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West Nile Virus

In Equids in the Northeastern United States in 2000

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West Nile Virus (WNV) in Equids in the Northeastern United States in 2000

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Introduction

Overview of the Case-Control Study and Spatial Data Analysis of WNV in Equids

The first new world outbreak of West Nile virus (WNV) in equids occurred in the Northeastern United States in 1999. Following investigations in 1999, it was recognized there were still some important questions regarding exposure and occurrence of disease among equids in this region that remained unanswered. Thus, a case-control study of premises was designed so that if cases of WNV in equids were identified in 2000, it could be implemented in a timely manner. States with confirmed WNV equine cases were requested to participate in the case-control study coordinated by USDA: APHIS: VS. This study was designed to gather information from premises with equids that developed clinical signs of WNV infection in 2000, as well as from premises that did not have confirmed disease due to WNV infection in their equids. In addition, a spatial analysis was conducted to describe the geographic and ecological aspects of case premises. Factors looked at included precipitation, temperature, and locations of case premises relative to equid inventories, WNV-positive mosquito pools, WNV-infected wild birds, elevation and ecoregions.

Materials and Methods

I. Case-Control Study

Objective

The objective of the case-control study was to identify potential environmental and management factors associated with equine exposure to WNV in the year 2000, and generate hypotheses that may direct future studies.

Case Definition

A case for this study was defined as any equid identified with exposure to WNV. A case premises was any premises with one or more cases.

Selection of Premises

During 2000, a USDA: APHIS: VS confirmed case was defined as any equid with clinical signs[1] plus one or more of the following (Ostlund, et. al, 2001):

- Isolation of WNV from tissue, blood, or cerebrospinal fluid (CSF)
- An associated 4-fold or greater change in plaque reduction neutralization test (PRNT) antibody (Ab) titer to WNV in appropriately-timed, paired sera
- Detection of both IgM Ab to WNV by IgM-capture enzyme linked immunosorbent assay (ELISA) and an elevated titer (positive at 1:10 or greater) to WNV Ab by PRNT in a single serum sample.

[1] Clinical signs included one or more of the following: ataxia (including stumbling, staggering, wobbly gait or incoordination), inability to stand, multiple limb paralysis, or acute death.

A goal of this case-control study was to include all premises with equine WNV cases confirmed by USDA: APHIS: VS. For each premises with a confirmed clinical case of equine WNV infection, data were collected from up to five premises, which were the nearest neighbors (that owned equids and were willing to participate) to the premises with confirmed cases. Participation of each premises was voluntary. Each participant signed an owner/operator agreement form and data collection was in compliance with Office of Management and Budget regulations. Questionnaire data regarding equine management and environmental conditions, as well as information on individual equids and serum samples on those equids were collected. Confidentiality regarding origin of data was assured with the exception of diagnosis or suspicion of dangerously infectious or exotic diseases such as African Horse Fever. States were alerted of the desire to gather such data when the first case in a state was identified. Premises in the following states were included in this study: Connecticut (n=40), Delaware (n=28), Massachusetts (n=9), New Jersey (n=59), New York (n=3), Pennsylvania (n=7), and Rhode Island (n=4).

Premises Data

A questionnaire was administered on each of the premises included in the study, and geographic coordinates were determined by global positioning system (GPS) methods. Questions were asked regarding the number and characteristics of the equids, whether they had any equids with signs consistent with WNV infection, and the management of equids with an emphasis on housing methods and insect control methods. Data were also collected regarding the bird and mosquito habitat, and the observation of dead birds on each operation. Lastly, there was an assessment by the data collector (a VS or State Veterinary Medical Officer, VMO) regarding the mosquito and bird habitat and the cleanliness of the premises based on described criteria. For more detail on the questionnaire and VMO assessment see Appendix A.

Animal Data

Data collectors were requested to collect serum from equids on the operation. The number of equids that were to be sampled was on a sliding scale based on the number of equids on the premises (up to 25 equids per operation). Data collectors were instructed to sample horses that represented the premises' equine population based on use, age and gender. Information gathered on each of the equids that was sampled included: housing method, insect control methods, overall health (hair/coat), color (light colored yes/no), age, sex, primary use, number of days out of the county in the past 45 days, and if it showed signs consistent with WNV infection since June 1, 2000. The serology test performed on all equids was a PRNT. Equids were considered seropositive if they had a titer of 1:10 or greater on the PRNT.

Sliding Scale for Sampling of Equids:

Number of Equids on the Operation	Number of Equids to be Sampled
Less than 20 equids	Sample all
20 to 49 equids	Sample 20
50 or more equids	Sample 25

Data Analysis Methods

Data from the questionnaires were entered into Lotus Approach databases and then exported into Statistical Analysis Systems (SAS) datasets. Using SAS, descriptive tabulations were run on all characteristics for which data were collected. Data were screened for associations of factors with WNV exposure. Chi-Square tests, or in some cases a Fisher's exact test, were run for selected characteristics to determine if the observed differences between groups were statistically significant. Odds ratios and 95 percent confidence intervals were calculated in EpiInfo. A p-value of <0.10 was considered an indication of potential association. Analysis of horse-level data was limited to equids on case premises.

Categories of Equid Infection Status

It was anticipated that there would be several different groups or categories of equids: those that had clinical signs of WNV and were confirmed to have been infected through virus isolation or serology (see confirmed USDA: APHIS: VS case definition, page 1), those that did not have signs of WNV infection but had evidence of exposure based on serologic testing, those that had clinical signs consistent with WNV but did not have evidence of exposure to this virus, and those that were clinically normal and had no evidence of exposure to WNV based on testing. For the purposes of the case-control study, a "case" equid was defined as any animal that had evidence of exposure to WNV through serologic testing performed at the National Veterinary Services Laboratories (NVSL) virology laboratory, or that was confirmed by USDA: APHIS: VS as a WNV case (see criteria, page 1). A "non-case" equid was defined as one that did not have serologic evidence of virologic evidence of exposure to WNV.

Categories of Premises

The premises were defined based on the status of the equids tested on that premises regarding their exposure to WNV. A "case" premises was a premises that had one or more equids that were identified to be exposed to WNV (serologic or virologic evidence of exposure to WNV). A "control" premises was a premises that had no evidence that the equids were exposed to WNV based on serologic testing of at least a portion of the equids on that premises.

Method of Reporting Case-Control Study Results

The results are reported in two tables **(Table 1 and Table 2)**.

Table 1 shows the premises-level results. The goal was to determine if there were any premises-level factors that were different between those premises that had evidence of exposure of their equids to WNV, and those that did not have evidence of exposure. There are two columns in Table 1. The first column is the percentage of premises with one or more equids that were confirmed WNV cases or with one or more equids with serologic evidence of WNV infection, i.e. case premises. The second column is the percentage of premises that had all tested equids with a negative serologic test, i.e. control premises. Each is broken out by various premises characteristics.

Table 2 shows equid-level results on premises with evidence of one or more equids with exposure to WNV, i.e. case premises. The goal of this analysis was to evaluate factors associated with individual animals. Using equids from only case premises avoids attributing to individual animals some of the variability that may be more likely due to differences between case and control premises. There are two columns for equids on case premises. The first column is the percentage of case equids on case premises broken out by equid characteristics or individual management factors. The second column is the percentage of non-case equids on case premises.

II. Spatial Data Analysis

Spatial Data Sources

Each data collector obtained the geographic coordinates of case and control premises, using standardized global positioning system (GPS) methods. On the premises information form (see Appendix A), coordinate data were recorded in decimal degrees, using the 1983 North American Datum (NAD-83). Premises coordinates and the survey database were imported into a geographic information system and this spatial information was displayed as point data, using ArcView, version 3.2a, (ESRI, Redlands, CA). In summarizing state and county data, point information was aggregated, using a point-in-polygon process, and then attributes were assigned to polygons representing administrative boundaries.

Premises with one or more infected equids in which questionnaire data and geographic coordinates were not obtained were geocoded using geographic coordinates of the nearest city. City coordinate data were obtained from an on-line gazetteer, the Getty Thesaurus of Geographic Names (www.getty.edu/research/tools/vocabulary) and added to the spatial database. Nearest city coordinates were used in analyses when comparisons involved all case equid sites, including those without a completed questionnaire. However, in analyses that included questionnaire-completed case premises, only the more accurate GPS coordinates were used.

Information on WNV infection in equids on premises not included in the case-control survey was obtained from the USDA: APHIS West Nile Virus Statistics web site (http://www.aphis.usda.gov/oa/wnv/wnvstats.html). This site also provided data on the approximate location of human cases, test results from the dead bird surveys, and virus detection results from field collected mosquitoes. For comparative purposes, a summary of information on the 1999 WNV outbreak was obtained from the USDA: APHIS: West Nile virus Summary web site (http://www.aphis.usda.gov/vs/ep/WNV/summary.html).

Temperature and precipitation data for August 1 through October 31 were obtained from the National Oceanic and Atmospheric Administration's (NOAA) National Climate Data Center. Records from 93 weather stations were used to calculate daily mean minimum temperature and data from 141 stations were used to determine the daily mean precipitation for the 32,900 mi^2, affected area.

Thirty-year average total precipitation data for 1961-1990 were obtained from The Climate Source, LLC (Corvallis, OR), in a grid format with a spatial resolution of 1.25 arc-minutes (about a 2-km^2 area). Average precipitation values, measured in millimeters, were based on reports from 7,700 National Weather Service stations. Precipitation data were interpolated between weather stations and variation due to topography was minimized, using the Parameter-elevation Regressions on Independent Slopes Model (PRISM) developed by Daly and Neilson (1992; Daly et al., 1994). In this regression-based interpolation process, precipitation values from weather reporting stations were weighted according to distance, elevation, cluster, vertical layer, topographic facet, coastal proximity, and effective terrain.

Administrative boundary data were obtained from ESRI Data and Maps, 1999 edition. County-level equid population data were obtained from the 1997 U.S. Census of Agriculture, Geographic Area Series (USDA: National Agricultural Statistics Service). Information on ecological regions was obtained from Bailey (1995). Terrain data were obtained from the Digital Terrain Elevation Data (DTED), Level 0, 30 arc second data blocks (DoD: National Imagery and Mapping Agency, formerly the Defense Mapping Agency).

Variation in vegetation conditions was assessed with image data from NOAA polar-orbiting satellites obtained from the U.S. Geological Survey's EROS Data Center (EDC), Sioux Falls, SD. Daily observations with the Advanced Very High Resolution Radiometer (AVHRR) sensor provided 1-km resolution data. Sensor data were calibrated to wavelength standards and processed into biweekly composites designed to exclude clouds and other forms of atmospheric interference. From these data, the maximum normalized difference vegetation index (NDVI) was calculated, using the method of Eidenshink (1992). The NDVI is the difference of near-infrared (channel 2) and visible (channel 1) reflectance values normalized over the sum of channels 1 and 2. Although the NDVI equation produces values ranging from –1.0 to 1.0, we used scaled values which were expanded over a zero to 200 range. Values are proportional to increasing amounts of vegetative biomass as measured by the property of light reflectance from chlorophyll pigment contained in plant chloroplasts.

Spatial Data Analysis

Thematic data layers were assembled using ArcView GIS, version 3.2a and analyzed with Spatial Analyst (ver. 1.1), Grid Analyst, X-Tools, and other ArcView extensions. Spatial Stats for S-Plus, version 2000 (Insightful, Inc., Seattle, WA) was used to create spatial regression models, using nearest neighbor methods. S-Plus Spatial Stats was also used in the analysis of spatial clusters, using Geary's C spatial autocorrelation methods (Geary, 1954; Cressie, 1993), and Ripley's K-function statistics (Venables and Ripley, 1999), and an intensity analysis (Diggle, 1983; Bailey and Gatrell, 1995). In addition, space-time clusters were evaluated using SatScan software, version 2.1.3 (Kulldorff, et. al, 1998, National Cancer Institute). The geographical center of the outbreak was determined using minimum polygon fitting methods (Harvey and Barbour, 1965). With this technique, line segments were drawn to connect the outermost case sites and then the resulting polygon's geometric properties were determined. Fourier polygon models were generated in TNTmips, version 6.5 (MicroImages, Inc., Lincoln, NE), using a point data fitting method described by Anderson (1982).

Vegetation characteristics were analyzed using biweekly composites of NDVI values. Three time periods that spanned the midpoint of the epizootic were used and these included: September 8 - 21, September 21 - October 5, and October 6 - 19. The ISODATA unsupervised classification algorithm (Tou and Gonzales, 1974) was applied to organize NDVI values into 10 statistically related classes. A correlation matrix, produced from a class co-occurrence analysis and a separability dendogram were constructed to assess class relationships.

Results

I. Case-Control Study

A total of 60 equine cases of clinical disease due to WNV infection was confirmed by USDA: APHIS: VS in the northeastern US in 2000. Of the 60 equids, 37 survived and 23 died or were euthanatized (38% case-fatality rate). Equids ranged in age from five months to 38 years with an average age of 14 years; 36 confirmed cases of WNV infection occurred in male horses and 24 in females. These cases occurred in seven states including Connecticut (seven confirmed cases of WNV infection in five counties), Delaware (four confirmed cases in two counties), Massachusetts (one confirmed case in one county), New Jersey (27 confirmed cases from 11 counties), New York (19 confirmed cases in four counties), Pennsylvania (one confirmed case from one county), and Rhode Island (one confirmed case from one county).

Forty-one (41) of the 60 premises with USDA: APHIS: VS confirmed cases referred to above participated in this case-control study. An additional eight premises were identified through serologic testing conducted as part of this study to have evidence of equids exposed to WNV. Of the 49 premises in this study with equids that had evidence of WNV exposure (case premises, see definition page 2 and 3), nine had more than one case equid: five premises had two case equids, three premises had three case equids, and one premises had five case equids. Eight of the 49 case premises had one equid that was seropositive but had no equids with clinical signs of WNV infection.

The eight premises which had equids with no clinical signs but serologic evidence of exposure (case premises, see definition page 2 and 3) had a mean of 22.0 equids bled per premises and a mean of one seropositive equid per premises. The 41 case premises with a clinical case had a mean of 10.3 equids bled per premises and a mean of 1.4 seropositive equids per premises (this includes 10 equids that were not available to be bled as part of the case-control study but were USDA: APHIS: VS confirmed as WNV infected). The 101 control premises had a mean of 9.0 equids bled per premises.

Table 1. Premises Results

Percent case premises and percent control premises by the following characteristics:

Table 1

Number of Premises	49	101
Characteristics of Premises:	**Percent Case Premises** (confirmed clinical case or seropositive equid)	**Percent Control Premises**

1-1. Types of Equids Present on Premises:

Horse	98.0	98.0
Mule	2.0	1.0
Donkey or Burro	12.2	2.0
Pony	28.6	37.6
Miniature horse	12.2	7.9

1-2. Acreage Available for Equine Turn-out:

Less than one acre per equid	12.2	6.9
One plus acre per equid	87.8	93.1
	100.0	100.0

- **Note:** The observed association between premises' infection status and available acreage was not statistically significant (p=0.35).

1-3. Other Types of Domestic Animals Present on Premises:

Cattle	12.2	5.9
Pigs	6.1	4.9
Sheep	14.3	2.0
Goats	24.5	16.8
Chickens	26.5	26.7
Turkeys	4.1	2.0
Domestic waterfowl	8.2	14.9
Other fowl *(Pheasants, Guinea Fowl, Peacocks)*	14.3	6.9

1-4a. Housing Type - Percent Premises that Housed 1 or More Equids by Day in:

Stalls only	34.7	23.8
Outdoor but some shelter *(either stalls with runs, dry lot with shelter or pasture with shelter)*	67.3	67.3
All outdoors *(dry lot or pasture only)*	32.7	38.6

- **Note:** The observed association between premises' infection status and stall housing only was not statistically significant (p=0.16).

