

**SPATIAL ANALYSIS OF WEST NILE VIRUS AND PREDICTORS OF
HYPERENDEMICITY IN THE TEXAS EQUINE INDUSTRY**

A Thesis

by

COURTNEY ANNE WITTICH

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

December 2007

Major Subject: Veterinary Public Health

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ABSTRACT

Spatial Analysis of West Nile Virus and Predictors of Hyperendemicity
in the Texas Equine Industry. (August 2007)

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West Nile Virus (WNV) first appeared in Texas equids during June 2002. It has since spread rapidly across the state and apparently become endemic. Data from outbreaks occurring between 2002 and 2004 were analyzed to determine hotspots of equine WNV disease, identify environmental factors associated with outbreaks, and to create risk maps of locations with horses at a higher risk of the disease. Kriging was used to model the smoothed WNV attack rates, and interpolated rates were mapped to describe the spatial distribution of WNV disease risk in Texas. A retrospective time-space analysis using a Poisson model was conducted on each year's data to identify clusters with high attack rates. The resulting overlapping yearly clusters were considered areas of hyperendemicity (hotspots). The counties identified as hotspots included Hockley, Lubbock, and Lynn (primary cluster) and Leon and Roberstson (secondary cluster). Environmental and geographic features were added to the disease maps and analyzed to determine possible environmental factors associated with outbreaks. Locations in close proximity to lakes, bird breeding routes, migratory flyway zones, crop farm and agricultural land, and all dense vegetation were found to be important environmental

predictors. Finally, risk maps were created that combined surveillance data on WNV positive mosquito collections and wild bird WNV cases with previously identified environmental risk factors to predict areas of high occurrence of WNV. These risk maps could be used to implement various preventative measures to reduce the transmission of WNV in the Texas equine industry.

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CHAPTER I

INTRODUCTION

West Nile Virus (WNV) is a flavivirus that is endemic in many countries in Africa, West Asia, and the Middle East, and most recently in the United States. WNV causes a vector borne disease that is maintained in nature by a bird-mosquito cycle (1). In humans and equids, WNV infection is usually asymptomatic or characterized by a mild febrile illness, although fatal meningoencephalitis or encephalitis may occur (2).

Since its introduction to North America (New York City) in August 1999, the geographic range of WNV has increased dramatically in a southern and western direction across the United States (3). The number of reported West Nile cases among horses dramatically increased in 2002, when 9144 equine cases were reported from 38 states (3). Research on WNV has covered a vast array of theories and methods including mosquito vector surveillance, spatial modeling, temperature dependency, dead bird surveillance, geographical and environmental status, and host-specific factors (age, breed, sex, herd size, vaccination history).

Mosquito vector surveillance information is important for designing WNV disease prevention programs. Although the vectors of WNV that maintain the transmission cycle within wild bird reservoirs have not definitely been identified, there is

This thesis follows the style and format of Emerging Infectious Diseases.

agreement that the genus *Culex* (specifically *Culex pipiens*) is probably important (4). However, with respect to equids, the bridge vector (a mosquito that feeds on birds as well as on horses) that transmits WNV to horses is unknown (4).

Within research on this topic, there are a few discrepancies or inconsistencies with respect to the vector of WNV. In their research, Brownstein et al. (5) stated that there were five mosquito species and genera found in NYC that were competent for WNV, the most prominent of which was *Ochlerotatus*. Research done by Apperson et al. (6) found that *Culex pipiens* L., *Cx. Quinquefasciatus* Say, *Cx. Salinarius* Coquillett, and *Cx. tarsalis* Coquillett appear to be the most important vectors of WNV in North America. Finally, in another study done by Nasci et al. (7) it was found through assay results that *Culex pipiens* L. and *Culex salinarius* Colquillett were the two most predominant vectors of WNV in horses in the United States.

While there is little agreement among studies conducted in North America concerning the primary bridge vectors for horses, there are similar contradictions between results of studies undertaken in Europe. One study done by Romi et al. (8) in Italy suggested that the main bridge vectors were *Cx. impudicus* and *Cx. pipiens*. Similar research by Porphyre et al. (9) found that in France the vectors from which WNV was most commonly isolated were *Culex modestus* and *Ochlerotatus caspius*. Based on previously conducted research it appears that further studies on already identified vectors would be helpful in a prevention program designed to control the spread of WNV.

Another avenue researchers have pursued to better understand the epidemiology of WNV is spatial analysis of the factors influencing occurrence of disease. The USDA used Geographical Information Systems (GIS) in a report in August 2001 (10) to find the

central location or starting point of a disease outbreak and, together with Ward et al. (11), to pin point hot spots of WNV using reported cases (equine, mosquito), and environmental clustering methods. GIS has proven to be a very important aspect of research for many vector borne diseases, and this applies equally well for WNV.

In another study, Mostashari et al. (12) used GIS to identify clusters of dead birds. This information helped lead to an early warning system for WNV activity, showing that an abundance of dead crows might precede an outbreak of WN disease in humans (12). Research done by Brownstein et al. (5) showed how GIS can be used in the spatial analysis of human infections when mosquito, human, and dead bird clusters were reported to estimate the risk of WNV in a population. All of these studies have used GIS to help further understand WNV epidemiology, so that future outbreaks can be predicted or prevented. The one area in which GIS could still help would be in understanding which vectors are responsible for spreading WNV to horses, and the specific environmental conditions necessary for those mosquitoes to survive and breed.

The climate and environment in which WNV is transmitted is another topic that has been researched. The general conclusion seems to be that WNV is likely to be abundant in areas where there is a lot of water (for example, lakes and rivers) and during periods of high temperatures (midsummer), however, not all of the evidence supports this theory. In an outbreak in Italy in 1998, Romi (8) found that the peak of the outbreak occurred in late September and the majority of the cases were within two distinct environments: wetlands, and hills (predominantly the hills). Upon further study, the primary habitat breeding sites (even in the hills) included: flooded prairies, canals, ponds, and marshes. While all mosquitoes breed in water, the type of water source determines

which species will be present and is therefore plays an intricate role in vector surveillance. More research done by Apperson et al. (6) in Tennessee also suggests that environments that bridge vectors inhabit are dense pine plantation with permanent fresh water supply, salt marshes, red maple freshwater swamps, and even urban areas with concrete drainage sewers.

Climatic (temperature) conditions are also important for WNV transmission, but there is some disagreement regarding the nature of the relationship. A study done in Coahuila State, Mexico (13) showed that the prevalent climatic conditions associated with seroconversion of horses to WNV were hot, dry, and arid with temperature ranging from 18 to 22 degrees Celsius, and an average rainfall of 100 to 300 mm per year. Epstein (4) also concluded that mild winters followed by hot, dry summers favor the transmission of infections (such as WNV) that cycle among birds, mosquitoes, and humans. It is reasoned that high temperatures speed up the incubation period of viruses within the infected mosquito vectors, increasing the probability that vectors will transmit the disease to humans or horses. Examining the specific environments of bridge vectors can help to detect and prevent future WN outbreaks.

The final set of factors that have been studied for association with WN disease are equine-specific host factors: breed, sex, age, management conditions, and herd environment. Several studies have been done with respect to breed categories, but not over a broad range of different breed categories. In their study of an outbreak in southern France, Durand et al. (14) included four breed classes (according to typical management conditions) defined: pure breeds, the Camargue breed, pony breeds, and others. This type of grouping does not give a very precise measure as to whether one breed has higher risk

than other specific breeds. In this particular study, no significant differences were found between serologic status of animals by age or sex, but seroprevalence was highest in the Camargue breed (14).

Whether or not age is a risk factor for WN disease also seems to be unclear. In a study of the clinical outcome of equids with WN disease and factors associated with death, Salazar et al. (15) concluded that females were 2.9 times as likely to die as males. In a similar study of encephalomyelitis in horses, Porter et al. (16) found that significantly more males than females were affected. Both studies were similar, yet there were opposite findings and conclusions. More research on age differences are necessary to determine if age is a risk factor for WN disease.

Although many studies have been conducted on the detection and prevention of WNV, there is still much more to be learned. Some areas that need further study are identification of bridge vectors responsible for transmitting WNV to horse populations (and specifically including their preferred environments) further spatial analysis to find hot spots of disease and matching those to environmental and horse-specific factors. WN is an important emerging disease that needs to be monitored and studied more thoroughly to create better detection and preventative measures.

CHAPTER II

CLUSTERING OF WEST NILE VIRUS DISEASE CASES, 2002-2004

Introduction

West Nile Virus (WNV) was first recognized in the United States in New York City in 1999 and has since spread across all 48 contiguous states. Disease due to WNV infection first occurred in Texas in 2002 in the northern panhandle area and has since spread towards the gulf coast.

Several studies have been conducted on WNV, its spread and distribution in Texas and cases in vertebrates and specifically horses. Previous research has used human cases in Geographical Information Technologies by geocoding addresses to describe their distribution (17). At the same time density kernel functions were performed on dead crow data to document geographic density of the cases. Maps of human cases and cluster analysis were used to show the grouping of cases that were validated using virus positive mosquito sample sites in those same areas (5).

In another study of human WN cases Theophilides used interpolation methods, specifically kriging, to identify high rate areas through GIS technologies. Many analyses can be performed in ArcMap version 9.0 that show spatial correlations including local Moran's autocorrelations. Epicenter or mean centers can be calculated to describe movement of the virus (18). When considering movement of WNV, research has shown

that cluster or hotspot movement tends towards mosquito prone habitats and specifically coastlines (19).

Spatial analysis of WN cases has been shown to aid in entry point identification and spatial prediction of risk of infection. If high risk areas can be predicted through spatial analysis then preventative measures can be implemented including mosquito control and education to decrease impact of the disease.

Methods

Case reports of equine West Nile disease occurring in Texas during 2002, 2003, and 2004, compiled by the Texas Department of State Health Services, were accessed. All cases displayed clinical signs suggestive of West Nile encephalomyelitis (ataxia, abnormal gait, muscle fasciculations, recumbency and depression) and had been confirmed by IgM enzyme-linked immunosorbent assay (MAC-ELISA > 1:400). Clinical signs and a positive MAC-ELISA are considered sufficient criteria for a probable case [12]. WNV-specific IgM antibodies are detectable 6 to 10 days post-infection, and persist for 2-3 months [Castillo-Olivares, J, Wood, J. West Nile virus infection of horses. *Vet Res* 2004; 35:467 – 483; Ostlund, NE, Crom, RL, Pedersen, DD, et al. Equine West Nile Encephalitis, United States. *Emerg Infect Dis* 2001; 7:665-669]. Vaccination is unlikely to produce false-positive IgM ELISA results [Hathaway D, Jennen C, Jennings N, et al. Serum antibody responses in horses vaccinated with West Nile Virus vaccines. In *Proceedings of the 85th Annual Meeting of the Conference of Research Workers in*

Animal Diseases, Chicago IL, 2004; Abstract number P61]. Data available included reported date of disease onset, residential address, date of collection, sex, age, and test results for cases in 2002, 2003, and 2004. Within the spreadsheet a column was added for each year to calculate the attack rates for each county. The attack rate is determined by dividing the number of cases in the county by the number of horses at risk (population) and multiplying by 100. For some reported cases, latitude and longitude coordinates (degrees North, and degrees West) were reported. Case report data was initially compiled, organized and checked within a spreadsheet program [Microsoft Office Excel. Microsoft Corporation, Redmond WA, 2003].

Data were imported into a geographic information system (ArcMap TM version 9.0. ESRI Inc., Redlands CA, 2004) for further formatting and analysis. A shapefile of Texas counties (Geographic Data Technology, Inc., ESRI, Redlands CA, 2004), was overlaid with a polyline file of highways and streets (Geographic Data Technology, Inc., ESRI, Redlands CA, 2004). While latitude and longitude coordinates were reported for some of the West Nile cases, some cases had only exact addresses, and some just had location descriptions. Cases with latitude and longitude coordinates were clearly placed on the Texas counties shapefile at the proper location without requiring further manipulation. Cases with exact addresses but no coordinates were geocoded using ArcMap. In ArcMap under ArcToolbox, Geocoding Tools was opened to allow access to the Geocode Addresses function. To geocode addresses an input table field and input address locator field are needed to run the program. The input table used was WNV cases for 2002, 2003, and 2004 which tells ArcMap what addresses to geocode with the

addition of fields: street, city, state, and zip. The input address locator is the file that will be run along with the input table to locate the address and input them as points on the Texas counties shapefile, in this case the address locator was Street Map USA, which contains all major streets in Texas. The geocoding function only works on cases that have addresses listed with them, so another method was required to input cases with only location descriptions. The remaining case locations were identified by using reported residential addresses and visually approximating the spatial position of each location. Using the select features aspect of ArcGIS street and highway names were highlighted, then using mapquest.com the specific addresses were located and transferred to the highlighted portion in ArcGIS where the coordinate position was recorded for that case. Reported addresses that consisted of post office boxes and rural route numbers could not be geocoded and were therefore excluded from the database. Similarly, some other addresses that could not accurately be geocoded (for example, missing street numbers for streets that exceed 1km in length) were also removed.

Using latitude and longitude coordinates, case locations were projected using the Texas counties shapefile, with a latitude/longitude projection of Geographic Coordinate System and North American 1983 datum (NAD83) which was created by Geographic Data Technology, Inc., ESRI and obtained through the United States Geological Survey website (usgs.gov) in the GIS (ArcMap version 9.0: Tools>Add XY Data).

The mean center of reported cases for each year was calculated (ArcMap version 9.0: Spatial Statistics>Measuring Geographic Distributions>Mean Center). The mean center is the location of a single latitude and longitude coordinate value that represents

the average x-coordinate value and the average y-coordinate value of all features in a study area, in this situation West Nile case locations. Directional ellipses were also calculated for each year of data (ArcMap version 9.0: Spatial Statistics>Directional distribution (Standard Deviational Ellipse). Directional influences are natural or physical processes (date of disease onset) that affect a measured trait or attribute so that the magnitude of the effects on the attribute vary in different directions. Here the directional ellipses show the direction of WN cases based on their date of disease onset around the mean center for each year. These procedures were repeated weighting mean centers and directional ellipses by the date of onset of each case. To further gain insight into the distribution of WN cases, case density maps were created for each year (ArcMap version 9.0: Spatial Analyst < Density). Density analysis takes known quantities of some phenomena and extrapolates it across the landscape based on the quantity that is measured at each location and the spatial relationship of the locations of the measured quantities. First the options under spatial analyst were set using no mask, extent same as Texas counties layer, and cell size maximum of inputs. Under density mapping input data was WN cases for each separate year, population field was Cases, density type simple, area units kilometers squared, output cell size .04265.

The spatial patterns of cases reported in each year of the study were further analyzed using spatial autocorrelation. The Moran's autocorrelation test was performed on each year of data, weighted by date of onset of WNV and the attack rate using the drop down arrow within ArcMap version 9.0. Moran's spatial autocorrelation is a measure of the degree to which a set of spatial features and their associated data values

tend to be clustered together in space (positive spatial autocorrelation) or dispersed (negative spatial autocorrelation).

More spatial analyses were run through ArcMap version 9.0 spatial statistics tools, using hi/low clustering (ArcMap version 9.0: Spatial Statistics Tools < Analyzing Patterns < High/Low Clustering (Getis-Ord General G)), and hot spot analysis with rendering (ArcMap version 9.0: Spatial Statistics Tools < Mapping Clusters < Hot Spot Analysis with Rendering) techniques. High low clustering calculates a general G statistic for an input class (WN cases) using a numeric field for analysis (cases) to measure the degree of clustering for either high or low values (in this situation looking at clustering of points with a high or low number of cases of WN). Inverse distance was used to conceptualize the spatial relationship, Euclidean distance was the distance method set, and there was no standardization with a distance band or threshold distance of zero. Hotspot analysis with rendering performs hot spot analysis on a feature class (WN cases) evaluated on a numeric count field (cases) calculating G_i^* statistics and applying a cold-to-hot type of rendering to the output z scores. The distance band or threshold distance, which specifies a distance cutoff value, was set at one.

Data were analyzed for clusters using spatial, temporal, and space-time scan statistics in SaTScan software. SaTScan is freeware that can be used to analyze spatial, temporal, and space time data using the spatial, temporal, or space-time scan statistics. It is designed for any of the following interrelated purposes: 1) perform geographical surveillance of disease, to detect spatial or space-time disease clusters, and to see if they are statistically significant; 2) test whether a disease is randomly distributed over space,

over time or over space and time; 3) evaluate the statistical significance of disease cluster alarms; and 4) perform repeated time-periodic disease surveillance for early detection of disease outbreaks.

Data were edited and text files were created (Microsoft Office Excel. Microsoft Corporation, Redmond WA, 2003) for each year of the study. These files were case files (case identification number, date of onset, number of cases), population files (identification number, year, population per county), and coordinate files (identification number, latitude, longitude). These text files were then used in the SaTScan analyses by opening the SaTScan program and adding them in the input tab as case file, population file, and coordinates file. Under this tab the control and grid files can be left blank for a poisson model analysis. Study period is defined as having a start date of year (2002, 2003, 2004), month (01), and day (01) with an end date of year (2002,2003,2004), month (12), and day (31). Finally the time precision is based on a day and the coordinates will be in latitude/longitude. Under the analysis tab the type of analysis is retrospective space-time, probability model is poisson, scan for areas with high rates, time aggregation is day with a length of 30 days, and Monte Carlo replications. Under the analysis tab there is an option for advanced parameters with should be set with a maximum spatial cluster size of 50% of the population at risk with a circular window shape, a maximum temporal cluster size of 50% of the study period, and no adjustments. Under the output tab the final output printout is saved and clusters should be reported with no geographical overlap.

Primary and secondary clusters were searched for in each study year. Each cluster was described by center (latitude and longitude coordinates) and radius of the cluster. Subsequent spatial analysis was undertaken in these areas (Chapter III).

Another form of spatial analysis, kriging, was performed on the WN case data within GIS (ArcMap version 9.0. ESRI Inc.) using attack rates to further visualize and confirm the SaTScan results. Kriging is an interpolation technique in which the surrounding measured values are weighted to derive a predicted value for an unmeasured location. Weights are based on the distance between the measured point, the prediction locations, and the overall spatial arrangement among the measured point. Kriging is unique among the interpolation methods in that it provides an easy method for characterizing the variance, or precision, of predictions. Kriging is based on regionalized variable theory, which assumes that the spatial variation in the data being modeled is homogeneous across the surface. That is, the same pattern of variation can be observed at all locations on the surface.

Kriging is performed within GIS (ArcMap version 9.0. ESRI Inc.) using the spatial analyst tools. Before you can use the spatial analyst tools you need to set the environment (spatial analyst >options), set your working directory and analysis mask as none, analysis extent is same as Texas Counties, and cell size of maximum of inputs. Next spatial analysis can be used to interpolate that data using attack rates for each year (spatial analyst>interpolate to raster>kriging). Input is the WN case files (2002, 2003, 2004), Z value field is the attack rates, kriging method is ordinary, semivariogram model is spherical, search radius type is variable, and number of points is 12. Under this method of kriging there is a tab for advanced parameters which gives the option to add

the major range, partial sill, nugget, and lag size for your particular data based on the semivariogram. Each year had different advanced parameters: 2002) major range = 6.42316, partial sill = 2.9822, Nugget = 1.0483, lag size = .94345; 2003) major range = 6.56609, partial sill = .69354, nugget = .39776, lag size = 1.0186; 2004) major range = 2.01323, partial sill = .36348, nugget = .036964, lag size = .17165. These parameters were chosen based on the results of semi-variograms of the WN case data. Once kriging was completed on each year the raster outputs were layered and visualized within GIS (ArcMap version 9.0. ESRI Inc.).

Results

Maps of the distribution of reported WN cases in 2002, 2003, and 2004 were created (Figure 1). With 2002 cases being distributed mainly in the panhandle region, 2003 cases were distributed through central Texas, and 2004 cases being concentrated along the Gulf coast.

Mean centers and association directional ellipses were added to the map of case distributions (Figure 2). These procedures were repeated weighting mean centers and directional ellipses by the date of onset of each case (Figure 3). These mean centers and directional ellipses showed a trend in case distributions moving from the panhandle in a southern and eastern direction toward the coast from 2002 to 2004.

Density maps of WN cases were layered over the Texas counties shapefile (Geographic Data Technology, Inc., ESRI) with a transparency of 70% to create case

density maps for years 2002 (Figure 4), 2003 (Figure 5), and 2004 (Figure 6). Also showing movement of cases from the panhandle to the Gulf.

Interpolation kriging analyses produced WN hotspot maps based on cases per county for each point (not taking into account equine population which was conducted using SaTScan software) for each year 2002, 2003, and 2004 (Figure 7). The hotspots produced from this map were located in the panhandle Lubbock area and the Leon area in central Texas along with other areas around the Gulf coast and specifically Galveston.

Moran's autocorrelations statistic (I), weighted by date of onset of cases, for 2002, 2003, and 2004 was 0.29 (Z-score = 15.5, $P < 0.0001$), 0.26 (Z-score = 3.8, $P = 0.0001$), and 0.16 (Z-score = 2.4, $P = 0.0164$), respectively. Thus, based on Moran's autocorrelation statistic, WNV cases in each year of the study were significantly ($P < 0.05$) spatially clustered by date of onset of the disease. In addition, this clustering was strongest in 2002, and was reduced in subsequent years.

Hi/low clustering (Getis-Ord General G) statistics (G^*), weighted by cases per county, for 2002, 2003, and 2004 was 0.98 (Z-score = 13.79, $P < 0.0001$), 1.22 (Z-score = 9.92, $P < 0.0001$), and 2.17 (Z-score = 4.77, $P < 0.0001$), respectively. Thus, based on Getis-Ord General G statistic, WNV cases in each year of the study were significantly ($P < 0.05$) spatially clustered for high values of cases. In addition, this clustering was weakest in 2002, and strengthened in subsequent years, showing a more confined and centralized location of WNV cases.

SaTScan results for the retrospective space-time analysis scanning for clusters with high rates using the poisson model gave coordinates, radius, relative risks, and p-values for primary and secondary clusters for all three years. 2002 primary cluster:

coordinate (-101.7428 S, 34.3458 E), radius 99.54 km, relative risk 15.792, P-value .001; secondary cluster: coordinate (-97.2983 S, 32.9312 E), radius 89.9 km, RR 5.628, P-value .001. 2003 primary cluster: coordinate (-102.166 S, 32.7 E), radius 99.83 km, RR 21.87, P-value .001; secondary cluster (-97.075 S, 31.333 E), radius 89.15 km, RR 7.932, P-value .001. 2004 primary cluster: coordinate (-102.0487 S, 31.96 E), radius 87.42, RR 64.244, P-value .001; secondary cluster: coordinate (-95.2125 S, 30.096 E), radius 60.49, RR 4.318, P-value .030. When mapped, these data show a primary cluster overlap in Cochran, Hockley, Lubbock, Terry, Lynn, and Garza counties with a secondary cluster overlap in Freestone, Limestone, Leon, and Robertson counties.

Using these parameters, primary and secondary clusters for each year were visualized within GIS and projected with a Texas counties shapefile to show cluster areas (Figure 8). The area of overlap of the primary clusters and secondary clusters were defined as “hotspots” of West Nile disease in Texas. Counties in areas of cluster overlap were noted (Figure 9) and overlaid with previous hot spot analysis maps (Figure 10). The kriging rasters of the attack rates were layered over the Texas counties shapefile and then the outlines of SaTScan primary and secondary clusters were added to the map (Figure 11).

Discussion

Study results suggest that the locations where WN cases occurred tended to change during the period 2002 to 2004. In 2002, the mean center of cases was in North Central Texas (Palo Pinto county). In 2003, the mean center had moved approximately

103.8 km south-east (Bosque county), and by 2004 the mean center was located in eastern Texas (Grimes county), approximately 340.3 and 232.3 km south east of the 2002 and 2003 mean centers respectively. Additionally, the case density maps show a progression of cases across Texas in a southeastern direction starting in the panhandle in 2002 and ending up near Galveston by 2004. The progressive (2002 to 2004) earlier occurrence of cases in the eastern parts of Texas suggests that these cases occurred alongside environments that were beneficial to the maintenance of the virus. These could include: areas that are good habitats for the mosquitoes spreading WNV (wetlands, lakes, rivers, reservoirs, stagnant water sources); areas that have a large susceptible equine population; or contained other factors that allowed the virus to flourish.

When WNV was first introduced into the US it started in New York and progressively moved in a southwestern direction over the years from 1999 to 2002 when it entered Texas. The first and earliest cases that were recognized in Texas were located in the panhandle during early 2002. These cases most likely spread to the panhandle from northern states where WNV was already endemic, such as Oklahoma. That would explain why when WNV was first introduced to Texas in 2002 there were so many cases in the panhandle region. Over the next year the virus moved in a southeastern direction, possibly continuing on to areas where mosquitoes and horses were more concentrated due to better mosquito habitats. There are very few rivers that run through the panhandle area in comparison to the density of rivers in central and eastern Texas. Then again in 2004 there was another more drastic movement and concentration of WN cases in southeastern Texas near Galveston and the gulf coast. The reason for this shift could again be the result of prime habitat locations for mosquitoes, there are lots of stagnant water sources

closer to the gulf coast than any where else in Texas and the humidity and precipitation levels are much higher on the coastline. Another possible cause of the shift in cases could be the practice of administering equine vaccinations. When cases broke out in the panhandle in 2002 owners may have been inclined to start vaccinating their horses whereas the need would not have seemed so great for owners in other parts of Texas. As the virus spread across the country equine owners may have chosen to vaccinate their horses after large numbers of horses in their area were infected, thus showing fewer cases in areas where the virus had already been in previous years. This would also explain the results of the hi/low clustering G statistic, showing a greater concentration of high case numbers in 2004 compared to previous years in the study.

SaTScan results indentified a primary hot spot location including Cochran, Hockley, Lubbock, Terry, Lynn, and Garza counties with a secondary hot spot location including Freestone, Limestone, Leon, and Robertson counties. Several other analytic methods (case density maps, hotspot analysis with rendering, and kriging of disease rates) also verify that those areas have greater rates of WN disease in equines than other areas of Texas. While it is true that cases gradually shifted away from the panhandle from 2002 to 2004, with very few cases occurring west of Mills county, case density maps still show a concentrated location within the primary hot spot. Reasons for these hotspots could include: environmental habitats ideal for the mosquitoes that spread WNV (hydrology, landscape, and climate factors); areas of increased equine populations in comparison to other counties; or a failure of owners to provide WNV vaccination to their horses for various reasons (economic, personal or livestock usage, fewer veterinarians

offering the vaccine in that area). All possible environmental influences in these locations will be analyzed in Chapter III.

CHAPTER III

ENVIRONMENTAL ANALYSIS OF EQUINE WEST NILE VIRUS CASES

Introduction

West Nile is a vector borne disease. Mosquitoes transmit infection from wild bird reservoirs to susceptible mammals, including humans and equids. As with most vector borne diseases, environmental factors influence the spread of WNV and therefore need to be taken into consideration when searching for hyperendemic foci (“hotspots”) of disease.

Previous research on WNV has shown that the most likely vectors involved in the transmission of the virus belong to the genera *Culex* and *Aedes*. The primary wild bird species used in dead bird surveillance belong to the *Corvidae* family (20). Within the genus *Culex*, *Cx. pipens*, *Cx. restuans*, *Cx. salinarius* and *Cx. quinquefasciatus* have been the species most commonly associated with WNV transmission to mammals (21). Species of the genus *Culex* also commonly feed on horses, and *Cx. salinarius* has been shown to be an effective bridge vector of WNV to mammals. *Cx. pipens* is a species in which WNV might overwinter in temperate climates (22).

Once a mosquito species involved in WNV transmission is identified, it then becomes easier to determine likely environmental factors that support the vector’s survival. Several environmental factors have been associated with potential WNV

vectors, including close proximity to water and forest cover (23). Distance to livestock and unmanaged pastureland, and proximity to agricultural runoff, has been shown to support *Culex* species habitats (5). Other habitats that appear to support mosquitoes include water flooded habitats with dense vegetation cover that are close to existing lakes, and water containing pollutants from agricultural runoff and irrigation from crop land (24). Precipitation, canopy cover, and proximity to the forks of rivers have also been noted as important seasonal dynamics for possible WNV vectors, specifically in Texas (23). Finally, standing water in basins near forests and wetlands and other permanent water sources showed the greatest concentration of mosquito breeding habitats, whereas locations with moving water produced the least amount of breeding habitats (25).

Based on the previous research, environmental factors that should be considered in the analysis of the association between WN disease and location include: climate (particularly temperature, precipitation, rainfall); vegetation (including crops, grasslands, forests, canopy cover); hydrology (such as lakes, rivers, reservoirs, water basins); avian populations (bird breeding routes, migration flyways); and finally agriculture (crops, irrigated land, beef cattle farms, horse populations). The objective of this study was to identify environmental factors associated with hotspots of reported WN disease in equine populations in Texas during the period 2002–2004. A range of potential environmental risk factors will be analyzed for their association with defined hotspots of equine WN disease (Chapter II) and a map showing potential high-risk areas will be created, based on identified environmental factors.

