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Environmental and ecological determinants of West Nile virus occurrence in horses in North Dakota, 2002

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SUMMARY

West Nile virus (WNV) outbreak in North Dakota in 2002 included over 569 horse cases, clustered mainly in the eastern and northeastern parts of the state. The pattern of occurrence observed suggested existence of specific environmental and ecological factors that increased the risk for infection and illness in those locations. We developed a predictive model with factors that explained the pattern of WNV occurrence observed. Results indicated that surface elevation, temperature, precipitation, reported WNV-positive birds, reported WNV-positive humans, and reported WNV-positive mosquitoes were important predictors of occurrence in horses. However, case distance from water bodies was not significant in the model. Future predictive models of WNV occurrence in horses should take into account these factors in order to improve accuracy and reliability. Research into other potential determinants such as horse management factors are required to determine more differential risk factors associated with WNV occurrence in horses.

INTRODUCTION

West Nile virus (WNV) is a Flavivirus first recorded in the United States in August 1999 in the borough of Queens, New York [1–6]. The virus is endemic in Africa, West Asia and the Middle East [7]. Since its introduction, the virus has spread virtually unimpaired across the North American continent [8, 9]. Its life cycle involves mainly birds and mosquitoes with a number of hosts (including humans and horses), considered accidental [1]. Birds are highly susceptible to the virus and can be good indicators of the start of a WNV epidemic [10, 11]. Although humans are considered an accidental host, once infected they form an integral part of the cycle and are an important indicative factor for risk of occurrence [1]. Mosquitoes are the main vectors that carry the virus from one host to the other and ensure the completion of the life cycle of the virus [2, 3].

Birds, equine species and other mammals have been reported to be the most susceptible hosts [10, 12]. Availability of these hosts is a possible reason for the rapid spread of the virus since its introduction in the United States [13]. The extent of spread is more often identified by the geographic distribution of infected animals through surveillance programmes [14, 15]. An epizootic can occur in an area without having a high risk for infection to humans [16].

In 2002, there were more than 15 257 laboratory-confirmed WNV equine cases reported in 43 states,

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with epizootic and epidemic activity intense in the central United States [17]. The outbreak of WNV in North Dakota in 2002 included over 569 horse cases, and they were notably clustered in the eastern and northeastern parts of the state [18]. The incidence of WNV infection in horses in North Dakota has been reported to be seasonal with cases occurring in the late spring/summer to mid-autumn months [17]. This period is associated with melting snow, intensive drainage discharge and relatively high temperatures. These conditions are closely associated with the life cycle of mosquitoes and the migratory habits of birds [19].

The virus thrives under specific environmental, seasonal and ecological conditions that maintain its life cycle and guarantee its spread; yet few studies have been conducted to describe the geographic and ecological determinants of WNV occurrence [20]. A case-control study conducted by USDA [21] using data from five states with confirmed cases of WNV in horses failed to find any associations between occurrence of WNV in horses and any environmental and management factors examined. Factors studied included precipitation, temperature, location of case premises relative to equid inventories, WNV-positive mosquito pools, WNV-infected wild birds, elevation and eco-regions. Another study [22] of environmental and social determinants of human risk to WNV during the outbreak of 2002 indicated that differential mosquito abatement efforts were especially important risk factors to occurrence of the infection in humans. The study [22] further indicated that human population characteristics could play a role in the pattern of occurrence of WNV seen in humans.

During the 2002 WNV epidemic in horses in North Dakota, cases were clustered in the eastern and northeastern parts of the state [17]. The pattern of WNV occurrence observed suggested the existence of specific environmental and social factors that increased the risk for WNV infection and/or illness in those locations. This investigation sought to develop a predictive model to account for these factors and to assess their importance in explaining the WNV pattern observed. The specific objective was to determine the risk factors that could best predict the pattern of WNV occurrence observed in horses in North Dakota in 2002.

MATERIALS AND METHODS

The area of study was the state of North Dakota. Sample population comprised confirmed horse cases of WNV, as reported by the North Dakota State University Veterinary Diagnostic Laboratory (VDL), Fargo, ND, USA.

Data on horses affected by WNV were retrieved from medical records for 2002 obtained from the VDL. Diagnosis of WNV infection in all horses was performed by staff at the VDL, using an immunoglobulin (Ig) M-capture enzyme-linked immunosorbent assay developed by USDA: APHIS [23]. Additional confirmatory diagnosis was performed on earlier submissions of horse specimens by virus isolation and (Ig) M-capture ELISA performed at National Veterinary Services Laboratory (NVSL) in Ames, Iowa.