1-4b. Categories of Housing Type - Percent Premises Housing by Night in:

Stalls only	44.9	39.6
Outdoor but some shelter *(either stalls with runs, dry lot with shelter, or pasture with shelter)*	63.3	65.4
All outdoors *(dry lot or pasture only)*	28.6	24.8

1-5. Categories for Insect Control Methods Currently in use in Equine Housing Area:

Screen on barn or stalls	18.2	11.7
Chemicals *(spray, foggers, topical repellent on horse, other chemicals)*	34.1	35.8
Mechanical *(zappers, tape, traps)*	40.9	36.2

1-6. Mosquito Habitat - Within ¼ Mile of Equids, in Past 30 Days:

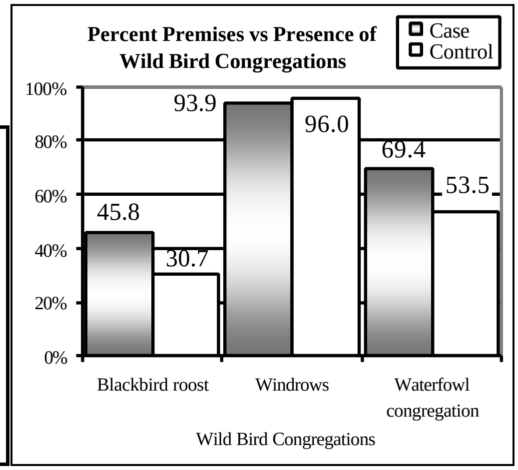
Ditches with standing water	44.9	47.5
Other standing water	87.8	72.3
If Other Standing Water is Yes, Specifically:		
Temporary pond	30.2	31.5
Waste lagoon	0.0	0.0
Fresh water marsh	34.9	34.3
Salt marsh	7.0	2.7
Swamp or pond	58.1	65.7

1-7. Primary Water Delivery Systems for Equids:

Ground water *(running water, ditch or surface)*	6.3	4.1
Man-made water delivery *(bucket, trough, bucket and trough, automatic waterer)*	93.7	95.9
	100.0	100.0

1-8a. If Barn = Yes, then (N=	**44**	**94)**
Barn is designed to minimize roosting birds	12.5	19.3
1-8b. Any Birds in Barn:	88.6	78.5

- **Note:** The observed association between premises' infection status and any birds present in the barn was not statistically significant (p=0.15).



1-9. Various Specific Wild Bird Congregations:

Blackbird roost within ½ mile of equids in last 30 days	45.8	30.7
Windrows within ½ mile of equids	93.9	96.0
Wild waterfowl congregations within ½ mile of equids in last 30 days	69.4	53.5

- **Note:** There appeared to be a marginally significant association between infection status of premises and the presence of a blackbird roost, p=0.07, OR= 1.91, 95% CI: (0.89 <OR< 4.12), and the presence of waterfowl congregations, p=0.06, OR=1.97, 95% CI: (0.90<OR<4.34) within ½ mile of equids in the last 30 days.

1-10. Dead Wild Birds Noted on Premises Since May 1, 2000:

Crows	2.0	1.0
Blue Jays	4.1	0.0
Hawks	0.0	1.0
Other *	28.6	17.8
Any dead birds	28.6	18.8

** 12 premises reported Sparrows, 4- Starlings, 4- Pigeons/Mourning Doves, 2- Geese, 2- Blackbirds, plus a few other types of birds only reported once.*

- **Note:** The observed association between premises' infection status and the percent of premises with any dead birds was not statistically significant (p=0.18).

1-11a. Clinical Signs in Equids on Premises Since August 1, 1999*:

Any of below	83.7	2.0
Apprehension	32.7	0.0
Depression	57.1	2.0
Listlessness	59.2	2.0
Head shaking	18.4	1.0
Lip paralysis	16.3	1.0
Ataxia	77.5	2.0
Weak hind legs	63.3	1.0
Inability to stand	42.9	1.0
Limb paralysis	18.7	0.0
Paresis	30.6	0.0
Combo for WNV *one or more of the following: increased apprehension, depression or listlessness **and** one or more of other 7 clinical signs.*	67.3	2.0

***Note:** This question was asked to determine if any equids on premises since 1999 (when WNV was first recognized in the US) had neurological signs.

1-11b. Of Premises with Equids Noted to have Above Signs, Percent of Premises Where:

(N=	41	2)
At least one equid died	39.0	0.0
At least one equid was tested for WNV	97.6	0.0
At least one equid was positive on test for WNV	97.6	0.0

1-11c. Percent of Premises (with Clinical Signs) by Date of Onset of Clinical Signs for 1st Equid:

August	9.8	100.0
September	53.7	0.0
October	36.5	0.0
	100.0	100.0

1-11d. Of Equids with Clinical Signs of WNV:

Earliest date noted	8/14/00	8/15/00
Latest date noted	10/30/00	8/15/00

1-12a. Data Collector Assessment of Mosquito Habitat:

(N=	49	100)
Low	16.3	25.0
Moderate or high	83.7	75.0
	100.0	100.0

1-12b. Data Collector Assessment of Bird Congregation Habitat:

Low	34.7	26.0
Moderate or high	65.3	74.0
	100.0	100.0

1-12c. Data Collector Assessment of Cleanliness of Operation:

Poor	2.1	0.0
Fair	23.4	14.1
Good or excellent	74.5	85.9
	100.0	100.0

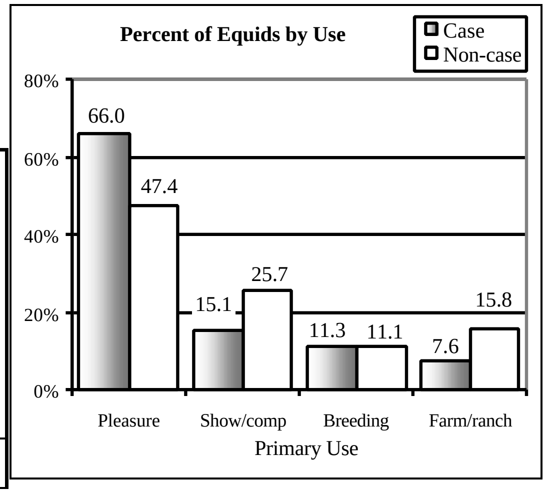
- **Note:** Equids with clinical signs of WNV were predominantly but not exclusively on case premises i.e., not all equids with clinical signs of neurologic disease were positive to serologic testing for WNV antibody. One or more equids died on 39 percent of case premises. The majority of case premises had WNV confirmed cases in September and October of 2000. Data collector assessment of mosquito habitat and bird congregation habitat was not different between case and control premises.

Table 2. Animal Results on Case Premises

Percent case equids on case premises and percent non-case equids on case premises by the following characteristics: the **bold** numbers for each characteristic are the number of equids with data for that characteristic.

Table 2

Total Number of Equids	54	540
Characteristics:	Percent Case Equids	Percent Non-case Equids



2-1. Primary Use:	**53**	**540**
Pleasure	66.0	47.4
Show, competition or race	15.1	25.7
Breeding	11.3	11.1
Farm, ranch or other	7.6	15.8
	100.0	100.0

- **Note:** There was an association between horses' infection status and use for pleasure vs. other use on case premises, p=0.01, OR=2.16, 95% CI: (1.15<OR<4.07).

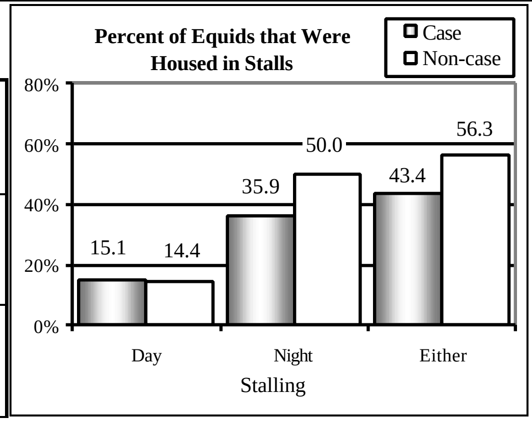
2-2. Age:	**34**	**213**
Less than 5 years	11.8	16.4
5 to 19 years	70.6	64.3
20 plus years	17.6	19.3
	100.0	100.0

- **Note:** There was no association between age and exposure to WNV status (p=0.7).

2-3. Gender:	**33**	**180**
Female	42.4	40.0
Male*	57.6	60.0
**There were no positive stallions in this study.*	100.0	100.0

2-4. Light Color:	**51**	**525**
Yes	23.5	19.8
No	76.5	80.2
	100.0	100.0

2-5. Housed in Stall:	**53**	**540**
Daytime:		
Yes	15.1	14.4
No	84.9	85.6
Night:		
Yes	35.9	50.0
No	64.1	50.0
Either:		
Yes	43.4	56.3
No	56.6	43.7



- **Note:** There was some evidence that case equids were less likely to be housed in stalls, p=0.07, OR=0.60, 95% CI: (0.32<OR<10.9). This appeared to be especially important at night.

2-6a. Insect Protection (Day):	**51**	**517**
Yes	52.9	50.7
No	47.1	49.3
	100.0	100.0
(Type of insect protection was almost exclusively insecticide/repellent)		

2-6b. Insect Protection (Night):	**51**	**517**
Yes	17.7	10.6
No	82.3	89.4
	100.0	100.0

- **Note:** The observed association between use of insect protection at night and exposure to WNV status was not statistically significant (p=0.13).

2-7. Signs Since 6/1/2000:	**54**	**540**
Apprehension	16.7	0.0
Depression	31.5	0.4
Listlessness	35.2	0.4
Head shaking	9.3	0.2
Lip paralysis	7.4	0.0
Ataxia	42.6	0.6
Weak hind legs	35.2	0.2
Inability to stand	14.8	0.0
Limb paralysis	5.6	0.0
Paresis	11.1	0.0
Any of the above	46.3	0.6
Combination for WNV	38.9	0.4

2-8. Travel History:	**51**	**454**
Yes	9.8	17.4
No	90.2	82.6
(Has been out of the county in previous 45 days)	100.0	100.0

- **Note:** Although there was an observed tendency for case equids not to have been out of the county, this was not statistically significant (p=0.17).

II. Spatial Analysis

A total of 64 premises were identified as having one or more equids infected with WNV. During the outbreak, 56 premises were confirmed to have one or more equids infected with WNV and, of these premises, 95% of the case sites were from New Jersey (48.2%), New York (26.8%), Connecticut (12.5%), and Delaware (7.1%). Rhode Island, Massachusetts, and Pennsylvania each had one case premises. Of the 56 premises identified during the outbreak, 41 premises were included in the case-control study. Serological testing of equids on farms selected as control sites resulted in finding eight additional premises with a subclinically infected equid. Figure 1 shows the number of case premises in relation to the states affected.

The geographic distribution of premises with case equids is shown in Figure 2. The geographic extent of equid infections in 2000 ranged from Middlesex County, Massachusetts, in the north and east, to Kent County, Delaware, in the south, to Dauphin County, Pennsylvania, in the west. Based on minimum polygon fitting methods, the estimated geographic center of the epizootic was at 40.76^{o} latitude and -73.99^{o} longitude, which is located in the vicinity of Manhattan, New York. Figure 2 also shows, as points in red, the location of premises used in the case-control study. The location of all case and control sites was determined from GPS observations, using standardized methods. However, in a few instances, premises coordinates were not available and, using the Getty gazetteer, geographic coordinates of the nearest town were used instead. Gazetteer-referenced locations are shown in Figure 2 as green points.

The temporal pattern of case incidence is illustrated by the epidemic curve, shown in Figure 3. The outbreak peak occurred about the first week of October followed by a sharp reduction in cases in mid-October. Figure 4 shows that the affected area received frequent rainfall during the time of the epizootic. The amounts received were most likely sufficient to maintain larval breeding sites for those mosquito species found associated with WNV (Nasci, 2001). Mean daily minimum temperatures in the affected area are shown in Figure 5. After August 6, the mean daily minimum temperatures remained consistently below 60^{o} F and by August 8 minimum temperatures were frequently below 50^{o} F.

A comparison between weather observations, averaged for August, September, and October, and expected 30-year climatic values for precipitation and minimum temperature is shown in Table 3. This table indicated that observed weather conditions were nearly normal relative climate expectations for each of the months compared. In October, there was a 53.1 percent decrease in observed precipitation from expected levels.

Table 3. Comparison of weather observations with expected climate values for precipitation and minimum temperature in the 32,900 mi^{2}, affected area.

Month	Climate Trend Average Total Precipitation (mm)	Actual Average Total Precipitation (mm)	Climate Trend Average Minimum Temperature (°F)	Actual Average Minimum Temperature (°F)
August	102.5	104.6	60.0	59.0
September	97.3	100.3	52.4	51.1
October	89.4	41.9	41.3	40.2

The geographic organization of the temporal and spatial pattern of case occurrence is illustrated in Figure 6. Temporal data were based on the day of onset of clinical signs. Of the first five cases reported during the outbreak's initial seven days, the first case occurred in Rhode Island, followed by southern New Jersey, then eastern Massachusetts, followed again by southern New Jersey, and then a case was reported in central Connecticut. Table 4 shows the straight-line distances between consecutive equid cases. The average distance between consecutive cases was nearly 100 miles (range 8 - 286 miles) and the average number of days between reported cases was slightly less than 2 (range 0 - 13; median = 1 day). The relationship between consecutive case distances and the time between case reports is shown in Figure 7. No specific spatial or temporal pattern was observed between consecutive cases, regardless of the duration between reported cases.

The location of control premises relative to each case site and to other WNV case premises is shown in Figure 8. The average distance between a case site and the respective control premises selected is shown in Table 5. Control premises were generally located about 2 miles (range = 0.29 to 4.59 miles) from each case site. Also, the mean distance between a case and associated controls was less than the mean distance between a case and the nearest other case by about 10 miles.

Table 4. Days and distances between pairs of consecutive equine cases

Case Order	Distance (mi)[1,2]	Days Between Cases[3]	Case Order	Distance (mi)[1,2]	Days Between Cases[3]
1-2	212	4	21-22	98	1
2-3	268	0	22-23	39	1
3-4	286	1	23-24	54	0
4-5	205	2	24-25	15	2
5-6	119	3	25-26	69	3
6-7	79	3	26-27	116	2
7-8	82	2	27-28	145	0
8-9	67	0	28-29	26	3
9-10	140	8	29-30	72	0
10-11	162	2	30-31	8	0
11-12	10	0	31-32	15	0
12-13	229	1	32-33	49	1
13-14	108	0	33-34	73	0
14-15	17	0	34-35	182	2
15-16	101	1	35-36	146	0
16-17	78	0	36-37	86	1
17-18	14	4	37-38	139	0
18-19	9	0	38-39	96	1
19-20	22	2	39-40	48	13
20-21	111	0	40-41	86	5

[1] Lambert Conformal Conic Projection for the continental United States used as the projection for distance calculations. Euclidean distances only were calculated.
[2] Mean (± SD) distance in miles was 97.0 (± 72.1).
[3] Mean (± SD) days between consecutive cases was 1.7 (± 2.5).

