Outcome of equids with clinical signs of West Nile virus infection and factors associated with death

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Objective—To determine outcome of equids in the western United States with clinical signs of West Nile virus (WNV) infection and identify factors associated with risk of death in infected equids.

Design—Cross-sectional study.

Animals—484 equids in Nebraska and Colorado.

Procedure—Owners of 484 equids with laboratory-confirmed West Nile virus infection in Nebraska and Colorado were contacted by telephone, and a questionnaire was used to obtain information on signalment, management, clinical signs, date of disease onset, duration of disease, WNV vaccination status, and health status at the time of the interview.

Results—137 of 482 (28.4%) animals died or were euthanatized. Ataxia, lethargy, muscle fasciculations, and weakness were the most common clinical signs of disease. Animals ≥ 3 years old were more likely to die than were animals ≤ 2 years old. Unvaccinated equids were twice as likely to die as were animals that had been vaccinated at least once prior to the onset of disease. Animals that were recumbent and unable to rise were 78 times as likely to die as were animals that never lost the ability to rise. Females were 2.9 times as likely to die as males. Two hundred seventy-one of 339 (79.9%) animals that survived recovered fully; mean duration of disease for these animals was 22.3 days.

Conclusions and Clinical Relevance—Among equids with WNV infection, age, vaccination status, an inability to rise, and sex were associated with the risk of death. (*J Am Vet Med Assoc* 2004;225:267–274)

West Nile virus (WNV) is a mosquito-borne flavivirus that can cause a wide range of neurologic abnormalities in humans and animals. Equids appear to develop clinical signs of WNV infection, including fatal encephalitis, more readily than do other domestic mammals, with the most common clinical signs in equids being weakness, incoordination, and ataxia.[1] In 2002, > 14,700 laboratory-confirmed equine cases of WNV infection in 43 states were reported.[2] During this time, however, there were more confirmed cases than initially predicted in the western United States, particularly Nebraska, which had 1 of the highest prevalences of equine WNV infection during 2002. The reason for this is still unclear, but it may have been attributable in part to warm, dry climatic conditions favorable for vector reproduction and maturation in the western United States[3-5] and the large numbers of *Culex tarsalis*, a highly efficient vector for the virus, around equine premises.[6-8] To our knowledge, the prognosis for equids with WNV infection and the factors associated with outcome for infected equids in the western United States have not been described. The purposes of the study reported here were to determine outcome of equids in the western United States with clinical signs of WNV infection and identify factors associated with the risk of death in infected equids.

Materials and Methods

Study population—A list of all equids in Nebraska or Colorado with clinical signs of WNV infection in which the diagnosis had been confirmed by means of laboratory testing during 2002 was obtained from the state veterinarian offices in these states. To be included in this study, equids had to have resided primarily in Nebraska or Colorado and have developed clinical signs of WNV infection during 2002. In addition, WNV infection had to have been confirmed serologically or by means of other testing (ie, virus isolation or polymerase chain reaction assay of tissues collected at necropsy).

A copy of the laboratory submission form for each animal eligible for inclusion in the study was obtained. For each animal, the owner's name and contact information from the laboratory submission form were entered into a computer spreadsheet. Each animal was entered separately, even if an owner had > 1 affected equid.

A sample of equids was randomly selected from the list of all affected equids in Nebraska and Colorado during 2002

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for further study by means of random numbers assigned to each animal. An attempt was made to select cases from all phases of the epidemic, and animals were purposefully selected in proportion to the number of infected equids in each state.

The required sample size was estimated on the basis of an a priori goal of estimating the true case fatality rate within ± 3% with 95% confidence; for this calculation, we assumed that the true rate was approximately 20% and that the total number of affected equids in the 2 states would be approximately 1,500.[9] The study was initiated prior to the end of the 2002 vector season; thus, the expected number of affected equids was estimated on the basis of the number of equids with confirmed infection at the time of the study and the proportion of affected equids in the 2 states. Our calculations suggested that information regarding approximately 500 valid subjects would need to be obtained to achieve the study goal. Pilot investigations suggested that 80% of owners could be contacted by telephone with information obtained from laboratory submission forms and that 97% of owners that could be contacted would agree to participate in the study. Thus, the initial sample size estimate was inflated to account for this nonparticipation rate.