Methods

A base map of Texas (Geographic Data System, North American Datum 1983) was imported into a Geographic Information System (ArcMap version 9.0. ESRI Inc.) using the Texas counties shapefile (Geographic Data Technology, Inc., ESRI). WN equine cases reported during 2002, 2003, and 2004 were projected using latitude and longitude coordinates. Next, a layer file of the primary and secondary hot spots (Chapter II) was added to the map to visualize areas of interest for environmental analysis. Data on hot spots by study years were merged into a single data base (ArcMap version 9.0 : Data Management Tools < General < Append). The input datasets were WN equine cases 2002 and 2003, while the target dataset was WN equine cases 2004. Append allows multiple input datasets to be combined into an existing target dataset to produce one dataset containing all of the data from the various input datasets. This allowed one analysis to be run on all three years of data.

While the primary and secondary hot spots show where equine WN disease rates were highest, it is helpful to know more about the equine population at risk in Texas. A table was created (Microsoft Excel) that contained county FIPS and equine population according to the 2002 census (United States Department of Agriculture). This table was joined to the Texas Counties shape file (ArcMap version 9.0: Data Management Tools < Joins < Add Join), with the layer name being Texas Counties, the join field being county FIPS, and the join table being TX Equine Population. This procedure joins a table to an existing layer based on a common field, so that the records in the layer and table are matched based on county FIPS when the values are identical. Next spatial analysis was

performed on the Texas Counties shape file using the joined equine population table to model the quantity or magnitude of point observations over a unity of area creating a continuous raster that shows the population per square kilometer (ArcMap version 9.0: Spatial Analyst < Density). Texas counties point data was the input data, and equine population was the population. The density type was simple, using square kilometers as the output area units to produce a density map of Texas equine populations.

Hydrology is an important environmental factor for mosquito population dynamics, so several hydrology metrics were added to the Texas shape files map. Hydrology shape files were downloaded from the United States Geological Survey website (www.mapping.usgs.gov) and imported into ArcMap version 9.0. Hydrology data were separated (ArcMap version 9.0: Selection < Select by attributes) into the following categories: lakes, rivers and streams, and reservoirs. These layers were exported as separate files.

The Euclidean (straight line) distance between each hydrology feature (lakes, rivers and streams, and reservoirs) and primary and secondary WN disease hot spots was calculated to determine the potential of hydrology features as environmental risk factors. The Euclidean distance determines how far each cell is from the nearest source. The source can be anything from a well to a road to a group of retail stores, or in this case a specific hydrology aspect (lake, river, or reservoir). This analysis was done using spatial analyst tools (ArcMap version 9.0: Spatial Analyst < Distance < Straight Line) the hydrology aspect (lake, river or reservoir) being the distance to field.

Another possible environmental risk factor for equine West Nile Virus is location or proximity to bird breeding sites, because wild birds are considered the reservoir for the

disease. A shape file of bird breeding survey routes was imported into ArcMap version 9.0 from the USGS Patuxent Wildlife Research Center via the National Atlas of the United States (nationalatlas.gov). The straight line (Euclidean) distance was calculated to determine the proximity or distance to bird breeding sites in Texas (ArcMap version 9.0: Spatial Analyst < Distance < Straight Line), with “distance to” being the bird breeding sites. Using the bird breeding site data, a map was created to display equine WN cases were within a 30 kilometer buffer of the bird breeding routes. A buffer creates polygons to a specified distance around an input feature, in this case bird breeding sites with a distance of 30 km, with an all dissolve command (ArcMap version 9.0: Analysis Tools < Proximity < Buffer). Then with the buffer, equine WN cases were chosen that intersected the buffered areas (ArcMap version 9.0: Selection < Select by Location < Select Features From = WNV Equine Cases < Intersect = Bird Buffer). Those cases in close proximity were exported and added to the existing map (ArcMap version 9.0: Display < Right Click WNV Cases < Data < Export Data < Add to Existing Map). Finally, a density map (ArcMap version 9.0: Spatial Analyst < Density) was created using bird buffer export as the input value with population as the field and square kilometers as the area units.

In previous research, farming and agriculture played an important role in the detection of WNV activity in certain areas (Hassan, 2004). To examine potential agricultural factors, several tables that contained census information on Texas farms were downloaded from the United States Department of Agriculture. All aspects of the census were put into one table (Microsoft Excel), identified by the county FIPS number, and then imported into ArcMap version 9.0. This table was then joined to the Texas counties

shape file (ArcMap version 9.0: Data Management Tools < Joins < Add Join) based on county FIPS. Density maps were created (ArcMap version 9.0: Spatial Analyst < Density) using the Texas counties shape file as the input data and the various agricultural features (farms, irrigated crops, cotton crop, beef cattle farms, and percentage of farms classified as crop farms) from the join as the population field with a simple density type and square kilometers as the output area units.

Along with hydrology features such as standing and moving water sources, precipitation and rainfall are also environmental factors that should be considered as potential risk factors for WN disease. Annual precipitation and rainfall shape files were downloaded from the U.S. Geological Survey website (mapping.usgs.gov) and imported into ArcMap version 9.0.

Additional hydrology features to consider as risk factors for WN disease are existing watersheds and aquifers, which might increase populations of mosquito vectors. Shape files were downloaded from the national atlas website with the originator being the USGS from a geological survey. Next the primary aquifers spatially connected to the two hot spot locations were selected (ArcMap version 9.0: Selection < Select by location < Select Features from = Tx aquifers < that intersect = Primary and secondary HS) and exported (ArcMap version 9.0: Display < Right click Tx aquifers < Data < Export Data < Add to existing map).

The final hydrology feature that was analyzed was the Texas river basins. Appropriate shape files were downloaded from the GIS Laboratory of the Texas Parks and Wildlife Department, Austin, Texas. Initially the shape file was displayed to show the locations of the various river basins located through Texas compared to WNV hotspot

locations. Then the river basin that ran through both the primary and the secondary hot spots was selected (ArcMap version 9.0: Selection < Select by location < Select Features from = Tx riverbasins < That Intersect = primary and secondary hot spots) and exported and added to the existing map (ArcMap version 9.0: Display < Right Click tx river basins < Data < Export Data < Add to existing map).

Additional environmental factors that were analyzed in the study were the natural land regions/soils, landcover, and vegetation associated with WNV hotspot locations. Shape files were downloaded from the Texas Parks and Wildlife Department GIS Laboratory and imported into ArcMap version 9.0. Then areas of both natural land regions and vegetation were selected (ArcMap version 9.0: Selection < Select by location < Select Features in = (tx land, vegetation) < Intersect = Primary and secondary hotspots), exported and added to the existing map (ArcMap version 9.0: Display < Right click (tx land, vegetation) < Data < Export Data < Add Data to Existing Map).

In addition to environmental factors, economic status of the human population in certain areas might be associated with the transmission of WNV. To analyze this factor, a table was downloaded from the U.S. Bureau of Census that contained information on the average income for each county in Texas. This information was then joined with the Texas counties shape file (ArcMap version 9.0: Data Management Tools < Joins < Add Join) with the layer being Tx counties and the table being Tx incomes with an input field of county FIPS. A density map was created (ArcMap version 9.0: Spatial Analyst < Density) with the input field of Tx counties and a population field of average annual income; density type was "simple".

The last environmental factors that were analyzed with regards to equine WNV transmission was the presence of existing migratory bird flyways. While mosquitoes are the vectors that transmit the virus, the reservoir for the disease is wild birds. Without birds, the virus is not maintained, making the presence of birds in any area an important risk factor. A shape file of the appropriate flyways was not identified but two images of north American migration flyways were located on the U.S. Fish and Wildlife Services website (www.usfws.gov) showing migratory patterns of wild birds.

All environmental factors that appear to increase the risk for equine WNV will be spatially analyzed and used to determine other potential hot spots of concern in Chapter IV.

Results

Texas counties point data was the input data, and equine population was the population. The density type was simple, using square kilometers as the output area units to produce a density map of Texas equine populations (Figure 12). The equine population density map does not appear to have a very strong correlation with either of the hot spot locations. A high population density of exists around the second hotspot near the Leon area, but appears to be less at the location of the hotspot. In the Lubbock county area there appears to be few equine in the vicinity of the hot spot, with only a slight increase in population density directly in the middle of the hotspot area.

The hydrology features density and distance to WNV hotspot maps showed some interesting patterns. The result was three maps showing distance to lakes (Figure 13),

rivers and streams (Figure 14), and reservoirs (Figure 15). In previous research, the proximity to lakes appears to be an important environmental factor in the transmission of WNV. The lakes around the secondary hot spot are extremely dense. The density of existing lakes increases dramatically in the center of the primary hotspot location near Lubbock. The distance to streams and rivers map shows a high concentration of rivers and streams across Texas with very little variation in the density, except for the north west panhandle area which appears to be void of rivers and streams. Although there are many rivers present at the secondary hot spot, there are none in and around the primary hot spot location. A high concentration of reservoirs exists across middle to eastern Texas, around the secondary hotspot, with very sparse reservoirs around the west and panhandle areas of Texas. There appears to be a few reservoirs near the southern edges of the primary hot spot, but not nearly as many as would have been expected. Despite expectations, reservoirs appear to have less impact on the transmission of WNV than do existing lakes.

Due to the importance of wild birds in the WNV transmission life cycle, it would be expected that relative close proximity to known bird breeding sites would have an impact on WNV transmission. A map was then created of the distance to bird breeding sites in Texas (Figure 16). The distance to bird breeding site maps appears to support this hypothesis. While the bird breeding sites are clustered throughout Texas, several clusters are located within both the primary and secondary hot spots, suggesting that this could be an important environmental risk factor. To further examine the proximity of WNV equine cases to bird breeding routes, a map was produced showing the density of cases that were within 30 km of the routes (Figure 17). This map also shows that both WNV

hotspots are closely associated with cases that were within proximity of the bird breeding sites.

Previous research had also shown a correlation between WN disease and farm land, agricultural irrigation and runoff. Several maps were created that show the locations of different agricultural features that could be important factors in the distribution of equine WNV (Figures 18 – 22). The farm density map shows high densities in both the primary and secondary hot spot locations. The primary location has a high density of farms compared to the surrounding areas. Examining the irrigated crop land map, again a strong correlation with the primary hot spot located in the Lubbock area was noted, with a slightly weaker correlation with the secondary hot spot near Leon. A map was produced displaying the percentage of crop-specific farms in Texas. This map shows a high density of crop farms at the primary hot spot location and, to a slightly lesser degree, at the secondary hot spot, suggesting crop farms are an environmental risk factor. Taking a closer look at specific crops, the density map of cotton crops shows a very high density in a few select areas. Those areas with high density cotton crops overlap the primary and secondary hotspots, with the highest density located in the Lubbock area. Beef cattle farms also appear to be associated with WNV hotspots, although not to the same extent as cotton crop farms. All agriculture and farm land factors appeared to be influential to the transmission of WNV to horses within Texas.

Despite findings from previous research, both annual rainfall and precipitation did not appear to be closely related to either hot spot location. Maps were then created to show trends in annual rainfall (Figure 23) and annual precipitation (Figure 24) in Texas. The secondary hotspot is located in a moderate to high precipitation and rainfall area.

However, the primary hotspot is located in one of the driest areas of Texas (discounting irrigation), suggesting that precipitation and rainfall are not as important in the transmission of WNV to horses as other environmental factors.

Elevation also does not appear to be an important environmental factor for the transmission of WNV to horses in Texas. Elevation shape files were also downloaded from the USGS website and imported into ArcMap version 9.0 to show possible elevation trends associated with the primary and secondary WNV hotspot locations (Figure 25). The primary hotspot is located in the middle of a high elevation area (3600 feet), while the secondary hotspot is located in the middle of a low elevation area (300 feet). There does not appear to be a strong correlation between the disease transmission and elevation.

Proximity to watersheds has been considered to have a positive influence on the transmission of WNV. After being imported into ArcMap version 9.0 a map was created that included the Texas watershed lines plotted against the WNV equine cases from 2002 – 2004, showing several trends of cases near watershed junctions (Figure 26). Then the Texas aquifers shape file was mapped with the primary and secondary hotspots highlighted (Figure 27). Examining the map of Texas watersheds overlapped with equine WNV case points it appears that most cases are located at or very near intersecting or junction points between watersheds. Therefore, proximity to watershed junctions appears to be an important factor in WNV transmission to horses.

The maps of Texas aquifers and ecological regions show some influence between WNV hotspots and the rock base in an area. A map was created showing the extent of the aquifers that were spatially important to the primary and secondary hotspots throughout Texas (Figure 28). The aquifers that intersect the two hotspots are both sand

aquifers, while the land regions that also intersect the WNV hotspots are mainly clay based. Although these features might be worth further study, the same features are present in other areas of Texas in which WNV hotspots were not identified.

River basins and catchment basins are a major feature of interest in WNV research because of their association with mosquito vectors. Initially the river basin shape file was displayed to show the locations of the various river basins located through Texas compared to WNV hotspot locations (Figure 29). The river basin was then displayed based on the various sub categories within that river basin and mapped along with the WNV hot spot locations (Figure 30). The maps produced suggest a strong connection between Texas river basins and hot spot locations. Both hotspots are situated on the same river basin, the Brazos watershed, that runs the length of the state from the panhandle to the Texas gulf coast at Galveston. Based on this association, together with the information gathered in Chapter II showing the changing location of mean cases of equine WN, this river basin appears to be an important factor in the spread and transmission of WNV to horses in Texas.

Vegetation, canopy cover, and ecological regions also showed interesting trends that supports previous research. Ecological regions of Texas (Figure 31) together with vegetation and landcover (Figure 32) were mapped with WNV hotspot locations to show the distribution of ground factors across Texas. The ecological region (Figure 33) and vegetation (Figure 34) areas were added to the Texas counties shape file together with the WNV hotspot locations to show the location and distribution of potentially important environmental factors. Either crop lands (primary hotspot) or dense vegetation with tree cover (secondary hotspot) were associated with hotspots. Both of these factors have

already been considered important risk factors in the transmission of WNV. The rock composition in both areas suggests that clay may be the best soil type for supporting WNV vectors.

Farm income was also related to the primary and secondary hotspot locations. A density map was produced that displays the average annual income in Texas by county (Figure 35). For both hotspots, income was much higher in the surrounding areas and reduced in the hotspot areas. Lower income areas seem to be at higher risk of WN equine disease than areas with higher average annual incomes.

Finally, wild bird migratory flyways appear to have a strong influence on WN in both of the hotspot locations in Texas. The Central flyway runs through Texas from north to south. A shape file of the appropriate flyways was not identified but two images of north American migration flyways were located on the U.S. Fish and Wildlife Services website (www.usfws.gov) showing migratory patterns of wild birds (Figure 36). These images were compared to the Texas shape file and WNV hot spot locations by inserting images while in the layout view of ArcMap version 9.0 (Figure 37). Examining more closely the overlap of the Texas counties shape file with the hotspots, this major flyway and one of the principal routes passes directly through or alongside a hotspot. The primary hotspot appears to have one of the principal routes of migratory birds pass through its southeast corner, while the secondary hotspot appears to have the major flyway pass through its center. Migratory bird flyways seem to be associated with WNV transmission.

Discussion

Based on the results of the environmental analysis, risk factors that appear to be associated with the transmission of WNV to horses are: proximity to lakes, location with respect to bird breeding sites and migratory flyways, proximity to farmland (particularly crop farms), location within the Brazos watershed river basin, and vegetation and soil types with tree cover and a clay base.

Lakes, unlike rivers and streams, do not experience much flow or current. While rivers and streams move and refilter water and water content constantly, lakes are existing standing water sources. It would be expected that larger mosquito populations will exist around standing water. Therefore, horses in close proximity to lakes that have suitable mosquito habitats are expected to be at a higher risk of developing WN disease.

Mosquitoes are not the only important part of the transmission cycle of WNV. Wild birds are the reservoir. Proximity to sites where mosquitoes are feeding on wild bird populations should increase the risk of WN disease in equine populations. Horses that are located closer to bird breeding sites and migratory flyways are at a greater risk of being bitten by infected mosquitoes than horses located farther away from such sites.

Previous research and the results of this study suggest a strong association between farms and crop land and equine WNV disease. Proximity to farms with cattle and other livestock could be a risk factor because mosquitoes (along with flies) tend to congregate around livestock where they can feed on the animals. There are also often various sources of stagnant water (for example, water troughs). Reasons for a higher risk

near crop lands could be in large part be due to the agricultural runoff and stagnant water created by the irrigation systems on the farms.

Many WN equine cases appear to have followed the migration along the Brazos watersheds river basin which stretches from the panhandle down to Galveston coast. Possible explanations for this could be that birds that were first infected with the virus in Texas came from northern states and entered Texas infecting mosquitoes near the head of this river basin. As the years progressed, infected mosquitoes occurred more commonly along this river basin south east toward the coast where there is more precipitation and vegetation cover. Location on the brazos river basin appears to put horses at a higher risk of WNV disease than being located on other river basins.

The last factor that appears to have an effect on WN disease is location within low income economic areas. There are two commercially-available vaccinations to prevent WNV infection in horses. If a horse is located in a low income area the likelihood that the owner is able to purchase and pay for vaccine administration might be dramatically decreased, leaving horses unprotected and increasing their risk of disease.

Based on the findings with regards to environmental risk factors, risk maps will be presented in Chapter IV that will show areas at high risk for equine WN disease cases due to proximity to certain environmental risk factors.

CHAPTER IV

CREATING WEST NILE VIRUS RISK MAPS FOR HORSES IN TEXAS

Introduction

Vector borne diseases tend to be limited to specific geographic locations, presumably because of one or more causes. This spatial variation tends to arise from variations in the environmental and geographical features of specific locations. Often these variations are directly related to the conditions that support the vectors and reservoirs that maintain and spread the pathogen (26). If the environmental factors that promote transmission of the disease can be identified, then those factors may be used to map areas of high risk of disease.

Spatial analysis in the geographic information system (GIS) can be used to show the relationships between vectors and hosts and their geographical environment, as well as to identify areas in time and space where exposure may occur (27). Previous research has been undertaken to map disease occurrences and the environmental features that contribute to the spread of disease (spatial epidemiology). Interpolation techniques are prominent in such research (28). In this research, dead and infected wild birds and the distribution of wild bird populations was used to weight the disease risk assessment. Both bird population distribution and bird habitats were used in mapping of the environmental and social determinants of human risk during a West Nile Virus outbreak in the greater Chicago area, 2002 (18).

Using environmental features known to be important in the spread of a disease, it is possible to use interpolation to generate maps and identify areas where the risk of disease is higher than other locations. Risk maps can be created for particular geographical areas because the probability of transmission declines dramatically with distance from an infected host (26). Vector borne infectious disease have a strong link to landscape features and GIS can be used to classify habitats in an attempt to understand associations with infectious diseases (29). Being able to layer environmental risk factors, such as those described in Chapter III, and then using interpolation methods allows the creation of risk maps. The risk maps produced allow prediction of the disease in specific areas, and allow preventive measures to be implemented in areas of high risk to reduce the impact of the disease on the population.

Methods

First, a base map of Texas (Texas counties shapefile; Geographic Data Technology, Inc., ESRI) was imported into a GIS (ArcMap version 9.0. ESRI Inc.), together with WN equine cases reported during the period 2002 – 2004. Next, environmental factors were added that had been identified as risk factors in Chapter III and in previous research. Sentinel and wild bird WNV positive case and WNV positive mosquito case shapefiles were downloaded from the National Atlas of the United States website (www.nationalatlas.gov). These shape files were imported into ArcMap version 9.0 and displayed on the base map at a global level. To identify points specific for Texas, the cases were selected (ArcMap version 9.0: Selection < Select by Attributes < Select

from = BirdCases, MosCases < Attribute = STATE = Texas) and then exported and added to the map (ArcMap version 9.0: Display < Right click BirdCases, MosCases < Data < Export Data < Add to existing map). The global points were removed from the map (ArcMap version 9.0: Display < right click BirdCases, MosCases < Remove).

Once the wild bird and mosquito WNV cases were added to the Texas map, density maps were created for each risk factor. Options had to be set for the spatial analyst tool (ArcMap version 9.0: Spatial Analyst < Options < Mask = Texas Counties < Display extent same as = Texas Counties), then density maps were created for wild bird cases and mosquito data (ArcMap version 9.0: Spatial Analyst < Density), with cases as the population and simple density type. Density analysis estimates the quantity or magnitude of point observations over a unity of area creating a continuous raster that shows the population (or cases) per square kilometer.

According to previous research, proximity to existing wild bird WNV cases and WNV positive mosquito collections increase the risk of a human testing positive for WNV. These risk factors, together with environmental factors identified in Chapter III, that appear to effect the risk of disease need to be overlaid to develop a risk map for WN disease in horses.

WN equine cases from 2002 to 2004 that were located within 30 km of WNV positive mosquito collections were selected (ArcMap version 9.0: Selection < Select by Location < Select from = WNV Cases < Within a Distance of = 30 km of MosCases). The selected cases (independent of date) in close proximity to WNV positive mosquito collections were then exported and added to the existing map (ArcMap version 9.0: Display < Right Click = WNV Cases < Data < Export Data < Add to Existing Map).

Next, WN equine cases reported between 2002 and 2004 that were located within 30 km of existing wild bird cases were selected (ArcMap version 9.0: Selection < Select by Location < Select from = WNV Cases < Within a Distance of = 30 km of BirdCases). The selected equine cases in close proximity to bird cases (independent of date) were then exported and added to the existing map (ArcMap version 9.0: Display < Right Click = WNV Cases < Data < Export Data < Add to Existing Map). To focus on areas at greater risk of WNV, equine cases were selected that were within 30 km of both WNV positive mosquito collections and wild bird WNV cases (ArcMap version 9.0: Selection < Select by Location < Select From = WNVmos30 < Intersect = WNVbird30). These selected cases were then exported and added to the existing map.

Another risk factor that appears to be important for the spread of WNV in previous research and in Chapter III analysis, is the proximity to lakes. The hydrology lakes feature shape file created in Chapter III from the United States Geological Survey (mapping.usgs.gov) data was added to the Texas counties map. The equine WN cases that were within 30 km of Texas lakes were selected (ArcMap version 9.0: Selection < Select From = WNVcases < Within a Distance of = 30 km of TxLakes), exported, and added to the existing map (ArcMap version 9.0: Display < Right Click = WNVcases < Data < Export < Add to Existing Map).

Proximity to WNV positive mosquito collections, WNV wild bird cases and existing lakes are probably the most important risk factors for the transmission of WNV to equine populations. WN equine cases located within 30 km of lakes and WNV positive mosquito collections and WNV wild bird cases were combined to show the highest risk areas that were within 30 km of all three of these factors (ArcMap version

9.0: Selection < Select by Location < Select from = WNVmosBird30 < Intersect = WNVlakes30). These selected cases were then exported and added to the existing map (ArcMap version 9.0: Display < Right Click = WNVmosBird30 < Data < Export Data < Add to existing Map).

Using environmental risk factors, areas of potential high risk of equine WN disease were selected. Environmental factors were converted into raster data (ArcMap version 9.0: Spatial Analyst < Convert < Feature to Raster < Feature Class = birdbreed, aquifers, riverbasins, vegetation, landreg, lakes, MosCases, BirdCases, TXFarms, Income < Field = routeleng, Name, Basin, Cover, Name, Ftype, Cases, Cases, Ftype, AnnIn). Calculations were then performed to select only areas of high environmental risk (ArcMap version 9.0: Spatial Analyst < Raster Calculator < BB = [Distance to Bird Breed] <= 30 km; Aqu = [TxAqu] == Texas Coastal Uplands | [TxAqu] == High Plains; RB = [TxRiverBasins] == Brazos Headwaters; Veg = [TxVeg] == Crops | [TxVeg] == Forest cover; LR = [TXlr] == High Plains | [TXlr] == Oakwoods Forest prairie land; L = [Distance to Tx Lakes] <= 30 km; MC = [Density of MosCases] >= 1; WB = [Density of WildBirdCases] >= 1; F = [Distance to Texas Farms] <= 30 km; I = [CensusIncome] <= 20,000 < Evaluate). The resulting data base described areas at which all environmental risk factors were present (raster cell = 1) and areas where one or more of the environmental risk factors were absent (raster cell = 0).

Areas that had all of the environmental risk factors in common with the WNV hotspots were selected (ArcMap version 9.0: Spatial Analyst < Raster Calculator < HSHighRisk = [BB] & [Aqu] & [RB] & [Veg] & [LR] & [L] & [MC] & [WB] & [F] & [I] < Evaluate). The process was then repeated for environmental aspects that are appear

to contribute to a high risk of disease transmission regardless of whether it was located in hot spot areas or not (ArcMap version 9.0: Spatial Analyst < Raster Calculator < $\text{HighRisk} = [\text{BB}] \& [\text{Veg}] \& [\text{L}] \& [\text{MC}] \& [\text{WB}] \& [\text{F}] < \text{Evaluate}$).

High risk values were assigned to WN positive equine cases, based on the presence of all environmental risk factors considered to be associated with WNV hotspots (Chapter III). A field was created in the equine WNV cases 2002–2004 data base for the high risk value (ArcMap version 9.0: Display < Right Click WNVcases < Open Attribute Table < Options < Add Field < Name = Risk Value < Type = Short Integer). For each environmental risk factor, WN cases were selected (ArcMap version 9.0: Selection < Select by Attributes/Location < Select From = WNVequineCases < That Intesect/Are Within a Distance of = (birdbreed, aquifers, riverbasins, vegetation, landreg, lakes, MosCases, BirdCases, TXFarms, Income)). A risk value was assigned to the selected cases based on their proximity to the various risk factors (ArcMap version 9.0: Display < Right Click = WNVCases < Open Attribute Table < Right Click = Risk Value < Field Calculator < = Risk Value + 5 (for lakes, mosquitocases, and birdcases) or + 2 (for all other factors)). By performing calculations when the various risk factors were selected, a cumulative risk score was derived that include several different risk factors. For example, an area that is only close to the important aquifers would have a risk value of 2, whilst an area that is close to aquifers, vegetation, and lakes would have a risk value of 9 (2+2+5). This process was repeated using only risk factors (lakes, income, irrigated crop farmland, mosquito cases, wild bird cases, vegetation of crops and dense forestation, and proximity to bird breeding sites) considered to be strongly associated with WNV disease

risk. Lakes, WNV positive mosquito collections, and WNV wild bird cases were given greater weighting in this scheme.

Using the risk values that were calculated, interpolation was performed using the kriging method (ArcMap version 9.0: Geostatistical Analysis < Geostatistical Wizard < Kriging < Data Set1 Input Data = WNVcases < Attribute = Risk Value < Next < Ordinary Kriging Prediction Map < Transformations = None < Major Range = .656, Partial Sill = .0906, Nugget .00499, Number of Lags = 12 < Next < Neighbors to include = 5 at least 2 < Finish). Parameters were determined through use of semivariograms of the case distributions. Kriging allows graphical investigation of spatial autocorrelation by using statistical models and creating isopleth maps of predicted values and the error of predictions. The two maps resulting from the raster calculations and the kriging interpolation show areas that are at high risk of equine WN disease.

Results

Locations proximal to lakes, WNV positive mosquito collections and wild bird WNV cases appeared to be at increased risk of factors of WNV. Maps were created that showed the density and distribution of WNV wild bird cases (Figure 38) and WNV positive mosquitoes (Figure 39) in Texas. Maps were created to show the distribution and location of equine WNV cases that were within 30 km of WNV positive mosquito collections (Figure 40), WNV bird cases (Figure 41), and that were within 30 km of both WNV positive mosquito collections and bird cases (Figure 42). A map was created showing the location and distribution of WNV equine cases from 2002 to 2004 that were

located within 30 km of Texas Lakes (Figure 43). A map was then created showing the location and distribution of equine WN cases located within 30 km of WNV positive mosquito collections, WNV wild bird cases, and existing lakes in Texas (Figure 44). These maps corresponded well with the locations of the 2002 – 2004 equine WNV case hot spots identified in Chapter II.

WNV equine risk maps were created using raster calculator methods and kriging interpolation of risk scores to identify high risk areas. Using raster calculator a few very small areas were selected that contained these risk factors and a map was made of these locations (Figure 45). Distinct areas that contained all of the above risk factors and therefore were considered locations at high risk of equine WNV disease were located and mapped in raster calculator (Figure 46). This procedure was repeated for hotspot environmental factors and for risk factors (using ordinary kriging method), to create maps of high risk areas for locations sharing environmental risk factors with the hotspot location (Figure 47) and for locations sharing environmental risk factors that might increase the risk of equine WNV disease (Figure 48). Both risk maps created showed areas of high risk in approximately the same locations; the raster calculator form was more restrictive since it did not allow for areas that have some, but not all, risk factors. The kriging interpolation identified high risk areas that included all, some, few, or none of the important environmental risk factors present.