Additionally, data were obtained on the following factors; precipitation, temperature, distance of cases to water bodies, and elevation, and served as independent or explanatory variables. Weather data on temperature and precipitation were obtained from the North Dakota Weather Network (NDAWN) [24]. NDAWN consists of a number of weather stations spread through out the state. Due to the uneven distribution of stations by counties, gaps with no data were found. This problem was addressed by using Geographic Information Systems (GIS) Arc Info 8 software, which uses the nearest neighbour principle to derive data for areas lacking data [25]. The predicted values assigned are based on the assumption that these areas have similar characteristics to those immediately surrounding them. The software gives the option of how many nearest neighbour points to consider for each location on the surface. A covariance model is then used to predict the values of each unmeasured point. In order to determine the model fit based on the datasets, the software carries out a cross-validation of the model. This process permits inherent adjustments to be made to the model until a best-fit model with better predictability is obtained. An adjusted standardized error prediction surface model is also provided by the software for comparison and further adjustment. This can be used to further enhance the accuracy of the prediction surface model using a combination of simple overlay and reclassification procedures.

Distance of identified horse cases from water bodies was defined as presence of a water body within a radius of $\leqslant 5$ miles of the city centre from where the horse WNV case was reported. These data were generated using GIS Arc Info 8 software. The approach of assigning a radius was applied due to the lack of exact coordinates for locations of the reported cases. Any city with a reported case close to a water body

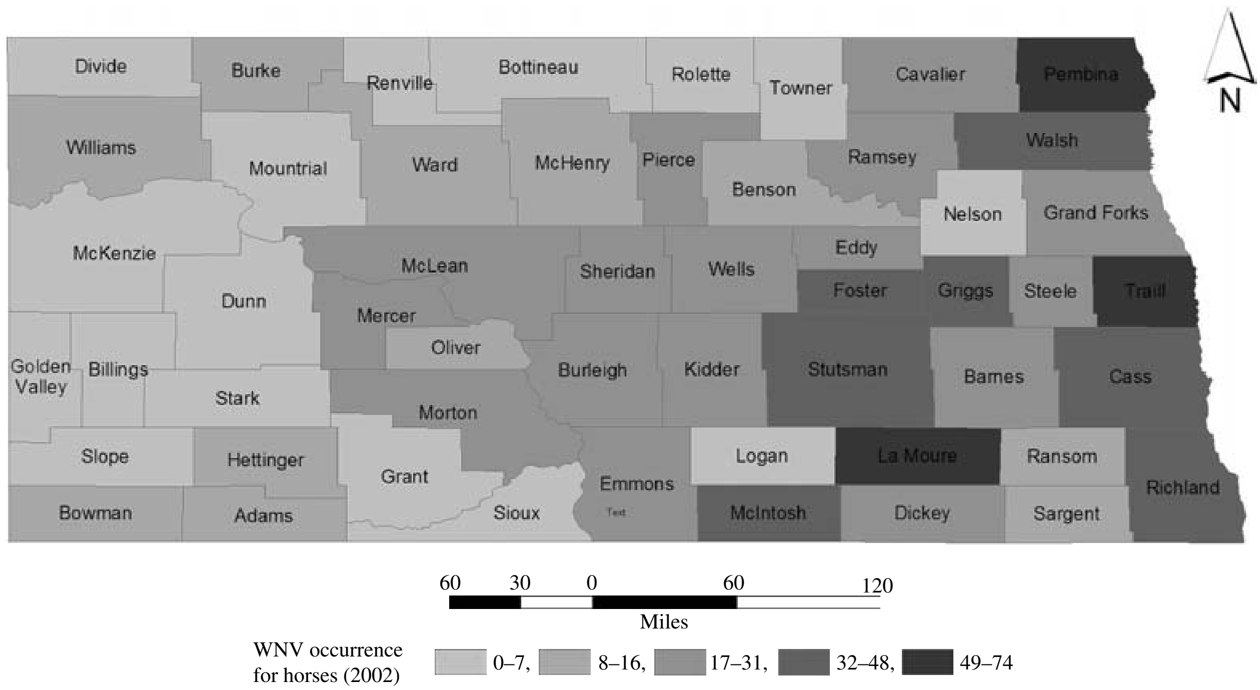

Fig. 1. Geographical distribution (by county) of WNV cases in horses in North Dakota, 2002.

was assigned the number 1; while 0 was assigned to a city with no nearby water body.

Elevation data for various parts of the state were generated using the Geostatistical Analyst extension software. Additional ecological data comprised populations of birds, mosquito pools, and humans that tested positive to WNV, obtained from the North Dakota State Department of Health Services website [26, 27]. A second set of data was collected for 2003, to validate the effectiveness of the model at predicting WNV occurrences in horses. Data from 2002 was for the months of June to September while 2003 data were from the months of May to November.