Table 5. Mean distance between a case premises and associated control premises, and the mean distance between a case premises and the nearest other case premises

Total number cases	Total number controls	Mean (± SE) number control premises per case	Mean (± SE) distance from a case to controls (mi)	Mean (± SE) distance from case to nearest other case
41	101	2.5 (± 0.4)	2.2 (± 0.2)	12.7 (± 2.6)

The relationship between county-level equid population estimates, displayed as a quintile distribution, and the location of affected premises is shown in Figure 9. Affected premises were generally found in those counties with greater estimated numbers of equids. Both linear regression and analysis of variance (ANOVA) comparisons of case numbers per county with equine census estimates showed statistically significant associations between number of cases and estimates of equid numbers ($p<0.01$), equid density ($p<0.05$), and the numbers of equid farms per county ($p<0.05$).

A comparison of the number of reported clinical human cases per county and the location of equid cases is shown in Figure 10. The geographic range of equine cases was considerably greater than the extent of human cases. It is noteworthy that, in spite of the wider spatial distribution of infected equids, the geographic center of equid cases was located near the geographic center of those counties reporting human cases. Linear regression and ANOVA comparisons between counties reporting clinical human cases and counties with infected equids did not show a statistically significant relationship ($p>0.05$).

Figure 11 shows a comparison between WNV isolations from mosquitoes collected from counties in the affected area and the location of case equine premises. About half of the affected premises were located in counties where no isolations of WNV were made from mosquitoes. Linear regression and ANOVA comparisons of equine cases per county with the number of virus isolations from mosquitoes did not show a statistically significant association ($p>0.05$).

A comparison between counties reporting one or more WNV positive dead birds and the location of premises with infected equids is shown in Figure 12. This illustration shows that counties with larger numbers of infected dead birds appear to be associated with areas having the most equine case sites. Regression models were created to determine if the number of infected dead birds reported from a county could be used to predict which counties would be most likely to have premises with infected equids. Both linear and spatial linear regression models showed that WNV infected dead bird numbers were statistically significant ($p<0.001$) in predicting which counties are most likely to have one or more equine cases. Illustrated in Figure 13 is a plot of the calculated predictive values from the spatial linear regression model to indicate the potential influence of dead bird numbers on the probability of having one or more WNV affected premises. This figure highlights those counties where equid cases might have been expected, based on the dead bird count.

As a preliminary characterization of the WNV affected region, a comparison of case premises relative to described ecological regions is shown in Figure 14. As can be seen here, most of the case premises are located in the Mid-Atlantic Coastal Plain and the New England Lowlands of the Eastern Broadleaf Forest (Oceanic) Province. Ecological characteristics of each ecoregion section with positive case sites are described in Table 6. Approximately 92 percent of all case premises were Eastern Broadleaf Forest (Oceanic) Ecoregion Province and, within the next smaller ecoregion classification, about 72 percent of case premises were found in plateau ecoregion sections of either the Lower (Southern) New England or the Upper Atlantic Coastal Plain.

Table 6. Ecoregion characteristics and the distribution of case premises by ecoregion section

Ecoregion Division	Ecoregion Province	Province Description	Ecoregion Section	No. (%) Case Premises
Hot Continental	Eastern Broadleaf Forest (Oceanic)	Mostly plateau with some hilly and mountainous landforms; climate has a strong annual temperature cycle with abundant rainfall greater in summer months; soils are characteristically Alfisols[1].	Hudson Valley	6 (10.0)
Hot Continental	Eastern Broadleaf Forest (Oceanic)	As above	Lower (Southern) New England	20 (33.3)
Hot Continental	Eastern Broadleaf Forest (Oceanic)	As above	Northern Appalachian Piedmont	6 (10.0)
Hot Continental	Eastern Broadleaf Forest (Oceanic)	As above	Upper Atlantic Coastal Plain	23 (38.3)
Hot Continental Regime	Central Appalachian Broadleaf Forest-Coniferous Forest-Meadow	Low mountain landforms, 300–6,000 feet, and dissected plateaus; distinct climate and seasons; precipitation is even throughout the year; soils are Ultisols[1] on ridges and Inceptisols[1] in steep areas.	Northern Ridge and Valley	1 (1.7)
Subtropical	Outer Coastal Plain Mixed Forest	Flat and irregular plain gently sloping to sea level, mostly less than 300 ft.; abundant rainfall distributed throughout the year; soils are mostly Ultisols[1], Spodosols[1], and Entisols[1].	Middle Atlantic Coastal Plain	4 (6.7)

[1] Soils classes (Donahue, et al., 1990)

Alfisols - High clay content soil that is not too highly leached, found in forested areas.
Inceptisols - Young soils that are in early stages of development. Only the more rapidly formed horizons present.
Ultisols - Clay soils that are leached, tend to be more red and yellow in color.
Spodosols - Light gray colored soils that are highly leached, found in humid forested areas.
Entisols - Very young soils with little or no horizon development.

Because terrain elevation is a principal characteristic of an ecoregion, we next compared case locations with terrain elevation data. Digital Terrain Elevation Data (DTED) in a grid/raster format were used to estimate land elevation and terrain features in and around each case equid site. Figure 15 shows the location of case sites relative to the DTED elevation grid. Most cases occurred in the coastal lowland areas. To compare quantitatively, grid cell values were extracted for the point location of each case premises. Figure 16 shows the elevation values associated with each case site. The mean (±SD) elevation value for all case sites was 192.4 (± 29.3) feet (range 10 – 784 feet).

Several methods were used to consider whether equid cases were clustered and to characterize the strength of spatial associations among any clusters found. Preliminary tests to detect spatial clustering used the K-function statistic as an estimate of case aggregation. The K-hat value was calculated for each case site and then compared with the results of a random, binomial simulation. Monte Carlo methods, with 99 iterations, were used to simulate expected K-hat values for an equal number of points that showed complete spatial randomness. Figure 17 shows a comparison between K-hat values calculated for case sites (circles) and predicted K-hat values (lines to show the mean, and upper and lower 95% confidence intervals), if the process were completely random. Most of the K-hat values calculated for case premises fell outside of the values predicted for a random process, suggesting that case sites are spatially clustered. It is possible that equine populations may be aggregated into clusters, however demographic information to assess population dispersal was not available.

The strength of the spatial associations within the case site cluster was measured by calculating Geary's C statistic of autocorrelation. An irregular lattice method was used in which the number of equid cases per county was evaluated over the entire, seven-state region. Also, the value of Geary's C for counties within the region was compared with a value calculated similarly if all counties were affected randomly. Geary's C index of spatial autocorrelation was 2.04, which was statistically significant ($p<0.001$) when compared with values estimated for a random association. To determine if case sites were clustered within the affected region, the SatScan method was employed, using a Bernoulli model applied to case and control premises. Results of this test yielded an overall relative risk of 1.6 for the region, plus a log likelihood ratio of 8.2. Comparison with stochastic calculations showed that case premises are clustered and that this cluster is statistically significant ($p = 0.025$).

The first-order properties of the stationary point pattern of case sites are demonstrated in an intensity plot shown in Figure 18. Intensity calculations were based on the mean number of case sites per square mile. The binning method was applied in these calculations, followed by a loess function to smooth the results. Although outlier points created some background noise, approximately five to six case clusters can be observed with this method.

Fourier polygon modeling methods were used to show which equid case sites might be included in potential clusters, depending on the level of sensitivity selected. Four levels of sensitivity were considered: low, low-medium, medium-high, and high. Going from low to high, each level was used to define a smaller and smaller center of WNV activity based on the distance between case premises. The location of polygons calculated from the Fourier model is shown in Figures 19 and 20. At a low sensitivity, six possible clusters of cases were bounded and these were located in: northern Delaware, southern New Jersey, central New Jersey, northern New Jersey, eastern Connecticut, and east-central Connecticut. Case premises in Rhode Island, Massachusetts, and Pennsylvania were considered as outliers in the clustering process. As the polygon model sensitivity was increased, fewer clusters of case sites were bounded. At the highest level of sensitivity, a single center of activity was located over central Monmouth County, New Jersey. These results correspond well with observations obtained from the intensity analysis.

Respondents were asked to report occurrence of blackbird roosts because land residents were most likely to observe these bird sites. Blackbird roosts were defined as small wood lots (generally three to five acres) where many blackbirds congregate at night. Because the case-control survey suggested possible associations between proximity to blackbird roosts and congregations of waterfowl, we compared the location of respondents who said yes to either of these situations with the location of the cluster polygons. Figure 19 shows the location of survey respondents who reported being within one-half mile of a blackbird roost. Five of six clusters, defined at the low sensitivity level, had one or more premises reporting proximity to a communal bird roost (CBR). Of the five clusters located in proximity to a CBR, two clusters had two CBR premises, one cluster had three CBR premises, and the cluster located near Monmouth County, New Jersey, had eight CBR premises.

The geographic distribution of case-control respondents reporting nearby congregations of waterfowl is shown in Figure 20. This figure also shows the spatial relationship between a predicted cluster, described above, and premises located in proximity to waterfowl congregations (WFC). In this case, all six clusters, defined at the low sensitivity level, had one or more premises with nearby WFC. Of the six clusters located in proximity to a WFC, one cluster had one WFC premises, two clusters had two WFC premises, three clusters had three WFC premises, and the cluster located near Monmouth County, New Jersey, had ten WFC premises.

The spatial pattern of vegetation types was examined using biweekly composites of NOAA satellite sensor data acquired during the time of the epizootic. NDVI values derived from AVHRR sensor information were used to compare shifts in vegetation biomass between September 8 and October 19. Shifts in NDVI values derived from vegetation in the affected area can be seen in Figure 21. Table 7 shows a comparison of central NDVI values (N=86,445 cells) obtained from the biweekly data. These measures indicate that, during the epizootic, there was a decline in chlorophyll reflectance, which was most probably due to a seasonal transition.

Table 7. Middle points in the distribution of scaled NDVI values

Time period	Mean (± SD)	Mode	Median
Sept 8 – 21	149.8 **(± 10.5)**	155	152
Sept 22 - Oct 5	146.3 **(± 9.6)**	150	148
Oct 6 – 19	134.1 **(± 7.9)**	136	135

Spectral patterns within the NDVI values for the WNV affected region were analyzed using statistical methods of the ISODATA classifier. Scaled index values from the three biweekly composites were grouped into 10 classes, a noise reducing 3x3 modal filter was applied, and each class was assigned a different color. The results, shown in Figure 22, show that each of the 49 case sites is associated with one or two specific classes. The hierarchical classification structure, based on the transformed divergence method, is also shown in Figure 22. Using grid analysis methods along with the geographic coordinates of case locations, classes 2, 4, 5, 6, 7, and 9 were identified as being near each case site. However, as shown in Figure 23, class 6 was most frequently associated with equid case sites. Grid analysis methods were also applied to the polygon boundaries of the predicted cluster outlines. Figure 24 shows the spectral patterns associated with each of the predicted clusters. In all but one of the clusters, class 6 occurred more frequently than other classes. Because the ISODATA classifier is an unsupervised method, predominate vegetation types in each class are presently unknown.

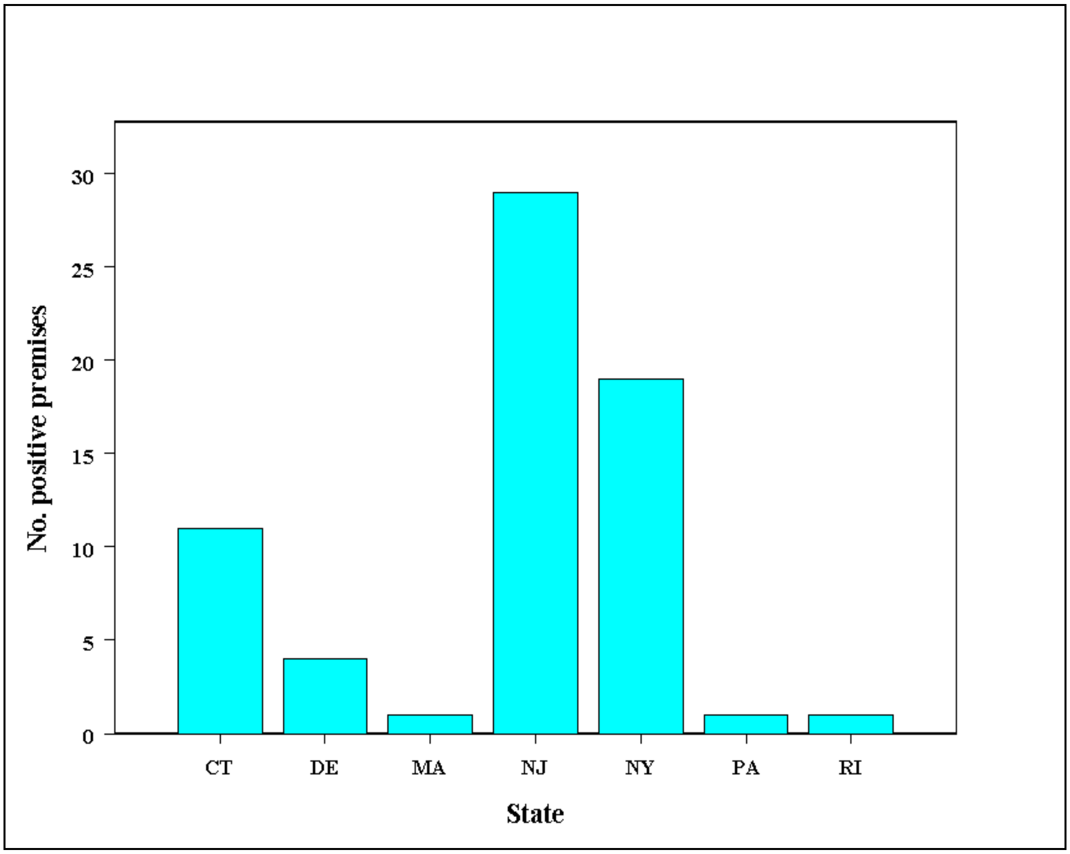


Figure 1. The number of case premises in each affected state

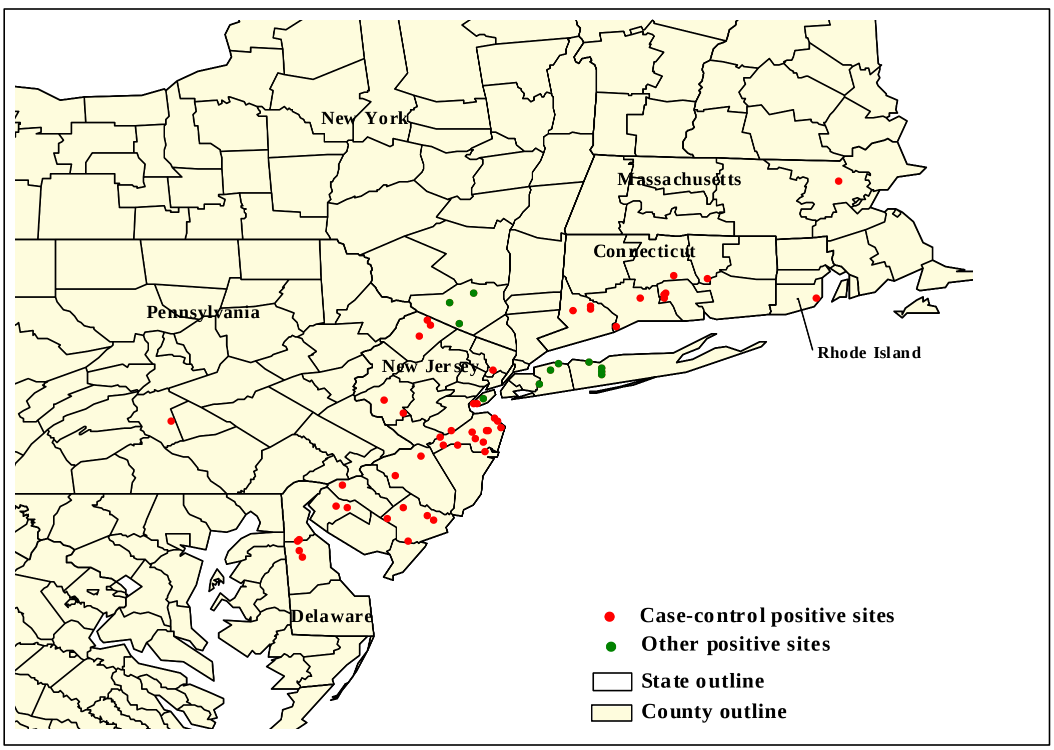


Figure 2. The geographic distribution of premises with one or more infected equids relative to county locations in affected states.