The raster calculator method using all attributes the same as those found in the hotspot areas identified several different high risk areas. In the panhandle areas were found in the primary hot spot and Deaf Smith county, areas found in north central Texas were Wichita, Archer, and Dallas counties. East Central Texas had high risk areas

located in the secondary hot spot with areas also in Harris and Galveston counties at the coastline. Finally, in south Texas high risk areas were found in Bexar, Cameron, and Nueces counties (Figure 45).

The second analysis performed with the raster calculator method used only environmental factors that were deemed important for the transmission of WNV regardless of factors found in the hotspot areas. A section of the panhandle was deemed a high risk area from Dawson county across the primary hotspot and up to Deaf Smith county. North central and eastern Texas showed several areas at high risk including Wichita, and Comanche counties and a block from Dallas across the secondary hotspot down to Brazos county. Finally, the coastline showed high risk areas in Fort Bend, Harris and Galveston counties and south Texas had areas in Bexar, Nueces, and Cameron counties (Figure 46).

Kriging interpolation of environmental factors associated with the primary and secondary hot spots showed two large areas of high risk for WNV transmission to horses. The first area of high risk was shown to be the panhandle area from Martin north up to Oklahoma. The second area of high risk was located in eastern Texas in a diagonal block from Van Zandt stretching south west across the secondary hot spot to Lee county (Figure 47).

Another analysis was performed with the kriging interpolation of environmental factors deemed important in the transmission of WNV regardless of whether they were found at the hot spot locations or not. The areas found with this technique were very similar when the same factors were used in the raster calculator technique used previously. The only risk area found in the panhandle area was the primary hot spot with

areas of high risk in the north in Wichita and Dallas counties. Central and east central Texas showed high risk locations at Erath and Comanche counties and the secondary hot spot including Brazos county. Finally, along the coastline high risk areas were found in Montgomery, Harris, and Galveston counties and Bexar, Nueces, and Cameron counties in the south (Figure 48).

Discussion

The most important spatial risk factors for equine WN disease in Texas are locations close to lakes and proximity to WNV positive mosquito collections and WNV wild bird cases. Other important factors are proximity to wild bird breeding sites, dense and forested vegetation cover, and proximity to farms with irrigated crop land.

Maps created using these risk factors consistently identified WNV hot spot locations in all of the analyses. Other locations that are high risk for equine WNV exist outside of the hot spot areas. Areas that were consistently identified along with the primary and secondary hot spots were Wichita, Dallas, Brazos, Harris, Galveston, Bexar, Nueces, and Cameron county areas.

While the reasons for WNV to be present in these areas are explained by the environmental factors used to determine these spots, there is a question of why they were not found in the analysis that identified the primary and secondary hotspots.

All of the areas found outside of the hotspots were located in high equine populated locations and all but Nueces and Cameron counties were also located in high income areas. Meanwhile, the primary and secondary hotspots were located in lower

populated horse areas with low annual incomes. The fact that these spots are located in high equine population areas could mean that equine farms are located in these areas where owners may be more likely to vaccinate horses than backyard owners would. Also, being located in areas of higher annual incomes also suggests that horse owners may be more inclined to vaccinate their horses because the cost may not be as much of a deterrent than it would be for lower income owners.

The 2002 data showed the main focus of WNV to be in the panhandle while its mean center shifted south east toward the gulf coast in subsequent years. When the SaTScan was calculated a large emphasis was given to the year 2002 because it was the first appearance of WNV and therefore was weighted higher with more cases, and lesser emphasis given to 2004 which had far fewer cases but included the areas in the south and eastern parts of the state. By the time the disease had spread to the vectors and reservoirs located near the other potential areas of high risk found in this chapter this study of equine cases had come to a conclusion. If this study were to be conducted again starting in 2004 using cases up to 2007, it may reveal that the potential areas of high risk were indeed hot spot locations as well.

Finally, the reporting of WN equine cases is done on a voluntary basis and not all cases are going to be reported or captured by this research. Some cases may exist and not be reported and some cases may exist and be misdiagnosed as another disease or encephalopathy. Misreporting of cases could explain why certain areas that are considered at high risk for transmission of WNV do not present as hotspots. Such areas should also be considered for further study and/or for the implementation of disease prevention.

CHAPTER V

SUMMARY AND CONCLUSIONS

Study results suggest that cases of equine disease due to West Nile Virus (WNV) infection in Texas, based on the reported dates of disease onset, tended to change during the period 2002 to 2004. In 2002, the mean center of cases was in north central Texas (Palo Pinto county). In 2003, the mean center had moved approximately 104 km south-east (Bosque county), and by 2004 the mean center was located in eastern Texas (Grimes county), approximately 340 and 232 km south east of the 2002 and 2003 mean centers, respectively. In addition, the case density maps show a progression of cases across Texas in a southeastern direction, starting in the panhandle in 2002 and ending up near Galveston by 2004. The progressive (2002 to 2004) earlier occurrence of cases in the eastern parts of Texas suggests that these cases occurred within environments that promote the transmission of WNV within reservoir species and possibly between reservoir species and susceptible species such as horses. Such risk factors could include: habitats that promote WNV infected mosquito populations (including wetlands, lakes, rivers, reservoirs, and stagnant water sources); locations with a sufficiently large reservoir species to sustain a WNV transmission cycle; and areas that have a large equine population that may not have been adequately vaccinated against WNV.

WNV was first introduced into the U.S. in the vicinity of New York City and Long Island, NY. It progressively spread in a southern and western direction during the

period 1999 to 2002, when it first entered Texas. The first and earliest equine cases that were reported from Texas were located in eastern parts of the state and in the Texas panhandle. In the panhandle, WNV infection most likely spread via wild bird migratory flyways from northern states (such as Oklahoma and Nebraska) where WNV was already endemic. If the Texas panhandle was one of the first sites of introduction, this would explain why so many cases occurred in this region during 2002. During the following year, the mean disease center moved in a southeastern direction: this possibly brought WNV into areas where mosquitoes and horses were more concentrated because of land use and habitat features. There are very few rivers that run through the panhandle area, compared to the density of rivers in central and eastern Texas. Again in 2004 there was another more dramatic movement and concentration of WN cases in southeastern Texas near Galveston and the Gulf Coast. The shift in this distribution could again be explained by prime habitat for mosquitoes located in coastal and subcoastal regions of Texas: many more stagnant water sources exist in this region than elsewhere in Texas and the humidity and precipitation levels are much higher. Another possible cause of the spatial shift in cases could be the practice of administering equine vaccinations. When cases first occurred in the Texas panhandle in 2002, owners may have been inclined to start vaccinating their horses whereas the need would not have seemed so great for owners in other parts of central and southern Texas where the risk did not seem as great. As the virus spread across the state, equine owners may have chosen to vaccinate their horses after large numbers of horses in their area were affected, thus resulting in fewer cases in these areas in the following year. This would also explain the results of the hi/low

clustering G statistic, in which a greater concentration of high case numbers were found in 2004, compared to the previous study years.

A primary hotspot location was located with the scan statistic; it included Cochran, Hockley, Lubbock, Terry, Lynn, and Garza counties. A secondary hotspot was located, including Freestone, Limestone, Leon, and Robertson counties. Several other analytical methods (case density maps, hotspot analysis with rendering, and kriging of attack rates) that these locations had higher rates of equine WN disease than other areas of Texas. Although cases gradually shifted away from the Texas panhandle during the period 2002 to 2004, with very few cases occurring west of Mills county, case density maps highlighted a concentration of cases within this primary hot spot location. Reasons for the occurrence of these hot spots could include: environmental habitats that are ideal for the mosquitoes that spread WNV (hydrology, landscape, and climate factors); areas of elevated equine populations, compared to other counties; or a failure of owners to administer the WNV vaccine, for various reasons (economic, fewer veterinarians offering the vaccine in that area), to protect their horses.

Based on the results of the environmental analysis, risk factors that appear to be associated with the transmission of WNV to horses include: proximity to lakes, proximity to bird breeding sites and a location within a migratory flyway, proximity to farms, particularly crop farms, location within the Brazos watershed river basin, and tree cover vegetation with a sand base soil type.

Lakes, unlike rivers and streams, do not experience much flow or current. While water is refiltered and water content is replaced constantly in rivers and streams,

lakes are permanent, standing water sources. Given the breeding habits of mosquitoes, standing water (rather than moving water), is likely to be the habitats where vectors might be found. More mosquito habitats mean an increased risk that a susceptible horse is bitten by a mosquito that might be infected with WNV. Therefore, horses in close proximity to lakes are likely to be at a higher risk of being infected with WNV and developing disease than horses located farther away from lakes.

Mosquitoes are not the only important part to the transmission cycle of WNV. Wild birds are the reservoir of WNV, so proximity to birds and bird breeding sites is likely to be a risk factor for WN disease, since these areas would have larger mosquito populations that are feeding on birds that can act as carriers of the virus. Horses that are located closer to bird breeding sites and within migratory flyways are at a greater risk of being bitten by WNV-infected mosquitoes than horses that are farther away from such sites.

Previous research and the results of this study suggest that farms and crop land have a strong effect on the transmission of WNV. Proximity to farms with cattle and other livestock could be a risk factor for equine WNV disease because mosquitoes (along with flies) tend to congregate around livestock where they can feed on the animals and have various sources of stagnant water (such as water troughs) as potential breeding sites. Reasons for a higher WN disease risk near crop lands could in large part be due to the agricultural runoff and stagnant water created by the irrigation systems at the farms. Horses located near farms with agricultural runoff from irrigation systems and with other stagnant water sources are at a higher risk of WNV disease than horses isolated from such spatial risk factors.

Most of the WN equine cases reported between 2002 and 2004 appear to have been located within the Brazos watersheds river basin, which stretches from the Texas panhandle to the Texas gulf coast around Galveston. Possible explanations for this could be that mosquito or wild bird reservoir species that were first infected with WNV in Texas came from northern states and entered Texas near the head of this river basin. Over time, infection might have spread (via wild birds) to mosquito populations along this river basin in a south east direction toward the coast, where conditions (rainfall, precipitation, and vegetation cover) are more conducive to mosquito breeding and survival. Location on the Brazos river basin appears to increased the risk that horses are infected by WNV, compared to being located on other river basins.

The last factor that appears to increase the likelihood of equine WN disease is locations within low agricultural income areas. The reason for this initially is not obvious. There are two WNV vaccines that are commercially available to protect horses against WN disease. If a horse is located in a low income area, the likelihood that the owner will pay for their horse to be vaccinated against WNV might decrease dramatically. Thus, horses located in such areas might be much more likely to susceptible to WN disease, even if the risk of WNV infection is constant.

The most important spatial risk factors for equine WN disease in Texas are locations close to lakes and proximity to WNV positive mosquito collections and WNV wild bird cases. Other important factors are proximity to wild bird breeding sites, dense and forested vegetation cover, and proximity to farms with irrigated crop land and agricultural runoff.

Maps created using these risk factors consistently identified WNV hot spot locations in all of the analyses. Other locations that are high risk for equine WNV exist outside of the hot spot areas. Areas that were consistently identified along with the primary and secondary hot spots were Wichita, Dallas, Brazos, Harris, Galveston, Bexar, Nueces, and Cameron county areas.

While the reasons for WNV to be present in these areas are explained by the environmental factors used to determine these locations, there is a question of why they were not found in the analysis that found the primary and secondary hot spots.

All of the areas found outside of the hotspots were located in high equine populated locations and all but Nueces and Cameron counties were also located in high income areas. Meanwhile, the primary and secondary hotspots are located in lower populated horse areas with low annual incomes. The population factor could mean that equine farms are located in these areas where owners may be more likely to vaccinate horses than backyard owners would and the higher incomes also suggest that horse owners may more inclined to vaccinate their horses because the cost may not be as much of a deterrent than it would be for lower income owners.

With the SaTScan analysis performed to determine the locations of the primary and secondary hotspots data from all three years, 2002 – 2004, were included in the analysis. The 2002 data showed the main focus of WNV to be in the panhandle while it migrated south east toward the gulf coast in subsequent years. When the SaTScan was calculated a large emphasis was given to the year 2002 which had the most cases, it was the first recognition of WNV, with lesser emphasis given to 2004 which had far fewer cases but also included the areas in the south and eastern parts of the state. By the time

the disease had spread to the vectors and reservoirs located near the other potential areas of high risk found in this research this study of equine cases had come to a conclusion. If this study were to be conducted again starting in 2004 using cases up to 2007, it may reveal that the potential areas of high risk were indeed hot spot locations as well.

Finally, the reporting of WN equine cases is done on a voluntary basis and not all cases will be reported or captured in this research. Some cases may exist and not be reported and some cases may be misdiagnosed as another disease or encephalopathy. Misreporting of cases could explain why certain areas that are at high risk for transmission of WNV do not present as hot spots while using positive cases to determine the hot spots. Such areas should be considered for further study and/or for the implementation of disease prevention.

Further research needs to be undertaken within the two hot spot locations identified to validate hyperendemic WNV transmission in these areas. Mosquito traps should be used in these locations to determine the species that might be involved in transmitting WNV to these equine populations. Unvaccinated horses should be serologically monitored (WNV IgE and IgG ELISAs) to determine the level of herd immunity and the history of exposure.