All data on the variables studied were standardized using the *Z* test procedure. The mean of each variable was subtracted from each value and the result divided by the standard deviation of values in the dataset.

GIS Arc Info 8 software was used to show the spatial distribution of reported infected horses in the state (Fig. 1). Trend analysis [20], which shows the actual and predicted spread direction of WNV, was performed using the same software. This analysis was used to identify the overall direction of spread, and to predict where the most WNV horse cases would occur. This analysis identifies and predicts where cases of WNV are probable by drawing an inference from areas that combine all factors which favour WNV occurrence. This is based on areas that have reported present infections of the virus, or where the virus has been identified in animal hosts. Any areas with no reported cases of occurrence or infection, but which have a similar combination of factors can be predicted as likely areas for occurrence. Based on trend analysis, the predicted spatial direction of flow of cases and the distribution of WNV in horses in North Dakota in 2002 was determined.

The number of WNV horse cases was used as the dependent or response variable in the analysis. A correlation was performed on independent variables to test for presence of multicolinearity. A number of independent variables were highly correlated so a principal component analysis (PCA) was performed on the data to create a new set of uncorrelated or orthogonal variables. A principal component regression (PCR) analysis was then run on the most significant principal components in order to obtain a prediction equation [28].

To test the effectiveness and usefulness of the model, a similar dataset of variables was obtained for 2003 and used to predict occurrence of WNV in horses. The data were collected for the months of May to November 2003. A PCA was performed on the 2003 data to create orthogonal variables, after which a PCR was run on the most significant principal components to obtain a prediction equation. The equation generated was used to predict occurrence of WNV in

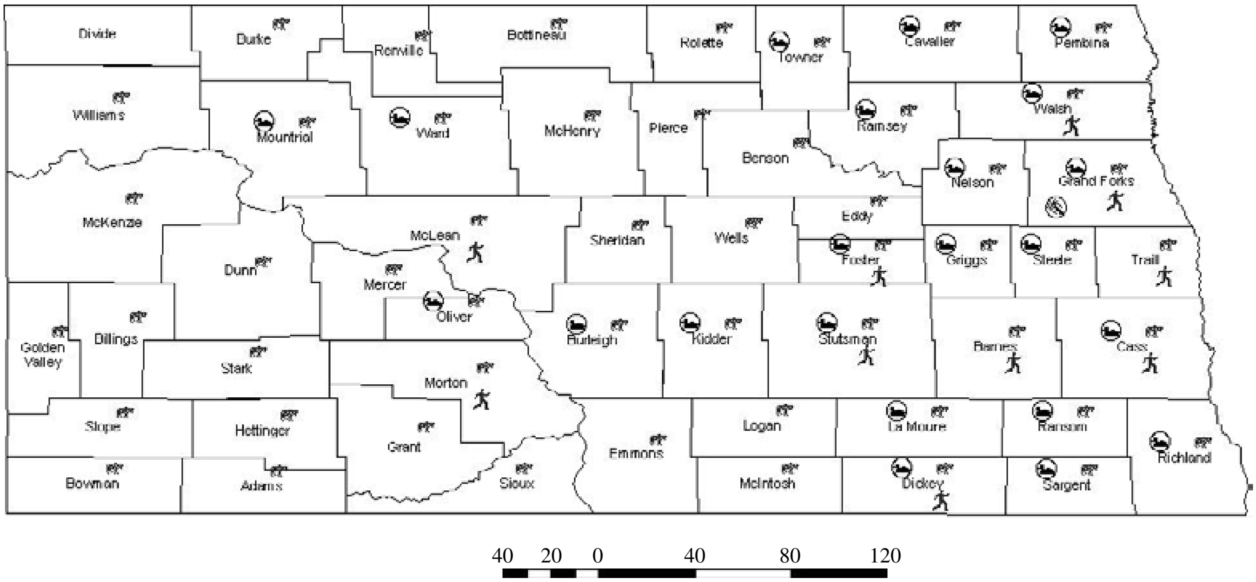

Fig. 2. Occurrence (by county) of WNV cases in horses, birds, humans and mosquitoes in North Dakota, 2002.

horses in North Dakota in 2003. Fisher's least significant difference (LSD) pairwise comparison test [29] was then performed on the 2002 WNV case distribution (by county) in North Dakota and the predicted WNV case distribution for 2003 to verify existence of differences in prediction between the two spatial distributions created.