Survey protocol—A survey instrument that could be delivered by telephone was developed for the study. The survey was specifically developed to gather information regarding signalment, primary use prior to infection, WNV vaccination status, date of onset of clinical signs, clinical signs, treatments, duration of disease, whether the animal returned to its previous use, health status of the animal at the time of the interview, total number of equids on the premises, number of equids on the premises that developed clinical signs consistent with WNV infection (reported and unreported), mosquito mitigation practices, and housing of animals on the premises. A script that served as a template for telephone interviews was devised and reviewed during several training sessions with individuals involved in data collection to ensure uniformity in communicating with owners and gathering data.

Owners were asked during telephone interviews to describe the clinical signs observed by themselves and others responsible for the care of affected equids. The survey instrument contained lists of specific signs of interest grouped into various descriptive categories. If an owner did not specifically mention the presence or absence of any 1 of these clinical signs when describing the affected animals, the interviewer asked about its occurrence during the course of the disease. The same approach was used to collect data regarding treatment (ie, open-ended interview questions followed by targeted questions for information on specific treatments not mentioned). If an owner was uncertain about details regarding treatment of his or her animal, the interviewer asked for permission to contact the owner's veterinarian for this information; veterinarians were not contacted without first obtaining permission from the owner.

All telephone interviews were conducted by 25 second-year veterinary students from the Colorado State University College of Veterinary Medicine and Biomedical Sciences as partial fulfillment of an honors project. Contact information for owners was initially obtained from copies of the laboratory submission forms supplied by the Nebraska and Colorado state veterinarian offices. If contact information was incomplete, attempts were made to find valid telephone numbers through Internet information resources or directory assistance. Telephone contact was only attempted a minimum of 30 days after laboratory confirmation of WNV infection to improve accuracy of data collected regarding outcome, presence of residual clinical signs, and return of animals to their previous use. A minimum of 3 attempts was made to contact each owner; calls were made on different days and at different times of the day in an attempt to maximize the chances of successfully contacting owners. Student interviewers routinely asked whether the person answering the telephone was the best person to provide information about the animal selected for inclusion in the study. For each owner the interviewers attempted to contact, whether the owner was ever successfully contacted and, if so, whether the owner participated in the study were recorded.

At the completion of each interview, the student interviewer rated the respondent on a scale from 1 to 4 regarding perceived knowledge of the management of the animal and medical aspects of the animal's disease. Criteria previously developed by the USDA Centers for Epidemiology and Animal Health were used.[10] A postcard was mailed to all respondents thanking them for their participation in the study. Owners who had questions regarding WNV infection were directed to information posted on the Colorado State University Web site and were mailed a fact sheet regarding WNV developed for this purpose.

Data management—Data collected during telephone interviews were initially recorded on paper forms and later entered into a computer database[a] by means of Web-based forms specifically designed for this purpose. Student interviewers attended training sessions regarding data entry prior to study initiation.

Information obtained through telephone interviews was merged with data obtained from the laboratory submission forms. All data were reviewed for consistency, accuracy, and uniformity of entry format by a single investigator (PS). Additionally, approximately 50% of computer records were compared with the paper forms to further validate the data. All corrections to the database were documented. When possible, the database was further verified by comparison with information supplied in laboratory submission forms. If an owner's response regarding a particular clinical sign in his or her animal could not be validated from the paper form or laboratory report, this field was left blank in the computer database.

Statistical analyses—Data were summarized and descriptive statistics were calculated. A χ^2 goodness-of-fit test was used to compare the sex distribution of study animals with the expected sex distribution; information from a 1998 national study[11] was used to calculate the assumed population sex distribution for adult horses in the general population. Categorical variables were analyzed with the Pearson χ^2 test; continuous variables were analyzed by means of ANOVA.[b]

Because the elapsed time from the reported onset of disease until the date of interview varied between 31 and 262 days and the long-term clinical status (ie, survival and recumbency) could have changed during this elapsed time, differences in elapsed time from onset to interview were compared with reported clinical status by means of the Wilcoxon signed-rank test.