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APPENDIX

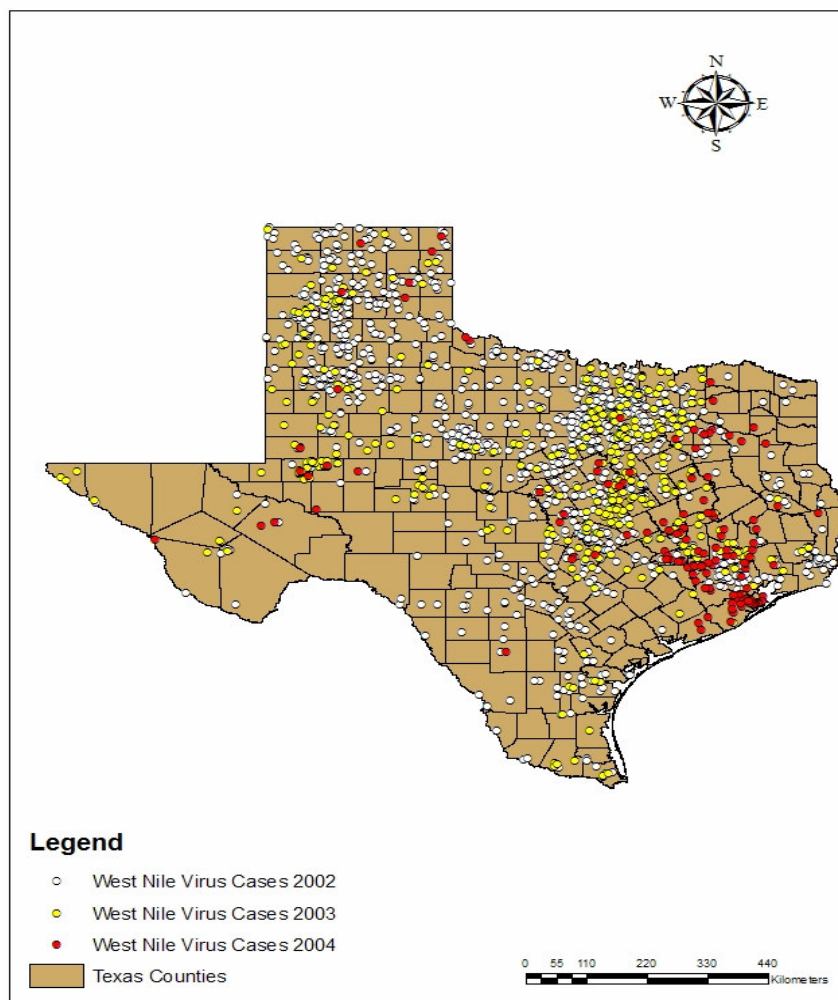


Figure 1

Map of Equine West Nile Virus Cases in Texas from 2002 to 2004

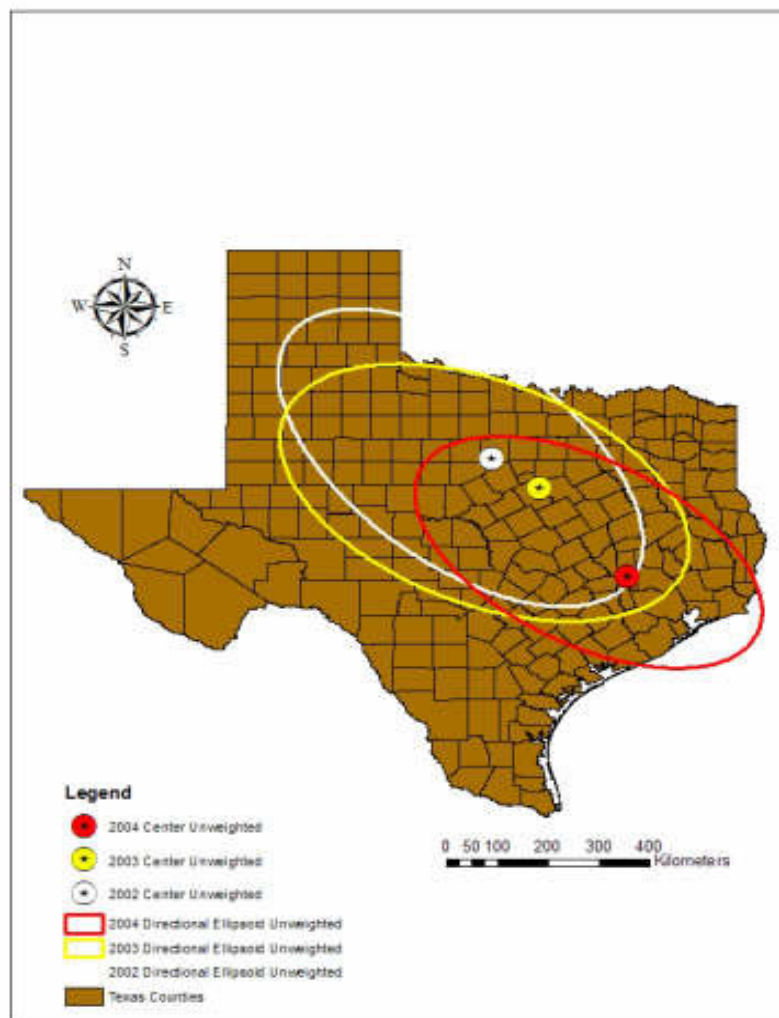


Figure 2

Map of Mean Centers and Directional Ellipsoids for 2002 – 2004, Unweighted

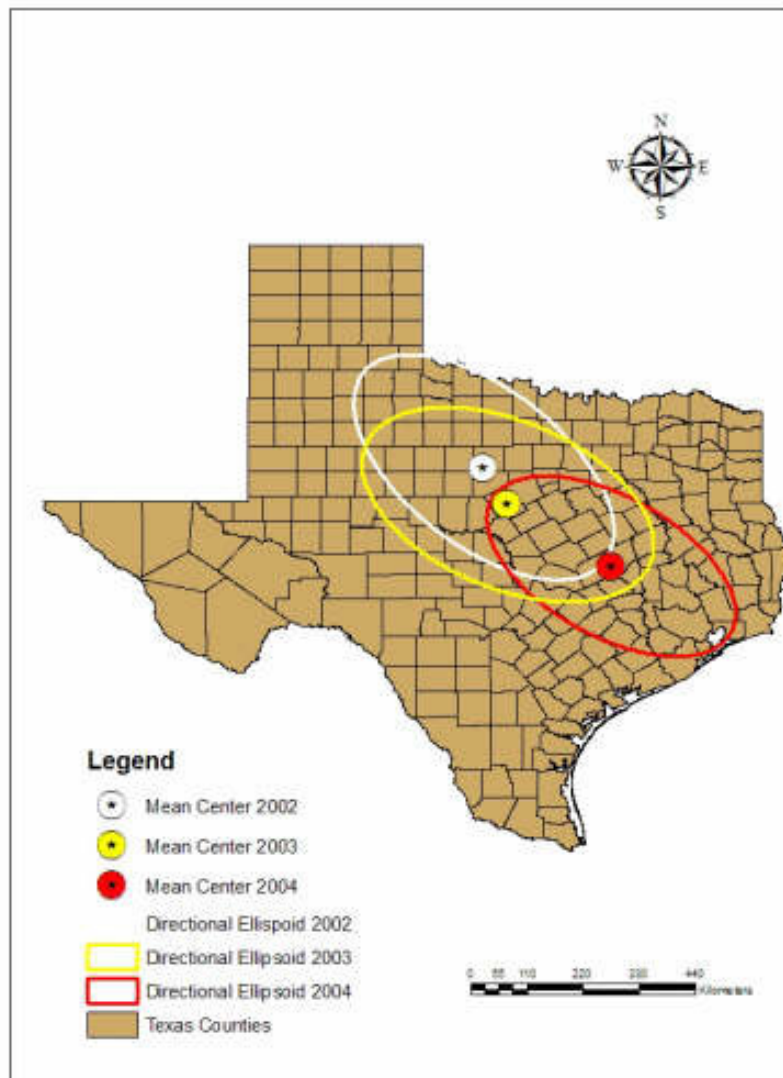


Figure 3

Map of Mean Centers and Directional Ellipsoids for 2002 – 2004, Weighted by DO

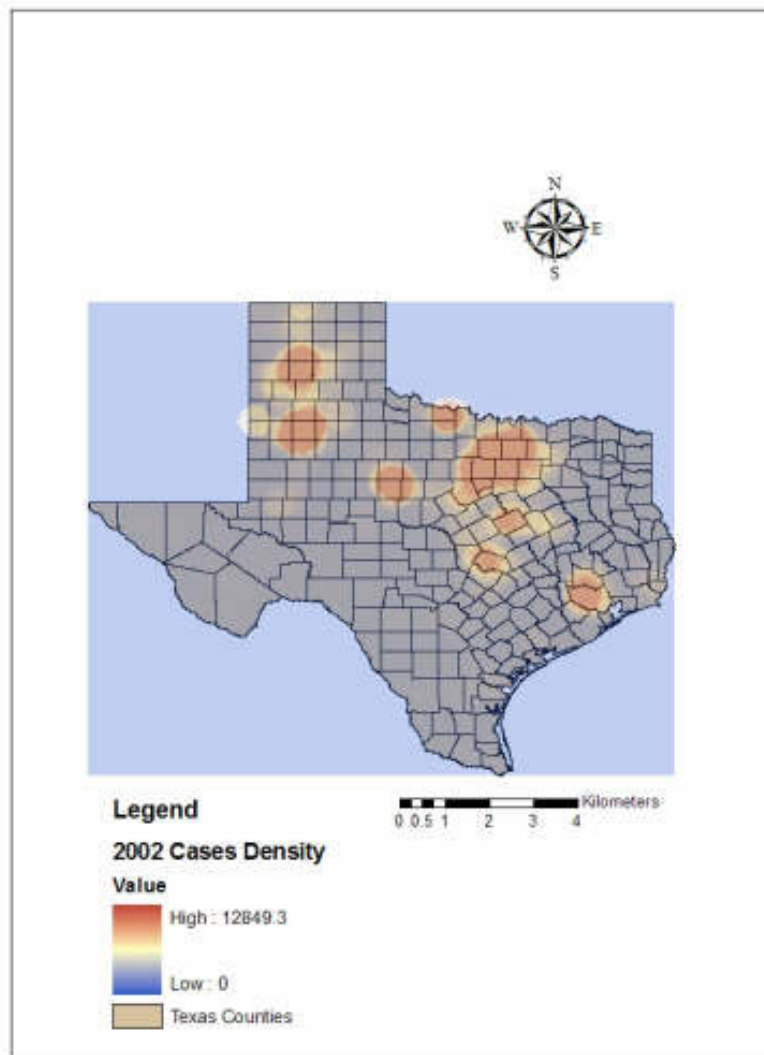


Figure 4

Equine West Nile Virus Case Density Map 2002

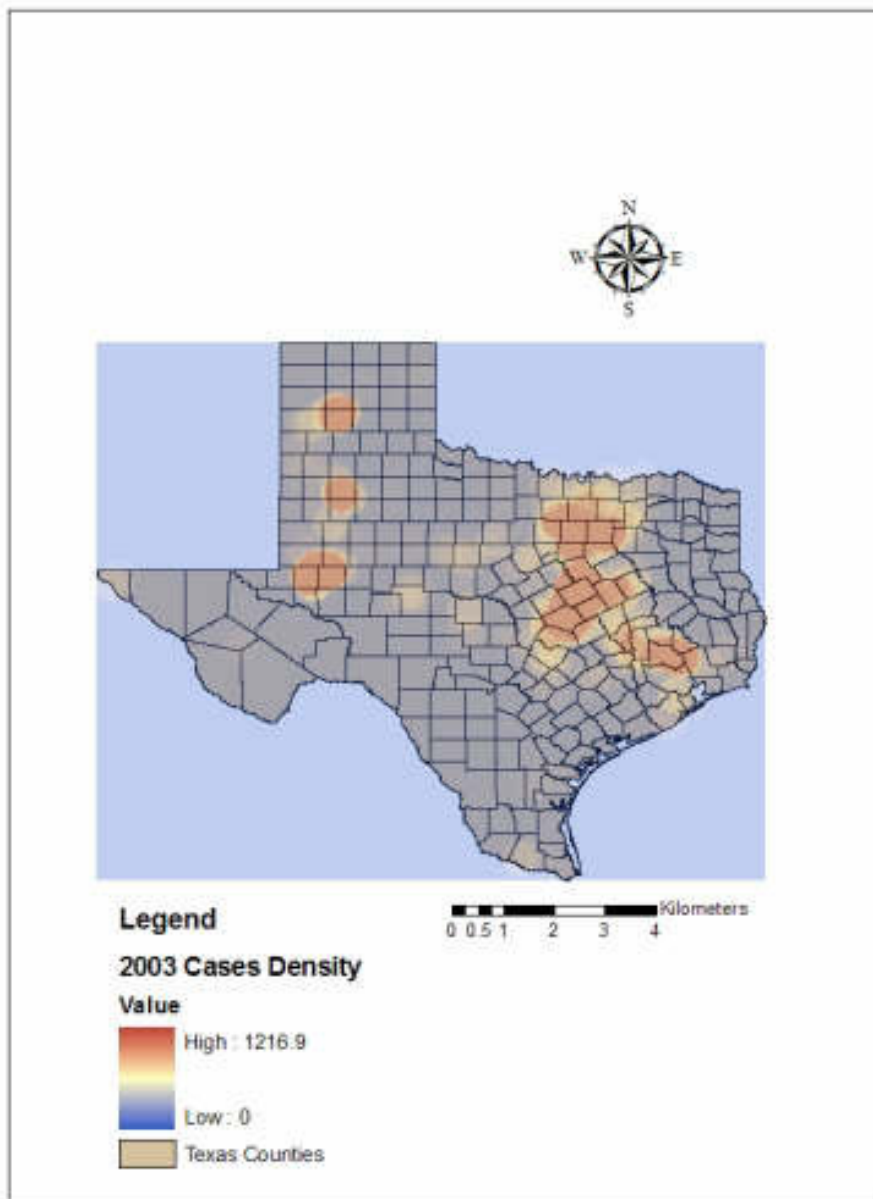


Figure 5

Equine West Nile Virus Case Density Map 2003

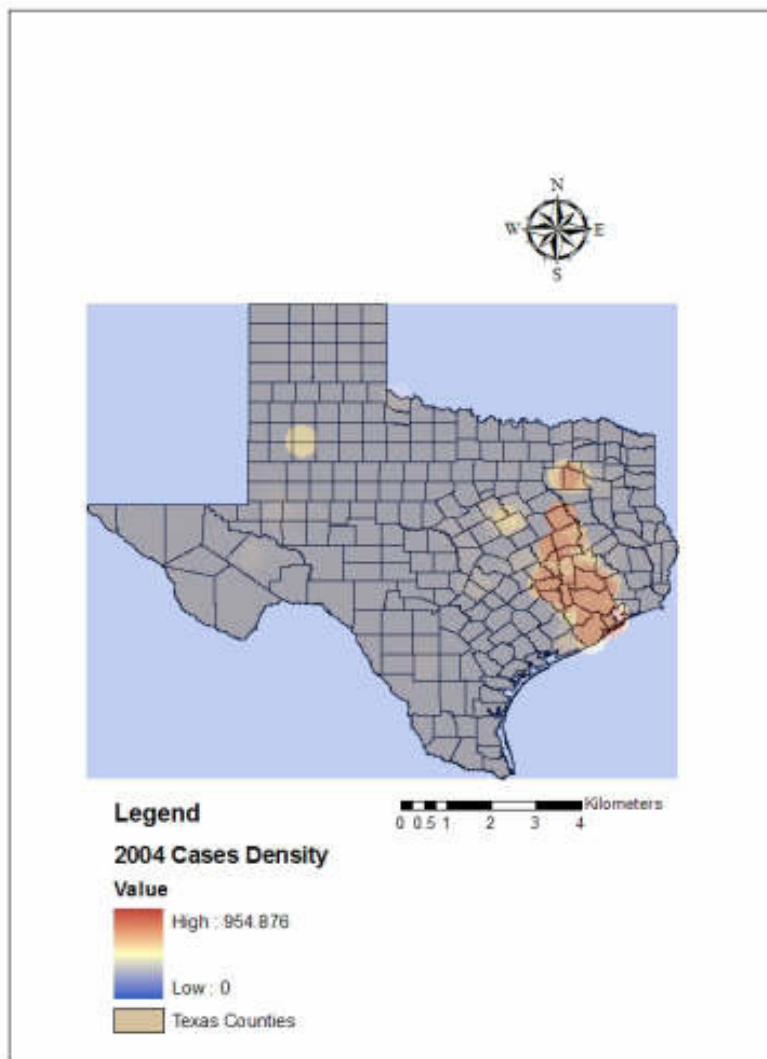


Figure 6

Equine West Nile Virus Case Density Map 2004

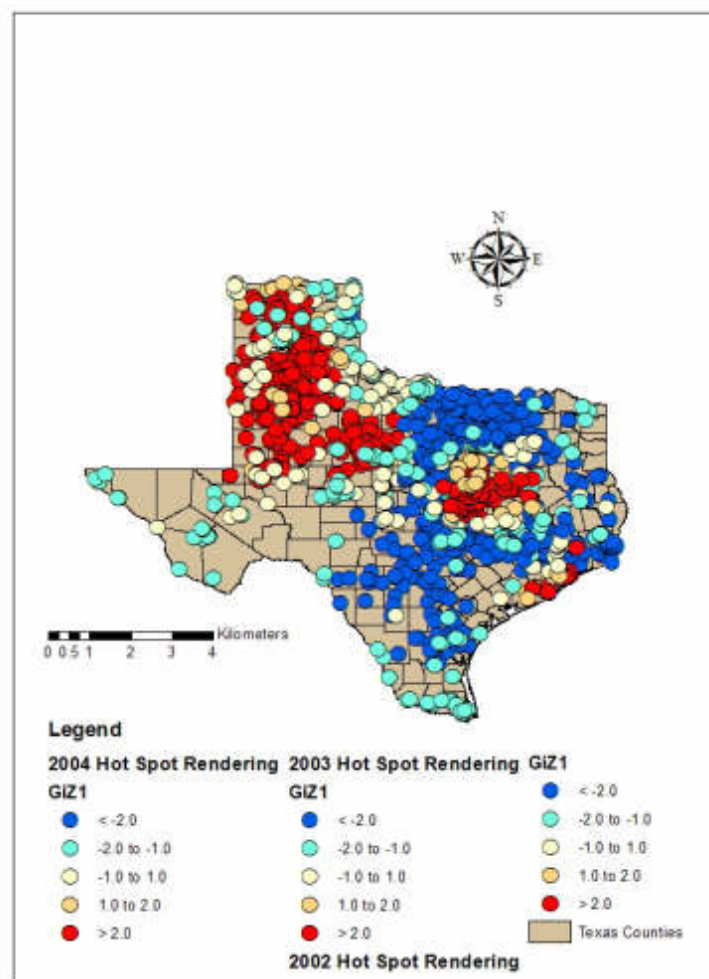


Figure 7

Hot Spot Analysis with Rendering 2002 – 2004

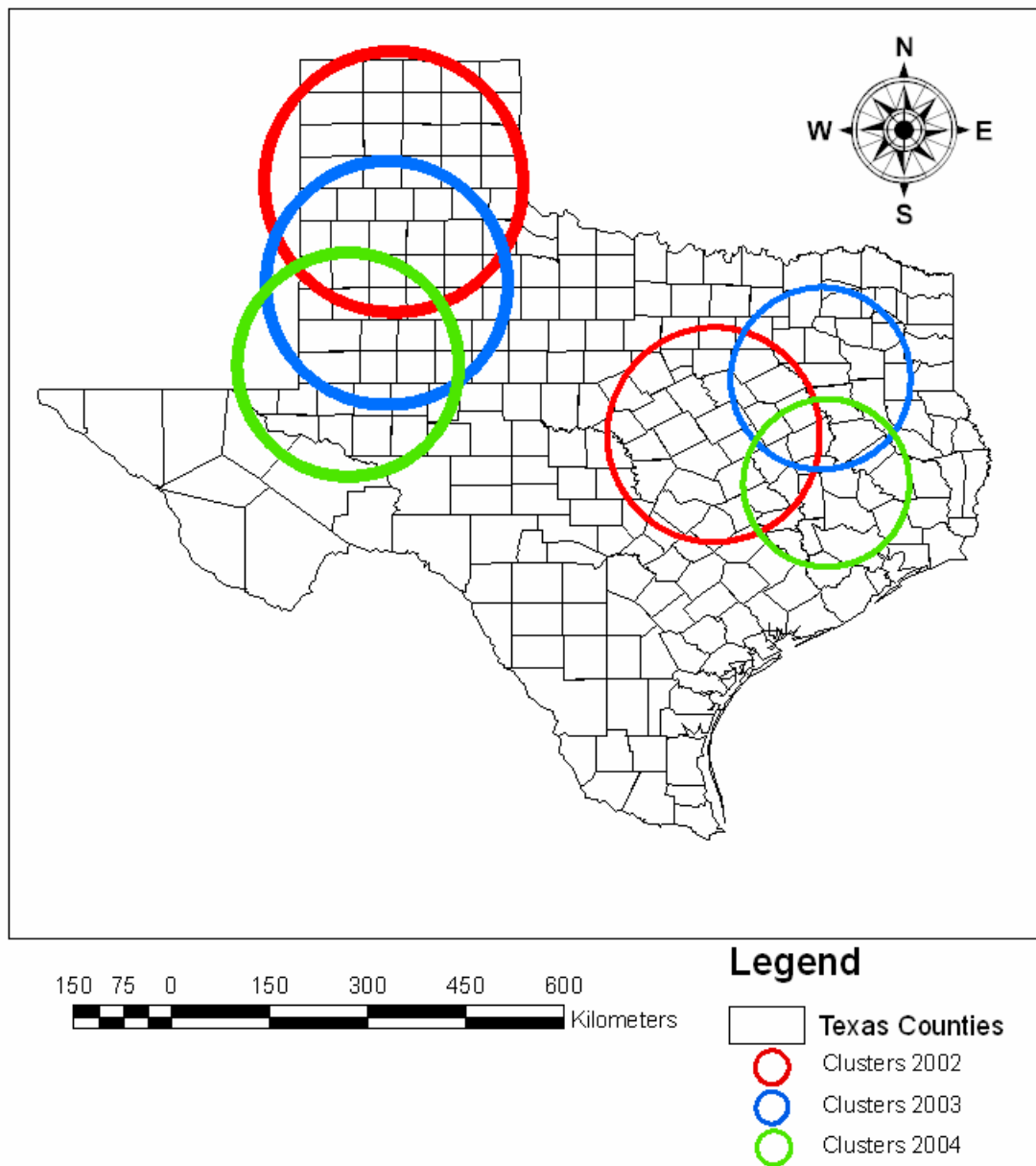


Figure 8

Map of SaTScan Primary and Secondary Cluster Output Analysis

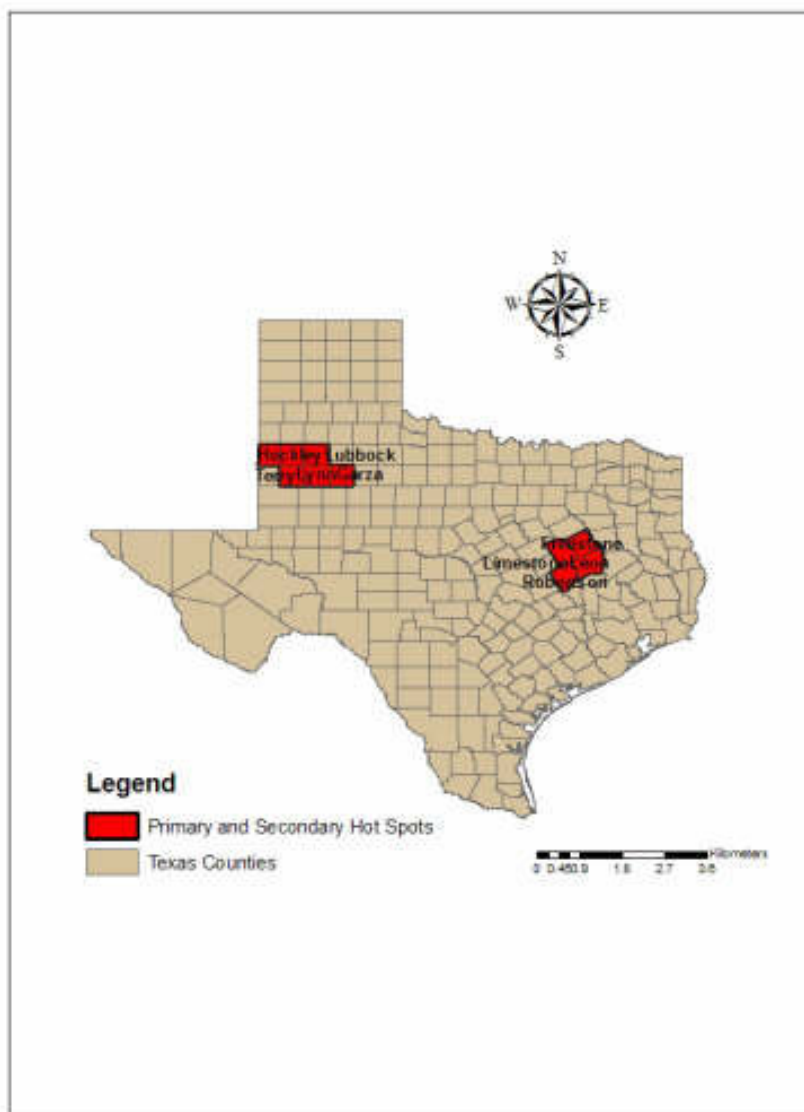