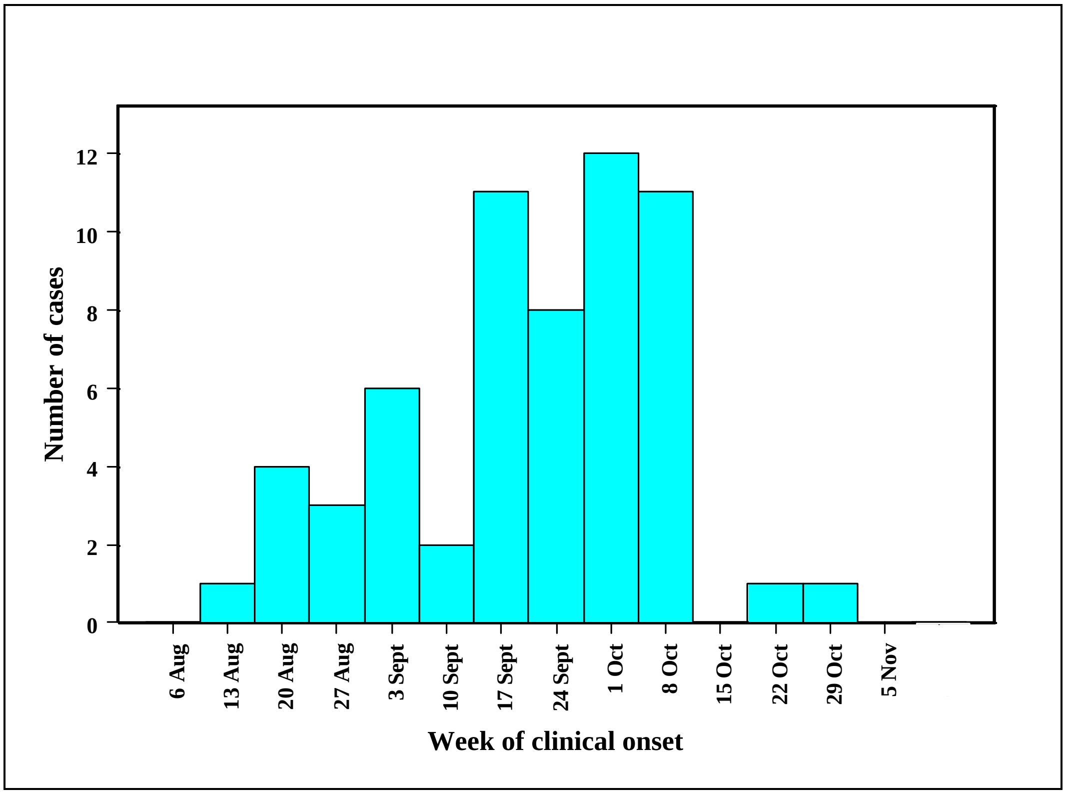


Figure 3. Epidemic curve for equid cases in the 2000 epizootic

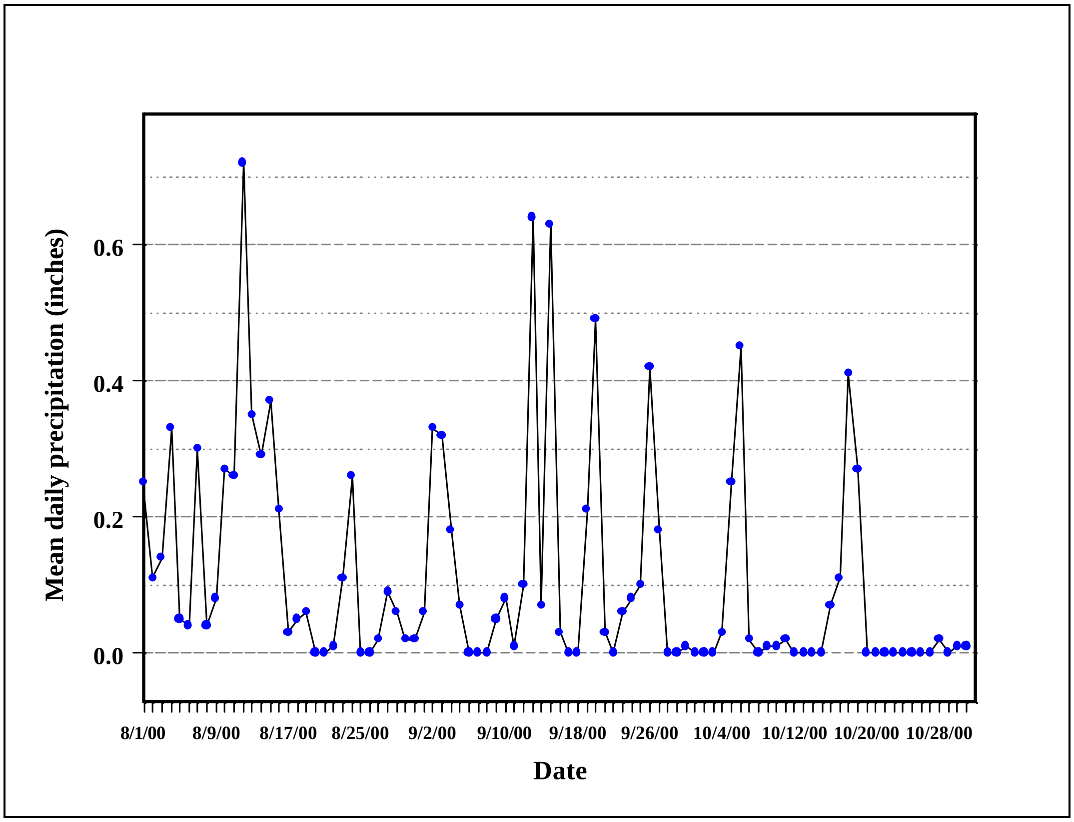


Figure 4. Mean daily precipitation in inches for the WNV affected area.

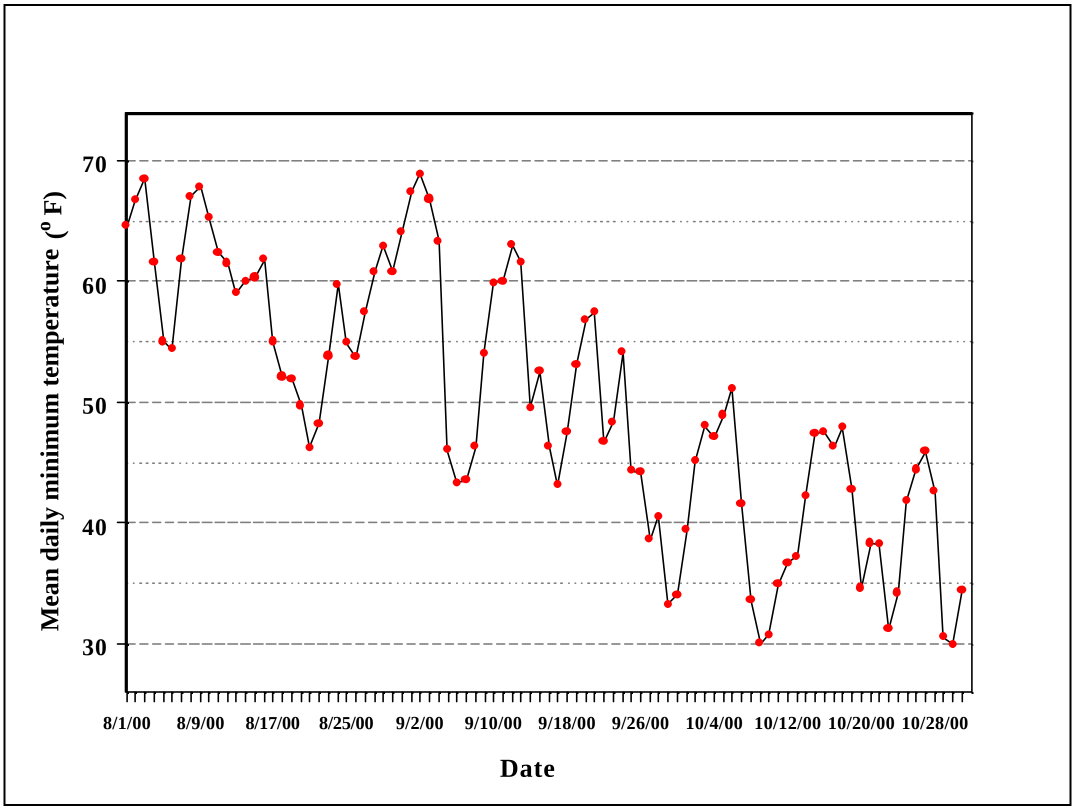


Figure 5. Mean daily minimum temperatures (°F) for the WNV affected area.

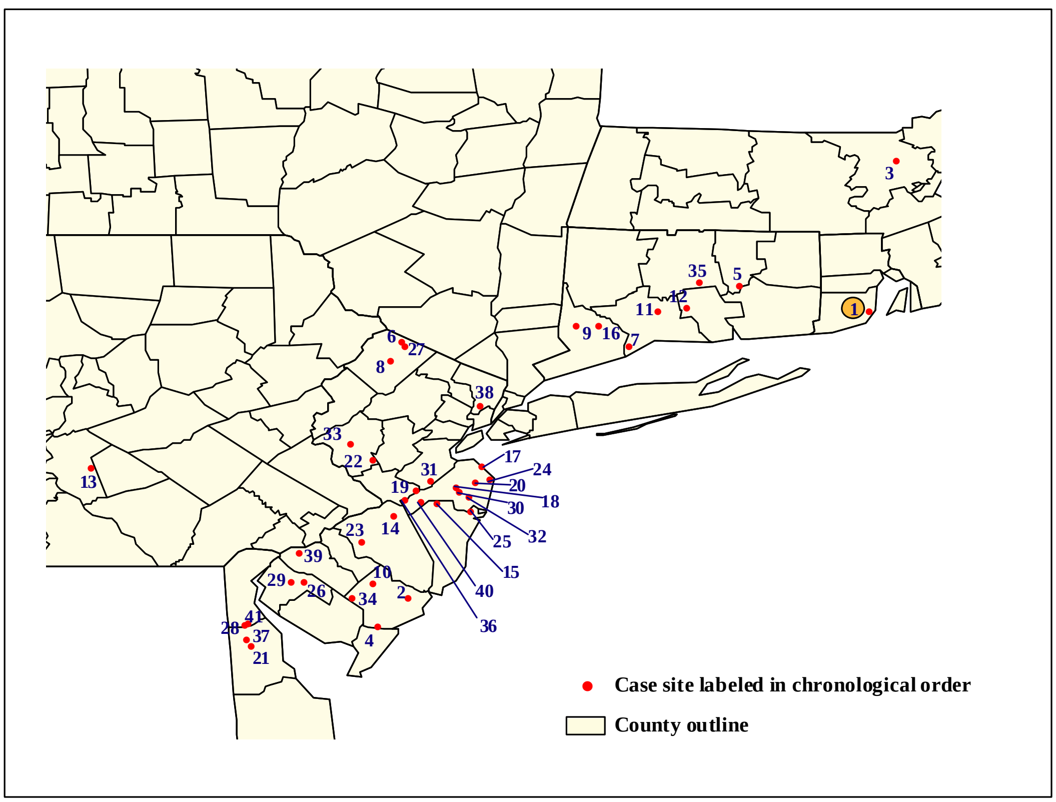


Figure 6. Chronological order of case premises being reported. The first case is located in southeastern Rhode Island.

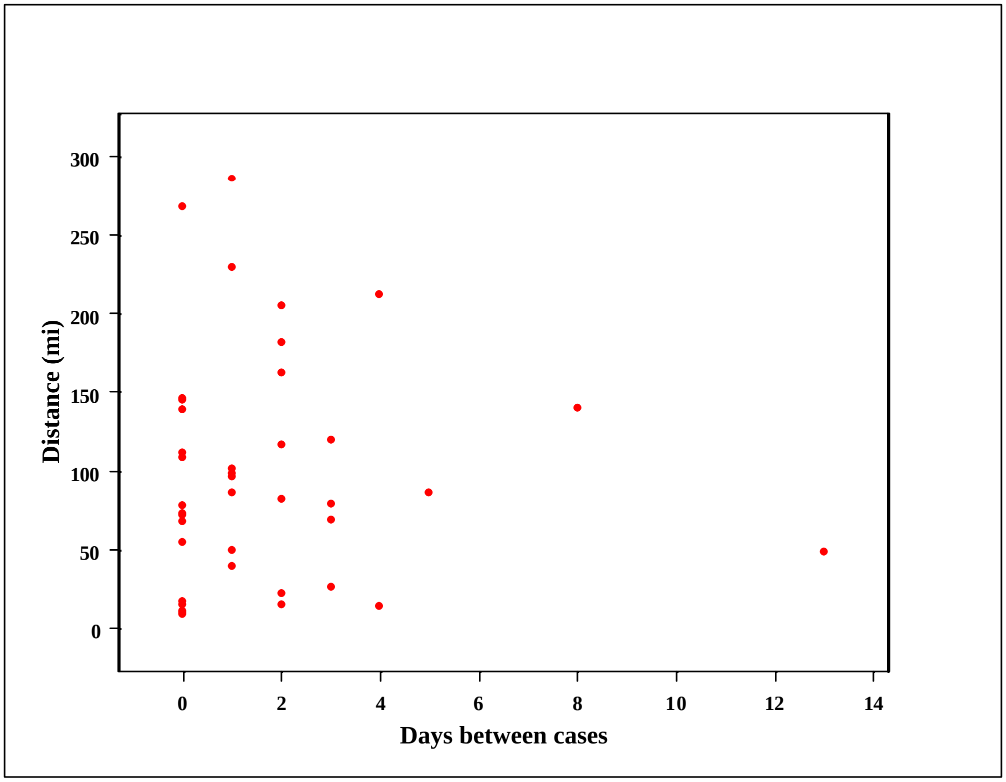


Figure 7. Distance (miles) between consecutive cases

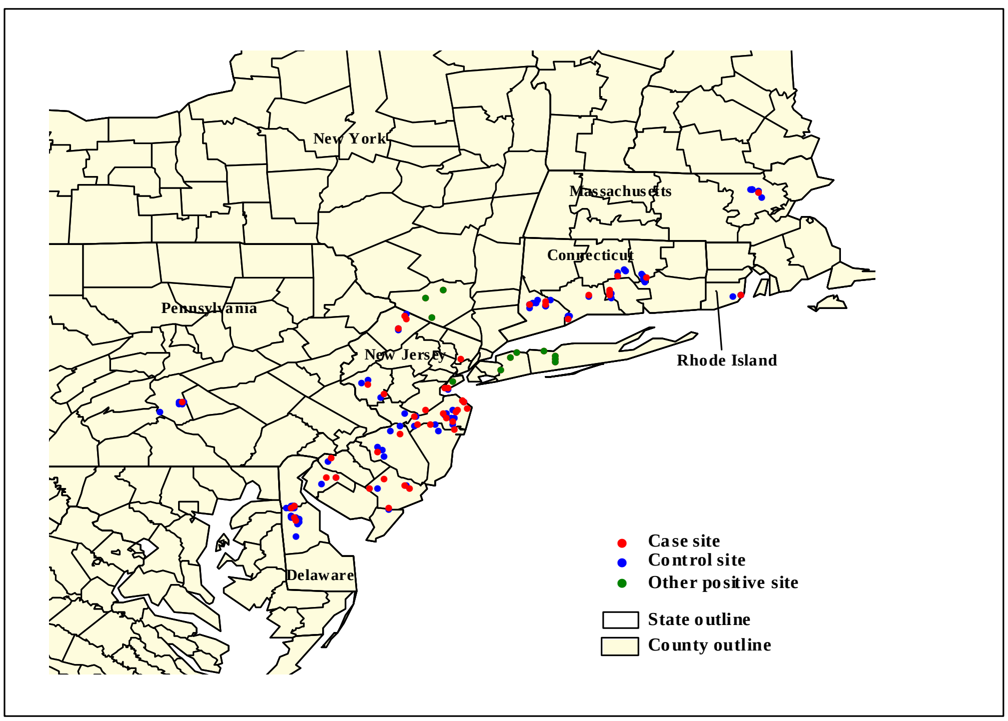


Figure 8. The location of premises used in the case-control study relative to other case premises not included in the study.