RESULTS

Fifty-two of the 53 counties of North Dakota reported WNV occurrence in 569 horses in 2002 (Fig. 1). The cases clustered around the central region of the state specifically around the counties of Stutsman and Burleigh (Fig. 1). However, after accounting for the total number of horses at risk per county, the incidence of equine cases (number of equine cases/1000 horses) was higher in the eastern and northeastern parts of the state [17]. In 2002, reported human, bird and mosquito WNV-positive cases followed a similar spatial pattern to that of the horse cases (Fig. 2). In 2003, only 23 of the 53 counties of North Dakota reported WNV occurrence in 42 horses (Fig. 3), and the majority of cases were located in the western region of the state.

In general, the average annual precipitation was considerably higher, and average annual temperatures were cooler, in the eastern and northeastern parts of the state compared to the western. Surface elevation decreased eastwards towards the Red River basin ranging from ~3000 ft in the western part of the state to ~780 ft in the Red River basin. Most reported cases (70%) of WNV in horses were within 5 miles of a perennial water body. The descriptive statistics for the variables studied are summarized in Table 1.

A correlation test on independent variables indicated the presence of multicolinearity among them. Most variables were correlated ($r > 0{\cdot}5$, $P < 0{\cdot}05$) with each other, much more than with the dependent variable. A PCA was therefore performed on the data to create a new set of uncorrelated or orthogonal variables. PCA identified six principal factors which accounted for almost 90% of variation observed (Table 2). Based on the maximum eigenvalues for the six principal factors, the following elements were the most significant components of the six factors: factor 1 (reported WNV human cases), factor 2 (reported WNV-positive mosquitoes), factor 3 (mean temperature), factor 4 (elevation), factor 5 (mean rainfall), and factor 7 (reported WNV-positive birds). The sixth factor under consideration (distance of cases to water body) did not load as a major factor in the study (Table 3).

A PCR analysis of the most significant principal factors identified was run on WNV horse cases in order to obtain a prediction equation. Results of the PCR analysis (Table 4) gave a model R^2 of 0·97 ($P < 0{\cdot}0001$), and factors that were significantly associated with WNV occurrence in horses in North Dakota

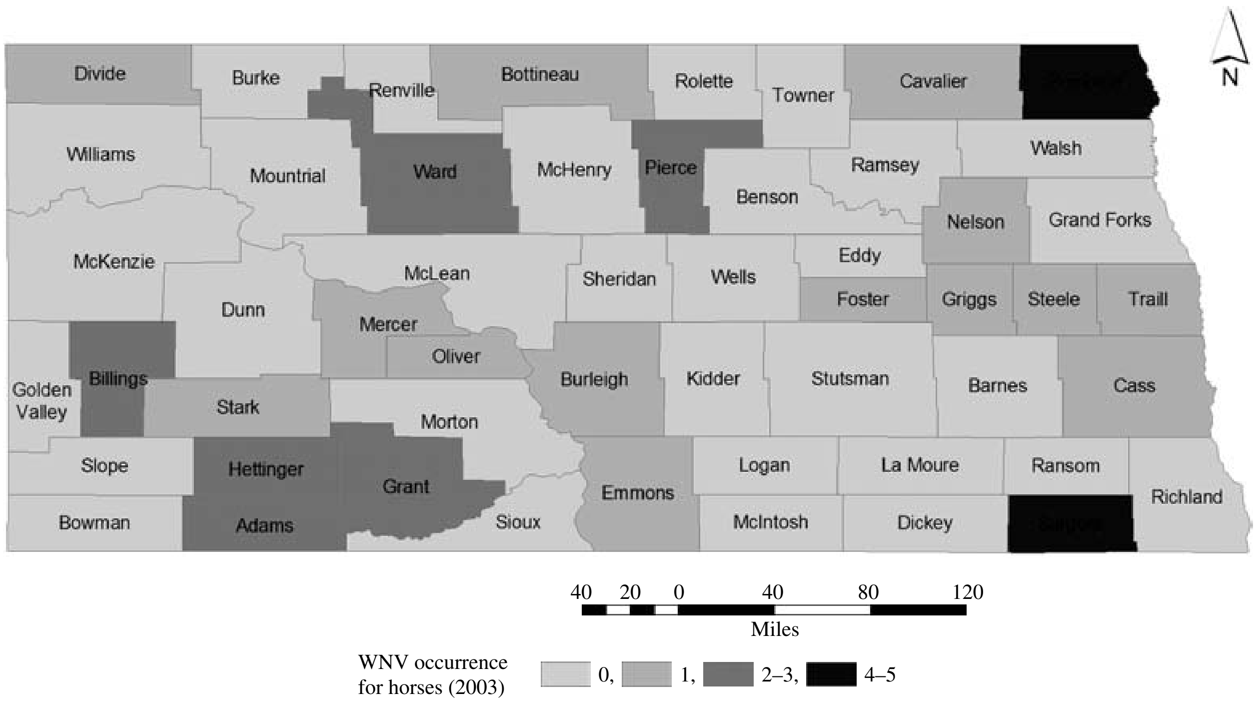

Fig. 3. Geographical distribution (by county) of WNV cases in horses in North Dakota, 2003.