Figure 9

Map of Corresponding Hot Spot Counties from SaTScan Results Overlap

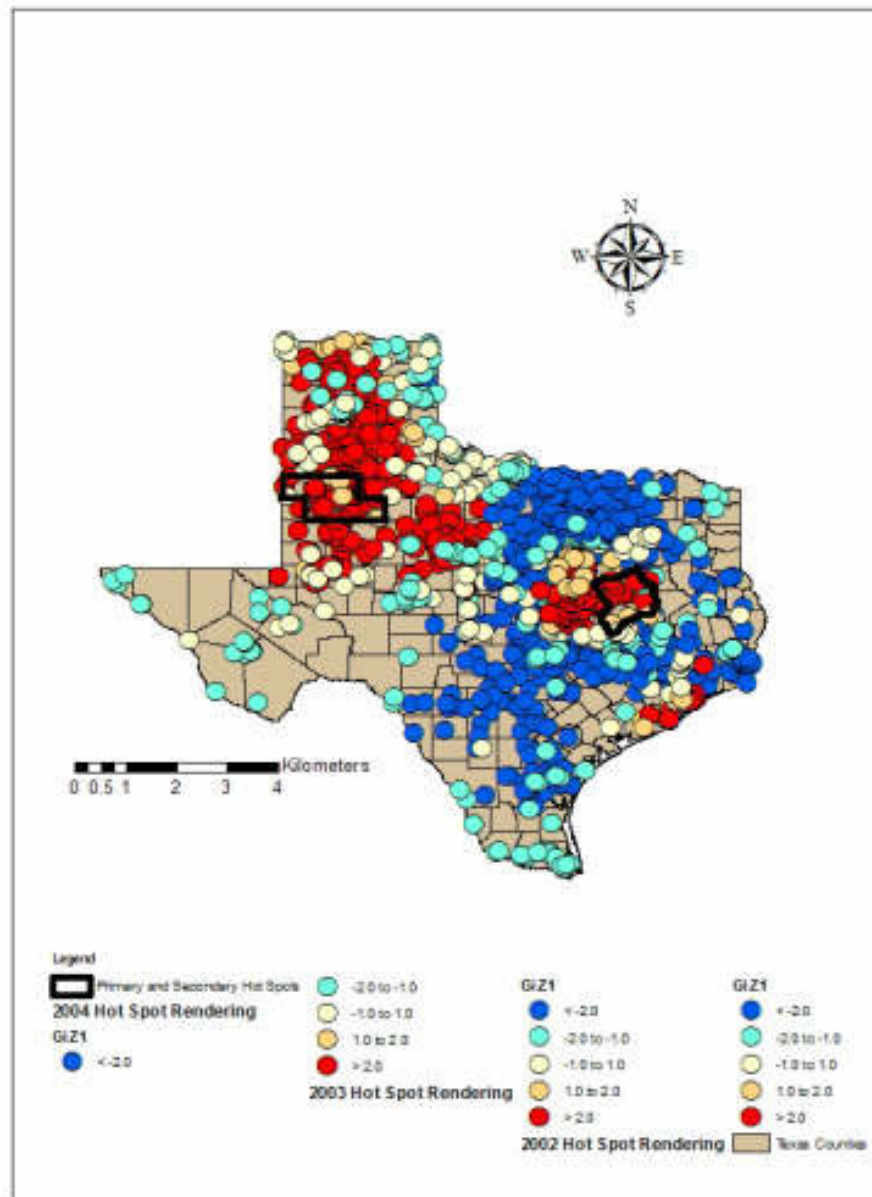


Figure 10

Hot Spot Analysis and Hot Spot Counties Overlay

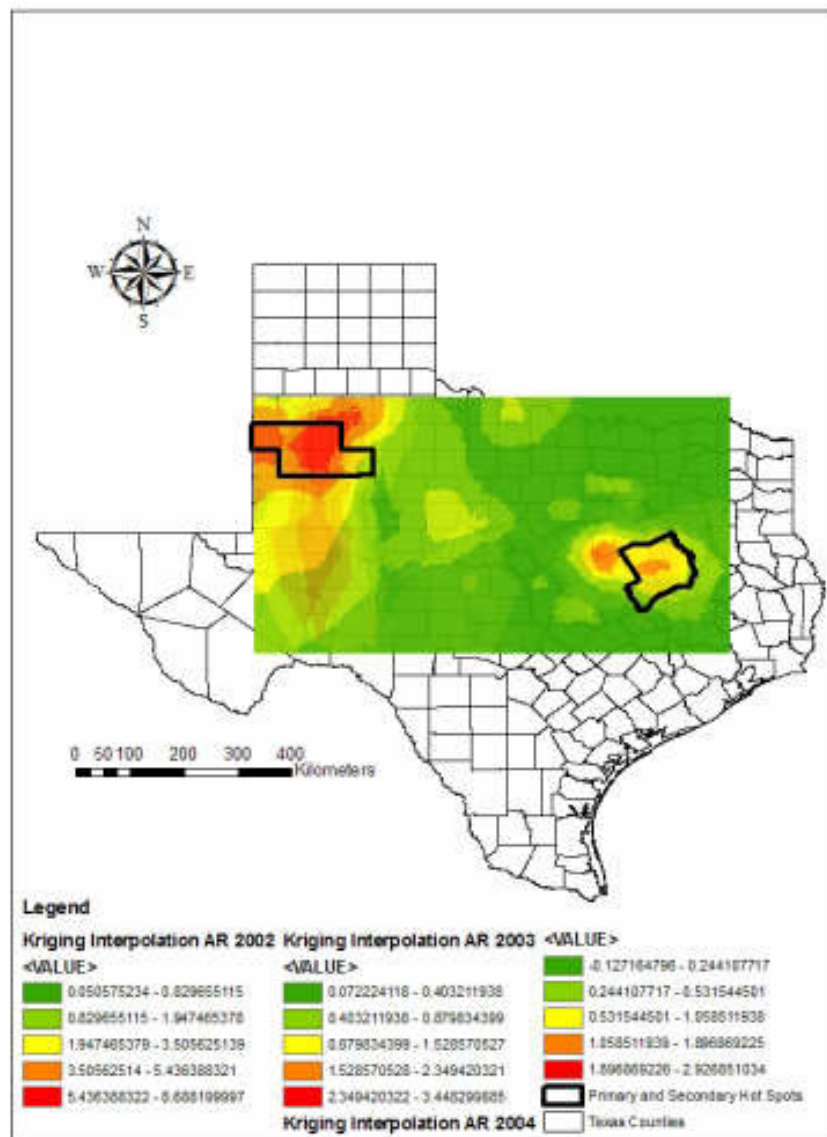


Figure 11

Map of Kriging Interpolation of WNV Attack Rates and Hot Spots Overlay

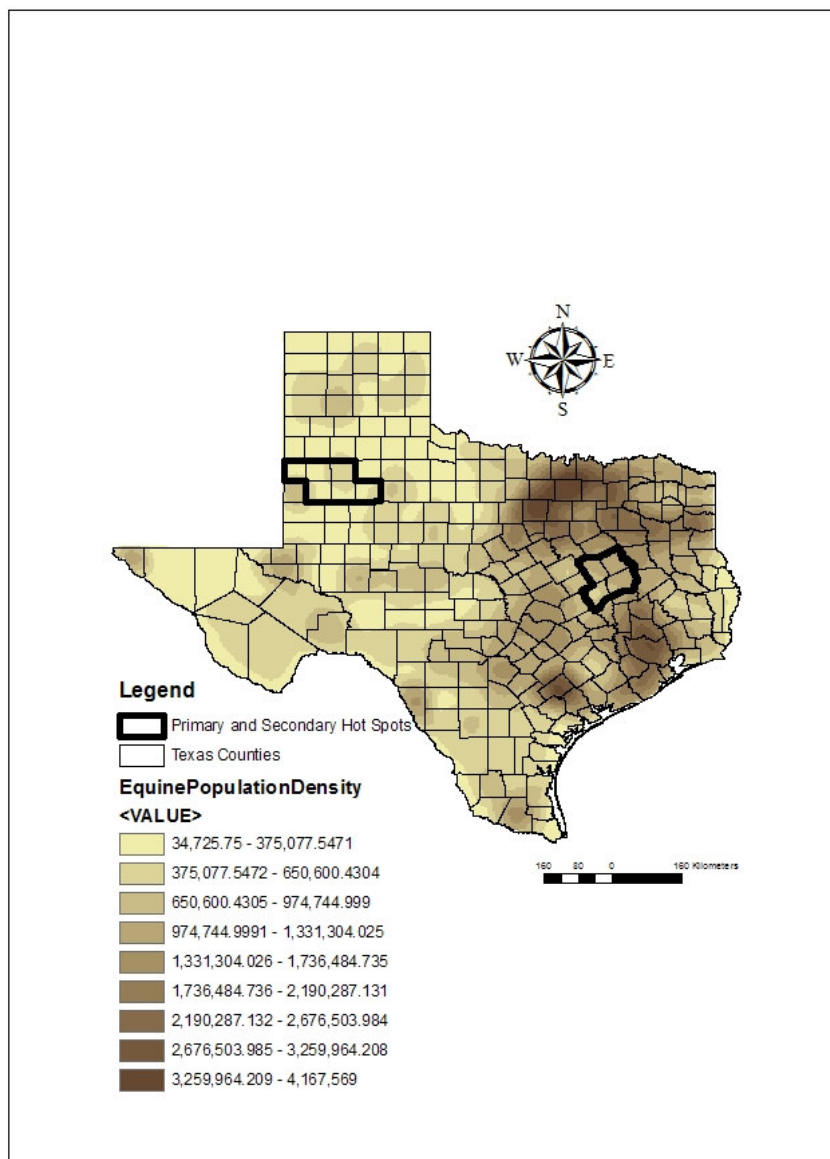


Figure 12

Equine Population Density in Texas

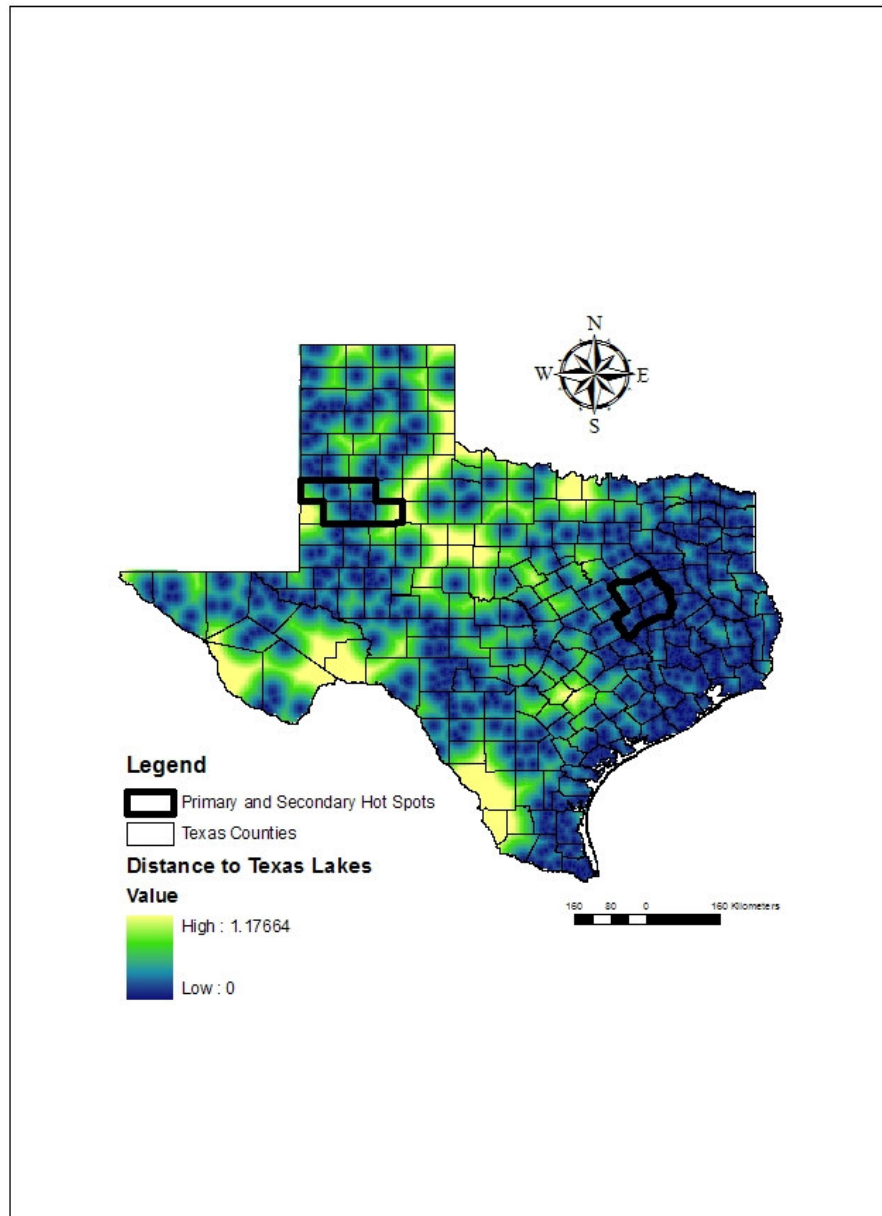


Figure 13

Distance to Texas Lakes

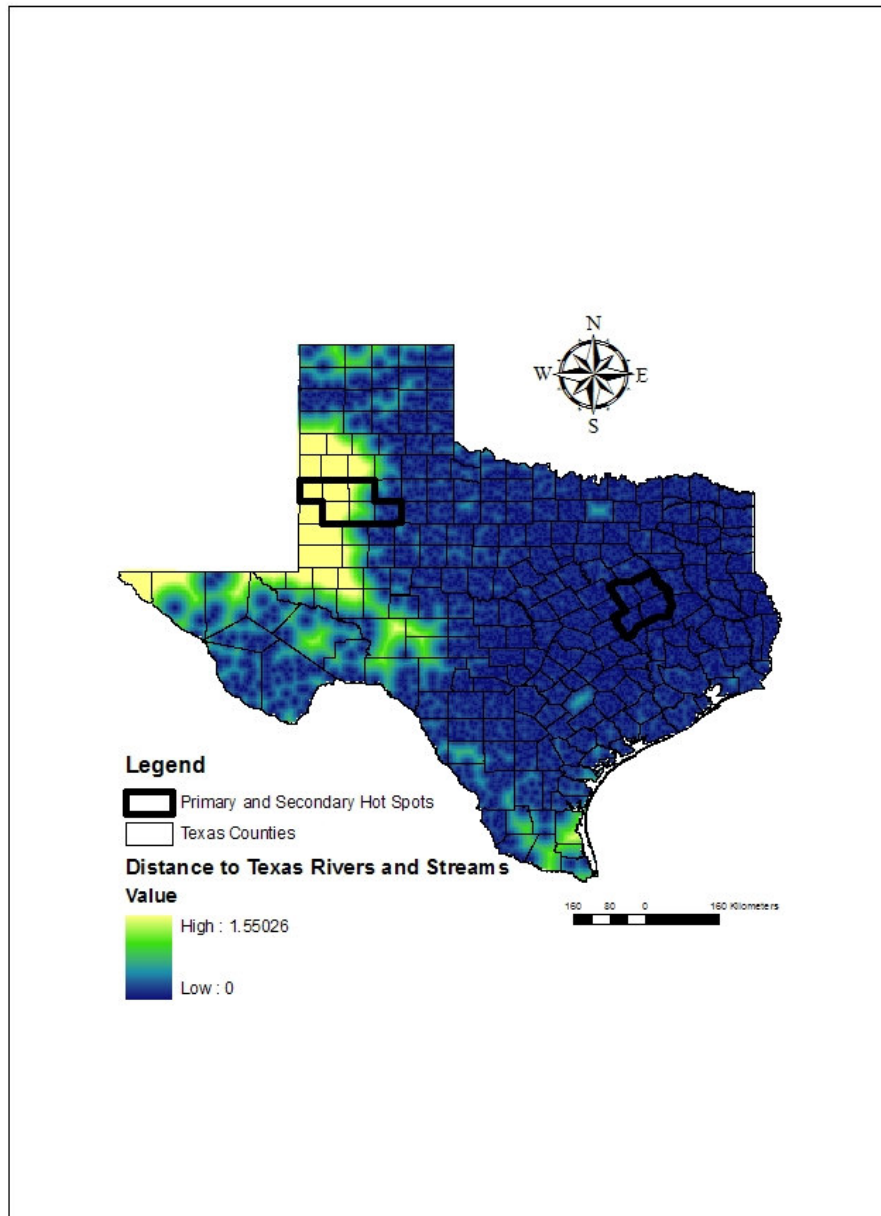


Figure 14

Distance to Texas Streams and Rivers

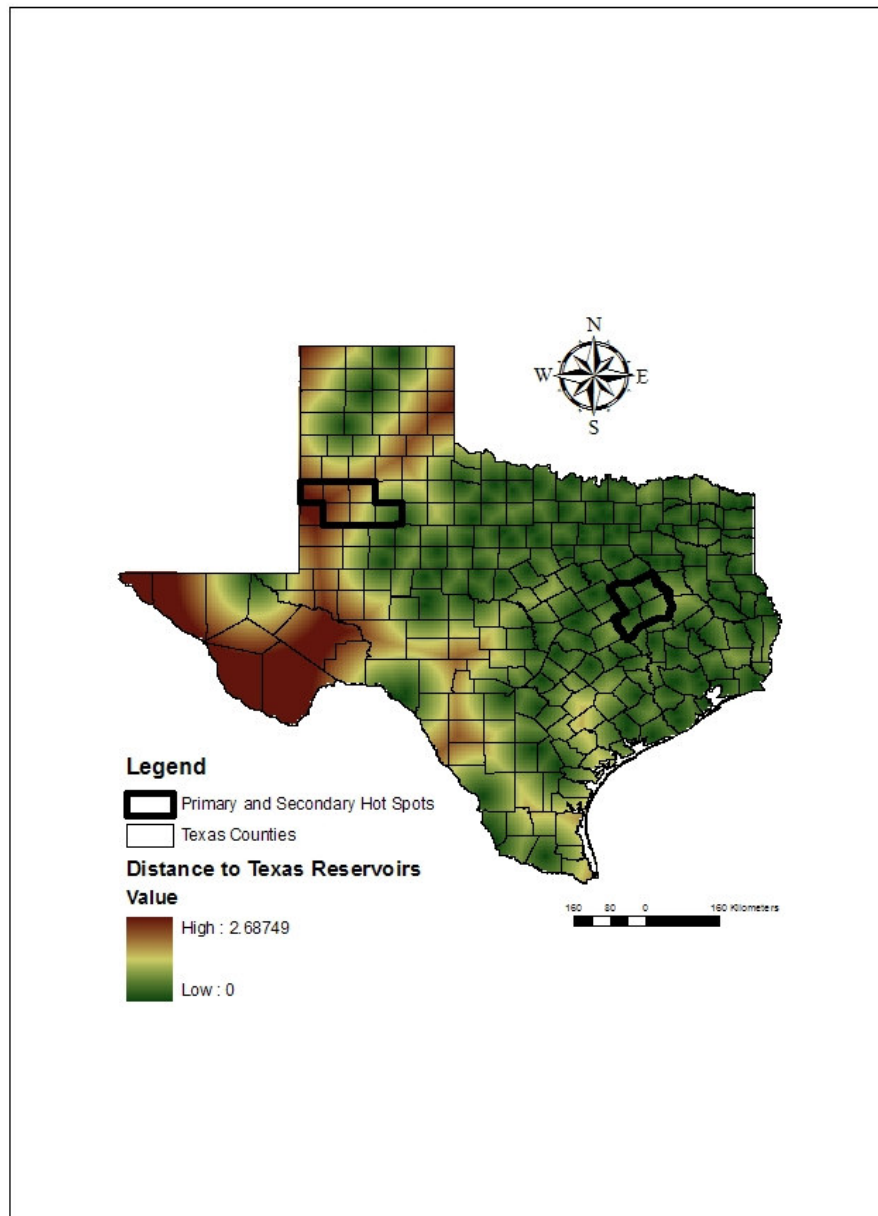


Figure 15

Distance to Texas Reservoirs

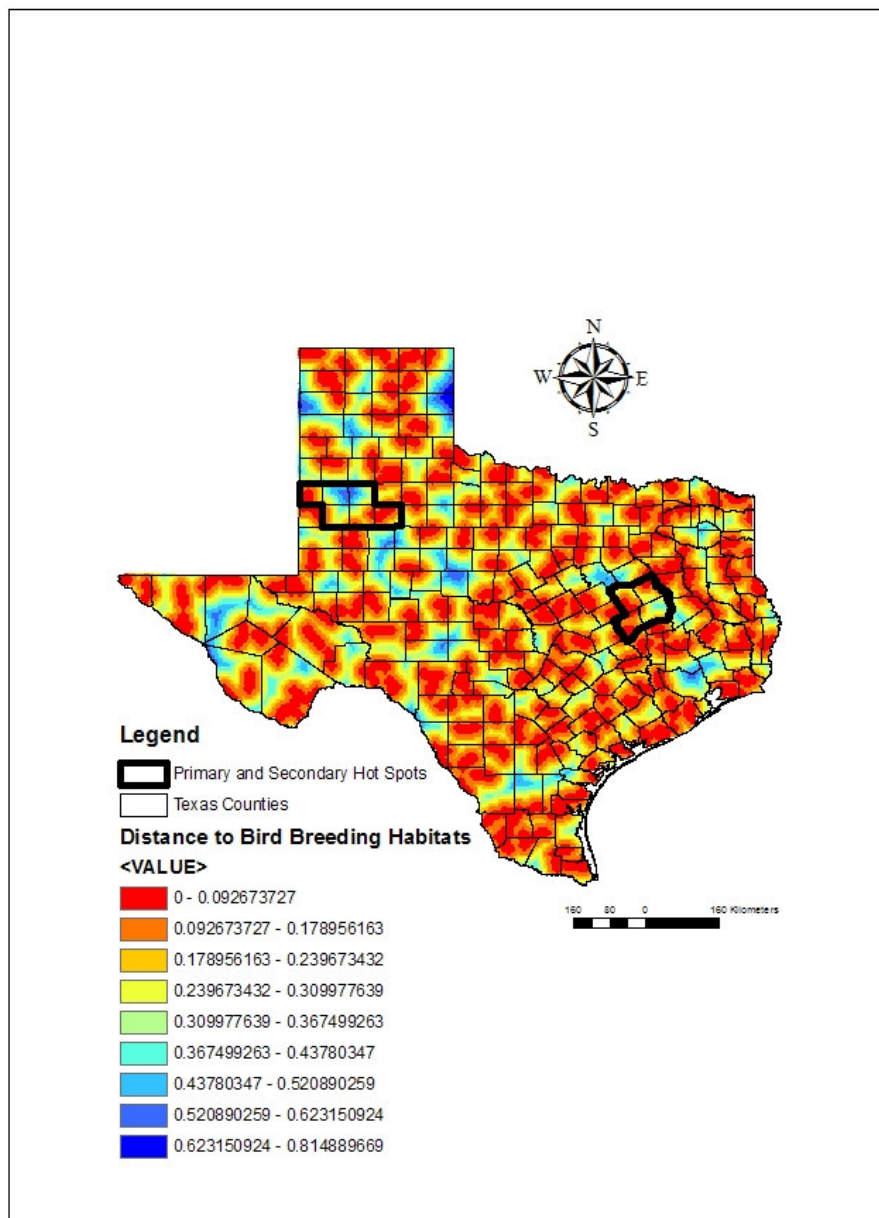


Figure 16

Distance to Bird Breeding Sites in Texas

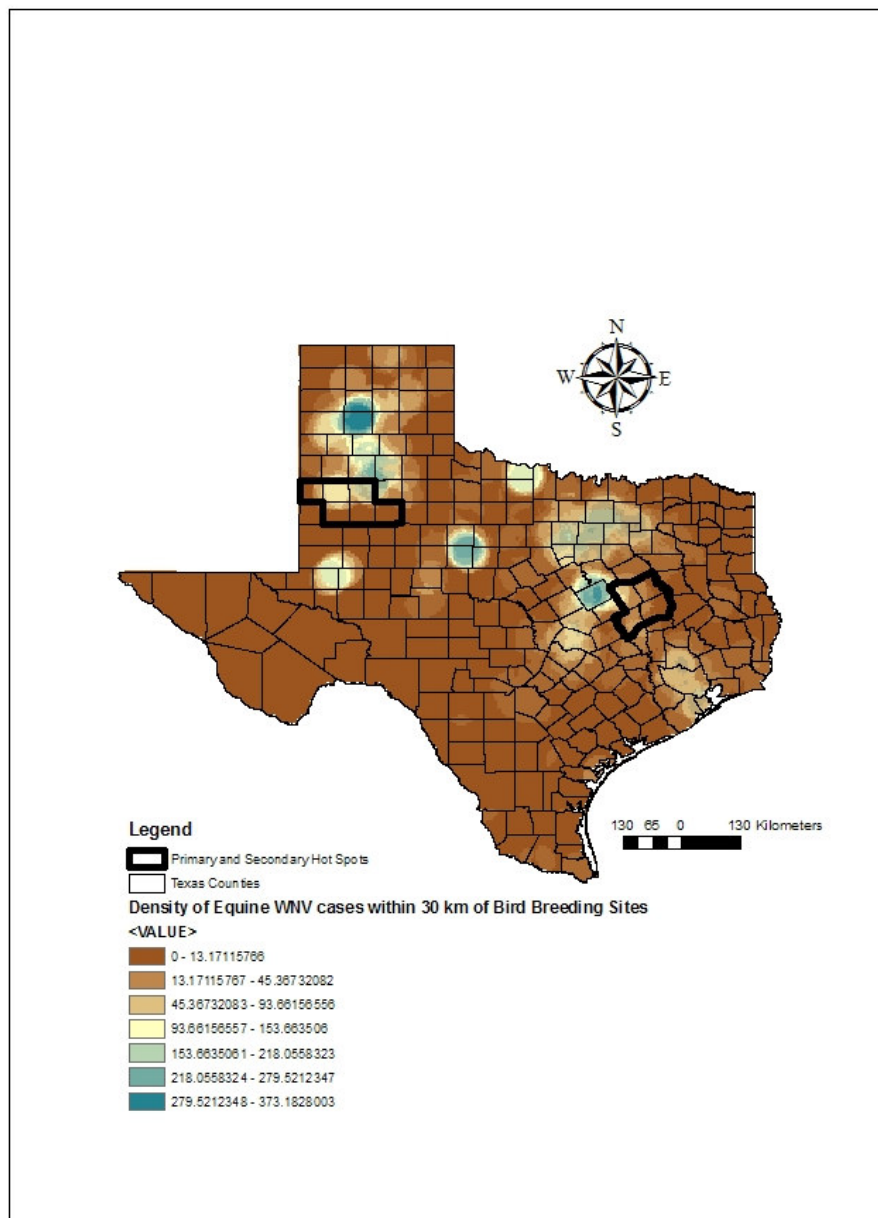


Figure 17

Density of Equine WNV Cases within 30 Kilometers of Bird Breeding Sites

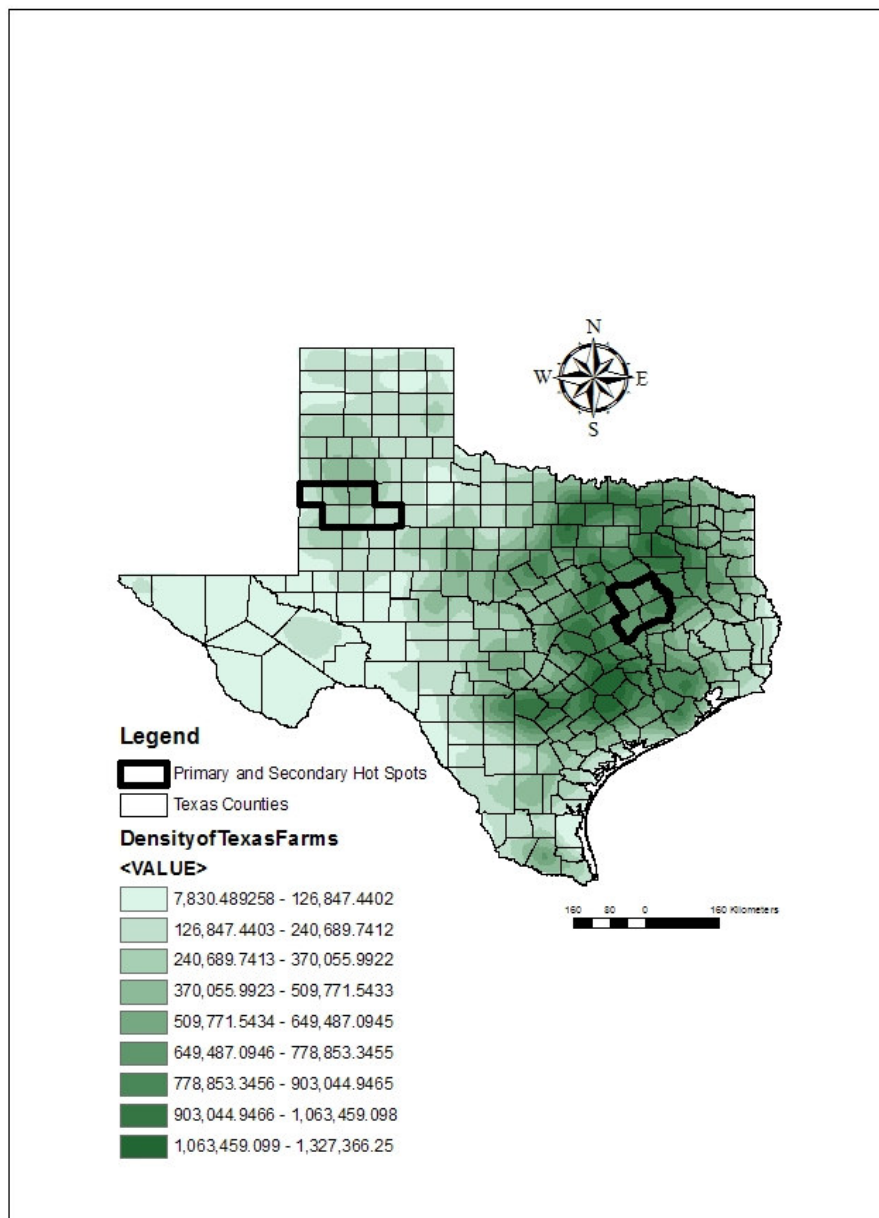


Figure 18

Texas Farm Density Map

