the incubation period of WNV disease in horses. Horses typically show symptoms 3 to 14 days after an infected mosquito bite (CFSPH, 2003). The time it takes for a *Culex* mosquito to develop from egg to adult is approximately 7 to 10 days (CDC, 2020). Total precipitation data was collected from using NOAA daily precipitation data and utilized the weather station in the county with the most complete data for the date range specified.

Analysis

Data was analyzed using R statistical computing software. Categorical variables were analyzed using chi-square tests of independence. Categorical variables were compared with quantitative variables through logistic regression.

Results

Characteristics of equine WNV testing

Between 2000 and 2020 a total of 2164 WNV tests were performed on blood samples from horses by the UKVDL. Table 1 (see Appendix) displays the characteristics of all equine WNV tests performed by the UKVDL during these years.

Most tests were performed in the fall and the spring had the fewest tests. Age was not recorded for almost 60% of the horses. The remaining 40% of horses did have age recorded, and of those, 45.1% of the tests were performed on fetuses and fewer tests were performed on horses in older age groups. More tests were performed on female horses than males, 56% versus 44% of tests with recorded sex, but sex was not reported for 73% of the horses. At 64% of the total number of samples, the breed most likely to be tested was thoroughbred. Likewise, the vast majority of tests (93.4%), were from the Bluegrass or Knobs regions of Kentucky, which is the major thoroughbred producing region of the state. The greatest number of tests was performed in 2002 (24.3%) followed by 2003 (12.5%).

Characteristics of equine WNV testing by result

Table 2 (see Appendix) displays the characteristics of equine WNV tests by test result as well as the positivity rate for each category. Table 2 includes only data recorded and does not include missing data.

While the results for 18 of the 2164 tests were not reported possibly due to inconclusive results, 152 (7.1%) of the tests were reported to be positive for WVN. Juvenile, adult, and senior horses had fairly comparable positivity rates while fetuses had the lowest positivity rate at 6%. Females were slightly more likely to test positive than males (13.3% versus 8.7%), however the sex was not reported on almost three quarters of the horses. Thoroughbreds and warmbloods had the lowest positivity rate at 3 percent and 0 percent respectively. Tennessee Walking Horses had the highest positivity rate of 25.6% while only comprising 5.6% of total tests. The Pennyrile region of Kentucky (comprising 2.6% of total tests) returned the highest positivity rate of 23%. The Pennyrile region had two predominant breeds comprising 72% of the tests from this region. The Quarter Horse comprised 40% of tests and the Tennessee Walking Horse comprised 32% of tests. The fall was the season with the highest positivity rate at 11%. There was no clear time trend in number of positive tests by year with the median positivity rate being 7.1%, but the positivity rate in certain years did stand out, such as 2002 (13.4%); 2010 (9.8%); and 2018 (8.7%) when compared to other years. Figure 2 (see Appendix) displays the equine WNV positivity rate by year.

Figure 3 (see Appendix) displays the number of tests submitted by county. Samples were received from only 61 of Kentucky's 120 counties. Most of the samples (70%) were received from Fayette County, and the second highest number of samples were received from Woodford County (6%). Less than 5 samples were collected from 31 of the 61 counties from which samples were received. Figure 4 (see Appendix) displays the number of positive equine WNV tests by county. The county with the highest number of positive tests is Fayette County at 61. Woodford, Boyle, and Shelby Counties each have the next highest number of positive tests with 12, 9, and 7 respectively.

Characteristics of equine WNV testing by environmental factors

Table 3 (see Appendix) displays the results of a logistic regression analyzing the impact of environmental factors (total precipitation, county land and water surface area, and number of horses per county) to the WNV positivity rate. The regression found that there is not a statistically significant relationship between the two variables. The total rainfall in the time period before the test including the life cycle of the mosquito and incubation period of disease in horses did not show a relationship with positive test results. Regression results were statistically significant for both county land and water surface area as well as the number of horses per county. There was a positive beta coefficient for land and water surface area indicating that as the land or water area of the county increased, positivity rate increased as well. The number of horses per county returned a negative beta coefficient indicating that as the number of horses in the county increased, the positivity rate decreased.