The case fatality rate was estimated by dividing the number of equids that died or were euthanatized as a result of WNV infection by the number of equids included in the study. The Student *t* test was used to compare ages of animals in various groups (eg, male vs female, vaccinated vs unvaccinated, and survived vs died). Animals' ages were divided into 4 categories to facilitate regression analysis (juveniles, ≤ 2 years old; young adults, 3 to 5 years old; adults, 6 to 18 years old; geriatrics, > 18 years old). Date of onset of clinical signs was grouped into quartiles. For individual clinical signs, responses recorded as "unknown" were grouped with negative responses. In regard to WNV vaccination status, animals were classified as having received at least 1 dose of vaccine

prior to the onset of clinical signs; having received at least 1 dose of vaccine, but only after the onset of clinical signs; or having not been vaccinated. An estimated attack rate for clinical WNV infection among equids in the study population was calculated by dividing the total number of equids with laboratory-confirmed WNV infection plus the number of additional equids in this population that had signs consistent with WNV infection, even if they were not tested, by the total number of equids at risk (ie, total number of equids on the premises where study subjects were housed).

Logistic regression was used to analyze the risk of death among affected animals and the risk that affected animals would become recumbent and unable to rise. Independent variables assessed in these models included age category, whether the animal was treated with corticosteroids (yes vs no), sex (male vs female), WNV vaccination status (vaccinated prior to disease onset vs vaccinated after disease onset vs not vaccinated), and date of disease onset (March 1 through August 19 vs August 20 through October 10). Repeated measures attributable to similarity of exposures for multiple subjects residing on a single premises were controlled for with generalized estimating equation methods.[c] Logistic regression models were used to estimate **odds ratios (ORs)** and their associated 95% **confidence intervals (CIs)**. Independent variables initially included in regression models were screened with the general hypothesis that they were potentially associated with survival or recumbency status of horses. Bivariable models were used to screen independent variables individually, and those associated with the outcome variable with a P value < 0.25 were included in multivariable modeling.[12] Final multivariable models were constructed by means of backward-stepping variable selection.

Results

Student interviewers attempted to contact owners of 819 affected equids between September 20, 2002, and January 31, 2003. Forty-four owners had > 1 equid randomly selected for potential inclusion in the study (range, 1 to 7 equids/owner). Owners of 283 animals could not be contacted because there was no answer despite ≥ 3 attempts to call (n = 120) or a valid telephone number could not be obtained (163). Owners of the remaining 536 animals (65%) were successfully contacted by telephone, and 493 of the 536 (92%) agreed to participate. During telephone interviews, it was discovered that 9 of the 493 animals (1.8%) had never displayed clinical signs of WNV infection, and these animals were excluded from the study. Therefore, 484 equids were included in the study.

For animals included in the study, median time from the onset of clinical signs to the time of the telephone interview was 70 days (interquartile range [25th to 75th percentile], 48 to 86 days). Differences in time elapsed from disease onset to interview were not associated with the study subjects' survival or recumbency status.

Owner knowledge—Median score for owner knowledge regarding their animals' medical treatment was 2 (mean, 2.1; SD, 1.0; range, 1 to 4). A score of 2 indicated accurate records and good knowledge of the facts pertaining to the questions asked, with little question about the data that the owner provided.[10] Median score for owner knowledge of their animal's management was 1 (mean, 1.7; SD, 0.9; range, 1 to 4). A score of 1 indicated thorough knowledge of the facts pertaining to the questions asked with no question about the validity of the overall data provided.[10] In general, scores for owner knowledge of their animals' medical treatment were higher than scores for owner knowledge of their animals' management because some owners could not recall every medication that their animal received; attempts were made to contact the veterinarian for this information if permission was provided.

Case fatality rate—Information on outcome was available for 482 of the 484 animals included in the study. Overall, 345 of the 482 animals (71.6%) were alive at the time of the telephone interview (at least 30 days after disease onset), and 137 (28.4%) were dead. Of the 137 animals that were dead, 32 (23.4%) had died, and 104 (75.9%) had been euthanatized because of disease. The remaining horse was reportedly dead, but the owner could not specify the cause of death.