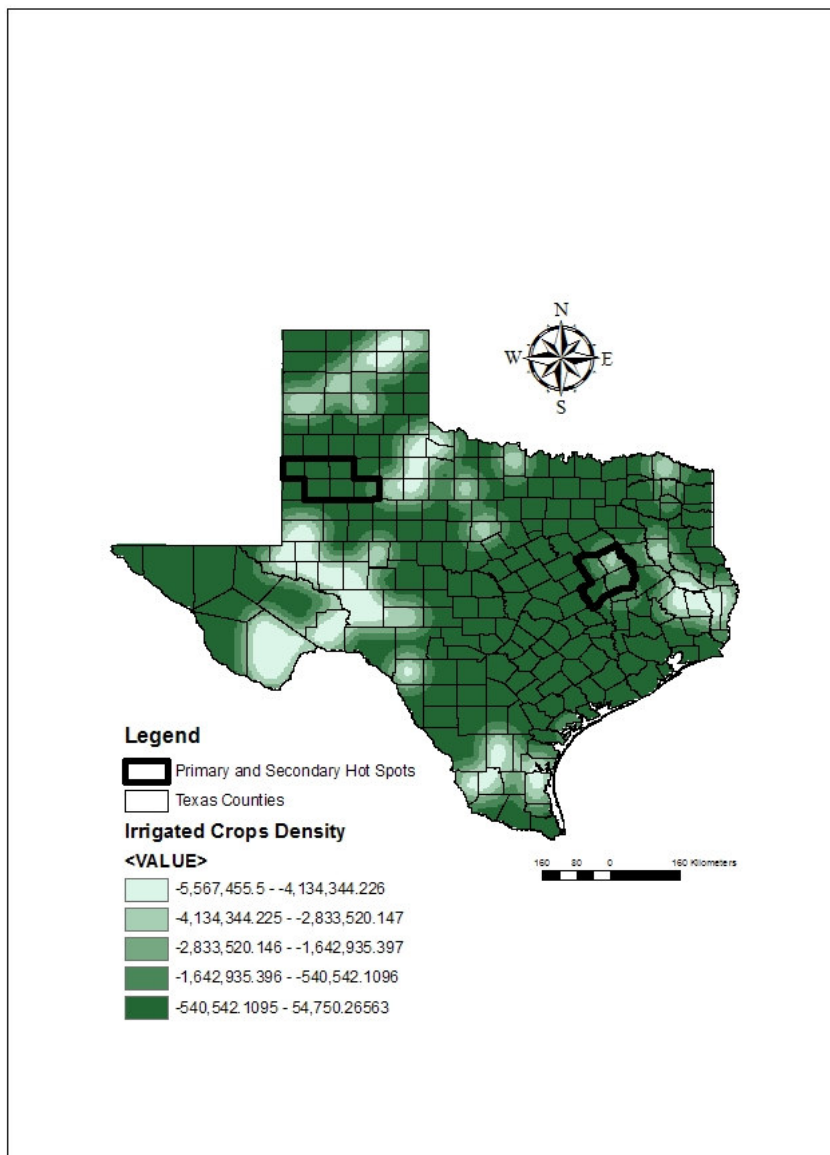


Figure 19

Irrigated Crop Farm Density

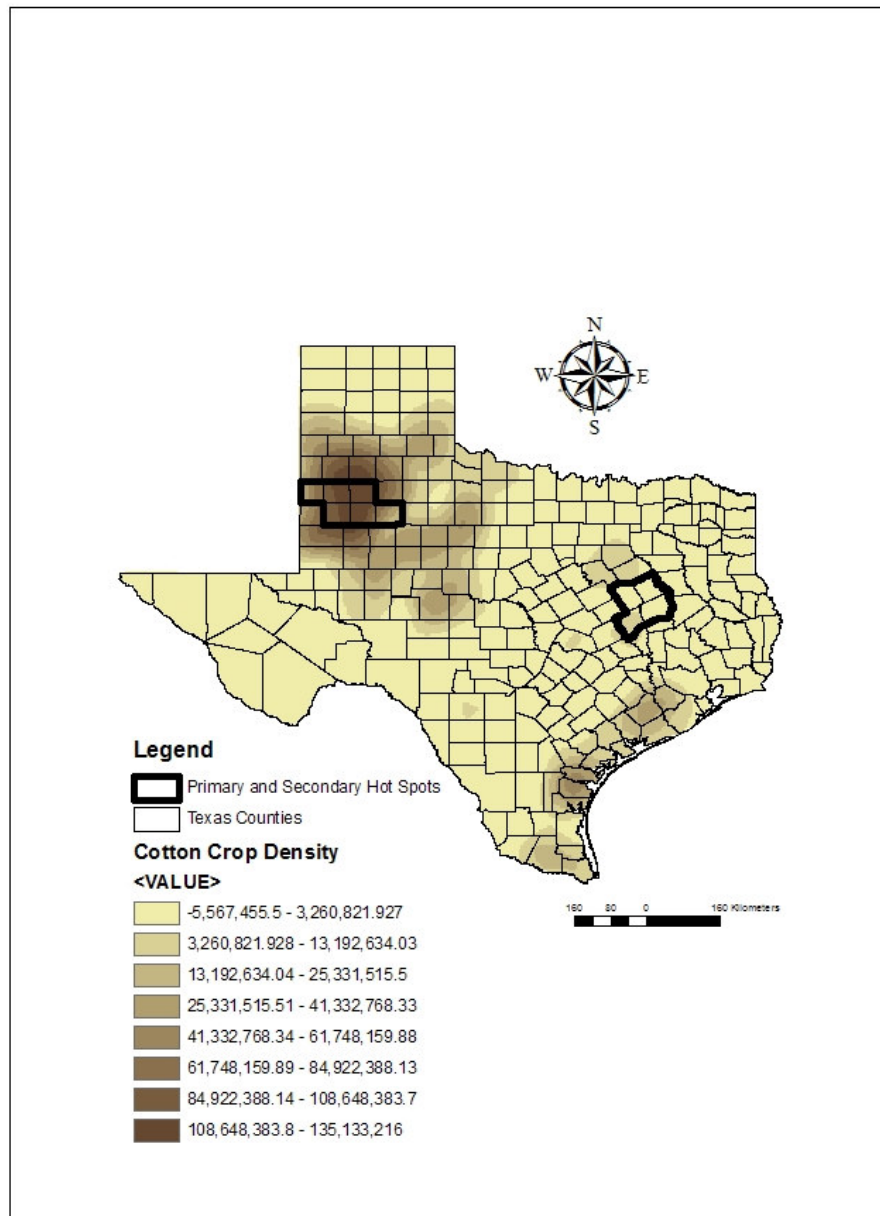


Figure 20

Cotton Crop Farm Density

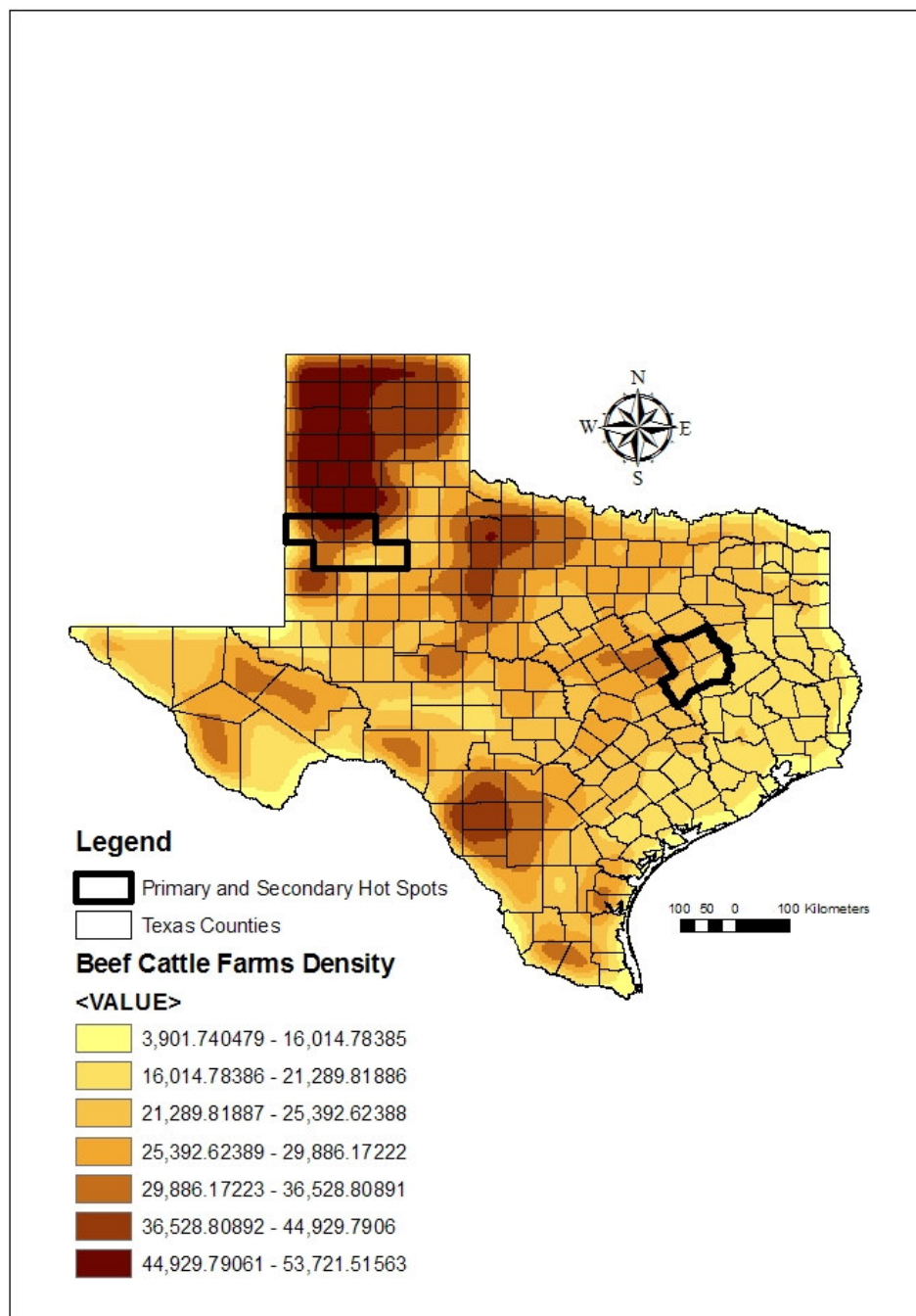


Figure 21

Beef Cattle Farm Density

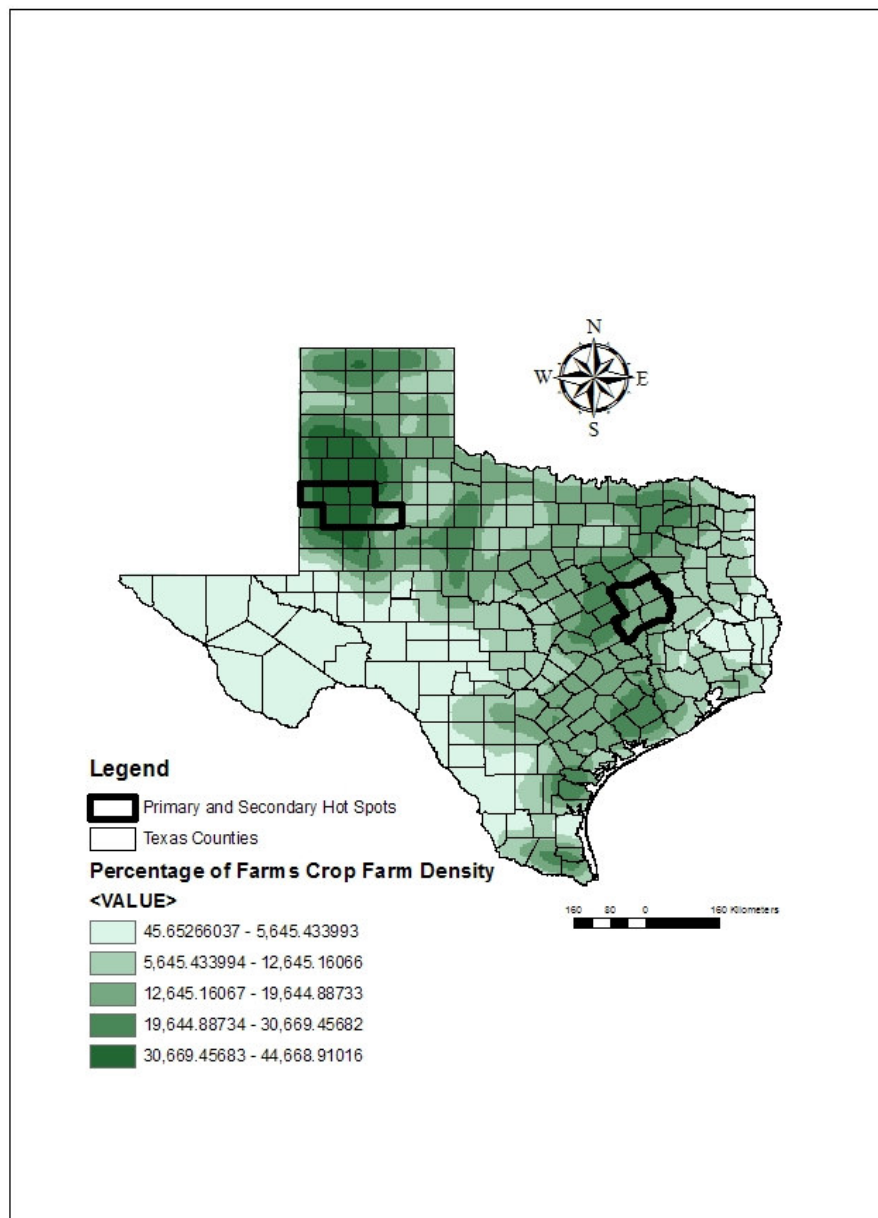


Figure 22

Percentage of Texas Farms That Are Crop Farms

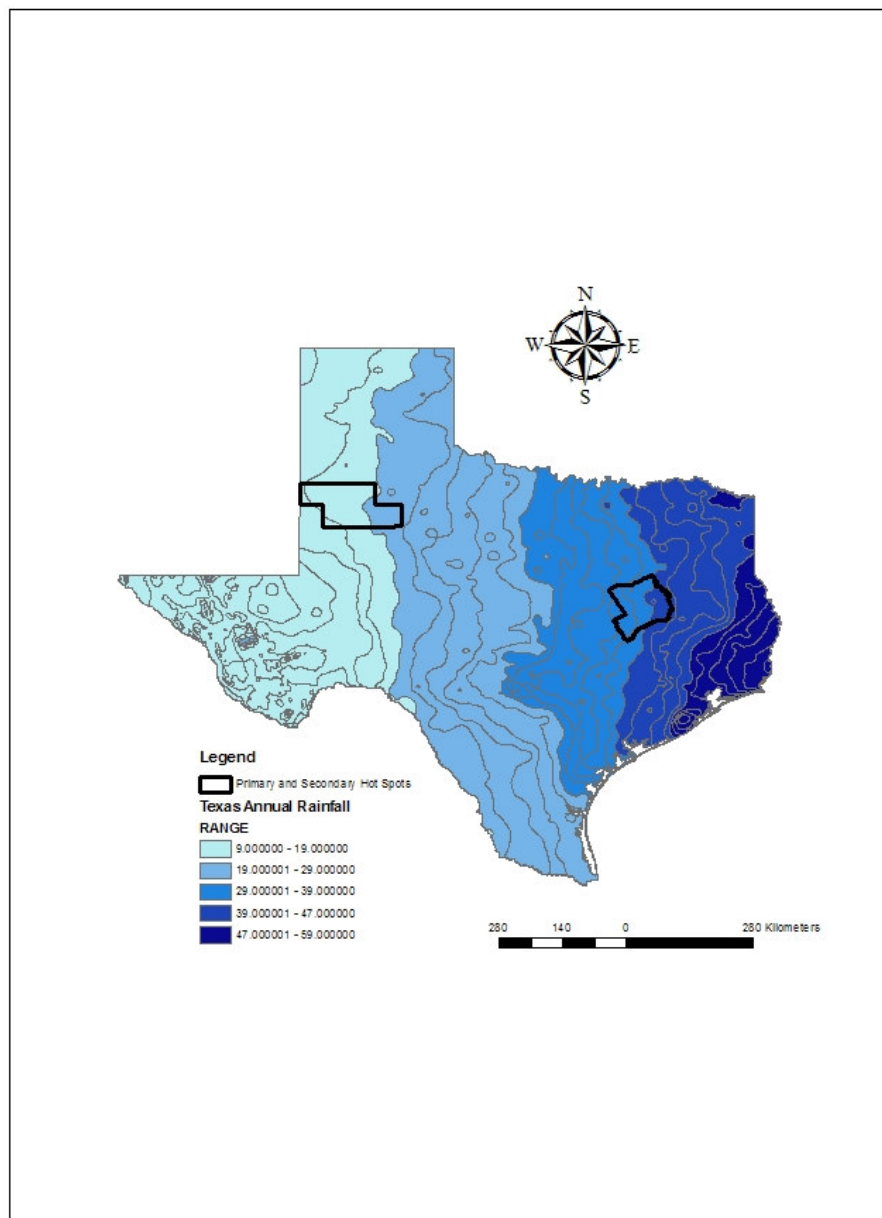


Figure 23

Annual Rainfall Across Texas

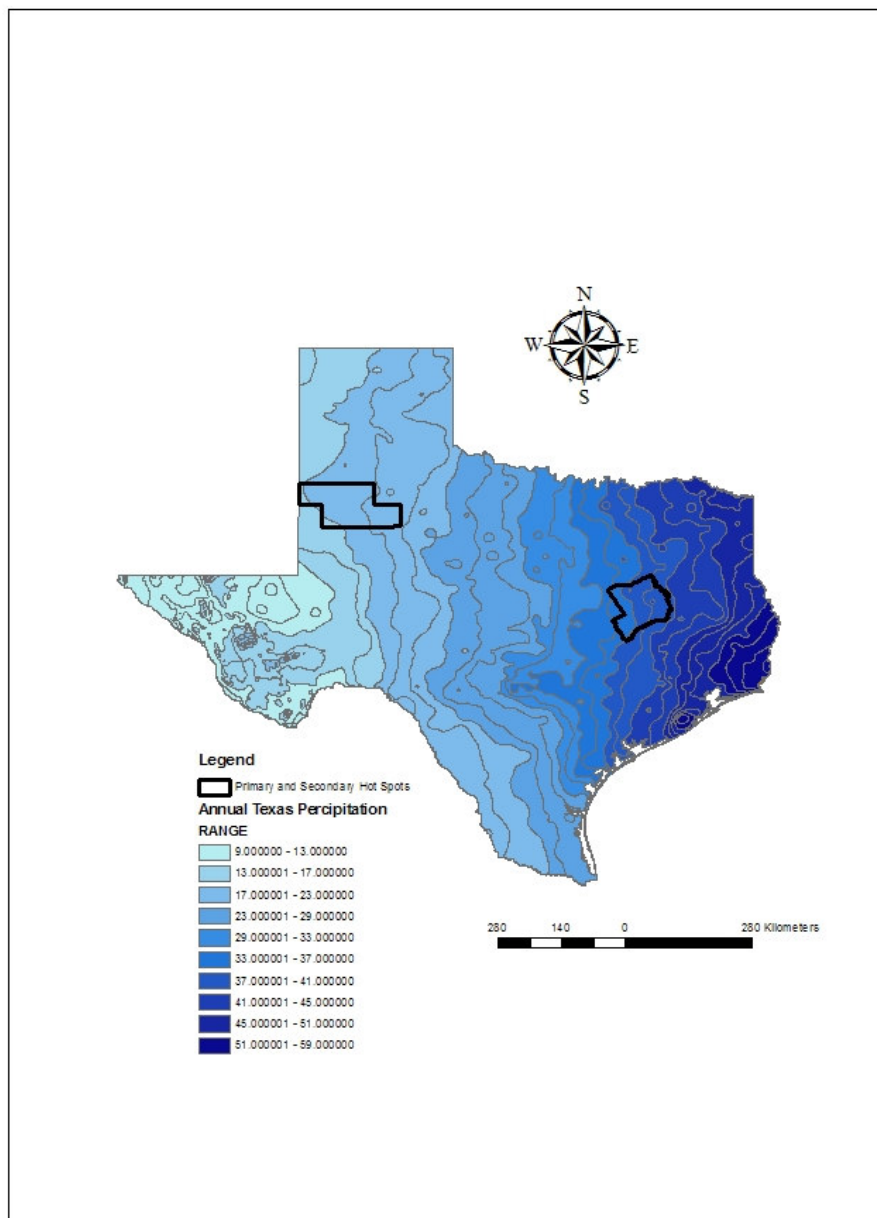


Figure 24

Annual Texas Precipitation

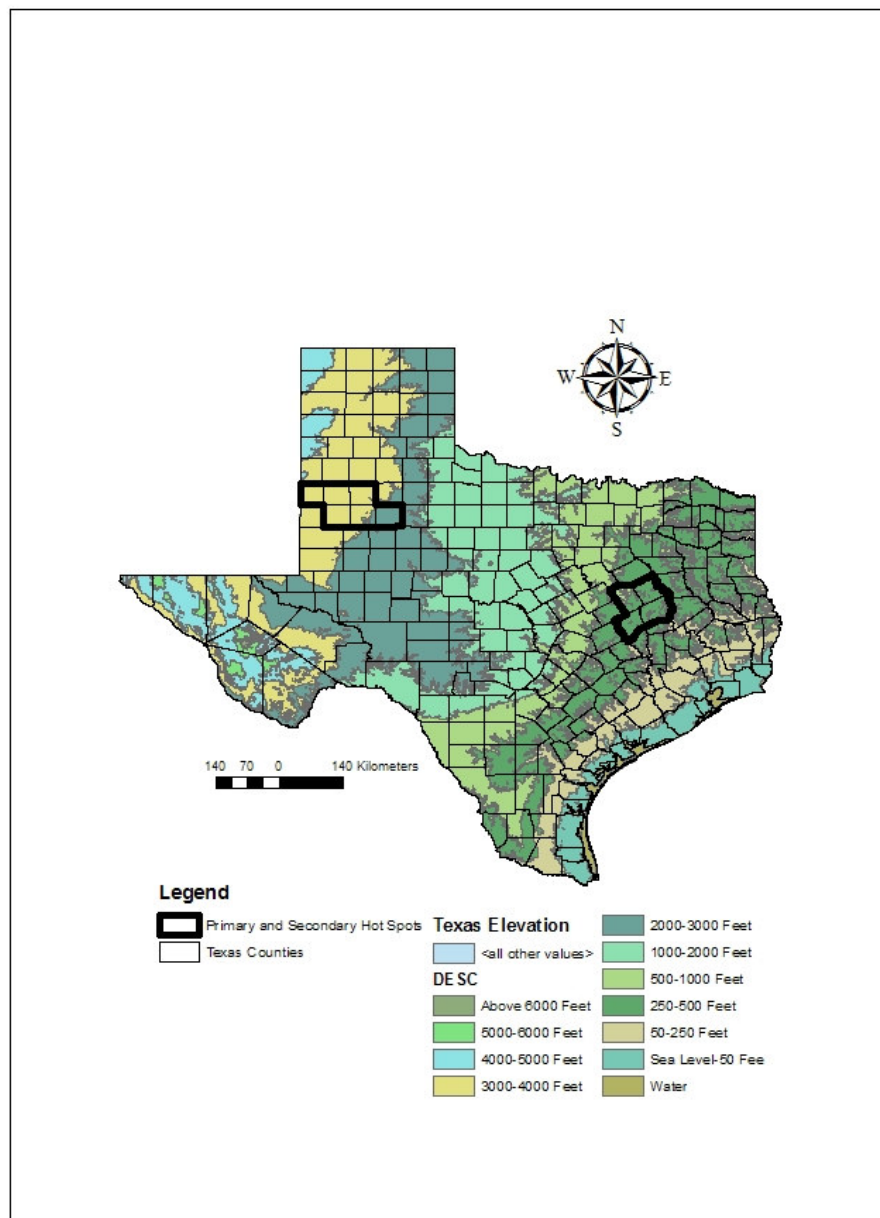


Figure 25

Texas Elevation

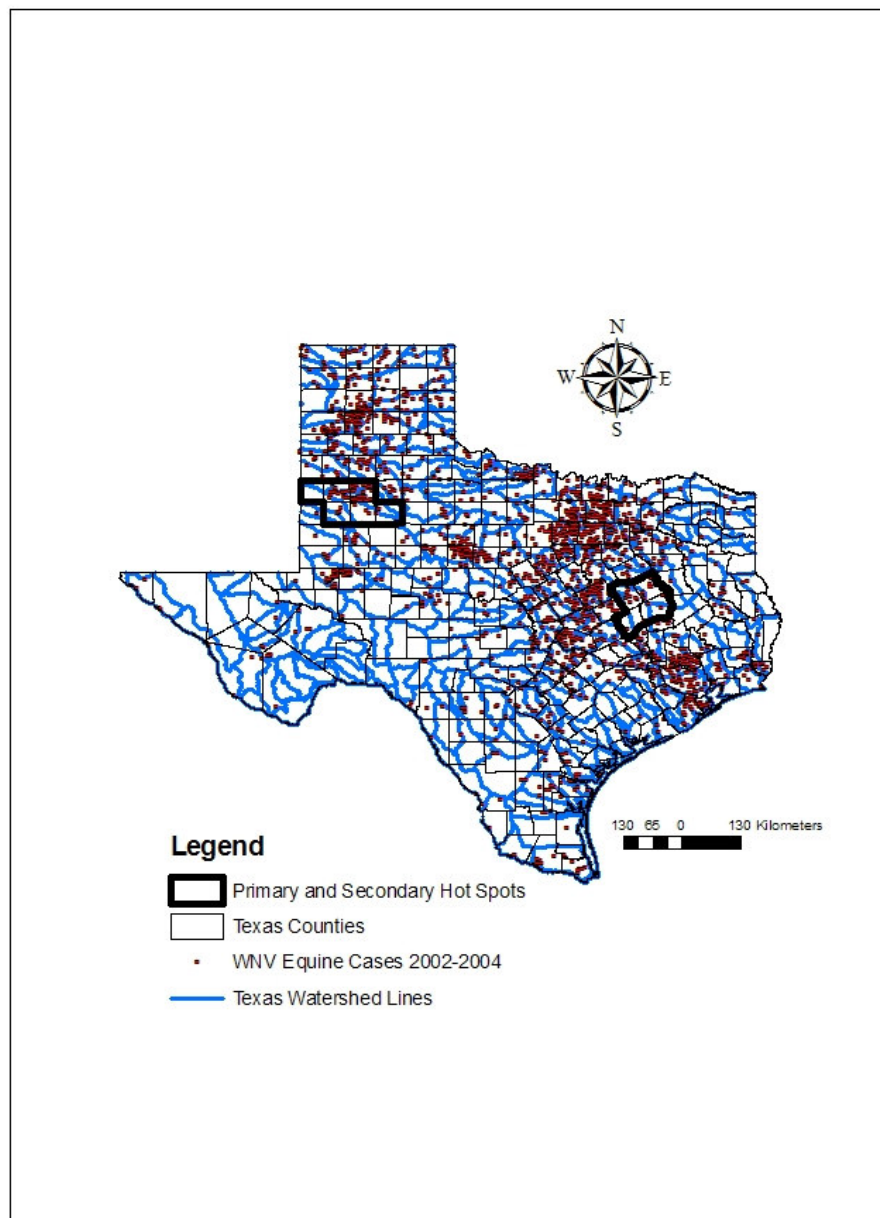


Figure 26

Texas Watersheds

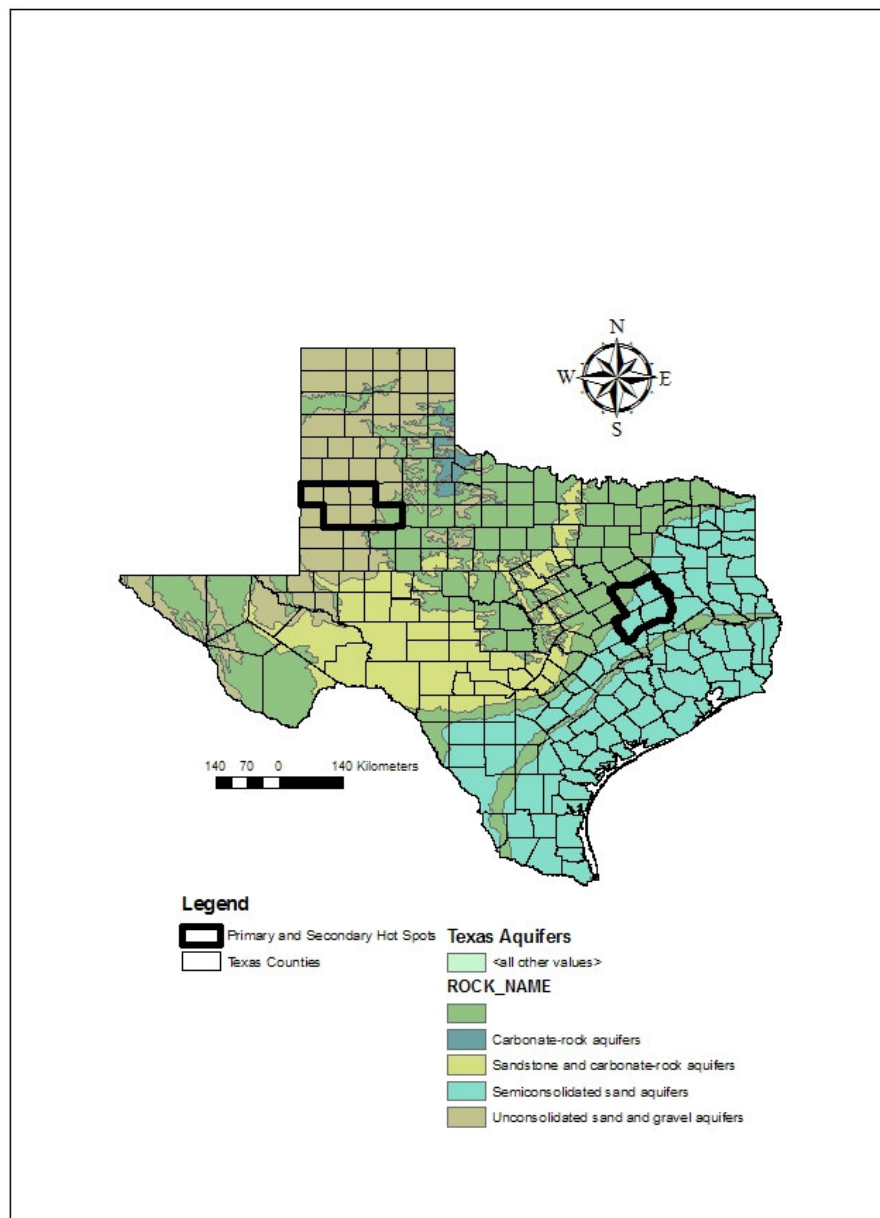


Figure 27

Texas Aquifers

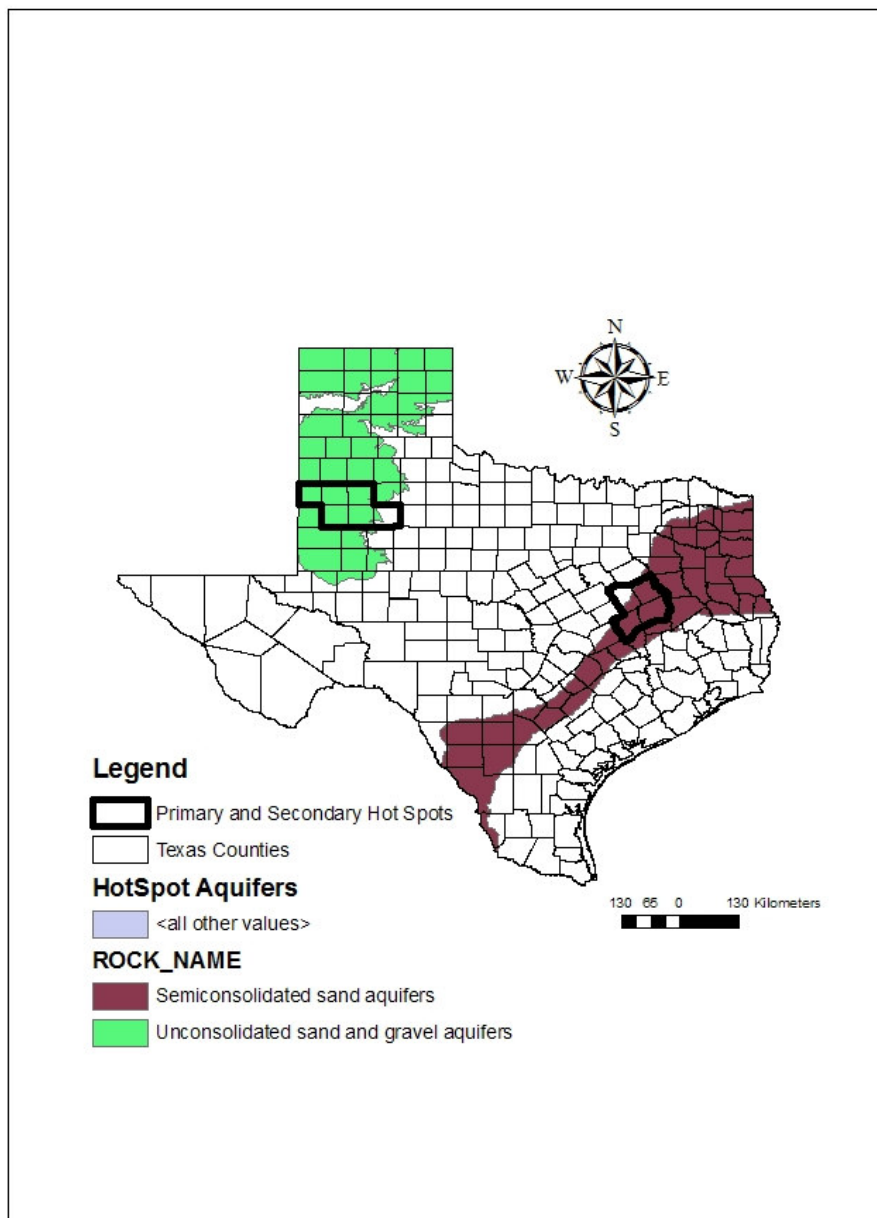


Figure 28

Primary and Secondary Hot Spot Aquifers

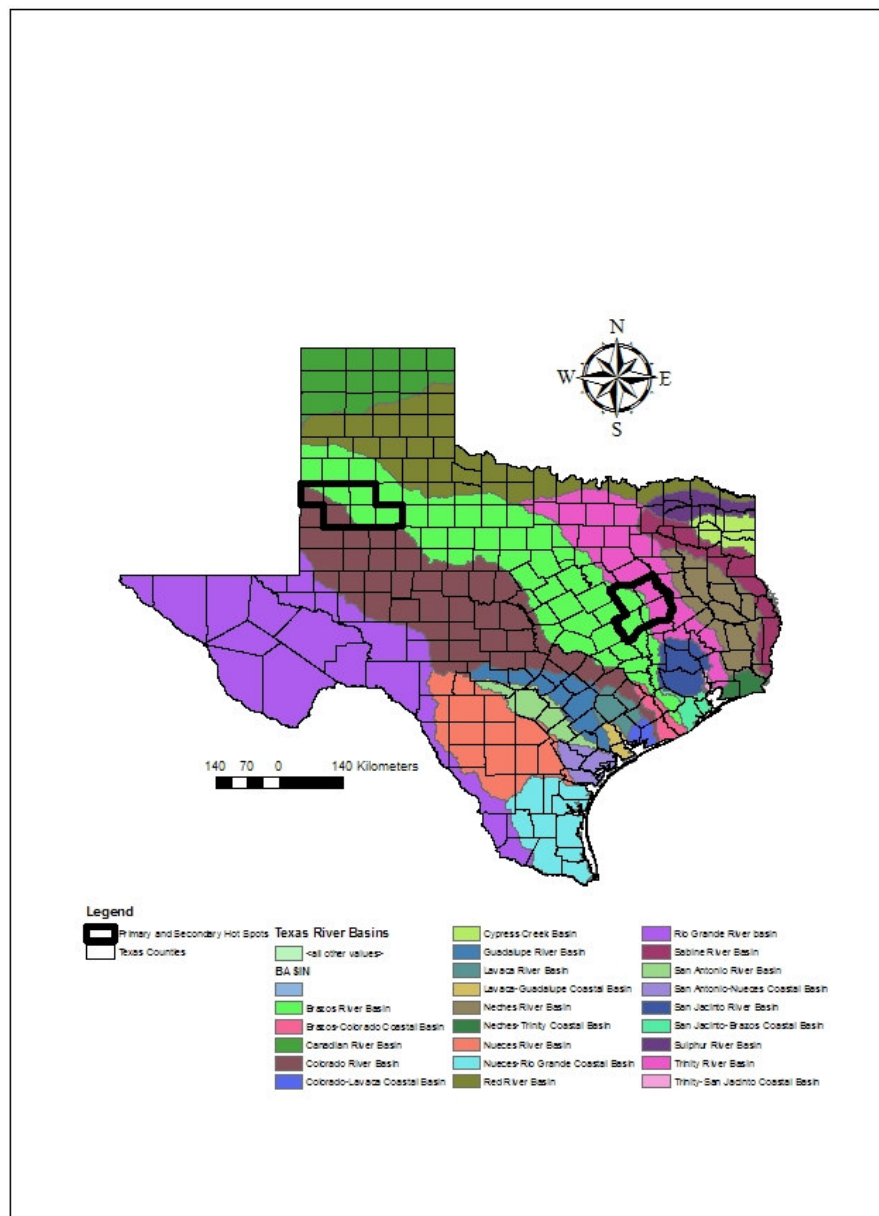


Figure 29

Texas River Basins

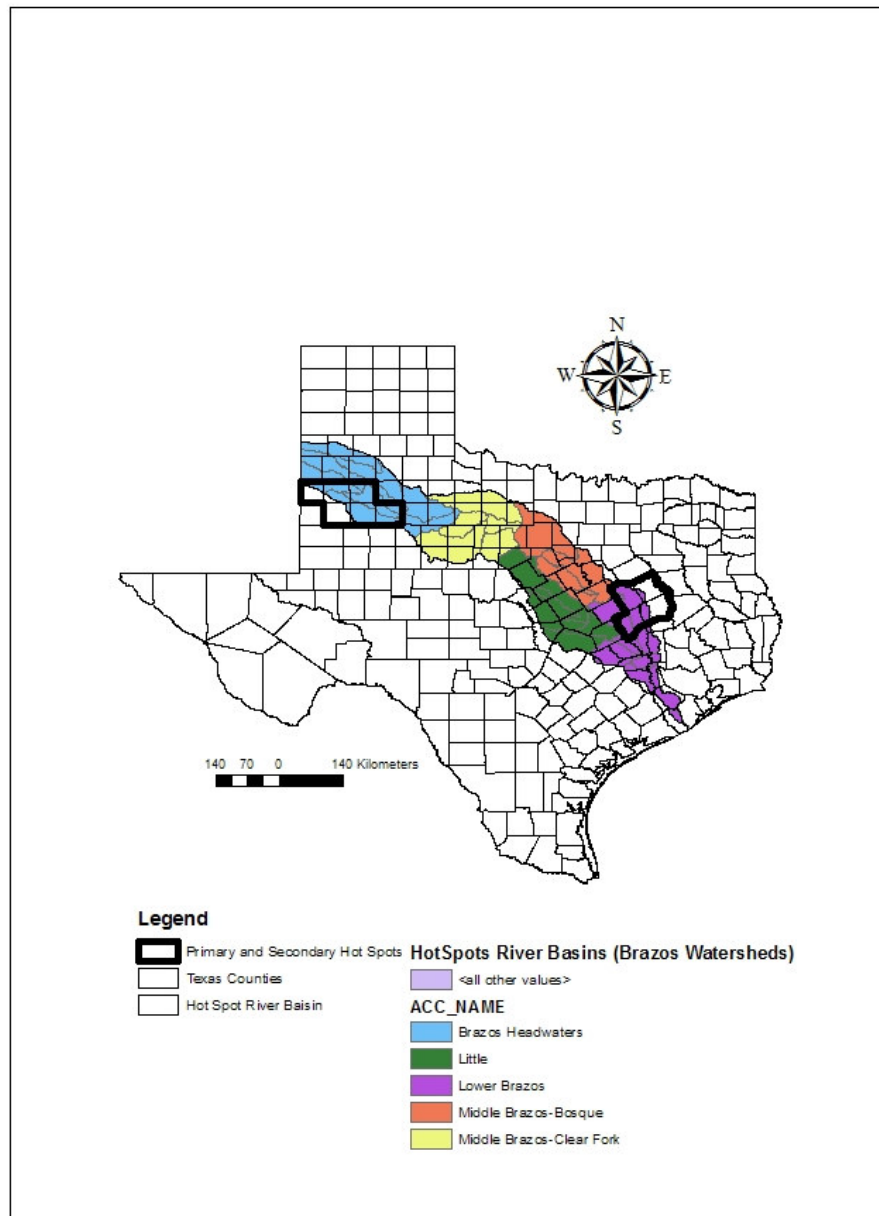


Figure 30

Primary and Secondary Hot Spot River Basins