were; factor 1 (reported WNV human cases, $P<0{\cdot}0001$), factor 2 (reported WNV-positive mosquitoes, $P<0{\cdot}0001$), factor 3 (mean temperature, $P<0{\cdot}0001$), factor 4 (elevation, $P<0{\cdot}0001$), factor 5 (mean rainfall, $P<0{\cdot}0001$) and factor 6 (distance of cases to water body, $P<0{\cdot}0001$). Factor 7 (reported WNV-positive birds) was not significant in the PCR ($P=0{\cdot}1733$). This model was used to generate a predicted distribution of WNV cases in horses in 2002 (Fig. 4). Fisher's least square difference pairwise comparison test between the actual (Fig. 1) and predicted (Fig. 4) WNV horse cases distribution in North Dakota in 2002 showed no significant difference; the model had a 97% fit or capacity in prediction.

The analysis was repeated using 2003 data and the PCR model showed only factor 1 (reported WNV human cases, $P<0{\cdot}0001$) and factor 2 (mean temperature, $P=0{\cdot}0134$) as significant in explaining the occurrence of WNV in horses. The model R^2 for the data of 2003 was 0·53 ($P<0{\cdot}001$). The predictive model developed from 2003 data was used to generate a predicted spatial distribution of WNV cases in horses in 2003 (Fig. 5). Fisher's least squares difference pairwise comparison of the actual (Fig. 3) and predicted (Fig. 5) WNV horse cases (by county) in North Dakota in 2003 showed a significant difference between them; the model had a 53% fit or capacity in prediction.

DISCUSSION

The study identified elevation as one of the factors significantly associated with occurrence of WNV in horses in North Dakota in 2002, with more cases occurring at lower compared to higher elevation. This finding concurs with what was reported earlier [17], whereby the highest incidence of WNV horse cases was in the eastern and northeastern parts of the state; surface elevation in North Dakota decreases eastwards towards the Red River basin. Also, it was reported that most WNV equine cases in Colorado and in the north-eastern US were located at lower elevations [21, 30]. This finding could also be explained by the fact that more mosquito populations (the main vectors for transmission of WNV to horses, humans and birds) are likely to be found at lower elevation compared to high lands. Low lands are usually cool places with temperatures that favour mosquito reproduction. Moreover, it has been reported that differential mosquito populations are especially important risk factors to occurrence of the infection in humans [22]. Horses acquire infection to WNV from mosquitoes in a similar manner as do humans.

It was not surprising to find that reported WNV-positive mosquito pools were associated with occurrence of the infection in horses since mosquitoes are the principal vectors of WNV. Also, for equids to

Table 1. *Descriptive statistics of variables investigated: 2002 and 2003*

Variable	*N*	Mean	Median	S.M.	S.D.	S.E. mean
Horses						
2002	53	10·74	8·00	9·51	9·73	1·34
2003	53	0·79	0·00	0·62	1·23	0·17
Birds						
2002	53	1·23	0·00	0·68	2·64	0·36
2003	53	3·17	1·00	2·51	4·36	0·60
Humans						
2002	53	0·32	0·00	0·19	0·78	0·11
2003	53	11·64	6·00	9·09	16·36	2·25
Mosquitoes						
2002	53	0·02	0·00	0·000	0·14	0·02
2003	53	0·19	0·00	0·04	0·76	0·11
Temperature (°C)						
2002	53	4·90	4·98	4·93	1·37	0·19
2003	53	4·94	4·97	4·95	1·50	0·21
Rain (in.)						
2002	53	2·27	2·18	2·26	0·64	0·09
2003	53	2·11	2·07	2·1045	0·53	0·07
Cum. water-body distance						
2002	53	10·96	8·00	9·66	10·20	1·40
2003	53	9·25	8·00	8·13	10·17	1·40
Elevation (ft)						
2002	53	1757·9	1696·1	1741·5	504·8	69·3
2003	53	1757·9	1696·1	1741·5	504·8	69·3

S.M., Standard mean; S.D., standard deviation; S.E. mean, standard error of the mean.