Comparative analysis of environmental factors

Because approximately 70% all WNV tests were from horses residing in Fayette County, it was difficult to assess positivity rates by the selected ecologic factors associated with each county--the number of horses, surface water area, or landmass by county. In Tables 4 and 5 (see Appendix) the positivity rates for Fayette County were compared to other county-level categories using a chi-square test. The percentage of positive tests by the total amount of rainfall from 10 to 24 days before the test date is shown by categories of precipitation in millimeters of water in Table 4 by approximate quintiles. There was no clear trend in positivity rate by amount of precipitation, indicating little association between these variables. The percentage of positive tests by land and water surface area is shown with Fayette County as the median value. While there is a statistically significant relationship between both land and water surface area, there is not a clear trend in positivity rate and surface area. Fayette County seems to be an outlier for both of these variables. The number of horses per county is displayed as approximate quartiles with Fayette County representing the highest number of horses. There was a statistically significant relationship between the number of horses per county where positivity rates decreased as the number of horses in the county increased.

Discussion

Characteristics of equine WNV testing

Seasonality of testing followed known positivity rates for equine WNV as found in past research with the fewest tests occurring in the Spring months of March through May. These months have historically returned the fewest positive tests (Murgue et al., 2001). The greatest proportion of testing occurred in the Fall months of September to November, the months that have historically been the most likely for an outbreak to occur. The overall positivity rate was a modest 7.1% with only 152 total positive tests out of 2146 total tests between 2000 to 2020. However, certain years such as 2002 with a positivity rate of 13% and 2010 with a positivity rate of 10% do stand out as years with relatively high positivity rates when compared to others.

While age of the horses was only recorded for about 40% of the tests, fetuses seemed to be more commonly tested, comprising 45.1% of the total tests. WNV in mares can cause spontaneous abortion. The high proportion of fetal testing could be explained by horse owners attempting to find a cause of pregnancy loss. Risk of abortion is increased in mares that are unvaccinated against WNV. Rates of testing declined as the population aged. This could possibly be explained by the economic value of the horse, which would decline as they get older (Freeman, 2016). As the value of the horse declines, the cost of the test for diagnosing WNV may not be considered a worthwhile investment by the owner. However, the emotional value of the horse would increase as age increases, which would be a testing. As treatment of horses infected with equine WNV is supportive care, testing may not be recommended due to cost. Supportive care without diagnosis may be recommended instead.

Equine WNV is associated with abortion in pregnant mares which could explain the higher rate of testing in female horses compared to males (Venter et al., 2011). Horse owners may test female horses to determine a cause for abortion if one occurred. As the Thoroughbred horse capital of the world, it is not surprising that the breed with the highest proportion of testing was the Thoroughbred at 69.25% (UK CAFE, 2013). Commonality of breeds in the area is the most likely explanation for these results. Quarter Horses, Tennessee Walking Horses, and Standardbred had the next highest likelihood to be tested as these breeds are also common in Kentucky (UK CAFE, 2013).

The Bluegrass region of Kentucky is where most of the horse industry is located which explains why the majority of tests come from this region (UK CAFÉ et al., 2013). Another likely explanation is that the UKVDL is in Lexington, KY, in the center of the

Bluegrass region. This could encourage horse owners in the Bluegrass region to use the UKVDL for their testing. The highest proportion of testing occurred in the years 2002 and 2003. This is around the time when the equine WNV vaccine was approved and began to be utilized. The years 2005 to 2009 showed very low testing rates for the state, potentially due to the vaccine. It is possible that vaccination rates during this time were high, so the need for testing was low (APHIS, 2018). Testing increased from 2012 to 2019, which could be explained by lower vaccination rates and an increased need to test for infection (APHIS, 2018).