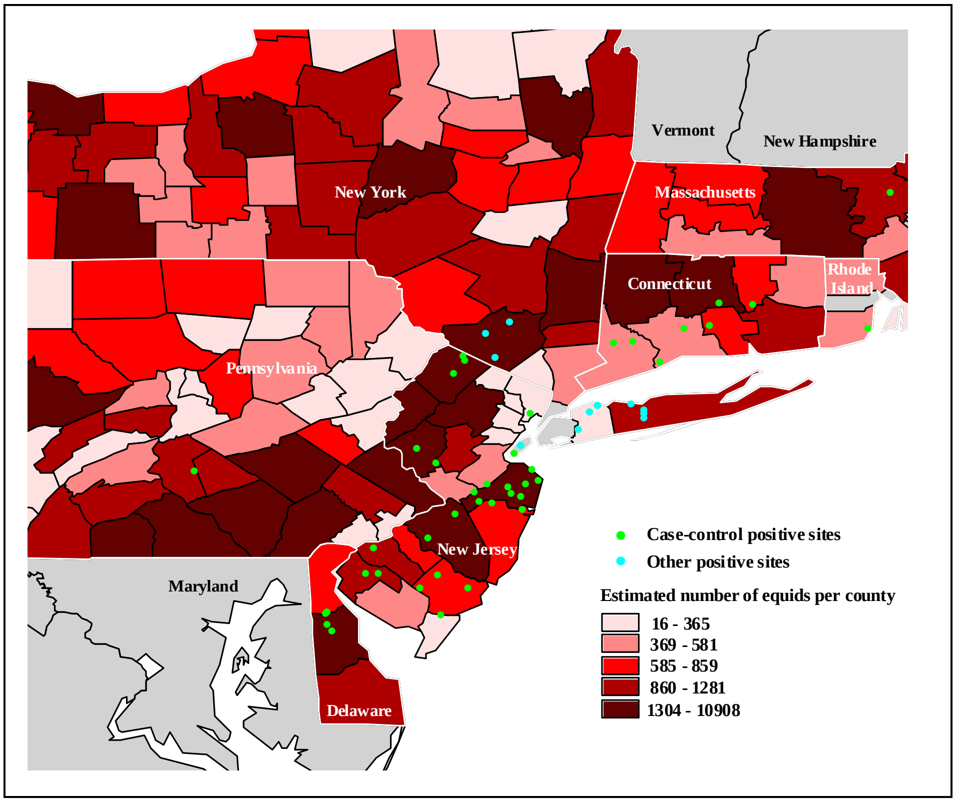


Figure 9. Estimated number of equids per county from the USDA's Census of Agriculture and the location of premises with infected equids

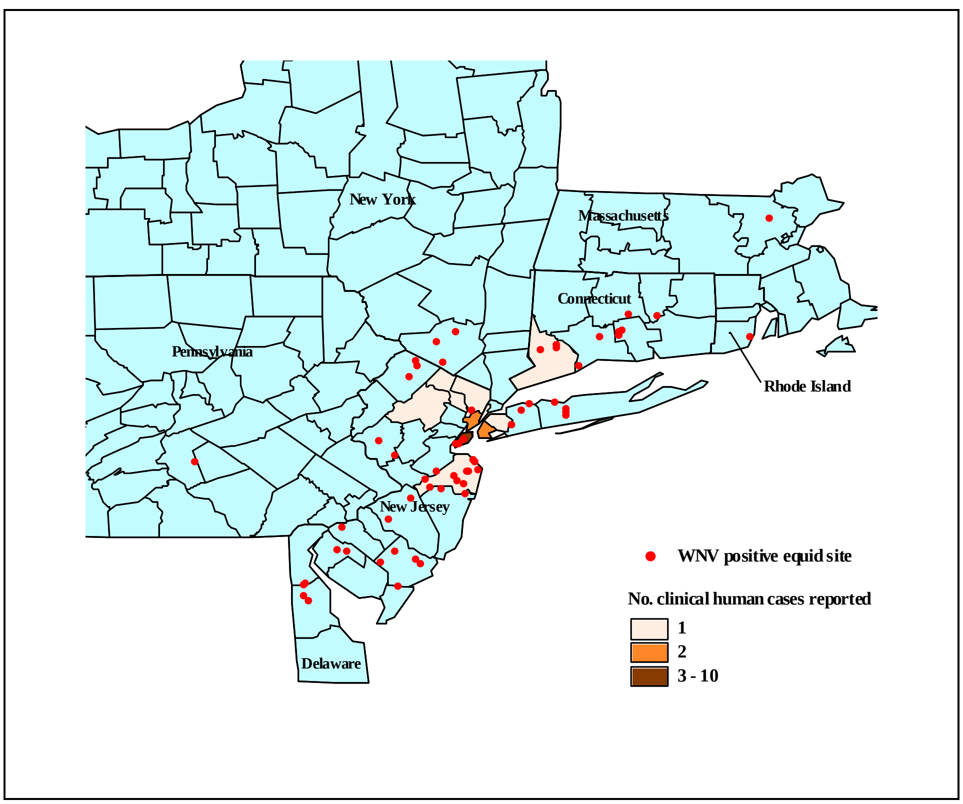


Figure 10. Counties reporting clinical human cases compared with the location of infected equids

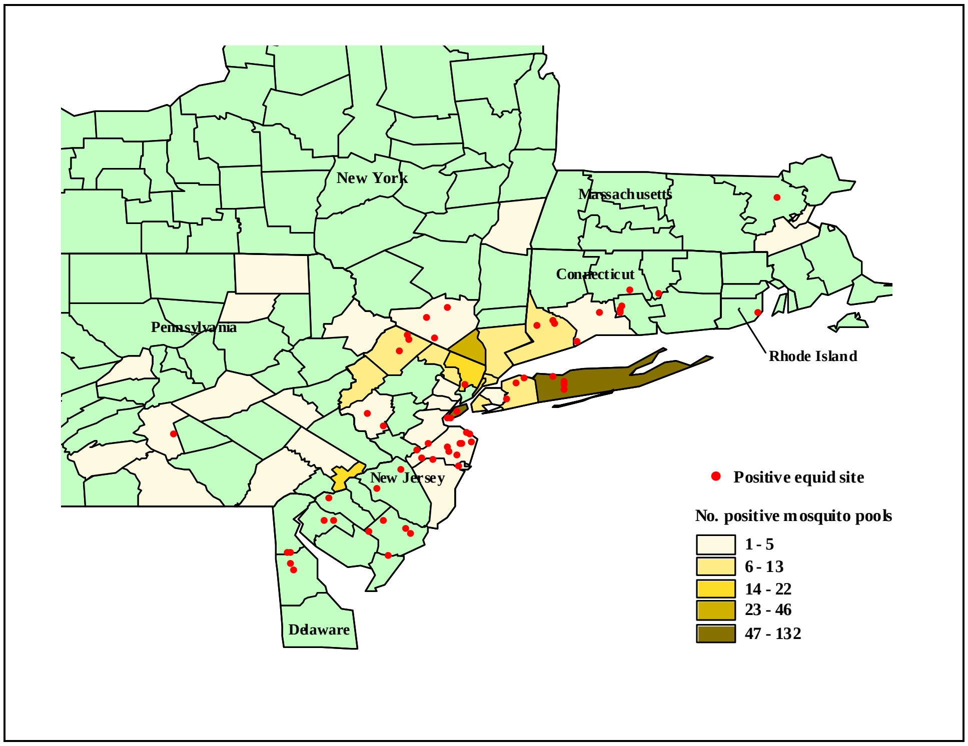


Figure 11. Counties reporting West Nile virus isolations from mosquitoes are compared with the location of infected equids. Not shown are virus isolates from two pools of mosquitoes collected in Erie County, Pennsylvania. Testing of mosquitoes for WNV varied among state and counties in the affected region.

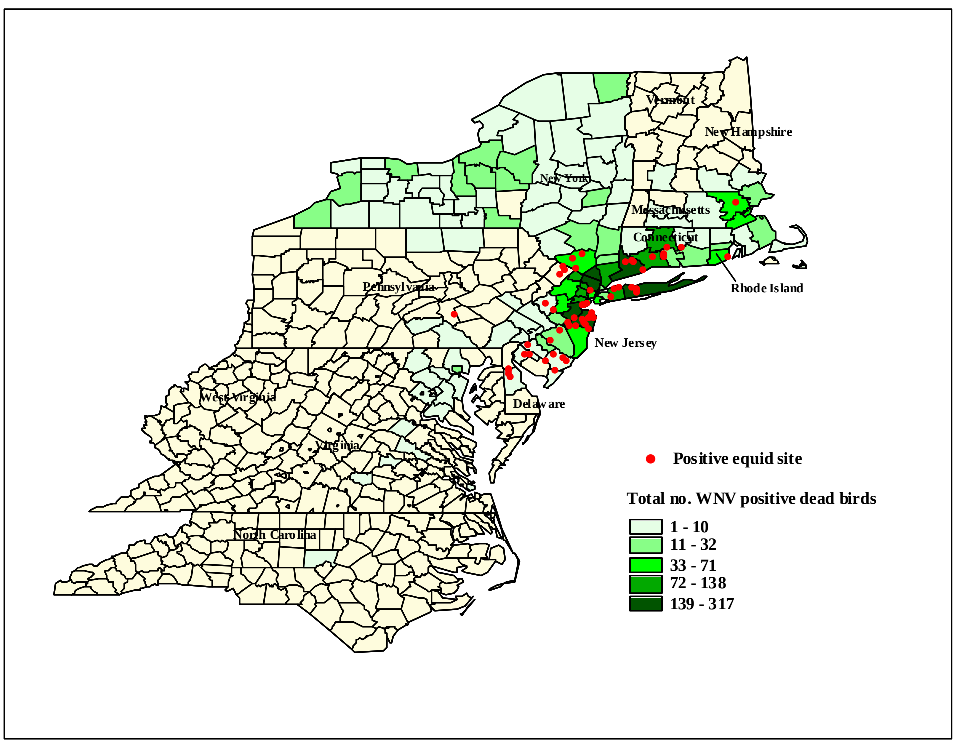


Figure 12. Counties reporting dead birds infected with West Nile virus compared with the location of infected equids. Submission and testing of avian samples varied among states and counties in the affected region.

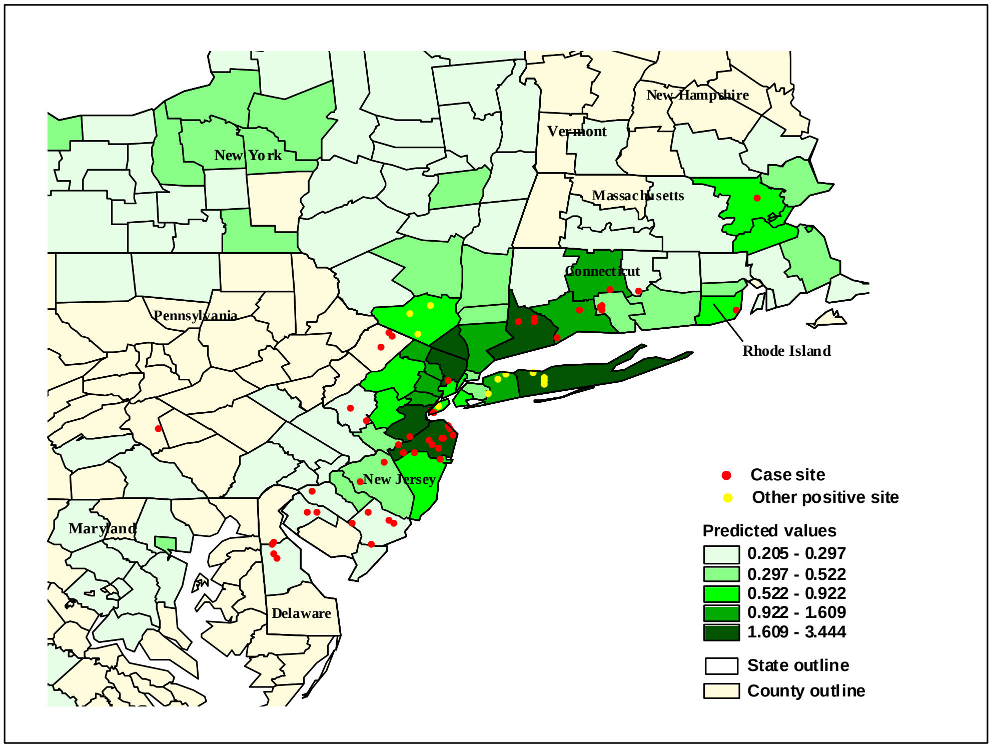


Figure 13. Predictive values of a spatial linear regression model compared with the locations of premises with case equids