Table 2. *Eigenvalues of the correlation matrix results* (*2002*)

Factor	Eigenvalue	Difference	Proportion	Cumulative
1	2·1002	0·5060	0·3000	0·3000
2	1·5941	0·4429	0·2277	0·5278
3	1·1511	0·3755	0·1645	0·6922
4	0·7756	0·1034	0·1108	0·8030
5	0·6721	0·2509	0·096	0·8990
6	0·4211	0·1356	0·0602	0·9592
7	0·2855		0·0408	1·000

The table shows the model is basically 4-dimensional based on the cumulative loading or contributions of the most important variables in the model.

become infected with WNV, they must be in contact with an adequate number of infected mosquitoes. Likewise the significant association observed between mean temperature and occurrence of WNV in horses was expected as warm temperatures are required for mosquitoes to breed in large numbers. Another study [31] of WNV in horses in northern Indiana reported a significant association between ambient temperature and the peak of the outbreak. Ambient temperatures were significantly lower after the peak of the outbreak, compared with prior to the peak.

Because of the time when the study was conducted (autumn 2004), we were unable to collect reliable data on temporary water bodies associated with the occurrence of WNV for the state. We therefore investigated the role of permanent water bodies in WNV occurrence. The model showed that permanent water bodies were not a significant factor associated with WNV occurrence. This was not a total surprise because a previous study [30] reported that smaller temporary and seasonal water bodies created during warmer months by melting snow or heavy rainfall did play a significant role as a breeding ground for mosquitoes, but not perennial water bodies.

It is possible the study's outcome would be different if common mosquito breeding sites that may hold or collect water were used because mosquitoes breed mainly in these small subset habitats. These habitats include cans, old tyres, barrels, and gutters; catch basins, standing water around structures, flat roofs, draining puddles, ditches, tree holes, and swampy pool areas [32]. There are studies [21, 33, 34] that have shown that incidence rates of WNV are usually high in

Table 3. *Principal components analysis results* (*2002*)

Variable	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6	Factor 7
X_1	0·54	0·32	0·16	−0·16	−0·04	−0·19	0·71
X_2	0·55	0·09	−0·17	0·11	−0·33	0·68	−0·23
X_3	0·13	0·62	0·42	−0·06	−0·00	−0·27	−0·57
X_4	0·10	−0·32	0·71	−0·10	0·45	0·38	0·01
X_5	−0·19	0·51	−0·35	−0·17	0·63	0·36	0·09
X_6	0·44	−0·14	−0·20	0·62	0·50	−0·29	−0·13
X_7	−0·36	0·30	0·28	0·72	−0·17	0·21	0·28

X_1, Reported WNV-positive birds; X_2, reported WNV-positive humans; X_3, reported WNV-positive mosquitoes; X_4, mean temperature; X_5, mean precipitation; X_6, distance of cases to water bodies; X_7, mean elevation.

Table 4. *Principal components regression results* (*2002*)

Variable	Estimate	S.E.	*P* value
Intercept	10·7	0·21	<0·0001
Factor 1	4·23	0·15	<0·0001
Factor 2	−0·79	0·17	<0·0001
Factor 3	−1·58	0·20	<0·0001
Factor 4	6·11	0·25	<0·0001
Factor 5	5·09	0·27	<0·0001
Factor 6	−3·19	0·34	<0·0001
Factor 7	−0·57	0·41	0·1733

Model $R^2=0·97$, $P<0·0001$; Factor 1 = X_2 (reported WNV-positive humans); Factor 2 = X_3 (reported WNV-positive mosquitoes); Factor 3 = X_4 (mean temperature); Factor 4 = X_7 (mean elevation); Factor 5 = X_5 (mean precipitation); Factor 6 = X_6 (distance of cases to water bodies); Factor 7 = X_1 (reported WNV-positive birds).

warmer months of the year that have more liquid precipitation and standing water bodies. Other studies have shown a positive correlation between winter precipitation and summer abundance of mosquitoes in the western half of the United States [30]. However, there is speculation that summer drought in the western United States may create more breeding sites for mosquitoes as more water sites become stagnant. Although it is known that the number of mosquitoes depends on environmental factors such as temperature and moisture, the correlation between moisture and mosquito abundance is the subject of ongoing research [30].

Interestingly, results of PCA showed 'reported WNV-positive birds' loading together with 'reported WNV-positive human cases' in principal factor 1. This factor was significantly associated with WNV occurrence in horses for the state in 2002. It is possible that 'reported WNV-positive birds' did not load strongly as a separate factor in this study due to the fact that passive bird surveillance in North Dakota started in June, 2002, at which time it was possible to have missed detecting earlier WNV-positive birds. Studies have reported that reports of dead birds preceded confirmation of viral activity in any species by several months, and WNV-positive birds were found more than 3 months before onset of human cases [35]. Moreover, it is possible that the small number of reported human cases (17 cases) within the period of interest, contributed to this observation.