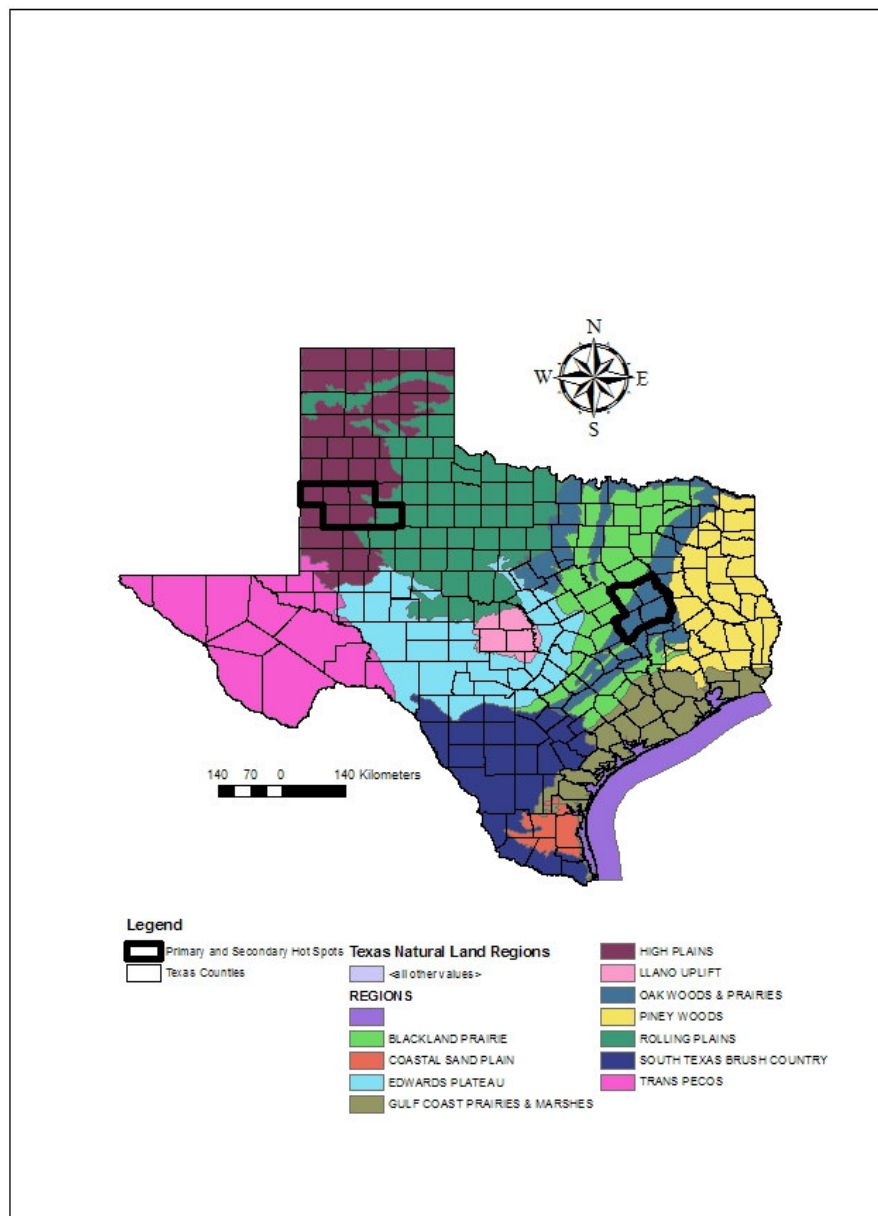


Figure 31

Texas Natural Land Regions

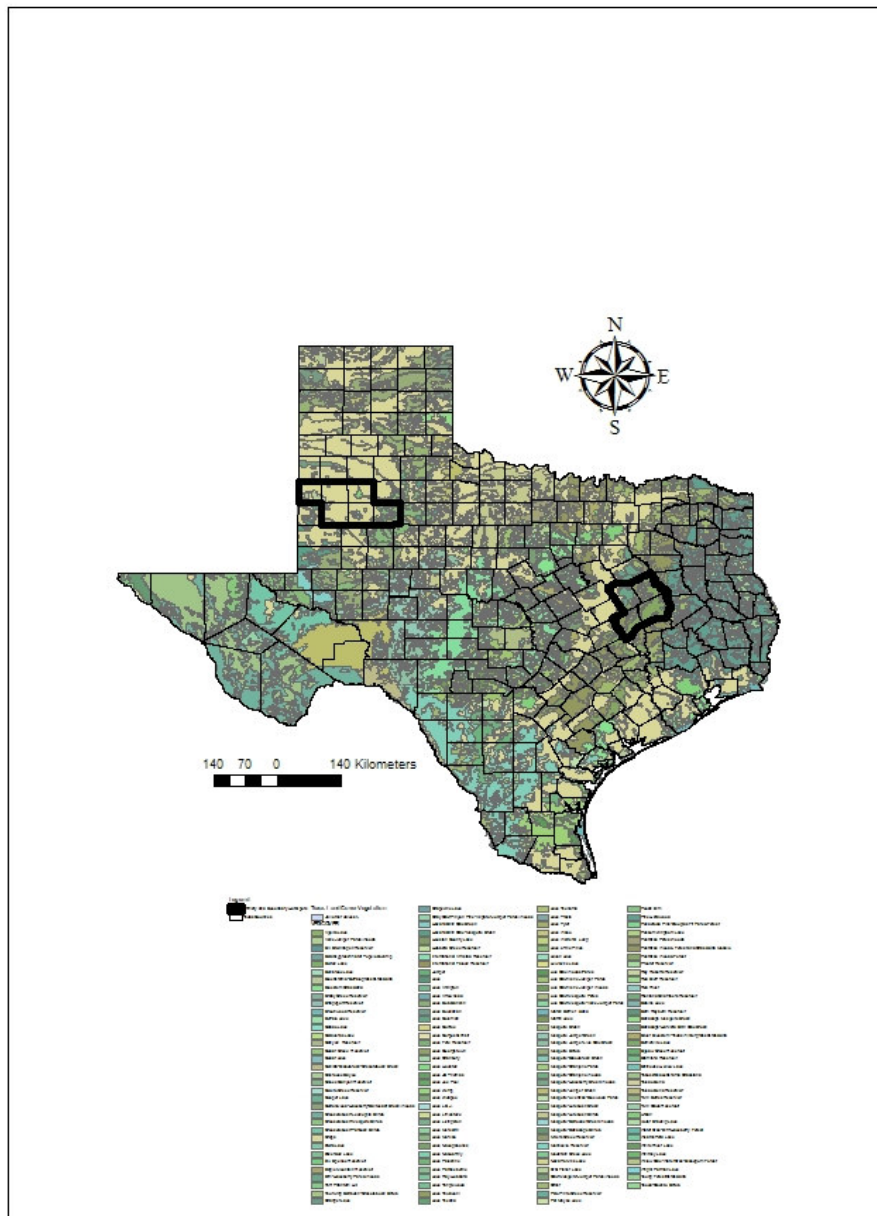


Figure 32
Texas Vegetation

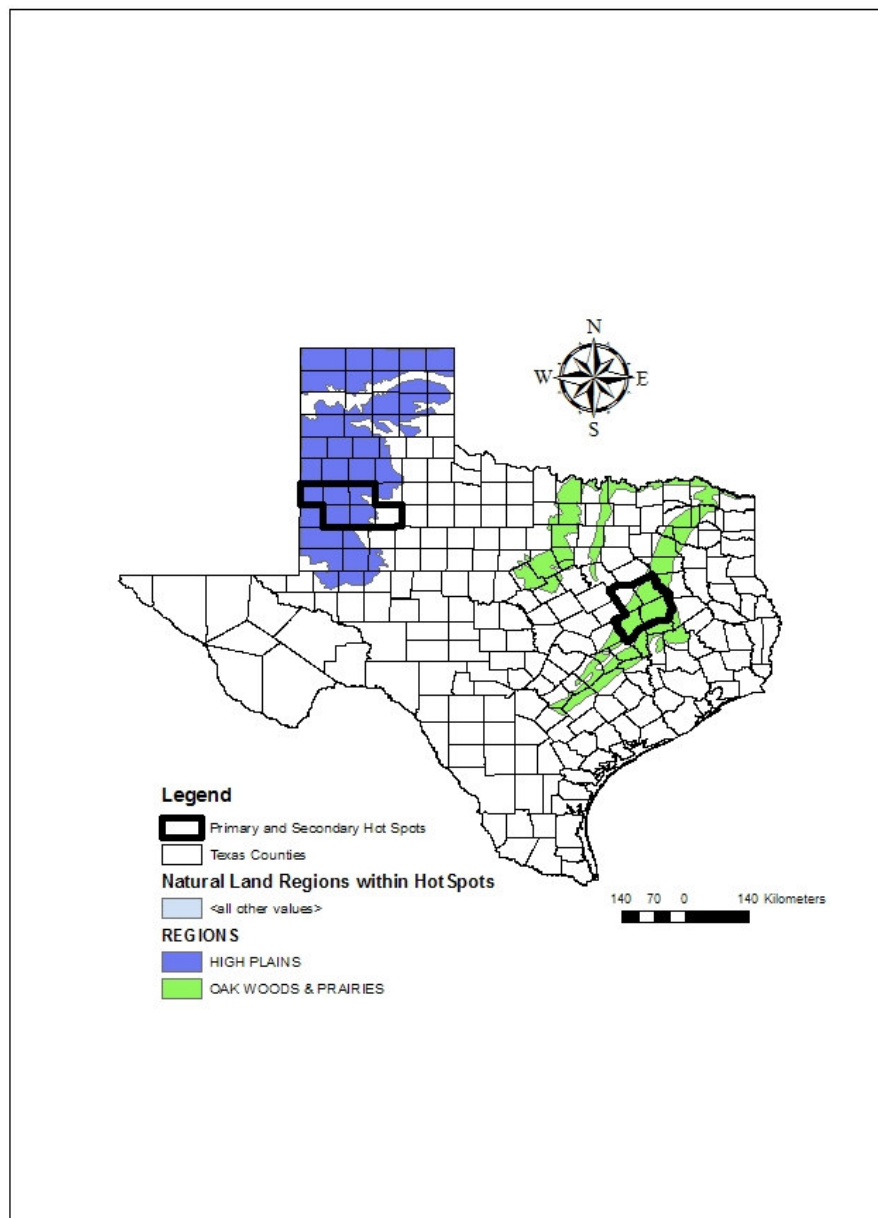


Figure 33

Primary and Secondary Hot Spot Land Regions

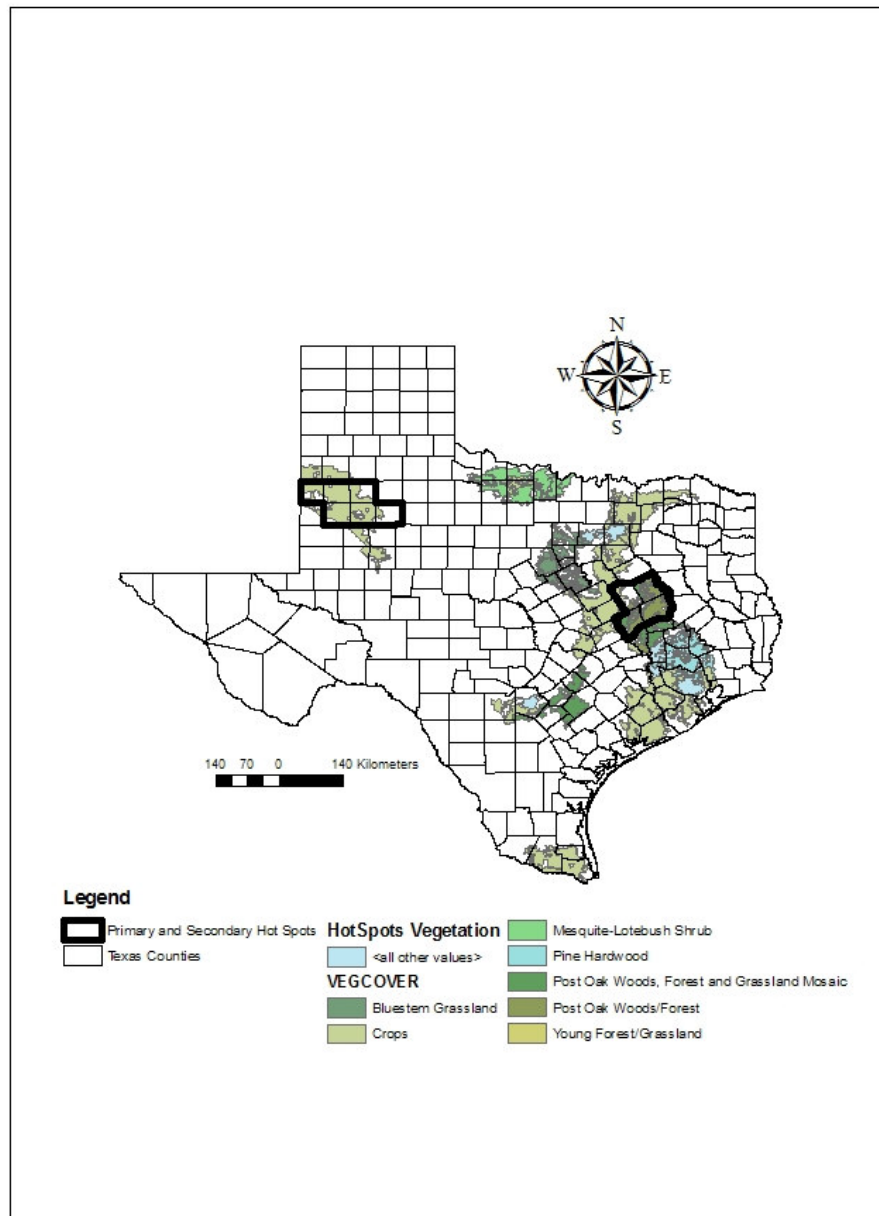


Figure 34

Primary and Secondary Hot Spot Vegetation

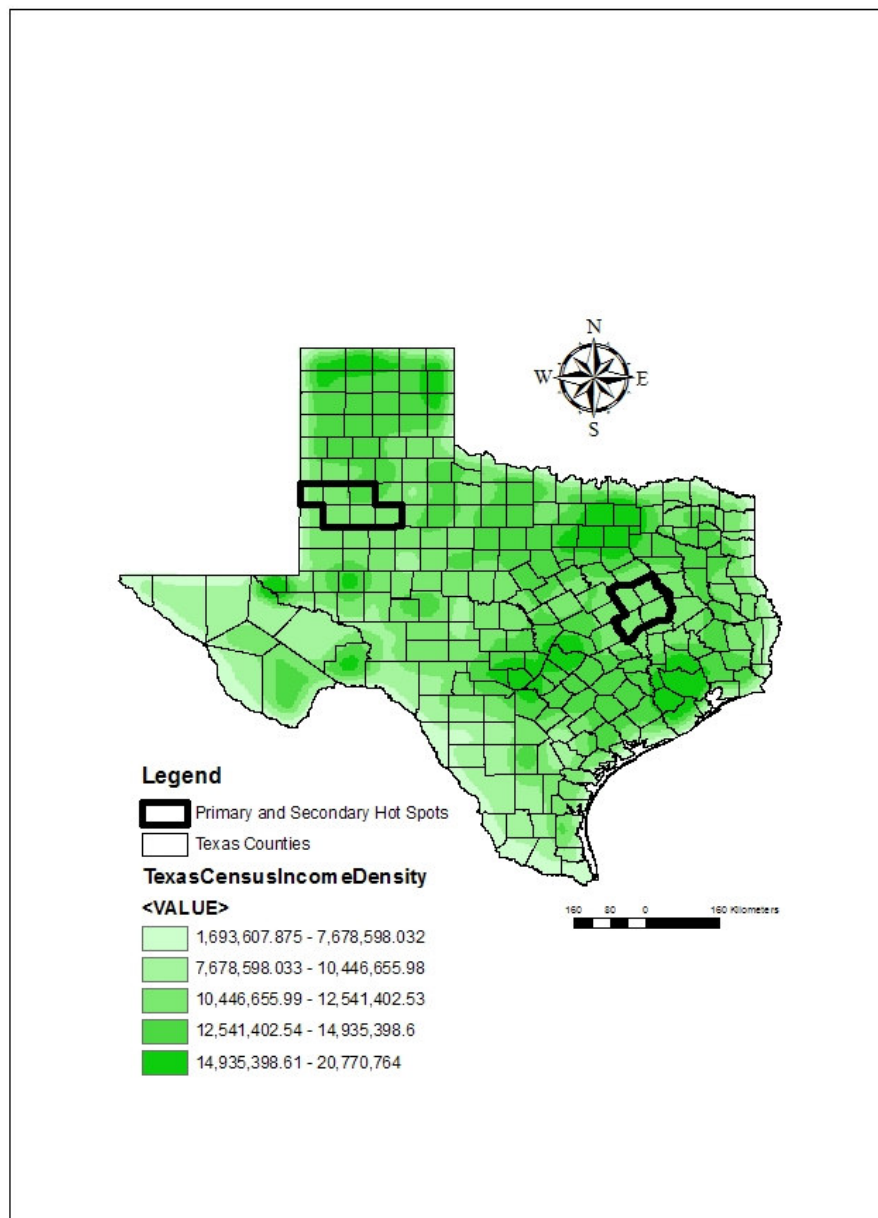


Figure 35

Texas Income Density

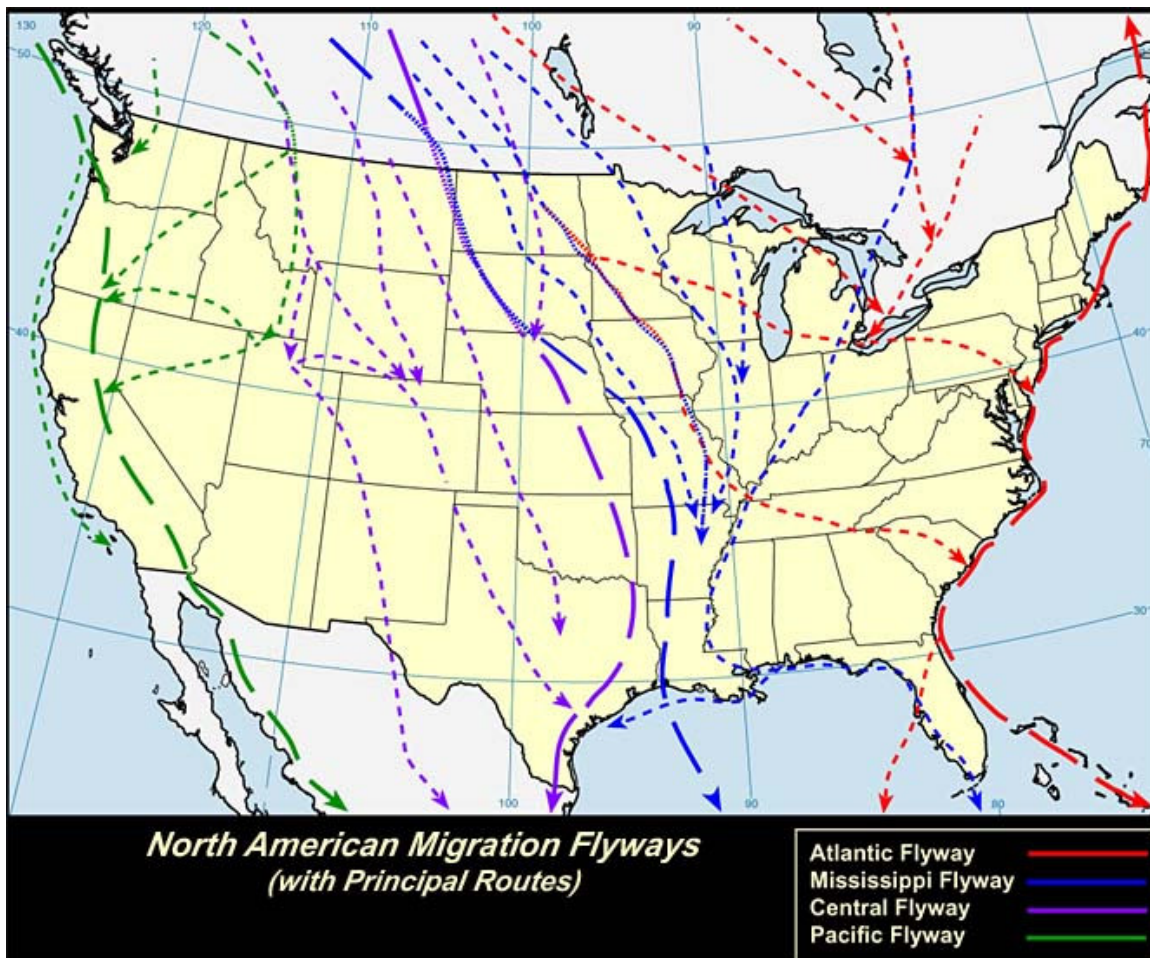


Figure 36

North American Migratory Flyways

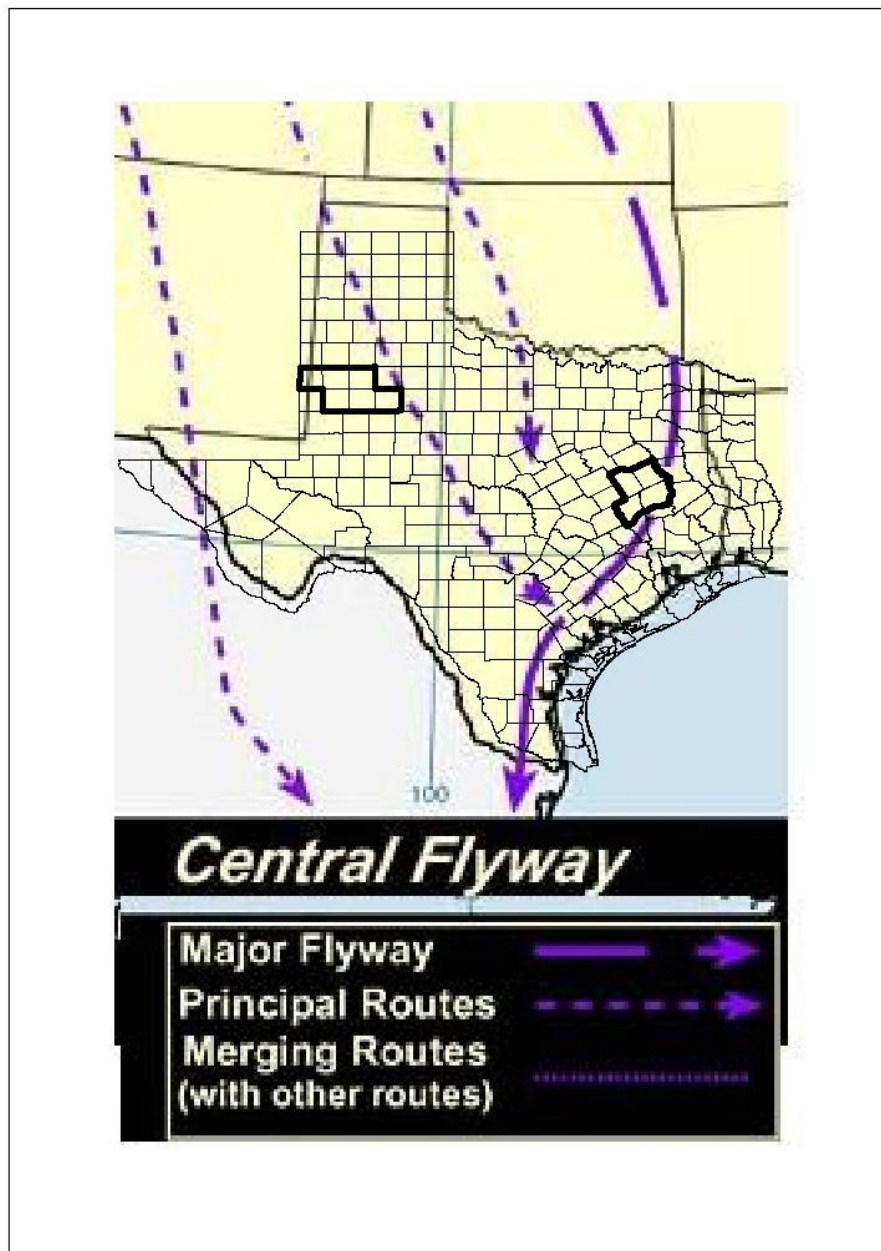


Figure 37

Central Migratory Bird Flyway Overlay with Texas Counties and Hot Spots

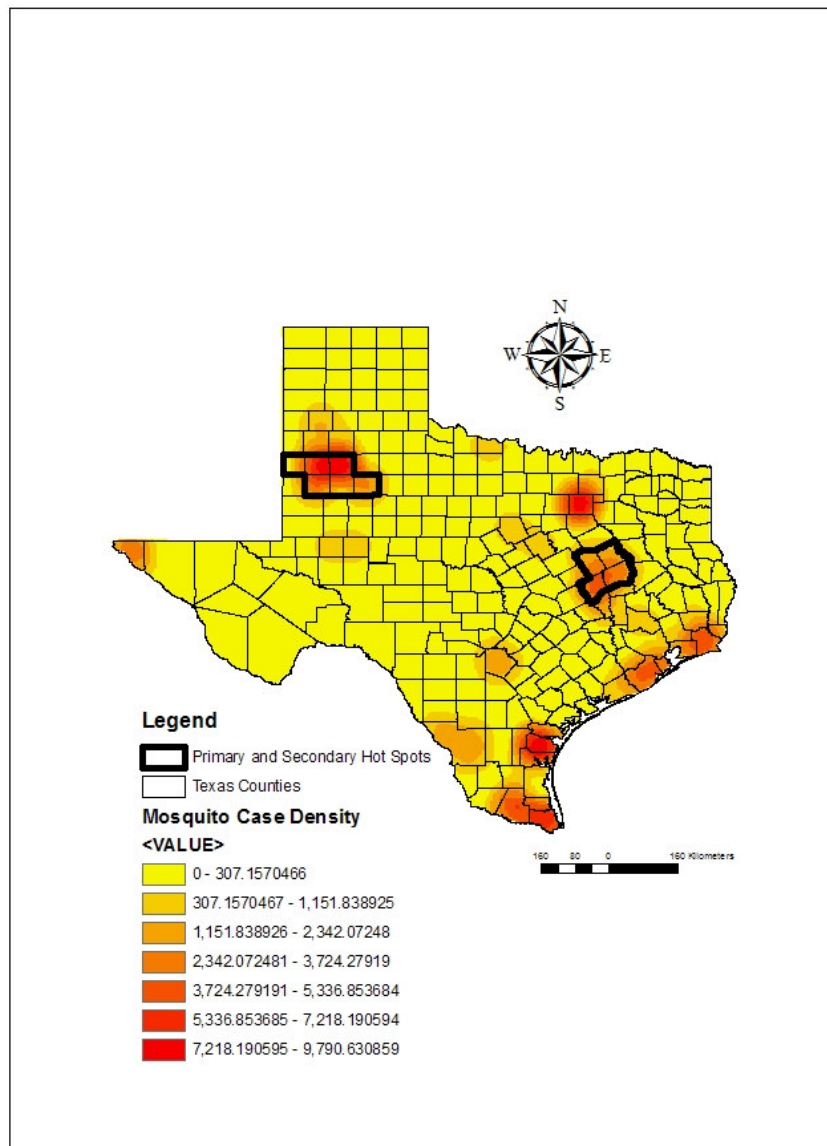


Figure 38

Mosquito WNV Case Density Map

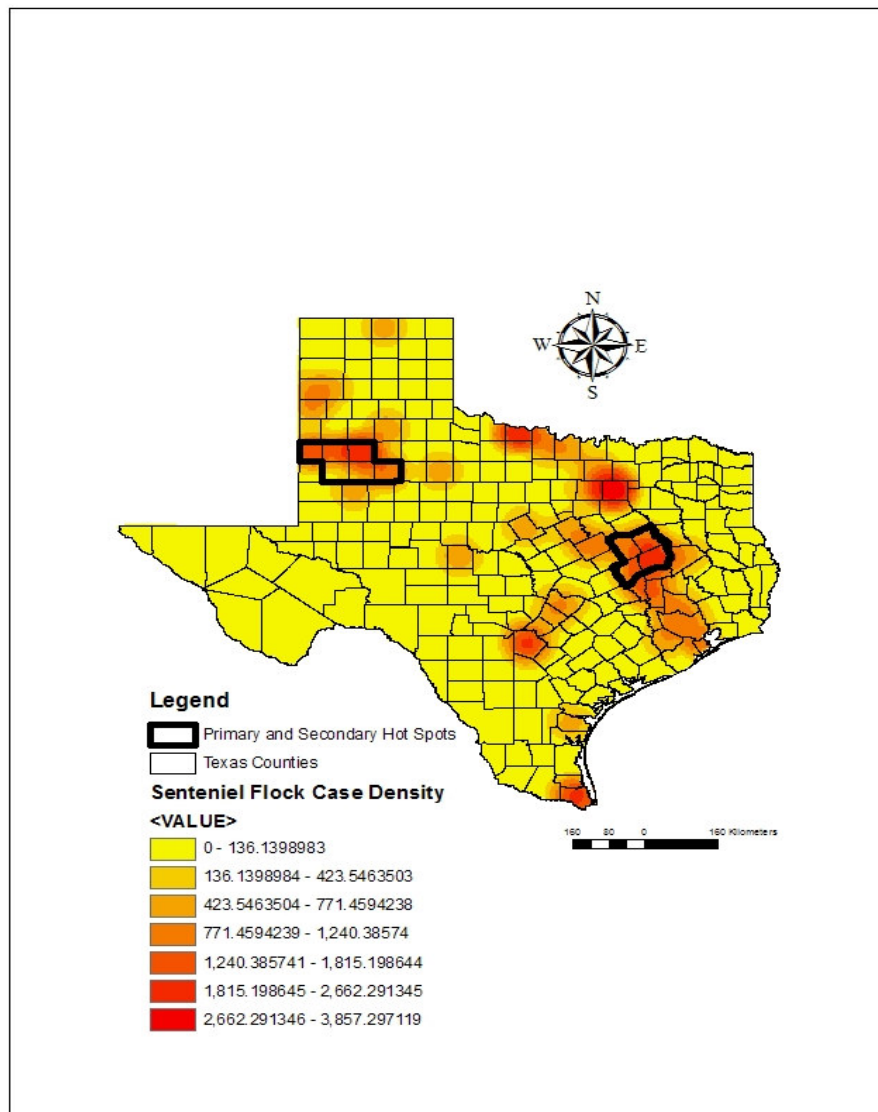


Figure 39

Wild Bird and Sentinial Flock WNV Case Density Map

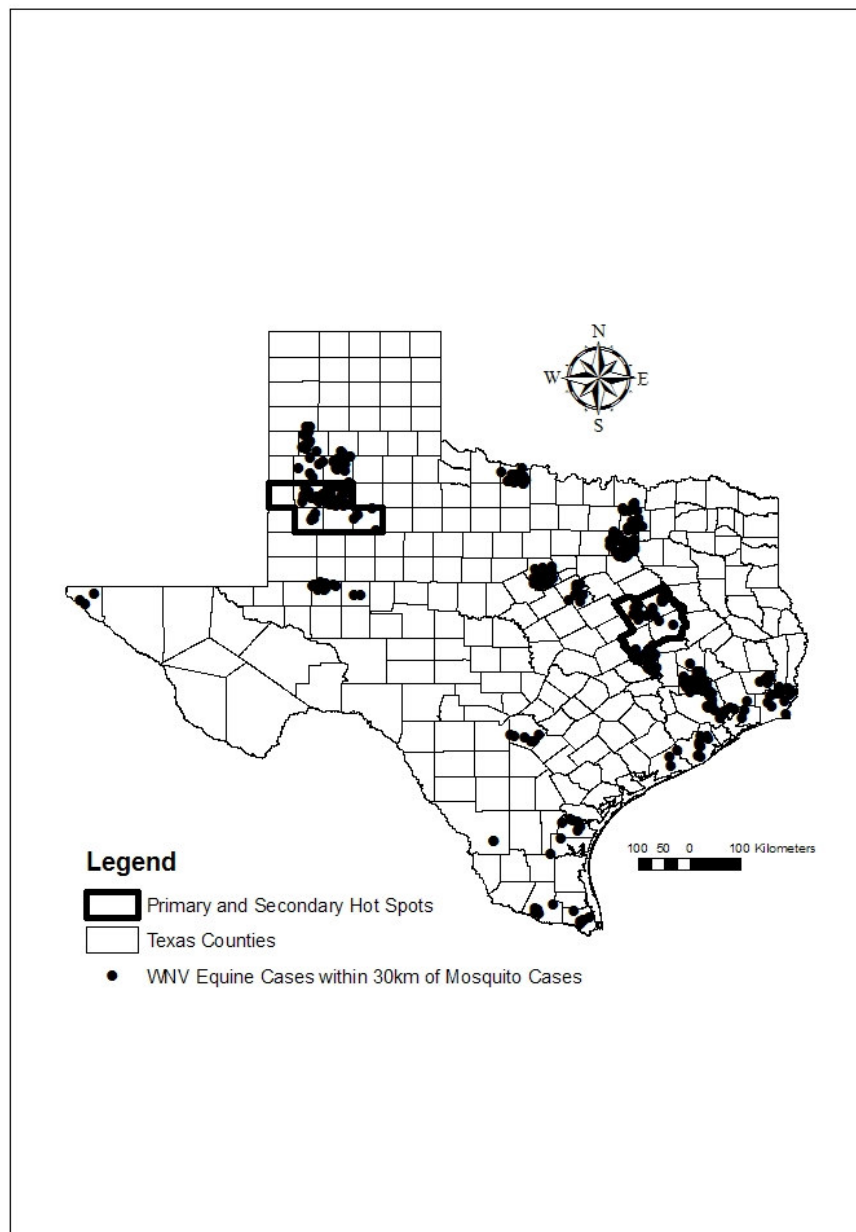


Figure 40

Map of WNV Equine Cases within 30 Km of WNV Mosquito Cases

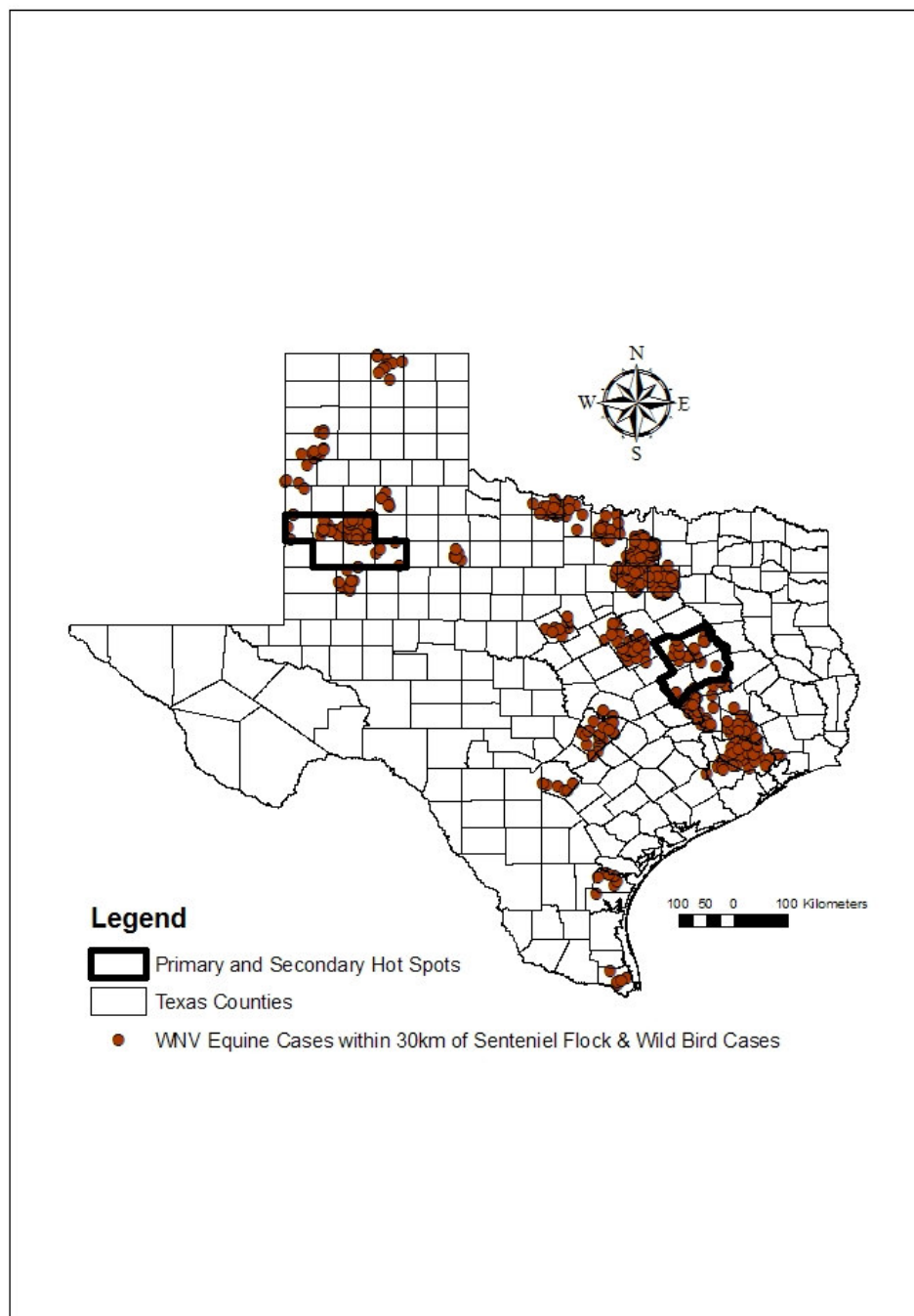