Characteristics of equine WNV test results

The seasonality of tests results reflects the outbreak patterns found in past research. Spring showed no cases of WNV while Fall had the highest positivity rate at 11%. This is explained by the climate of the state and the life cycle of the mosquito vector. These results follow mosquito season, the time of the year where mosquitos are most active. Examining the positivity rate among age groups, positivity increased as age increased. The positivity rate for adults was 1% lower than seniors. As discussed with the proportion of total tests performed, this could be due to the economic value and risk of infection. A high number of tests were performed on fetuses in an attempt to explain the cause of abortion. More tests were being performed on younger horses, who would have a higher economic value due to opportunities for racing or show (Freeman, 2016). This higher number of tests being performed would lower the positivity rate. Inversely, adults and seniors have a higher positivity rate potentially because fewer total tests were performed on these age groups. Since the value of these horses is lower, owners may require more severe illness before testing in order for the cost to be worthwhile, which could lead to a higher positivity.

While females did have a higher positivity rate than males, this difference was not statistically significant. Thoroughbreds, the breed with the highest proportion of tests performed, interestingly had one of the lowest positivity rates at only 3%. The breeds with the next highest proportions of tests performed did have some of the highest positivity rates. These breeds included Tennessee Walking Horses, Quarter Horses, and Standardbreds. This could suggest that the higher rates of testing of Thoroughbreds could be explained by testing requirements from the equine industry or testing on a "just in case" basis because of the value of the horse. The higher positivity rates in Tennessee Walking Horses, Quarter Horses, and Standardbreds could be explained by their use in the state. These breeds are more likely to be used for trail riding and pleasure riding, which would cause the horse to be exposed to rural, wooded environments. This would increase their risk of mosquito exposure and their likelihood of becoming infected with WNV, explaining their high positivity rates.

While the Bluegrass/Knobs region had the highest proportion of total tests, the positivity rate was the lowest among regions with positive results. The proportion of tests from the Bluegrass region can also be explained by the region containing a higher proportion of farms, more breeding animals, and large competition venues. The Eastern Coal Fields had the highest positivity rate, which again could be explained by the cost of the test versus the value added. Those in other regions where the horse industry is not as significant are less likely to submit tests, which could make the tests they do submit more likely to be positive. The high positivity rate could also be explained by the environment the horse is in and what the horse is being used for. If the horse is being used on trails and for riding, their exposure would increase which would also increase their risk of being infected with WNV.

2002 was the year with the highest positivity rate at 13%. This again was the time when equine WNV vaccines were still fairly new and not in widespread use. There were also concerns about the safety of the vaccine, especially in pregnant mares (Connell, 2003). After vaccine implementation from 2005 to 2009, positivity rates were very low, with only one case seen in 2006. Positivity rates increased and have stayed consistent from 2011 to 2020 at approximately 4% to 5% per year. This could be explained by the general acceptance level of horse owners and perceived value of the vaccination. Data on estimated mosquito numbers by year are not available.

Equine positivity rate

The positivity rate by county did not reflect the counties with the highest number of submitted tests. Counties in the Pennyrile and Eastern Coal Field regions had the highest positivity rates while counties in the Bluegrass/Knobs region had lower positivity rates that were almost equal. This could again be attributed to economic value and cost of the test as well as the use of the horse. The Bluegrass region has shown to have a higher inventory value of horses per county, making this region likely to take on the expense of performing the test (UK CAFE et al., 2013). Those in other regions with lower inventory values of horses may not take on the expense of the test unless the horse had more severe illness and higher need for the test (UK CAFE et al., 2013). While the overall number of positive tests is small (152), counties in the Pennyrile and Eastern Coal Field regions that have more woodland and rural areas did show higher positivity rates. While the overall number of tests ordered from each region would impact the positivity rate, the exposure of the horse to mosquito habitats and the frequency of exposure, which would be higher for horses in these regions, should be considered.

Environmental factors

Total rainfall was not shown to have a statistically significant relationship to positive WNV results. When looking at total precipitation, there was an even distribution of precipitation for both positive and negative results. The insignificant relationship could be due to the criteria tests were submitted. If tests were submitted specifically for routine surveillance, likely in the Bluegrass region, rather than diagnostic purposes (likely in other regions), this would lead to no significant relationship.

Regarding the test result and surface area of land and water, there was a significant positive relationship. This indicates that as the surface area of land and water increases, positivity rate increases. While data on estimated mosquito populations by county is not available, an increase in area of a mosquito's habitat may explain this relationship. Counties with more water area especially may be more likely to have increased infected mosquito populations, which would increase positivity rates. This would follow trends shown in past research where increased grassy areas and waterways were associated with the spread of WNV (Liu & Weng, 2008).