Several reasons could explain why the predictive model developed in 2002 when applied to 2003 data showed only reported WNV human cases and mean temperature to be significantly associated with WNV occurrence in horses. First, there was a significant increase in the number of reported human cases with WNV in 2003 (617 cases) compared to 2002 (17 cases) [18]. There were also significantly fewer horse cases (41 cases), in 2003 compared to 2002 (569 cases reported) [17, 18]. The drop in WNV cases in horses could have been caused by horses developing immunity to WNV after the 2002 exposure. Furthermore, a large percentage of equids are likely to have been vaccinated against WNV; a WNV vaccine provisionally licensed by USDA first became available for use in horses in August 2001 [30]. Moreover, increased mosquito mitigation measures could have contributed to fewer WNV equine cases during 2003 than occurred in 2002. There is a possibility that weather differences between the years 2002 and 2003 could have explained the inability of the model developed for 2002 to predict occurrence of equine cases in 2003. Seasonal variations observed in WNV occurrence between 2002 and 2003 could have, in part, influenced the ability of the model to predict occurrence of WNV in horses in 2003.

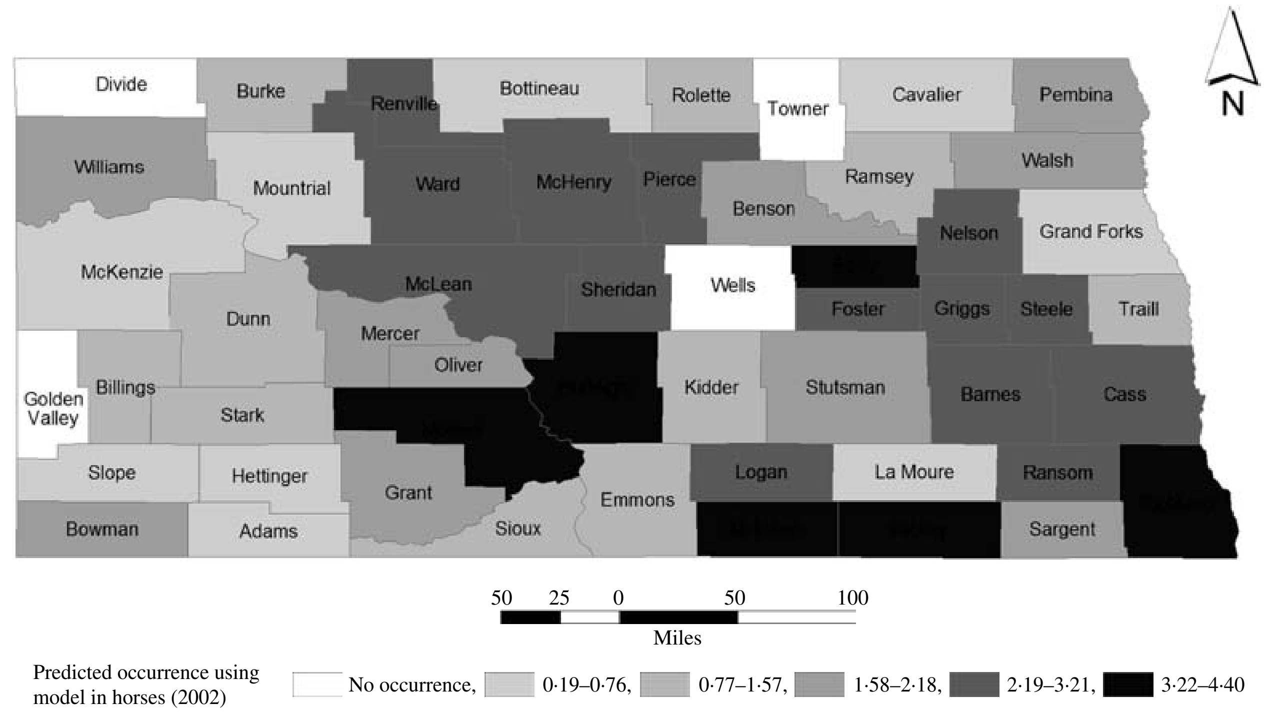

Fig. 4. Predicted occurrence of WNV cases in horses in North Dakota, 2002, using the model. The equation for this model is: $y = 10{\cdot}7 + \text{Factor 1 } (4{\cdot}23 * \text{HUMAN CASES}) - \text{Factor 2 } (0{\cdot}79 * \text{MOSQUITO CASES}) - \text{Factor 3 } (1{\cdot}58 * \text{TEMPERATURE}) + \text{Factor 4 } (6{\cdot}11 * \text{ELEVATION}) + \text{Factor 5 } (5{\cdot}09 * \text{PRECIPITATION}) - \text{Factor 6 } (3{\cdot}19 * \text{DIST WATERBODIES}) - \text{Factor 7 } (0{\cdot}57 * \text{BIRDS CASES})$. Percentage of values are used to create the prediction maps to enable comparison between maps.