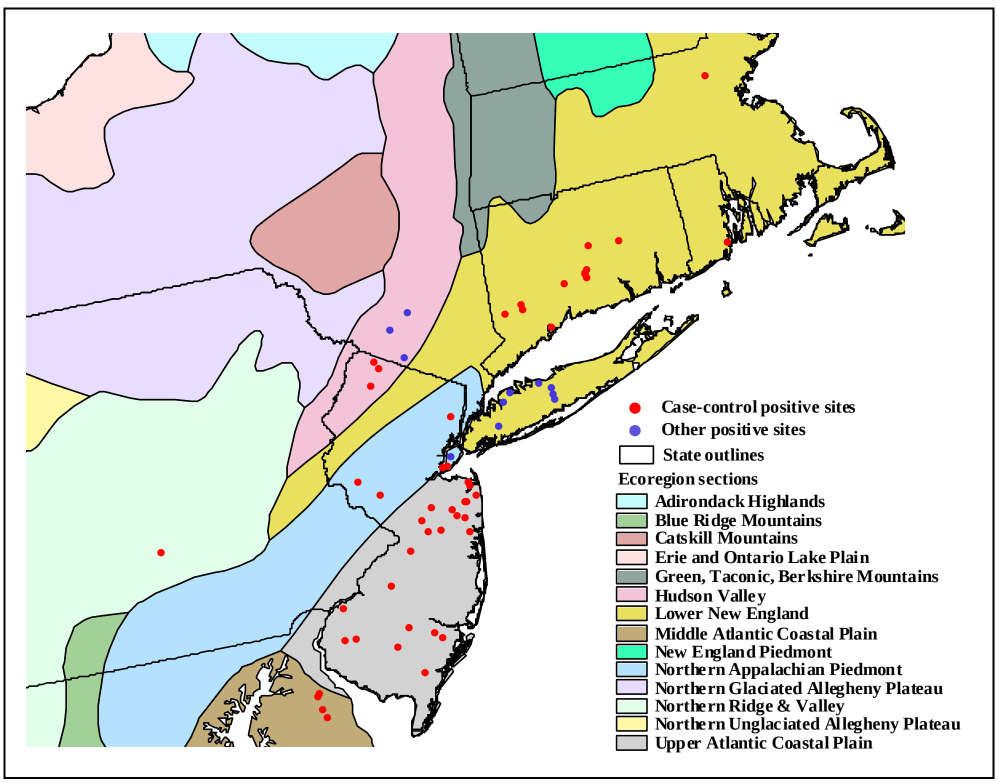


Figure 14. Ecoregion sections in the affected area compared with case equid sites

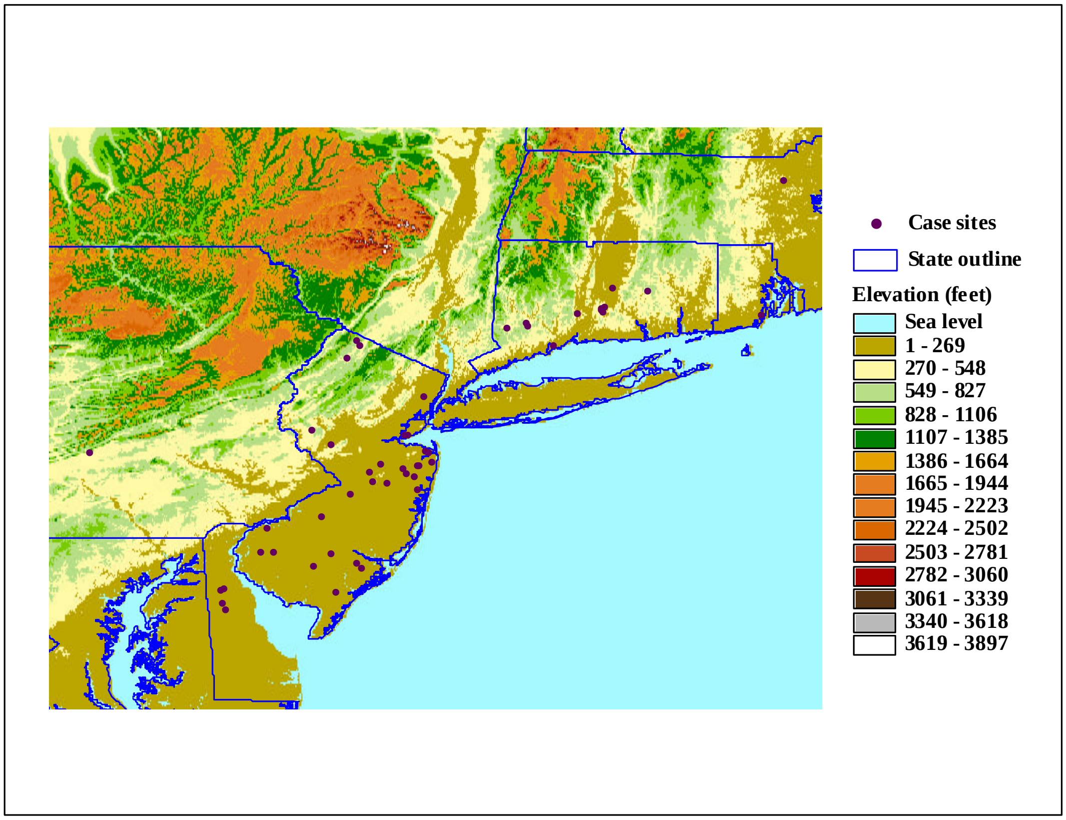


Figure 15. Elevation in feet of the affected area compared with case site locations

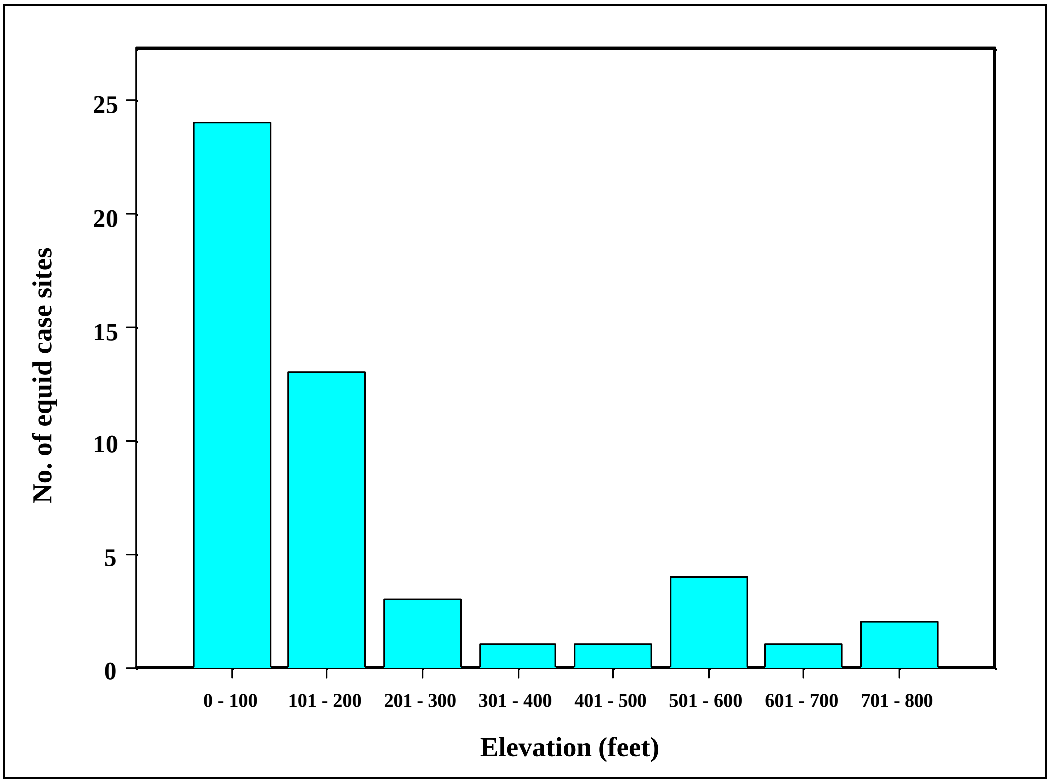


Figure 16. Elevation in feet of equid case sites

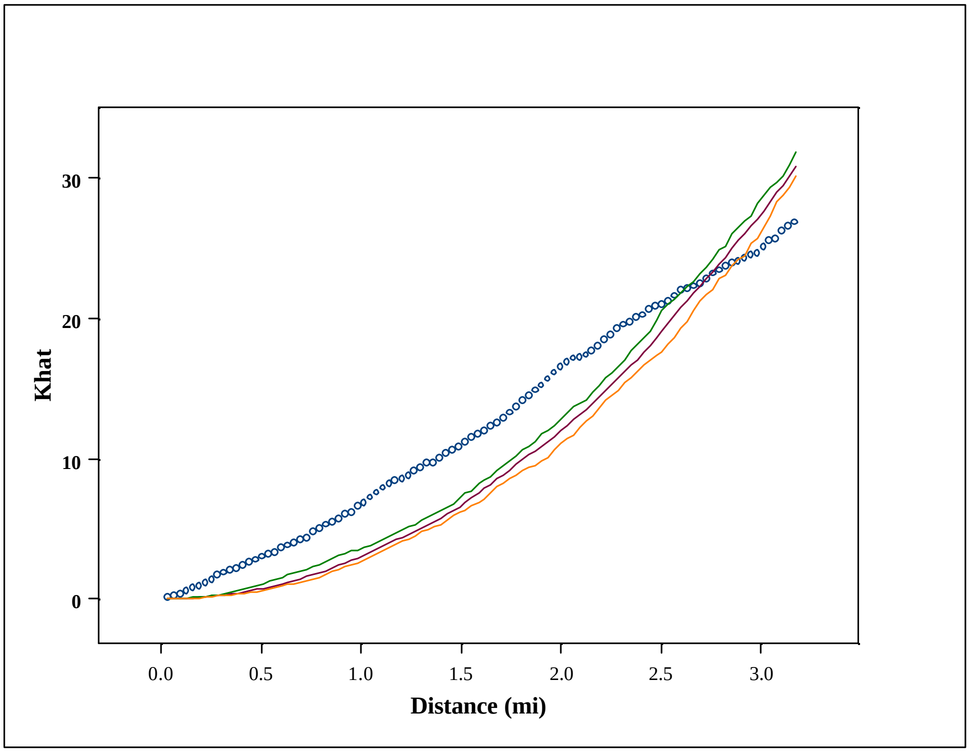


Figure 17. K-hat statistic calculated for each case site (circles) and a binomial simulation (lines: mean, upper CL, lower CL) of K-hat values for a random pattern

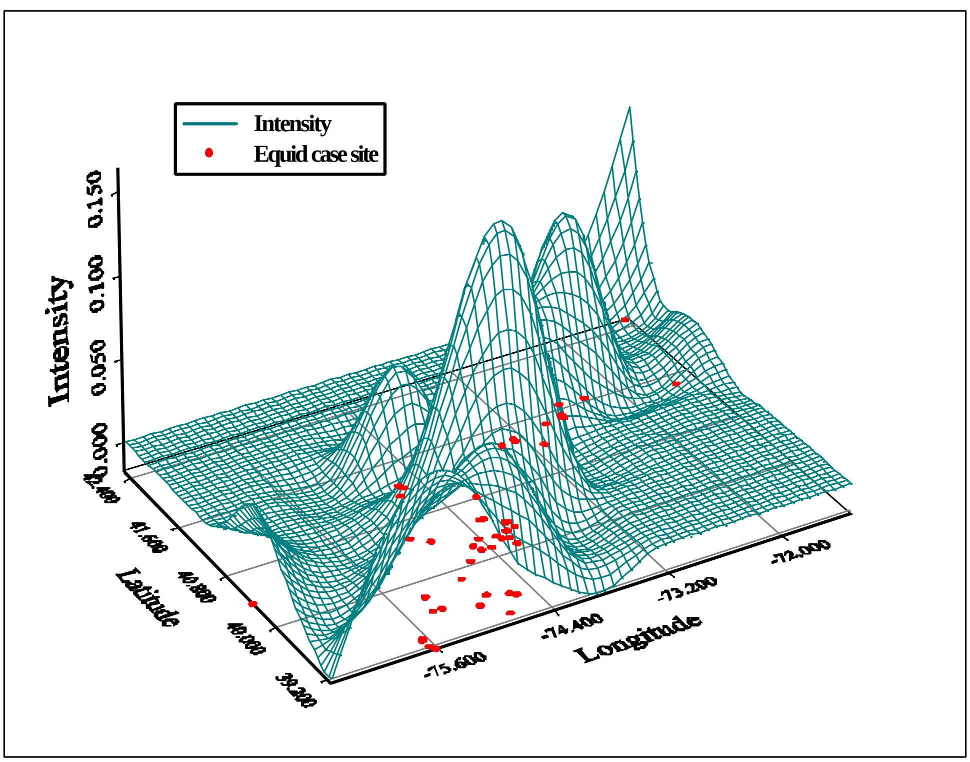


Figure 18. Intensity plot of equid case sites showing the mean number of equid case sites per square mile

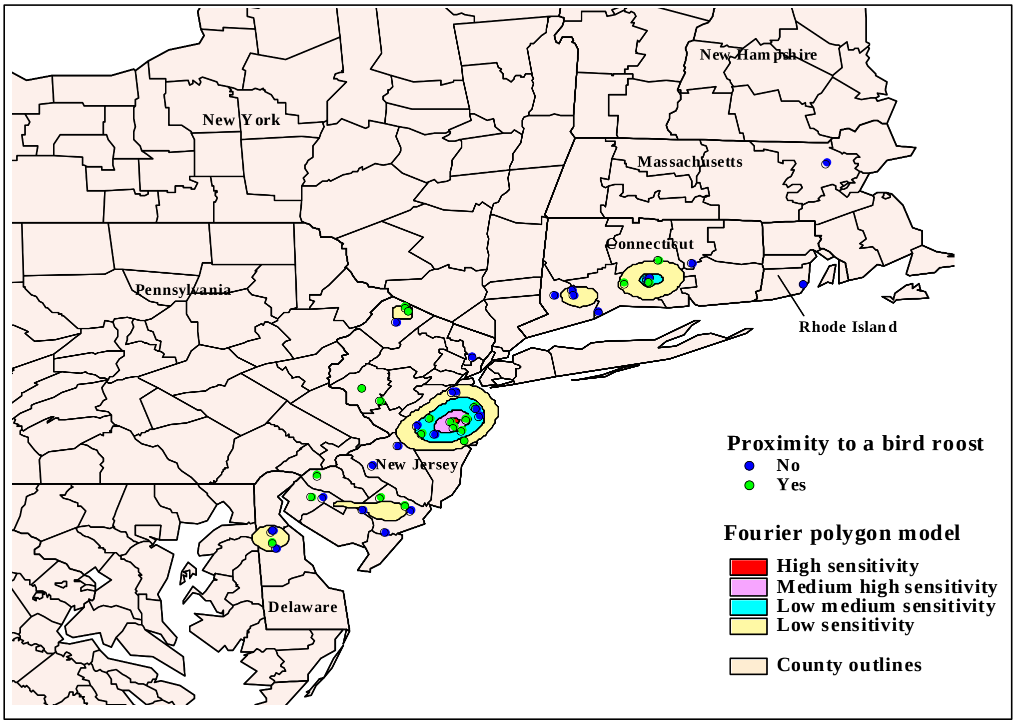


Figure 19. Fourier polygon models of case clusters compared with case sites reporting proximity to a blackbird roost