The relationship between number of horses per county and test results was also statistically significant. There was a negative relationship shown where positivity rate decreased as the number of horses per county increased. This could be because of the differences in the horse industry by county. Counties with a larger horse industry may participate in more preventative testing while counties with a smaller horse population may only conduct testing when horses are symptomatic or visibly ill. It has been shown through the 2012 Kentucky Equine Survey that counties with a larger horse industry have higher inventory values, making surveillance testing a more worthwhile investment whereas low value horses in counties with a smaller horse industry may not be worthwhile to test unless they are visibly ill (UK CAFE et al., 2013).

Comparative analysis of environmental factors

When comparing the impact of environmental factors on positivity rate between Fayette County and other counties, the number of horses per county as well as county land and water surface area were found to have statistically significant relationships. Total precipitation was not found to be statistically significant and there was no clear trend in positivity rate. As the number of horses in the county increased, the positivity rate decreased. As previously discussed, this could be explained by horse industry per county and the cost of preventative testing. Fayette County seems to be an outlier for both county land and water surface area. Fayette County surface area for both land and water was placed as the median value and the positivity rates for counties larger and smaller were compared. The positivity rate for the larger and smaller counties are similar, but Fayette County itself is very different. While these results were found to be statistically significant, there does not seem to be a real relationship between land and water surface area and positivity rate since the positivity rate remains similar when excluding Fayette County.

Limitations

This dataset has several limitations. Only samples received by the UKVDL were tested. This data does not represent every equine WNV test performed in Kentucky.

UKVDL client non-compliance when requesting laboratory testing was high, resulting in a high number of missing data fields. Since fields were not required, the variables of sex, age, and breed were missing large portions of data. In order to reduce the number of missing fields for future tests, the UKVDL is exploring methods for electronic submission of testing requests with required fields in order to submit the request. The geographic location of the horse before and during testing is unknown. For the purposes of this study, it was assumed that the horse was located at the home address of the person that ordered the test, but it is possible that the horse was in a different location. Having geographic location and history of travel for the horse would allow for more precision in determining where the horse was located when their potential WNV infection occurred. It would also aid in identifying clusters of disease and allow for more targeted mosquito surveillance and control. The data itself is not evenly distributed and 70% of the data is from Fayette County. This, combined with only having data from 61 of Kentucky's 120 counties, makes it difficult to compare testing between counties since there is not even distribution of tests and results.

The symptom status or symptom onset of the horse was also unknown. There were also a small number of positive tests collected during the study period (152). This makes it difficult to identify significant characteristics of positive tests. Knowing the symptom status of the horse as well as symptom onset would allow for tracing of encephalomyelitis outbreaks and more precise estimation of the date the horse was infected. The purpose of the test was unknown, whether the test was performed as routine surveillance or because of illness. If this data was collected, it would allow for analysis of only symptomatic tests, which would give a greater understanding of the causal factors of disease.

The vaccination and booster status of the horse is also unknown. It is important to understand if the horse had received vaccination against WNV to know if the vaccine is still protective against the virus as well as provide an opportunity for education of horse owners on the importance of the vaccine. The control measures of the horse owner would be additional valuable data. Vaccination status of the horse as well as mosquito control measures taken including spraying for mosquitoes would allow for a more accurate assessment of risk for the horse. The economic value of the horse was also not provided. If provided, it, along with the morbidity and mortality outcome of the horse could be combined to understand the total economic impact of equine WNV at both the state and county levels. This data could be used to justify increased mosquito control programming or state funded vaccination programs to decrease cases.

Additional sources of external data would allow for better understanding of equine WNV in Kentucky. Data on mosquito populations by county would allow for examination of risk. Species of mosquitoes by county as well as the percentage of mosquitoes infected with WNV would have been interesting variables to compare with positivity rates by county. Data on the percentage of infected birds or other amplifying hosts by county would have also provided more insight for analysis. Standardized weather data over time would have also been helpful for analysis. Daily weather data provided by NOAA is comprised of many weather stations in each county, each with varied and inconsistent data over time. A weather station with daily data for an area would have prevented missing data for this variable and allowed for a more complete understanding of historical weather and its impact on WNV infection.