Factors associated with death and inability to rise—Bivariable logistic regression analyses indicated that several variables were associated with the risk of death and the risk that the animal would be recumbent and unable to rise (**Table 1**). However, the risk that an animal would be recumbent and unable to rise was also strongly associated with the risk of death. Therefore, only results from multivariable logistic regression in which risk of death was used as the outcome are reported (**Table 2**).

Signalment—Age was recorded for 474 of the 484 equids included in the study. Mean age was 9.5 years (SD, 6.4 years; range, 3 months to 35 years). Equids that survived infection (8.9 ± 5.8 years) were significantly ($P = 0.003$) younger than equids that died or were euthanatized (10.8 ± 7.8 years). Mean age of equids that died (8.7 ± 6.1 years) was not significantly ($P = 0.06$) different from mean age of equids that were euthanatized (11.5 ± 7.8 years). After controlling for other factors associated with death, juveniles (ie, animals ≤ 2 years old) were less likely to die or be euthanatized as a consequence of WNV infection than were older animals (Table 2). However, the odds of dying were not significantly different among the 3 older age categories.

Sex was recorded for 479 of the 484 animals included in the study, of which 40 (8.4%) were sexually intact males (including adults and juveniles), 220 (45.9%) were castrated males, and 219 (45.7%) were females. The proportion of males in the study population was significantly ($P = 0.03$) greater than expected, compared with the proportion of males in the general equine population in the United States.[11] However, after controlling for other variables associated with death, female equids were more likely to die or be euthanatized than were male equids (Table 2).

Breed information was available for 474 of the 484 animals in the study. Most (n = 299 [63.1%]) were Quarter Horses. Other breeds included American Paint Horse (n = 46 [9.7%]), Arabian (29 [6.1%]), Thoroughbred (23 [4.9%]), Appaloosa (18 [3.8%]), American Mustang (5 [1.1%]), Morgan Horse (5 [1.1%]), Shetland Pony (4 [0.8%]), Miniature Horse (2 [0.4%]), Welsh Pony (2 [0.4%]), Missouri Fox Trotter

Table 1—Results of bivariable logistic regression analyses of factors associated with death among 484 equids in Nebraska and Colorado with laboratory-confirmed West Nile virus infection during 2002.

		Death			Recumbency and inability to rise		
Variable	**Category**	**OR**	**95% CI**	***P* value**	**OR**	**95% CI**	***P* value**
Vaccination				0.005			< 0.001
	None	2.3	1.5–3.5		2.4	1.6–3.8	
	After onset of signs	1.2	0.5–3.9		0.8	0.2–3.2	
	Prior to onset of signs	NA	NA		NA	NA	
Age (y)				0.008			0.007
	> 18	2.6	1.3–5.3		2.2	1.0–4.7	
	6–18	0.9	0.5–1.5		0.6	0.4–1.1	
	3–5	NA	NA		NA	NA	
	≤ 2	0.6	0.3–3.3		0.9	0.5–1.9	
Corticosteroid administration				0.01			0.60
	Yes	NA	NA		NA	NA	
	No	1.4	1.1–2.2		0.9	0.6–1.4	
Unable to rise				< 0.001			
	Yes	51.8	26.9–99.8				
	No	NA	NA				
Clinical disease onset*				0.02			0.11
	Early	1.7	1.1–2.7		1.5	0.9–2.6	
	Late	NA	NA		NA	NA	
Sex				0.14			0.59
	Female	1.3	0.9–1.9		1.1	0.7–1.7	
	Male	NA	NA		NA	NA	

*Disease onset was classified as early (March 1 through August 19) or late (August 20 through October 10).
OR = Odds ratio. CI = Confidence interval. NA = Not applicable (reference category).

(1 [0.2%]), Pony of the Americas (1 [0.2%]), and Tennessee Walking Horse (1 [0.2%]). One horse was reported to be a Warmblood breed, and 11 (2.3%) were reported to be draft horse breeds. Twenty-three equids were categorized as nonregistered breeds, including crossbred horses (n = 14 [3.0%]), grade horses (7 [1.5%]), ponies (1 [0.2%]), and 1 horse of unspecified breed (1 [0.2%]). The remaining 3 equids (0.6%) were mules.