Figure 41

Map of WNV Equine Cases within 30 Km of WNV Wild Bird Cases

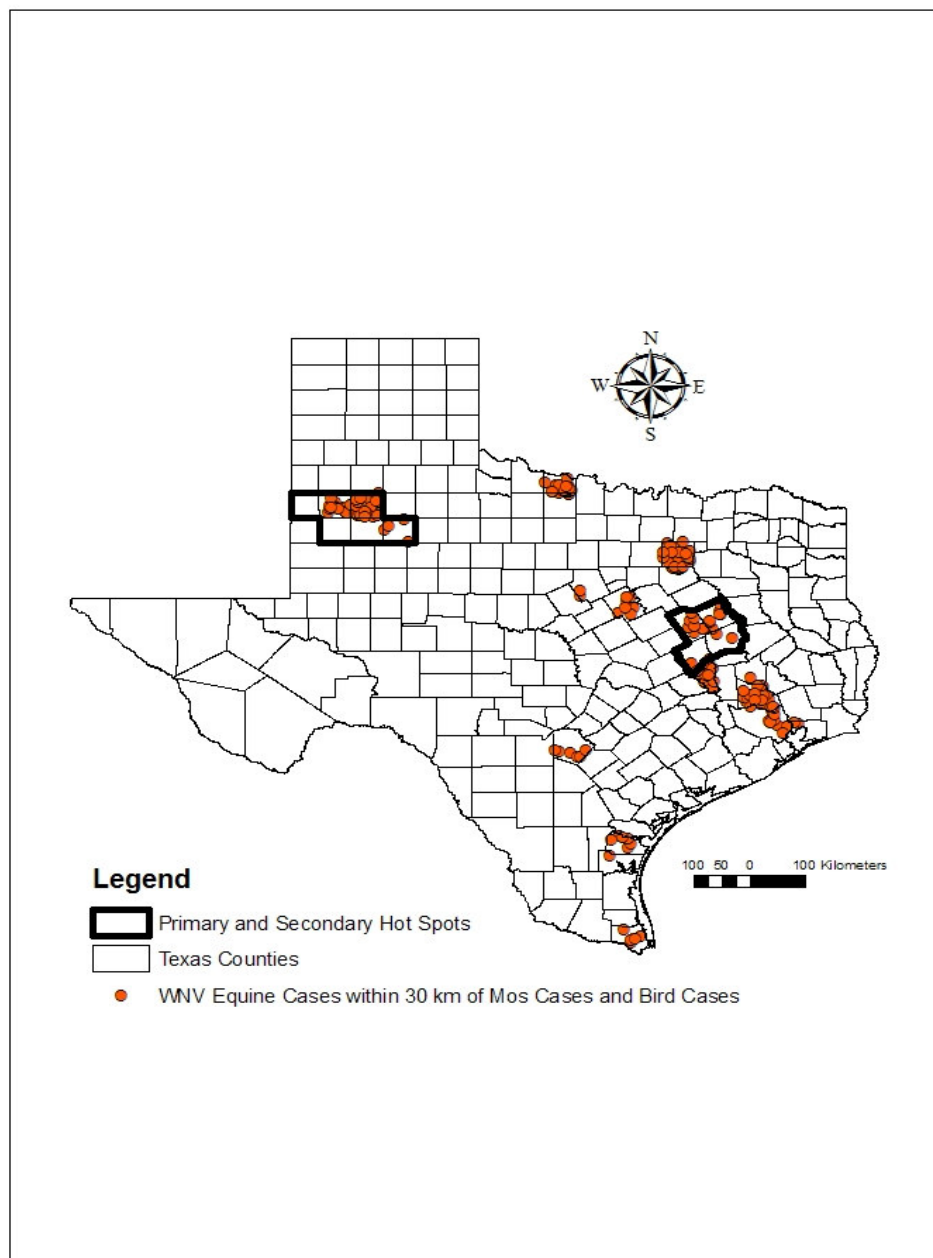


Figure 42

Map of WNV Equine Cases within 30 Km of WNV Mosquito and Wild Bird Cases

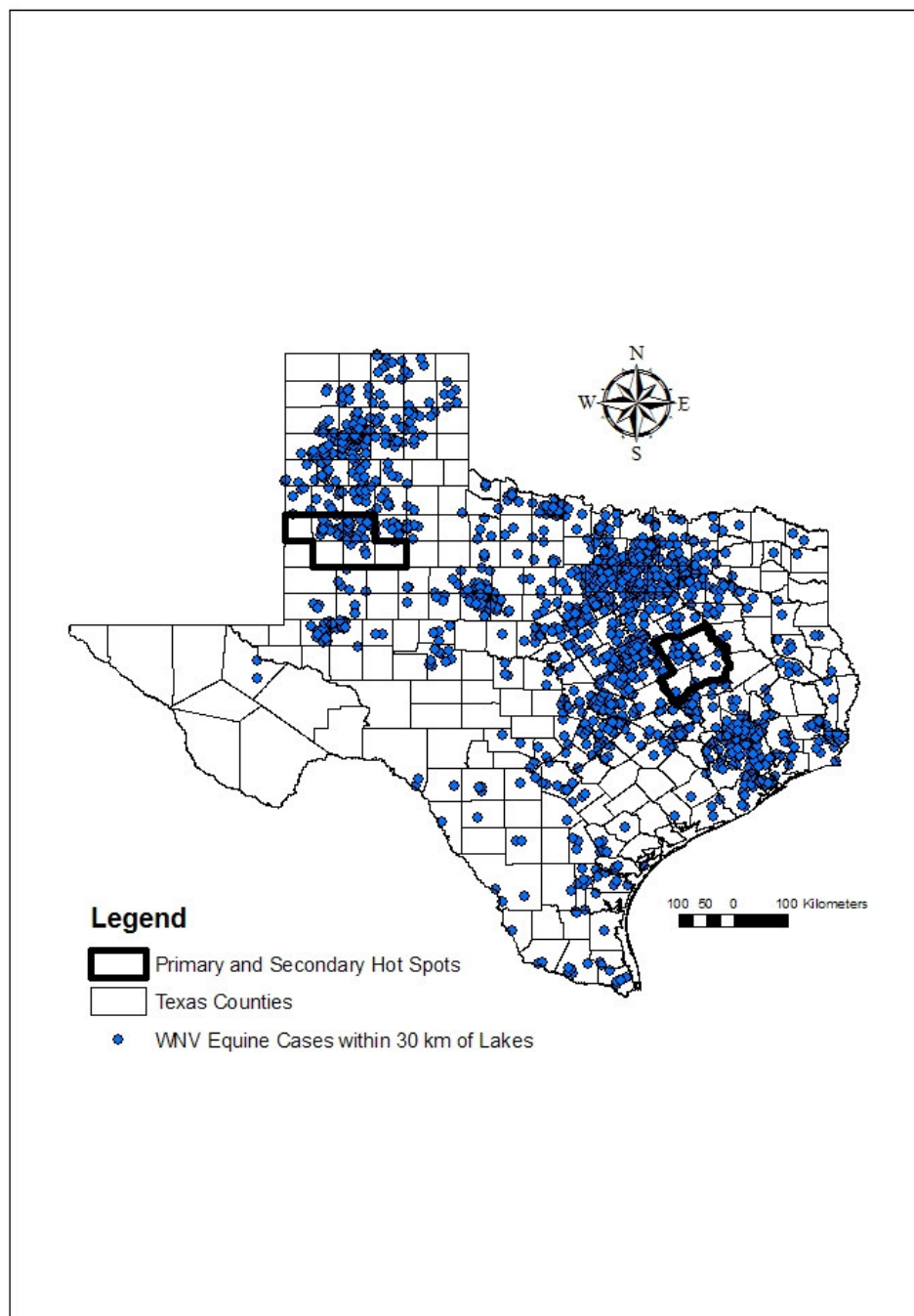


Figure 43

Map of WNV Equine Cases within 30 Km of Texas Lakes

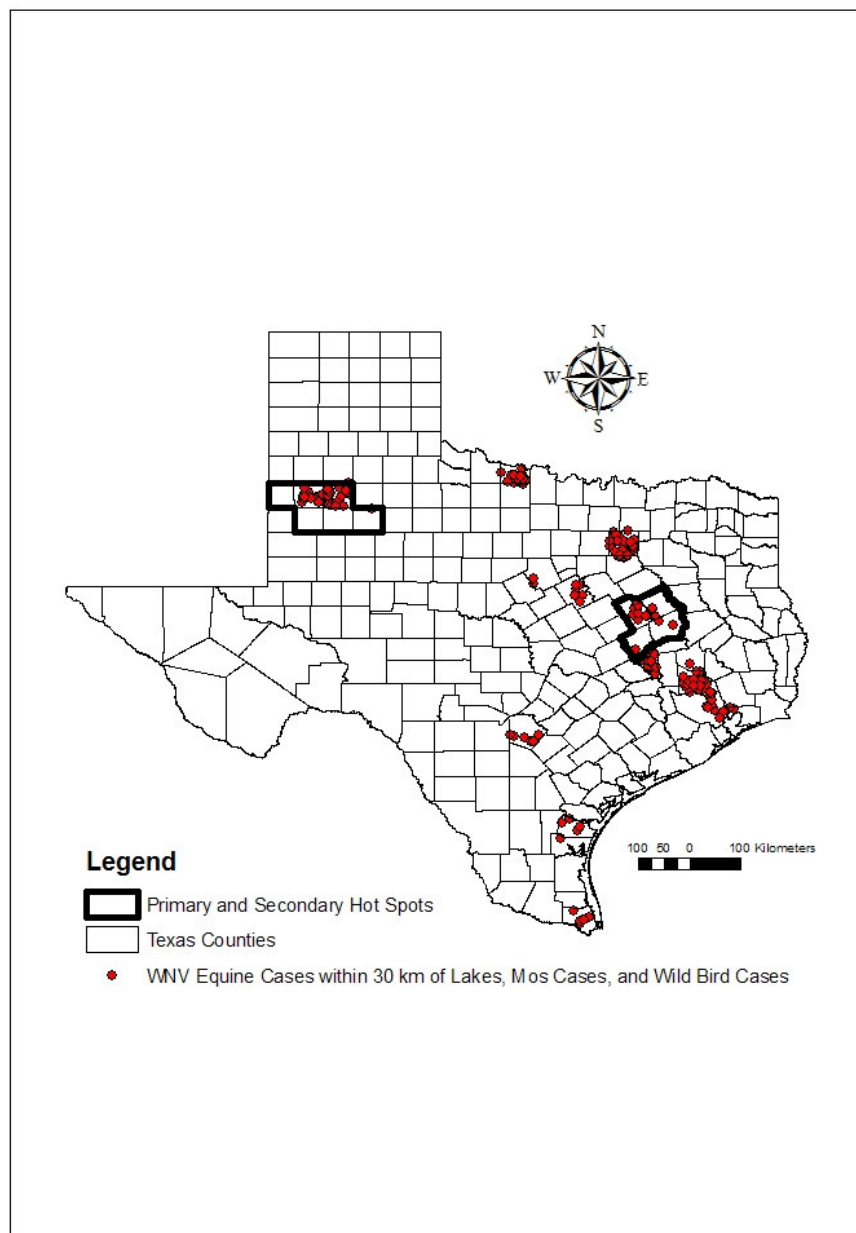


Figure 44

Map of WNV Equine Cases within 30 Km of WNV Mosquito and Wild Bird Cases and Texas Lakes

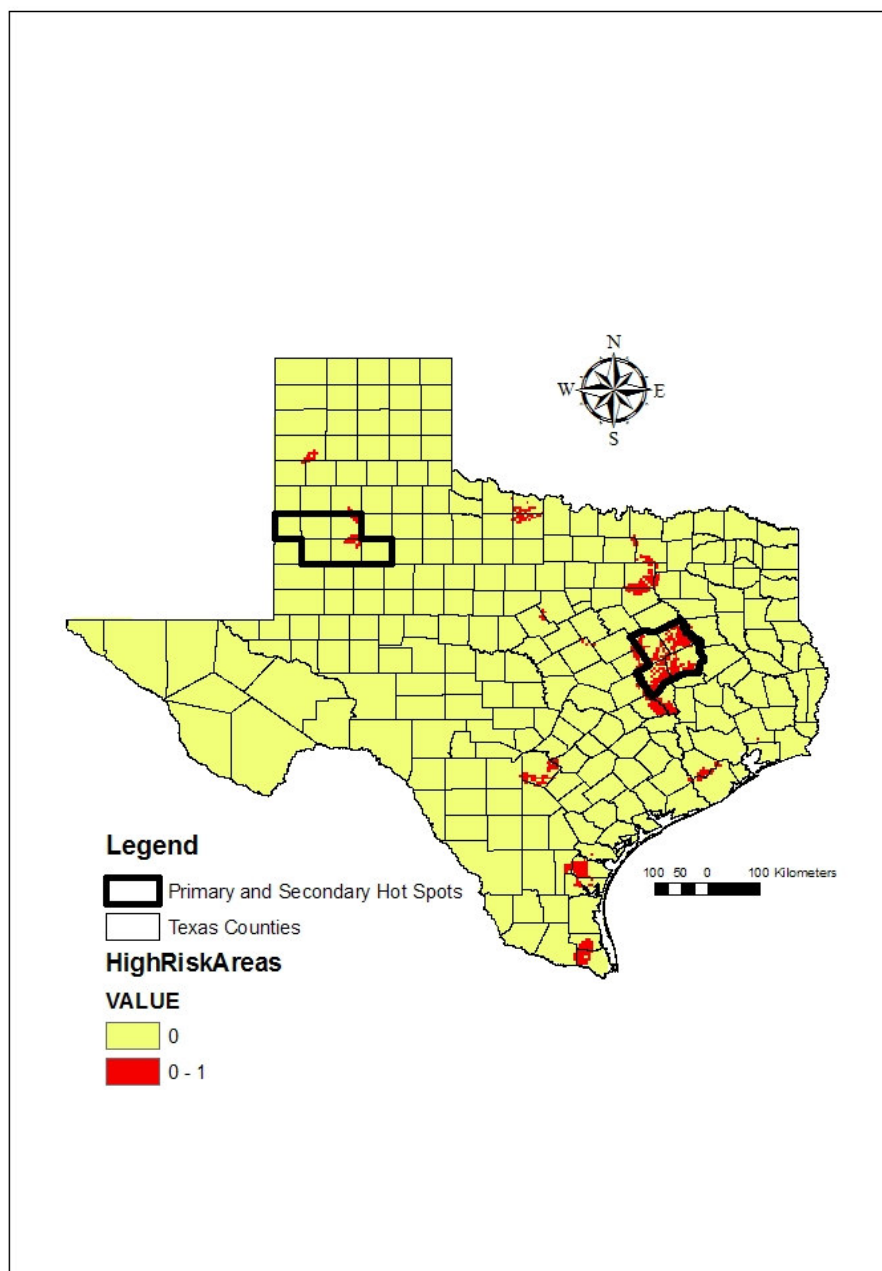


Figure 45

Raster Calculator High Risk Areas (All Hot Spot Factors)

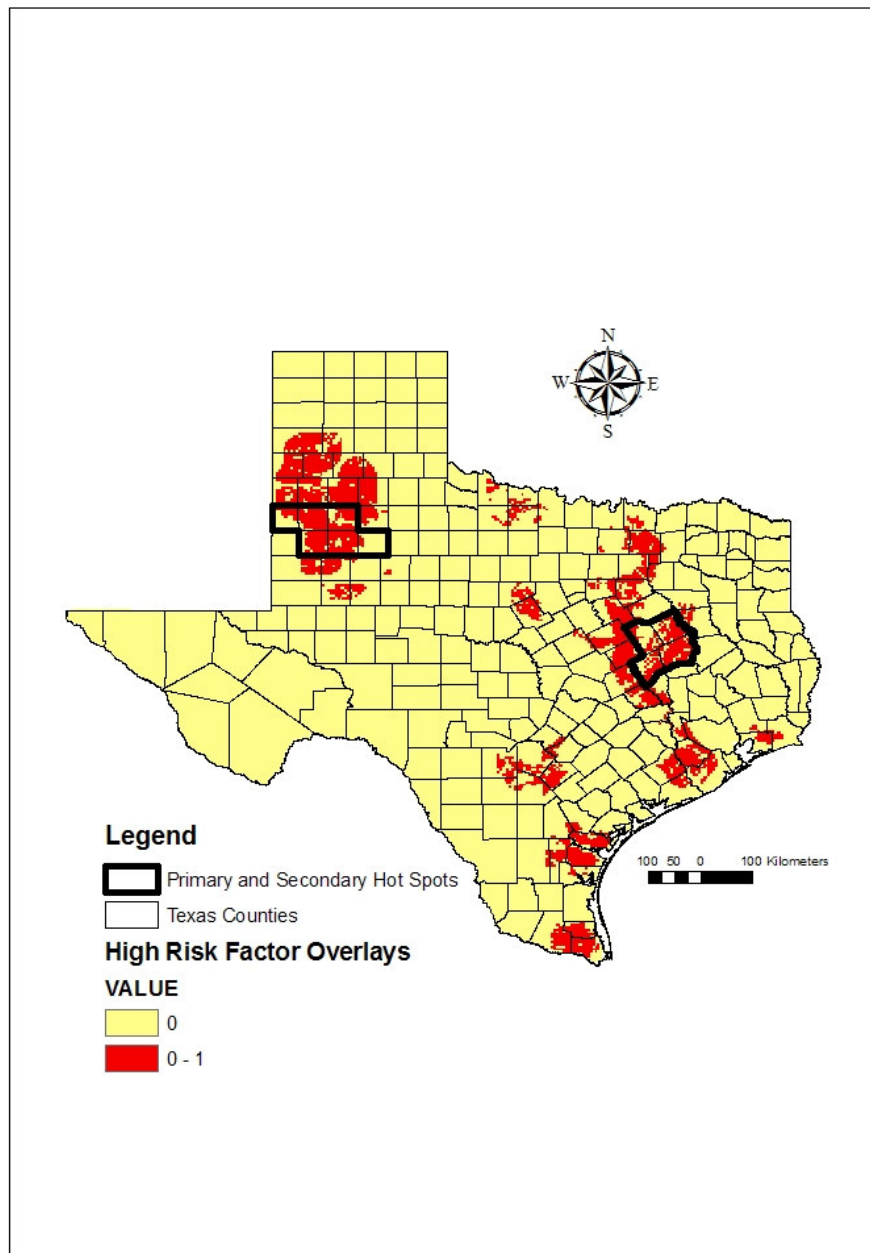


Figure 46

Raster Calculator High Risk Areas (WNV Risk Factors)

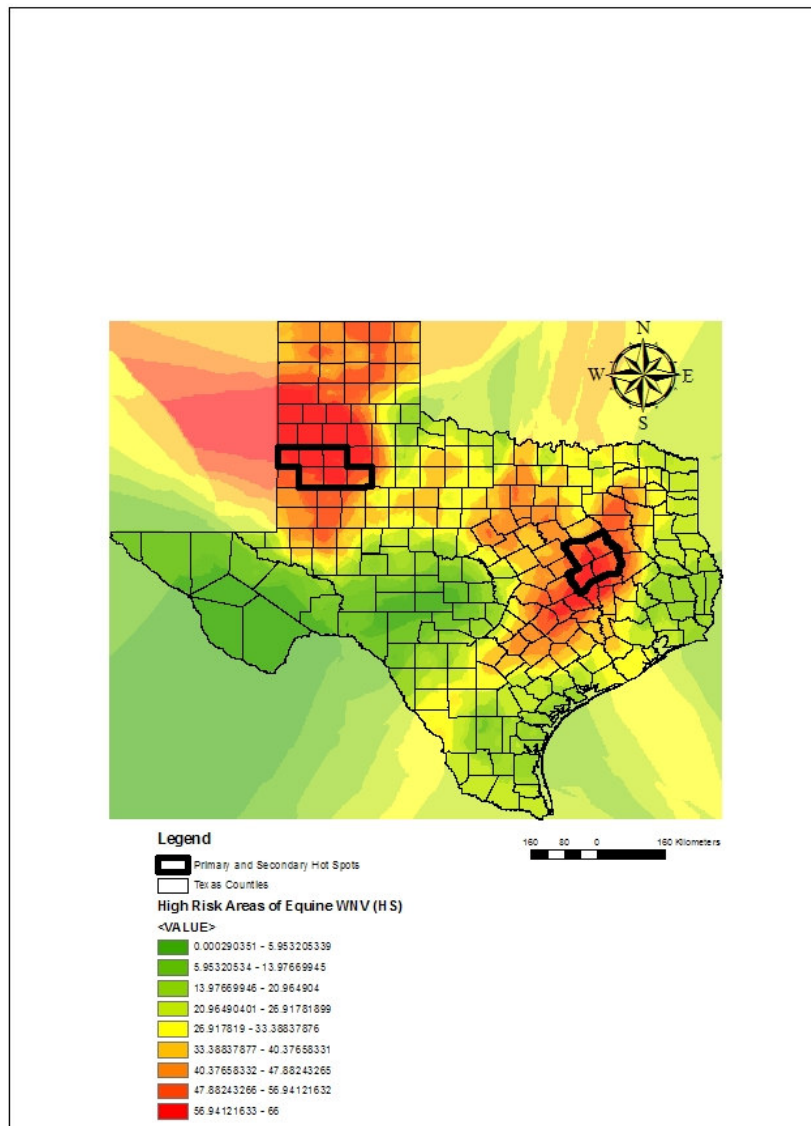


Figure 47

Kriging Interpolation Map of High Risk Areas (All Hot Spot Factors)

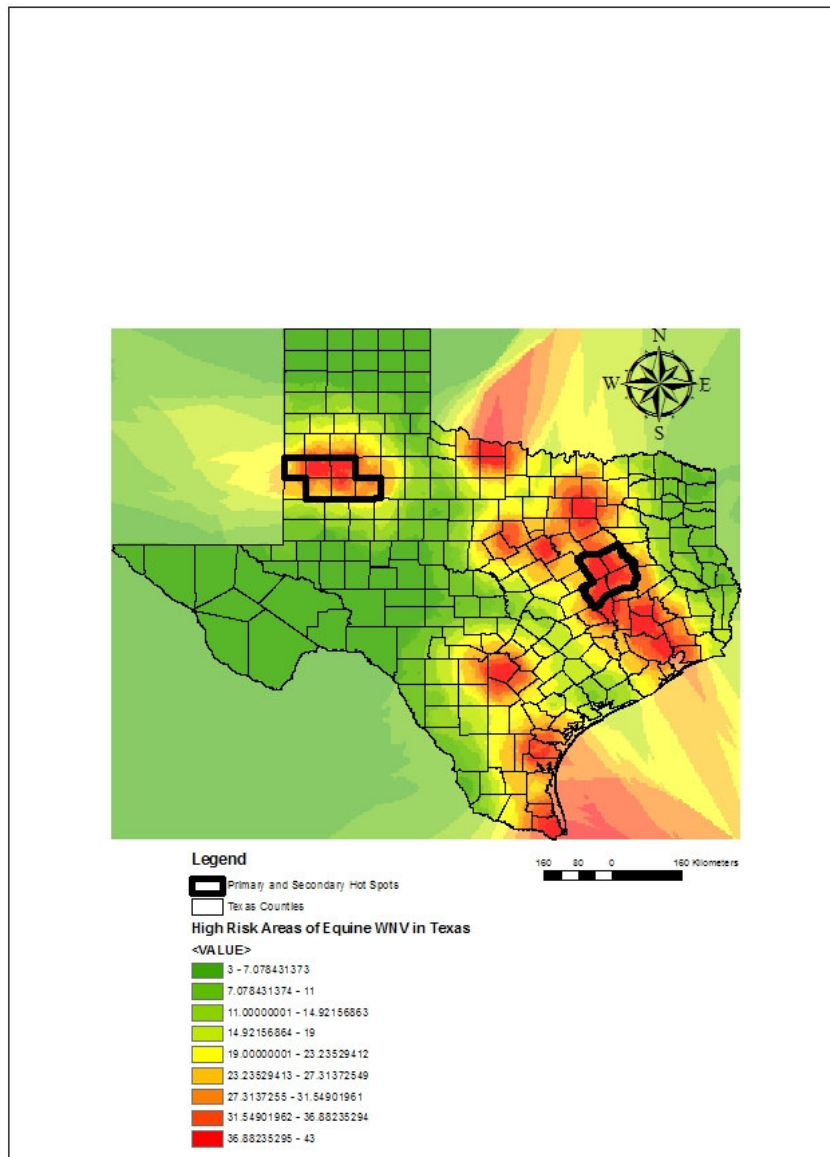


Figure 48

Kriging Interpolation Map of High Risk Areas (WNV Risk Factors)

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