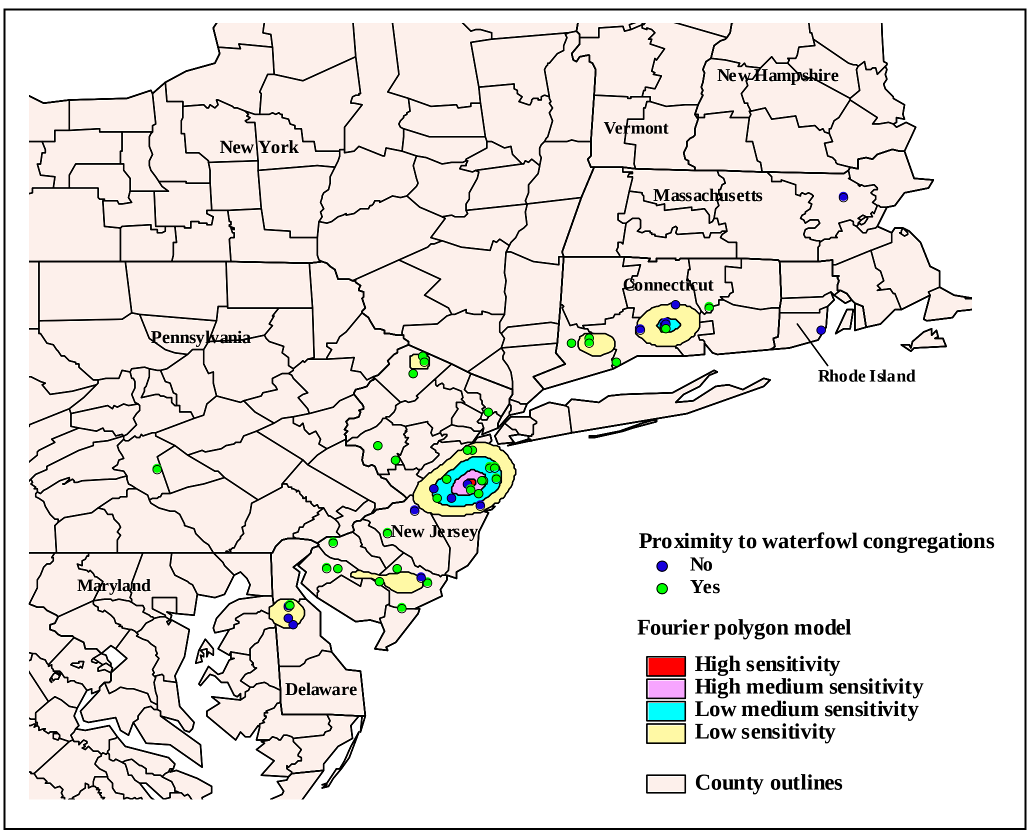


Figure 20. Fourier polygon models of case clusters compared with case sites reporting proximity to waterfowl congregations

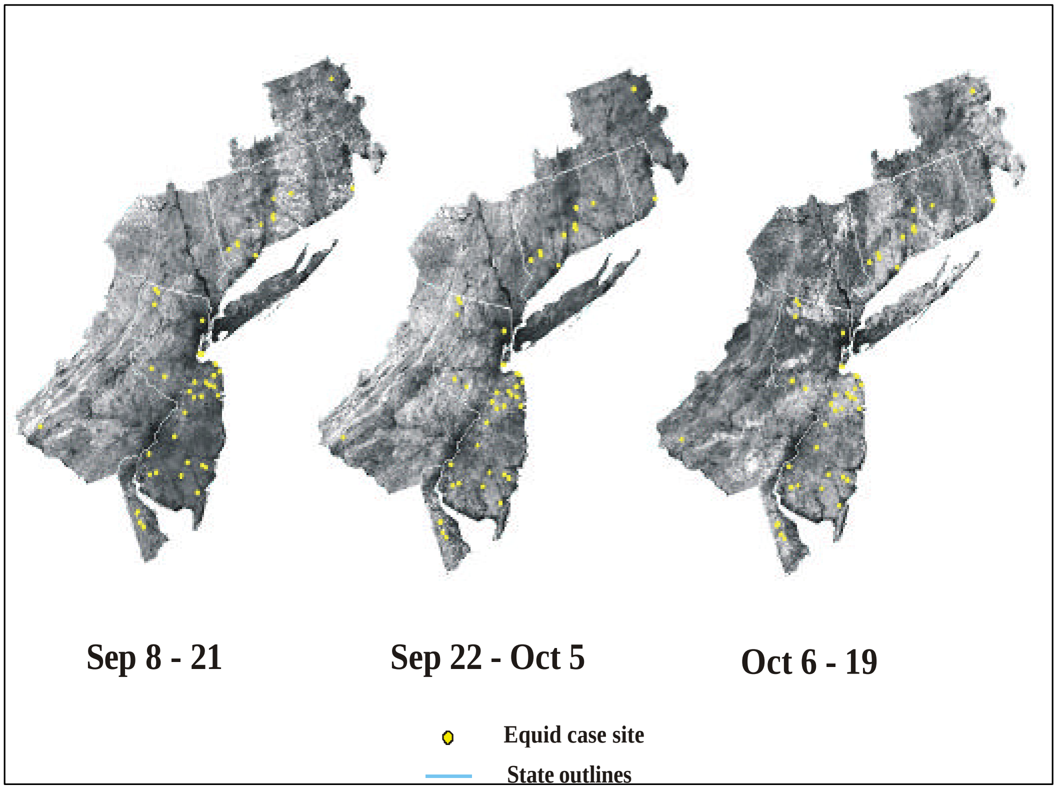


Figure 21. Biweekly composite NDVI values for September 8 – October 19, shown in gray-scale, compared with the location of equid case sites and state boundaries.

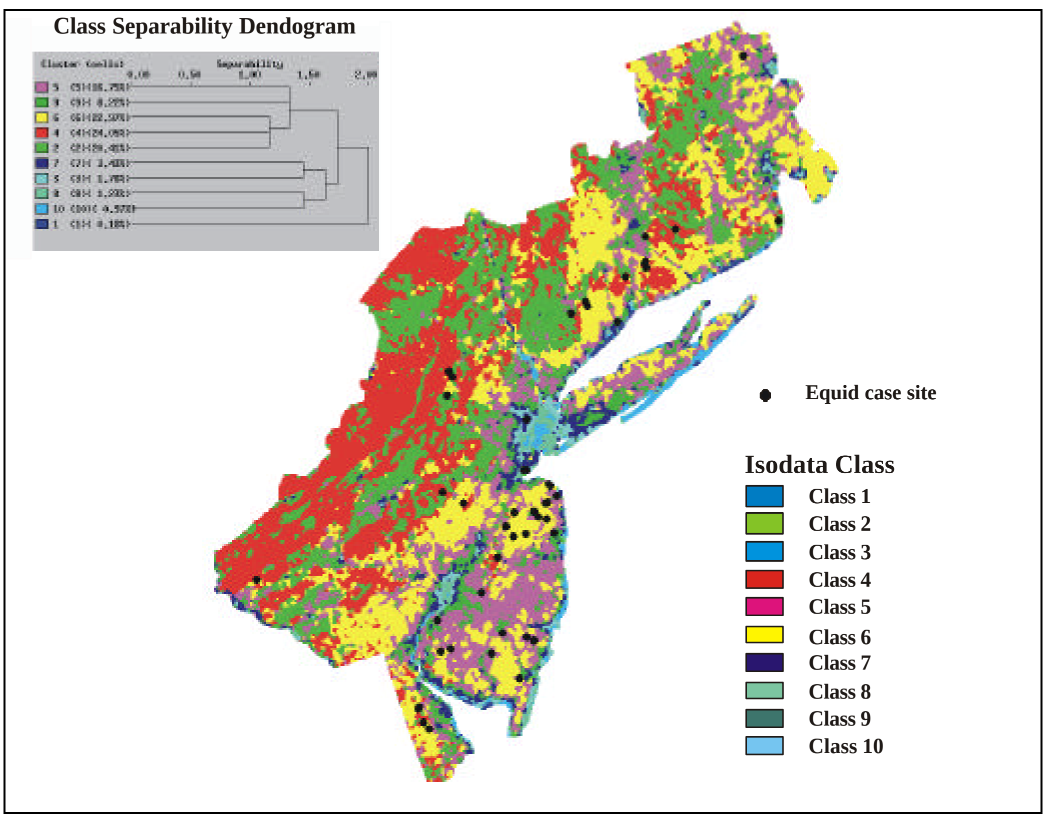


Figure 22. Isodata classified NDVI values from September 8 – October 19 and compared with the location of equid case sites. Inset shows class relationships and separability.

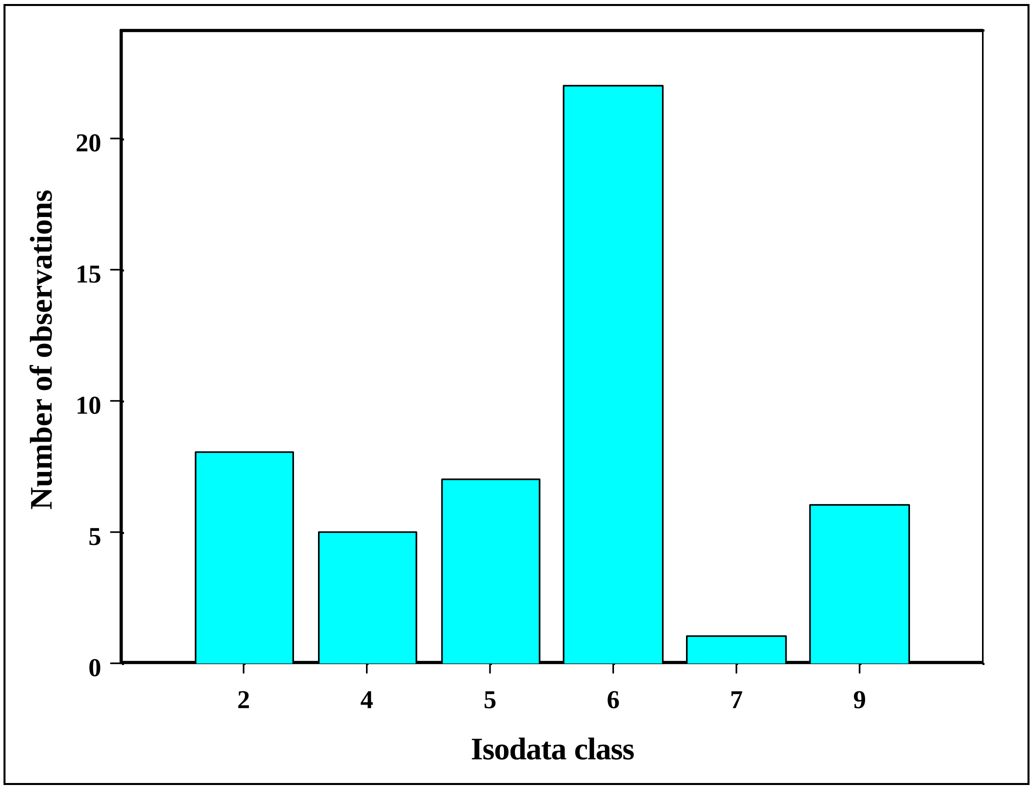
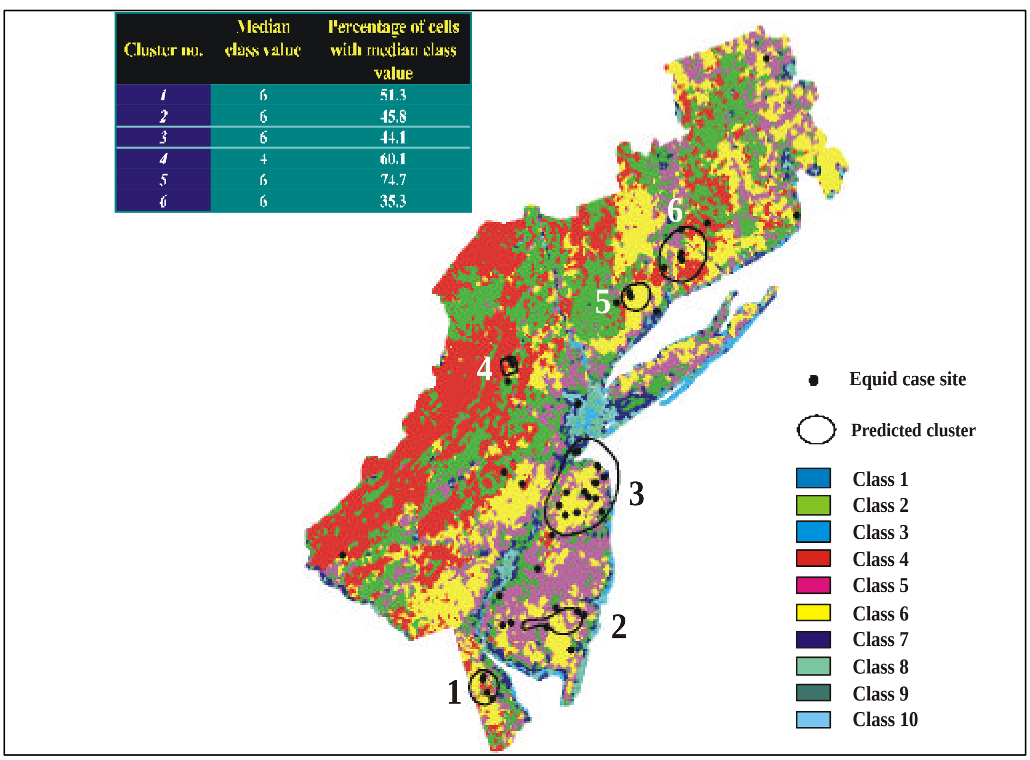


Figure 23. Distribution of Isodata class assignments relative to observations made for each equid case site.



Cluster no.	Median class value	Percentage of cells with median class value
1	6	51.3
2	6	45.8
3	6	44.1
4	4	60.1
5	6	74.7
6	6	35.3

Figure 24. Isodata classified NDVI values from September 8 – October 19 combined with the location of equid case sites and outlines of predicted clusters. Inset shows the median class value for each cluster.

Discussion

I. Case-Control Study

One of the strengths of this study is that it represents a large number of equine premises and equids. As part of the case-control study a total of 150 premises contributed data and a total of 1,487 equids were tested for presence of exposure to WNV. The number of equids bled per premises ranged from one to 73, with a median of seven equids per premises bled as part of the case-control study.

This study also represents a joint effort of the field veterinary personnel in the northeastern region, personnel from the USDA: APHIS: VS Area offices, the USDA: APHIS National Veterinary Services Laboratories, the USDA: APHIS: VS Centers for Epidemiology and Animal Health, State Veterinary Offices, and from USDA: APHIS: VS Emergency Programs. Major contributions to the study were made by data collectors in the field, by equine owners who contributed information about their premises and their equids, by private veterinary practitioners who reported suspect cases to state veterinary offices and assisted in collection of blood samples on some control premises, and by the equids themselves that contributed biologic samples (i.e., blood samples).

A limitation of the study is the lack of complete data. Some information for individual equids was not provided and data were available from only a limited number of premises from one geographic region that had approximately one third of the clinical cases (for a description of these cases, see: Trock, et. al, 2001).

Findings of note include a marginally significant association between the presence of a blackbird roost and the presence of waterfowl congregations within ½ mile of the equine premises and having one or more equids with evidence of exposure to WNV. It may be useful to map blackbird roosts and wild waterfowl congregations to identify geographical areas of greater risk. Potentially focusing vector-control efforts at these sites (blackbird roosts and waterfowl congregations) may reduce the risk of exposure for equids in areas where WNV is present. Insect control methods at the premises or at the animal level were not significantly different between cases and controls. This could be the result of a failure to request data in enough detail, (i.e. determine ingredients of insect sprays, etc.) or a true lack of benefit of insecticide applied to horse (difficulty in complete coverage and requires frequent application). Based on survey results, it would appear that most insect control efforts were directed at fly control which may not be effective in reducing exposure to mosquitoes (likely vector of WNV).

For case premises (one or more equids with evidence of exposure to WNV) at the animal level, there was a difference in use of the equid and housing between those equids with evidence of exposure to WNV and those with no evidence of exposure. Perhaps pleasure horses were more likely to be exposed to the vector for various reasons. For example, one potential reason could include trail-riding activities.