WNV vaccination status—Information on WNV vaccination status was available for 478 of the 484 animals in the study. Of these, 221 (46.2%) had received at least 1 dose of WNV vaccine before the onset of clinical signs; 17 (3.6%) had received at least 1 dose of WNV vaccine, but only after the onset of clinical signs; and 240 (50.2%) had not been vaccinated. Of the 221 animals vaccinated before disease onset, 74 (33.5%) had received 2 doses of WNV vaccine. However, information regarding vaccination interval and timing prior to disease onset was unknown for 5 of these animals. Forty-three of the remaining 69 animals (62%) had completed the vaccination series at least 1 day before disease onset. In the remaining 26 (38%), the second dose of vaccine was given the day that clinical signs were first reported or up to 6 weeks later. Among the 43 animals that had received 2 vaccine doses prior to disease onset, 30 received the second dose between 1 day and 3 weeks before disease onset. Only 13 had received the second dose ≥ 4 weeks before disease onset (range, 4 to 20 weeks). Mean ± SD interval between the 2 doses of vaccines in these 13 animals was 4 ± 1.4 weeks (range, 2 to 6.4 weeks), and mean time between administration of the second dose of vaccine and disease onset was 12 ± 4.4 weeks. Only 11 animals had been vaccinated in accordance with the manufacturer's instructions (2 doses 3 to 6 weeks apart) and had completed the vaccination series a minimum of 4 weeks prior to disease onset; all 11 survived. No single age category of equids in the study (juvenile, young adult, adult, or geriatric) was significantly ($P = 0.26$) more likely to be vaccinated against WNV than another.

Table 2—Results of multivariable logistic regression analyses of factors associated with death among 484 equids in Nebraska and Colorado with laboratory-confirmed West Nile virus infection during 2002.

Variable	**Category**	**OR**	**95% CI**	***P* value**
Vaccination				0.04
	None	2.1	1.0–4.5	
	After onset of signs	0.2	0.03–1.6	
	Prior to onset of signs	NA	NA	
Age (y)				0.03
	> 18	9.4	1.6–55.5	
	6–18	4.8	1.5–15.2	
	3–5	6.0	1.8–20.1	
	≤ 2	NA	NA	
Unable to rise				< 0.001
	Yes	77.9	33.5–180.9	
	No	NA	NA	
Clinical disease onset			0.06	
	Early	2.8	1.0–8.2	
	Late	NA	NA	
Sex				0.006
	Female	2.9	1.3–6.2	
	Male	NA	NA	

See Table 1 for key.

Because only clinically affected equids were included in the study, it was not possible to evaluate the efficacy of vaccination in terms of reducing the risk of disease occurrence. However, it was possible to evaluate the relationship between vaccination and some aspects of disease severity. Information on WNV vaccination status and outcome was available for 476 of the 484 animals in the study, of which 221 had had been vaccinated prior to disease onset, 17 had been vaccinated after disease onset, and 238 had not been vaccinated. Overall, 177 of the 221 (80.1%) animals vacci-

nated prior to disease onset survived, compared with 13 of the 17 animals (76%) vaccinated after disease onset and 151 of the 238 (63.4%) that were not vaccinated. After controlling for other factors associated with death, equids that were not vaccinated were 2.1 times as likely to die as were equids that were vaccinated (Table 2).

Clinical signs of disease—Information on the date of onset of clinical signs was available for 468 of the 484 animals in the study. After controlling for other factors associated with death, equids that became affected early in the epidemic (March 1 through August 19) were 2.8 times as likely to die as were equids that became affected later in the epidemic (August 20 through October 10; Table 2). Geriatrics were not significantly (P = 0.63) more likely to be affected early in the epidemic than were younger animals.