Conclusions

Characteristics of testing followed trends previously discussed in literature. Trends in positivity followed the implementation of the equine WNV vaccine. Positivity rates can be explained by economic factors. While rainfall was not found to have a significant relationship with positive test results, greater surface area of both land and water were found to be associated with higher positivity rates and a higher number of horses per county was found to be associated with lower positivity rate.

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Appendix

Table 1. Characteristics of equine WNV tests (UKVDL, 2000-2020)

	Number	Percent	p-value*
Total Number of Tests	2164	100.0%	
Procedure			
IgM Capture ELISA	1089	50.3%	<0.0001
Real-Time PCR or WNV-PCR	900	41.6%	
WNV-Screening	175	8.1%	
Result			
Negative/Not Detected	1994	92.1%	<0.0001
Positive	152	7.0%	
Missing	18	0.8%	
Age			
Fetus	395	18.3%	<0.0001
Juvenile (Under 5 Years)	205	9.5%	
Adult (5 to 14 Years)	182	8.4%	
Senior (15 or older)	93	4.3%	
Missing	1289	59.6%	
Sex			
Male	254	11.7%	<0.0001
Female	324	15.0%	
A	1	0.05%	
Missing	1585	73.2%	
Breed			
Arabian	13	0.6%	<0.0001
Belgian	11	0.5%	
Miniature Horse, American	23	1.1%	
Mixed Breed	50	2.3%	
Morgan	11	0.5%	
Paint Horse, American	21	1.0%	
Pony	16	0.7%	
Quarter Horse, American	134	6.2%	
Rocky Mountain Horse	17	0.8%	
Saddlebred, American	49	2.3%	
Standardbred	66	3.0%	
Tennessee Walking Horse	121	5.6%	
Thoroughbred	1403	64.8%	
Unknown Breed Equine	10	0.5%	
Warmblood	13	0.6%	
Other	68	3.1%	
Missing	138	6.4%	

Region			
Bluegrass/Knobs	2022	93.4%	<0.0001
Eastern Coal Fields	81	3.7%	
Pennyrile	57	2.6%	
Western Coal Fields	2	0.1%	
Jackson Purchase	2	0.1%	
Season			
Spring	207	9.6%	<0.0001
Summer	536	24.8%	
Fall	937	43.3%	
Winter	484	22.4%	
Year			
2000	2	0.1%	<0.0001
2001	105	4.9%	
2002	523	24.2%	
2003	268	12.4%	
2004	33	1.5%	
2005	19	0.9%	
2006	18	0.8%	
2007	8	0.4%	
2008	4	0.2%	
2009	3	0.1%	
2010	62	2.9%	
2011	45	2.1%	
2012	129	6.0%	
2013	151	7.0%	
2014	52	2.4%	
2015	111	5.1%	
2016	144	6.7%	
2017	124	5.7%	
2018	196	9.1%	
2019	130	6.0%	
2020	37	1.7%	

*p-value from Chi-squared test

Table 2. Characteristics of equine WNV tests by test result and positivity rate (UKVDL, 2000-2020)

	Result			
	Negative	Positive	Positivity Rate	p-value
All Tests	1994	152	7.6%	---
Procedure				
IgM Capture ELISA	1070	58	5.1%	<0.0001*
Real-Time PCR or WNV-PCR	808	99	10.9%	
WNV-Screening	175	0	0.0%	
Age				
Fetus	372	24	6.1%	0.008*
Juvenile (Under 5 Years)	183	21	10.3%	
Adult (5 to 14 Years)	157	26	14.2%	
Senior (15 or older)	84	12	12.5%	
Sex				
Male	231	22	8.7%	0.1878†
Female	280	43	13.3%	
A	1	0	0.0%	
Breed				
Arabian	10	3	23.1%	<0.0001†
Belgian	10	1	9.1%	
Miniature Horse, American	21	2	8.7%	
Mixed Breed	47	3	6.0%	
Morgan	10	1	9.1%	
Paint Horse, American	19	2	9.5%	
Pony	15	1	6.3%	
Quarter Horse, American	111	22	16.5%	
Rocky Mountain Horse	14	3	17.6%	
Saddlebred, American	44	4	8.3%	
Standardbred	55	11	16.7%	
Tennessee Walking Horse	90	31	25.6%	
Thoroughbred	1342	47	3.4%	
Unknown Breed Equine	8	2	20.0%	
Warmblood	13	0	0.0%	
Other	58	9	13.4%	
Region				
Bluegrass/Knobs	1875	129	6.4%	0.0003†
Eastern Coal Fields	71	10	12.3%	
Pennyrile	44	13	22.8%	
Western Coal Fields	2	0	0.0%	
Jackson Purchase	2	0	0.0%	
Season				
Spring	222	0	0.0%	<0.0001*
Summer	506	46	8.3%	
Fall	850	101	10.6%	
Winter	475	10	2.1%	