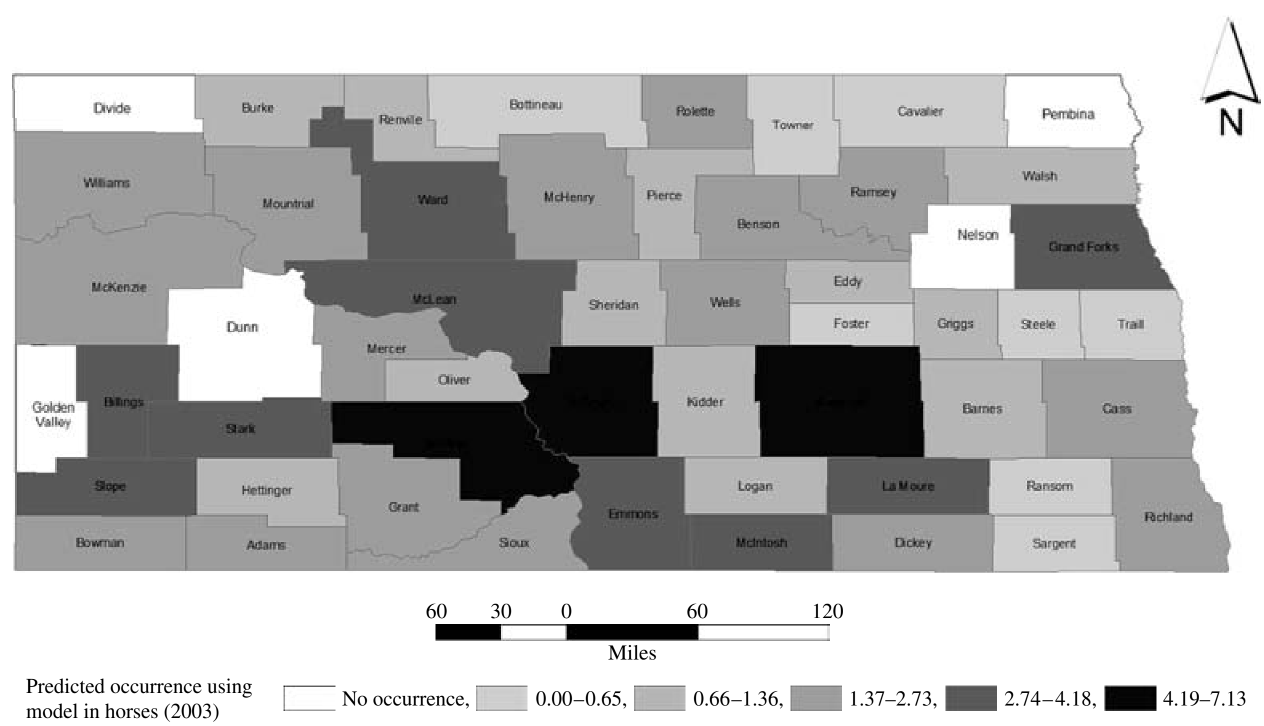

Fig. 5. Predicted occurrence of WNV cases in horses in North Dakota, 2003, using the model. The equation for this model is: $y = 0{\cdot}79 + \text{Factor 1 } (0{\cdot}52 * \text{HUMAN CASES}) + \text{Factor 2 } (0{\cdot}27 * \text{TEMPERATURE}) - \text{Factor 3 } (0{\cdot}07 * \text{ELEVATION}) + \text{Factor 4 } (0{\cdot}03 * \text{MOSQUITO CASES}) + \text{Factor 5 } (0{\cdot}10 * \text{PRECIPITATION}) + \text{Factor 6} + \text{Factor 7 } (0{\cdot}42 * \text{DIST WATERBODIES})$. Percentage of values are used to create the prediction maps to enable comparison between maps.

This study highlighted some environmental determinants of WNV occurrence in horses. However, further research into other potential determinants of WNV occurrence in horses such as horse characteristics and management factors (vaccination and immunity-related factors) and social factors related to mosquito control is warranted. For equids to become infected with WNV, they must be in contact with an adequate number of infected mosquitoes, so implementing mosquito-control measures is an important factor to consider when predicting future WNV cases. Other significant factors such as the presence of vegetation, age, income, race of the population, distance to a WNV-positive dead bird specimen, age of horses, mosquito abatement effort, geological factors, vaccination status and some clinical signs have also been identified as predictors of WNV severity. It is important that future predictive models of WNV occurrence in horses should take into account most of these factors in order to improve their accuracy and reliability.

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DECLARATION OF INTEREST

None.

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