The clinical signs consistent with WNV infection were observed in a small percent of serologically negative equids. This is not surprising as the neurologic signs of WNV infection are not unique and can be caused by other agents or diseases. However, in the northeast region, any horse with neurologic disease should be investigated for a diagnosis of WNV infection based on the rarity of neurologic signs in seronegative horses. Even seropositive horses though, do not necessarily show neurologic signs. Less than half of the case horses in this study showed signs suggestive of WNV infection.

II. Spatial Analysis

The geographic extent of equids infected in the 2000 WNV outbreak was considerably greater than the area involved in the 1999 outbreak when only Suffolk and Nassau counties, located on Long Island, New York, were affected. In that outbreak, 19 premises with equids were involved compared with the 64 premises in 2000 outbreak. The temporal pattern of clinical onset was similar in both epizootics, beginning in mid- to late August and ending approximately the last week of October. Meteorological data for the affected region showed that this area received sufficient rainfall to support the development of aquatic stages of mosquitoes. However, low temperatures in mid-October may have reduced mosquito-biting activity to a minimal level, thus bringing an end to the outbreak.

The geographic tracking of new case sites showed that consecutive cases appeared to occur randomly. Predicting where a new case will occur, based on the location of previous case sites, is presently not possible without knowing the specific factors that influence disease spread. Although the temporal and spatial occurrence of new cases appears to be haphazard, a key event that must occur first is the establishment of a focus of infection and transmission in an area near susceptible equids. The habitat within a focus must be suitable to establish both mosquito-to-bird and bird-to-mosquito transmission cycles. It is possible that the timing of consecutive cases is related to the establishment of WNV in areas where equid exposure to infected, mammal feeding, mosquitoes is most likely to occur. If this is the situation, then it may be possible to identify WNV foci based on the presence of specific habitat components, such as vegetation and standing water. However, predicting when a case is likely to occur will probably remain unreliable.

In the case-control study, all premises selected as control sites were located near a case premises. It is probable that case and control premises shared similar environments and risks of exposure. The serological detection of eight previously unrecognized, subclinical WNV infections as part of the control group evaluation raises the idea that infection risk may be increased for equids located near a natural focus of infection. To support or refute such a relationship, additional testing of horses on premises not located near an equine clinical case of WNV infection needs to be conducted.

Equid population estimates, based on animal numbers, equid density, and the numbers of farms with equids, suggest that when more animals are exposed there is a greater chance that one or more equids will become infected. The accuracy of the U.S. Census of Agriculture demographic data for equids is unknown; however, it may represent an underestimate of the actual population size and number of equid farms. Accurate and timely equid census data would provide a better means of estimating vector-equid contact rates in an area. Vector-host contact information is important in planning outbreak mitigation efforts.

Considering the relatively wide geographic range of sites reporting wild bird and equid infections, it is surprising that WNV infections in humans and mosquitoes were not found over a more extensive area. However, the number of dead birds reported infected with WNV seemed to have predictive value in indicating where equid cases would be expected to occur. In our analysis, all free-ranging, avian species were combined as a total number of WNV infected dead birds per county. It is possible that one or more bird species may be a better indicator of virus activity in an area rather than using combined totals for all avian species. Another consideration is that accuracy and consistency in reporting dead birds is dependent on surveillance systems developed by states and counties. These reporting systems may not be timely enough to provide sufficient data for predictive modeling. Further studies are needed regarding how to interpret dead bird counts relative to the risk of WNV infection in equids.

Most case premises were within ecoregions characteristic of Bailey's (1995) Eastern Broadleaf Forest category and these sites were generally situated in a coastal plain. Comparisons with the DTED model showed that most cases occurred in areas below 200 feet in elevation. Hydrologic basins in these areas are often poorly drained and are frequently characterized by well-developed wetlands. The specific role that wetlands might serve in the transmission of WNV to equids was not be determined by the case-control study. It is possible that marshes, swamps, or similar wetland habitat might act as focus of infection that establishes, at least temporarily, WNV activity in a suitable area. Virus transmission to equids could then occur if competent vectors and a sufficient number of susceptible hosts are present.

Evidence of spatial clustering was supported by several separate measures. Ripley's K-statistic showed that the pattern of case sites was not randomly arrayed. A high level of spatial autocorrelation was demonstrated by Geary's C index. This indicated a strong spatial association among all case sites. In addition, the SatScan statistic, based on the use of likelihood ratios, provided further evidence of clustering. According to Kulldorff and Nagarwalla (1995), the SatScan method can detect clusters of any size, located anywhere within a study region. This method is not restricted to clusters conforming to administrative boundaries. Further work with the SatScan method is needed to identify those case sites that can be assigned to specific sub clusters.

A possible association between case sites and proximity to a CBR or a WFC was identified in the case-control survey. Additional support of this association was provided by the spatial analysis. Most case premises reporting proximity to a CBR or a WFC were related to case clusters, as defined by the Fourier polygon modeling method. When two or more case respondents replied affirmatively to the CBR or WFC questions, it is not known whether each one is referring to the same object of risk. It is possible that CBR and WFC are indicative of another, as yet unknown, risk factor that is associated with virus transmission. Recent evidence by Hodgson, et al. (2001) suggests that defensive behavior by European starlings (*Sternus vulgaris*) in response to mosquito (*Culiseta melanura*) biting frequently ends in interrupted feeding. Consequently, a partially fed mosquito is more likely to feed on more than one bird in a communal roost, leading to a more rapid transmission of an ornithonotic arbovirus, such as WNV. This mosquito feeding behavior could lead to the swift dissemination of WNV throughout a population of avian hosts and produce a focus of infection in short amount of time.

Satellite imagery was used to characterize vegetation in the affected region. Biweekly composites of NDVI data showed a seasonal, downward shift in chlorophyll biomass. These data were used to classify vegetation in the affected region. This approach was used to convert highly variable sensor data into specific classes of vegetation based on standardized statistical methods. Identification of vegetation classes in the vicinity of affected premises showed categories common to each site. Because the ISODATA classification algorithm is an unsupervised technique, it is necessary to establish the identity of each class from field observations. Therefore, information about the vegetation feature common to each sub-cluster has yet to be determined.

Conclusions

Based on the spatial analysis of case-control and other data, exposure of equids to WNV is a geographically clustered event. Within regions of virus activity, exposure of individual equids appears to be chance event. Consequently, immunoprophylaxis is warranted for equids in regions where foci of WNV are likely to be found. This is similar to the approach used in protecting equids from infections with the viruses that cause eastern equine encephalitis and western equine encephalitis. Other recommended mitigation methods include reducing the size of vector mosquito populations, especially in areas near communal bird roosts or waterfowl congregations.

Because there are many unanswered questions about equid exposure to infected mosquitoes in and around epizootic foci, future studies of affected, equid premises should include an ecological assessment of the surrounding area. It is important to determine which species of mosquitoes feeding on equids are also infected with WNV. In addition, the infection status of free-ranging birds in the vicinity of an affected premises needs to be determined. The location of communal bird roosts or congregations of waterfowl relative to a site with an infected equid needs to be determined more precisely.

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Appendix A: Sample Questionnaire

Section 1 – Premises Information

United States
Department of
Agriculture

Animal and
Plant Health
Inspection
Service

Veterinary Services

West Nile Virus
Data Collection

Section 1 - WNV Premises Information

Premises Identification:

Premises Identification Number: ____

Latitude (in decimal degrees)	Longitude (in decimal degrees)

Interviewer name and telephone number: ____

Premises Information

1) How many of the following equids are present on the premises?

Type	Number on premises
Horses	
Mules	
Donkey/burro	
Ponies	
Miniature horses	
Other (Specify ____________________________)	
TOTAL	

2) How many total outdoor acres have been available for equine turnout since June 1, 2000?	Acres.

According to the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number. The valid OMB control number for this information collection is 2 hours. The time required to complete this information collection is estimated to average 2 hours per response, including the time to review instructions, search existing data resources, gather the data needed, and complete and review the information collection.

3) How many of the following **domestic** animals are present on the premises? How are they housed?

Species	**Number**	**Housing:** **Use code 1-3** 1 -Outdoors only 2 - Indoors only 3 - Access to both	**Distance from equids** (in feet)
Cattle			
Pigs			
Sheep			
Goats			
Chickens			
Turkeys			
Waterfowl			
Other fowl (specify____________)			

Mosquito Abatement in Equid Building

4) How many equids were **primarily** housed in each of the following ways, during the day and/or twilight to midnight, since June 1, 2000?

Housing Type	**Number Equids – Day**	**Number Equids - Twilight to Midnight**
Stalls only		
Stalls with runs		
Dry lot paddock only		
Dry lot paddock with shelter		
Pasture only		
Pasture with shelter		
Other		
TOTAL		

5) Which of the following insect control methods are currently being used in equid housing area?

	Control Method - (Use codes 0 - 2) 2 - No 1 - Yes 0 - No barn
Screens on barn openings (windows, doors, vents)	
Screens on stall	
Bug zappers	
Insect foggers	
Number of times daily________________________	
At what time of day usually? *(check all that apply)* ? morning ? ? afternoon ? dusk ? night	
Sticky tape without sugar	
Sticky tape with sugar	
Other (specify_________________________________)	

Mosquito Breeding Habitat

6) Within ¼ mile (about 500 yards) of where equids are kept, are there: (or has there been in the past 30 days?)

a) Roadside or irrigation ditches where water is standing? Yes ? No ?
b) Sources of open, standing water present? Yes ? No ?
If so, indicate the type of standing water (mark all that apply): ? temporary pond ? waste lagoon
? freshwater marsh ? salt marsh ? swamp

7) What was the **primary** water delivery system for most equids for most of the time since June 1, 2000? Enter code in the box at right (from the list below) for **primary** water delivery system used.

➔ ☐ code

1 - Running surface water (such as a: creek, stream, spring, river, or irrigation ditch
2 - Ditch
3 - Non-running surface water (such as: pond or lake)
4 - Buckets only
5 - Water trough only
6 - Buckets and water trough equally
7 - Automatic waterer
8 - Other, specify____

Bird Habitat

8) Is there an equid barn on the premises? ? Yes ? No
a) Is the building constructed to minimize roosting wild birds? ? Yes ? No
b) Are barn swallows nesting in the barn? ? Yes ? No

Type of bird	Average Number in barn (circle category closest to estimated number)				
House sparrows	0	1-9	10-49	50-99	100 ?
Swallows	0	1-9	10-49	50-99	100 ?
Pigeons	0	1-9	10-49	50-99	100 ?
Other (specify)	0	1-9	10-49	50-99	100 ?

9) Wild Birds in the area:

a) Are there any blackbird roosts within ½ mile of where equids are kept (within last 30 days)? ? Yes ? No

b) Is there any dense vegetation, row of bushes or trees (hedgerows, windbreaks, large willow trees, etc.)? Within ½ mile of where equids are kept? ? Yes ? No

c) Are there congregations of wild waterfowl within ½ mile of where equid are kept (within last 30 days)? ? Yes ? No

10) How many dead birds were noted on the premises since May 1, 2000? How many were submitted for laboratory testing? To whom were they submitted?

Type	Number	Number Submitted	Submitted to whom?
Crows			
Blue Jays			
Hawks			
Other (specify)			

11) Have any equids on the premises shown any of the following signs since August 1, 1999

a)

Increased apprehension	? Yes	? No
Depression	? Yes	? No
Listlessness	? Yes	? No

b) **If any are yes, did they also show:**

Head shaking	? Yes	? No	If so; how many equid ____________
Flaccid paralysis of lower lip	? Yes	? No	If so; how many equid ____________
Ataxia	? Yes	? No	If so; how many equid ____________
Weakness of hind limbs	? Yes	? No	If so; how many equid ____________
Inability to stand	? Yes	? No	If so; how many equid ____________
Limb paralysis	? Yes	? No	If so; how many equid ____________
Paresis	? Yes	? No	If so; how many equid ____________

How many equids total are counted in 11b? _________________Did any die? ? Yes ? No

For equids counted in 11b:
What was the approximate date of onset for 1st equid? _____________________
Were any tested for West Nile virus? ? Yes ? No
Where were the tests done? _______________________________________
Were any tests positive for West Nile? ? Yes ? No ? Don't Know

12) Additional comments: Use the area below (or on the back) for any additional information that was not covered in the questions above.

Section 2 - VMO Evaluation Sheet

Section 2 - WNV VMO Evaluation Sheet

1. Rate mosquito breeding habitat: ? low ? moderate ? high

2. Rate bird congregation habitat: ? low ? moderate ? high excellent

3. Rate overall cleanliness of the operation: ? poor ? fair ? good or excellent

Any additional comments about premises or equids?

Section 3 – Blood Collection Record

Section 3 - WNV Blood Collection Record

Premises Identification # __________________ Collection Date: __________________ Page __________ of __________

Interviewer's Name: ____________________________ Interviewer's Phone Number: ____________________________

Number of Equids on the operation today: ________________ Total Samples Submitted ______________________

Tube #	A. Primary Use Code 1-6	B. Is equid light in Color? (White, gray, buckskin, palomino, etc.) Yes/No	C. # of days equid has been out of county in past 45 days If>0, complete travel log	D. Housed in stall - daytime? Yes/No	E. Housed in stall dusk to midnight? Yes/No	F. Insect protection used during daytime? Use code 1-5	G. Insect protection used dusk to midnight? Use code 1-5	H. Overall health status of equid Use code 1-2	I. Showed signs since 6/1/2000 Record all signs (from list below) shown since 6/1/2000 If none, enter 0
		Y1 **N**3		**Y**1 **N**3	**Y**1 **N**3				
		Y1 **N**3		**Y**1 **N**3	**Y**1 **N**3				
		Y1 **N**3		**Y**1 **N**3	**Y**1 **N**3				
		Y1 **N**3		**Y**1 **N**3	**Y**1 **N**3				
		Y1 **N**3		**Y**1 **N**3	**Y**1 **N**3				
		Y1 **N**3		**Y**1 **N**3	**Y**1 **N**3				
		Y1 **N**3		**Y**1 **N**3	**Y**1 **N**3				
		Y1 **N**3		**Y**1 **N**3	**Y**1 **N**3				

Primary Use of Equid: (if young, what is intended use)	**Insect Protection:**	**Overall Health Status of Horse:**	**Signs:**
1 - Pleasure 2 - show or competition 3 - breeding 4 - racing 5 - farm/ranch work 6 - other	1- Chemical insecticide repellant 2 - Fly sheets 3 - Screened stall 4 - Other (specify) 5 - None	1 - Normal 2 - Abnormal - (Specify)	1 - increased apprehension 2 - depression 3 - listlessness 4 - head shaking 5 - flaccid paralysis of lower lip 6 - ataxia (including stumbling) 7 - weakness of hind limbs 8 - inability to stand 9 - limb paralysis 10 - paresis ***If none, enter 0***

Section 4 – Equine Travel Log

Section 4 - WNV Equine Travel Log

Premises ID # __________Interviewer's Name______________________Phone # ______________

Date Completed _______________

List travel history for each equid that has left the county in past 45 days.

Tube #	Traveled to (give city, county & state)	Purpose of travel Use code 1 - 8 (See below)	Number of visits in past 45 days

Purpose of travel:

1 **-** trail ride
2 **-** show/competition
3 **-** race
4 **-** hospital
5 **-** breeding
6 **-** other horse premises
7 **-** sale
8 **-** other (specify)