Year				
2000	2	0	0.0%	<0.0001†
2001	99	5	4.8%	
2002	452	70	13.4%	
2003	252	16	6.0%	
2004	31	2	6.1%	
2005	19	0	0.0%	
2006	17	1	5.6%	
2007	8	0	0.0%	
2008	4	0	0.0%	
2009	3	0	0.0%	
2010	55	6	9.8%	
2011	44	1	2.2%	
2012	108	8	6.9%	
2013	148	3	2.0%	
2014	50	2	3.8%	
2015	106	5	4.5%	
2016	139	4	2.8%	
2017	115	9	7.3%	
2018	179	17	8.7%	
2019	128	2	1.5%	
2020	35	1	2.8%	

*p-value from Chi-squared test

†p-value from Fisher's exact test

Figure 2. Equine WNV positivity rate over time (UKVDL, 2000-202

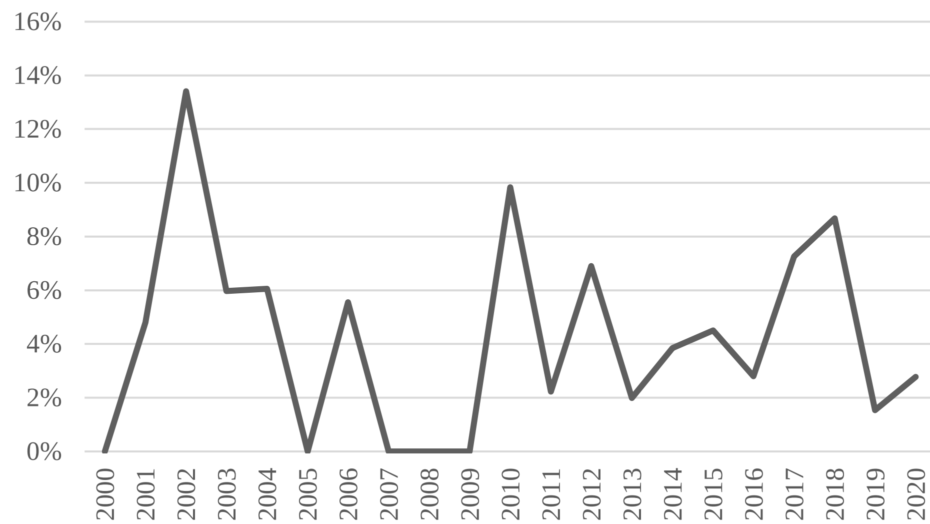

Figure 3. Total number of equine WNV tests submitted by county (UKVDL, 2000-2020)

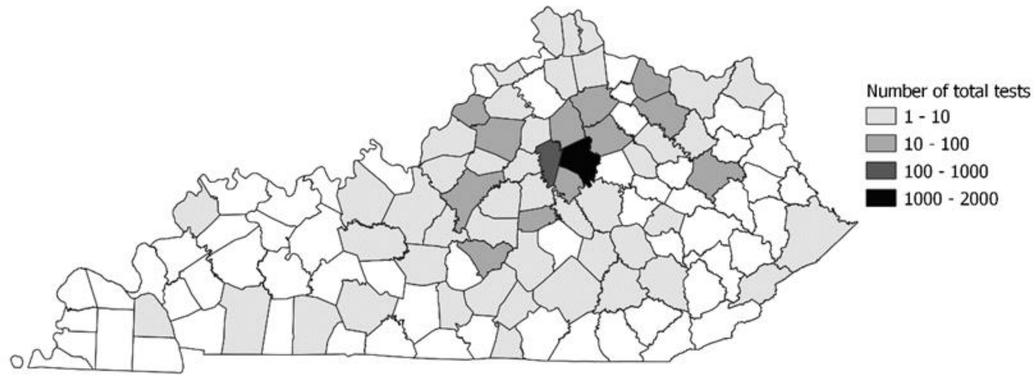

Figure 4. Number of positive equine WNV tests by county (UKVDL, 2000-2020)

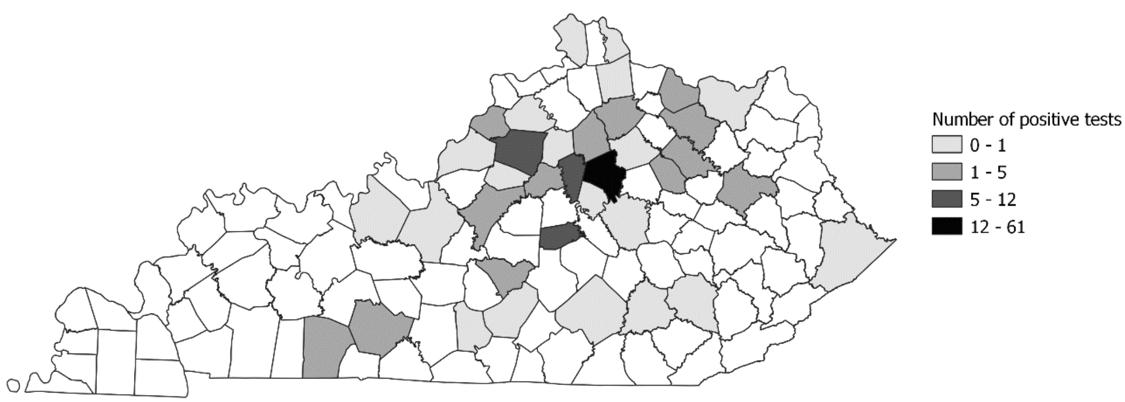

Table 3. Logistic regression analysis of environmental factors and positivity rate

Variable	β Coefficient (Std Err)	p-value
Total Precipitation (mm) to WNV Positivity Rate	-0.0004276 (0.0023665)	0.857
County Surface Area of Land (km^2) to WNV Positivity Rate	0.0008686 (0.0004329)	0.0448
County Surface Area of Water (km^2) to WNV Positivity Rate	0.036268 (0.008386)	0.0000153
Number of Horses in the County to WNV Positivity Rate	-0.0001375 (0.00001607)	<0.0001

Table 4. Number and Percent of Positive Samples by Categories of Total Amount of Precipitation 10 to 24 Days before the Test Date

	Negative	Positive	Positivity Rate (%)	p-value*
Total Precipitation in Millimeters (mm)				
≤12	351	18	4.9	0.312
>12 – 30	304	28	8.4	
>30 – 50	411	33	7.4	
>50 – 80	370	22	5.6	
>80	381	26	6.4	
Total All Samples	1817	127	6.5	

*p-value from Chi-squared test

Table 5. Number and Percent of Positive Samples by County-Level Categories of Number of Horses, Land Surface Area, and Water Surface Area

	Negative	Positive	Positivity Rate (%)	p-value*
County Land Surface Area (km^2)				
< 735	285	47	14.2	
735	1441	61	4.1	<0.0001
> 735	268	44	14.1	
County Water Surface Area (km^2)				
< 4.9	53	6	10.2	
4.9	1441	61	4.1	<0.0001
> 4.9	500	85	14.5	
Number of Horses in the County				
≤ 1200	172	36	17.3	
> 1200 – ≤ 2400	152	31	16.9	<0.0001
> 2400 – ≤ 12,600	229	24	9.5	
> 12,600	1441	61	4.1	

*p-value from Chi-squared test