Owners reported that many animals in the study had > 1 clinical sign of WNV infection. The most commonly reported clinical signs included ataxia (278/484 [57.4%]), generalized weakness (259/484 [53.5%]), lethargy or signs of depression (208/484 [43.0%]), and muscle fasciculations (206/484 [42.6%]). Other commonly reported signs included frequent stumbling (177/484 [36.6%]), stiffness or reluctance to move (143/484 [29.5%]), recumbency and an inability to rise (133/484 [27.5%]), poor appetite (135/484 [27.9%]), recumbency for prolonged periods but able to rise (117/484 [24.2%]), altered mentation (107/484 [22.1%]), fever (102/484 [21.1%]), lameness (101/484 [20.9%]), and an abnormal head carriage (100/484 [20.7%]). Less common clinical signs included cranial nerve deficits (92/484 [19.0%]), hyperesthesia (89/484 [18.4%]), a dog-sitting posture (50/484 [10.3%]), dysphagia (39/484 [8.1%]), compulsive behaviors (33/484 [6.8%]), hypermetria (31/484 [6.4%]), muscle atrophy (30/484 [6.2%]), seizures (24/484 [5.0%]), a praying posture (15/484 [3.1%]), and head pressing (13/484 [2.7%]).

Thirty-four animals (7%) reportedly developed 1 or more abnormal behaviors not specifically described in the questionnaire, among which apprehension (13 animals) and agitation or restlessness (8) were the most commonly reported. In addition, 57 animals (11.8%) reportedly developed 1 or more additional clinical signs that were not behavior related and had not been listed on the survey. The most common of these were ocular abnormalities, including blindness (13 animals), localized or generalized edema (7), and abnormalities in respiratory effort or rate (7).

One hundred six of the 132 animals (80.3%) that became recumbent and were unable to rise died (n = 20) or were euthanatized (86), compared with 31 of the 350 animals (8.9%) that were able to rise throughout the course of the disease. After controlling for other factors associated with death, animals that became recumbent and were unable to rise were 77.9 times as likely to die as were animals that were able to rise throughout the course of the disease (Table 2).

For animals that became recumbent and were unable to rise, mean duration of recumbency was 2.3 days (range, < 1 to 7 days). However, true duration of recumbency could not be determined in 16 of 25 animals because of euthanasia. Mean duration of recumbency for animals that survived was 2.8 days, although 1 horse was recumbent for 7 days and survived. One animal became recumbent and unable to rise on 2 separate occasions for 2 days each time. Mean onset of recumbency was 2.1 days (range, 0 to 14 days) after the first signs of disease. All 4 animals in which an inability to rise was the first observed abnormality were euthanatized. Vaccination against WNV infection was associated with a decreased likelihood that equids would become recumbent and unable to rise. Unvaccinated equids were 2.4 times as likely to become recumbent and be unable to rise as were equids vaccinated prior to the onset of clinical signs (Table 1).

Twelve equids were reportedly managed in slings after they became recumbent. Two of these animals were eventually able to stand unassisted after they were lifted in the sling, 2 were eventually able to rise unassisted after use of the sling was discontinued, and 8 were not able to stand unassisted prior to death or euthanasia.

Treatment—Information on treatment was available for 473 animals in the study, of which 464 (98.1%) were evaluated by a veterinarian because of clinical disease related to WNV infection. Of these, 265 (57.1%) were examined within 6 hours of the onset of clinical disease, 52 (11.2%) were examined between 6 and 12 hours after the onset of clinical disease, 43 (9.3%) were examined between 12 and 24 hours after the onset of clinical disease, and 104 (22.4%) were examined > 24 hours after the onset of clinical disease.

Medical treatments administered were reported for 417 animals in the study. Flunixin meglumine was the most commonly administered treatment, with 303 of 417 animals (72.7%) being given flunixin. Other common treatments included dimethyl sulfoxide (223 [53.5%]), corticosteroids (223 [53.5%]), fluids PO or IV (167 [40.0%]), antimicrobials (115 [27.6%]), phenylbutazone (89 [21.3%]), vitamins (33 [7.9%]), and immune stimulants (12 [2.9%]).

Overall, 395 of the 417 animals (94.7%) for which information on treatment was available received a **nonsteroidal anti-inflammatory drug (NSAID)**, corticosteroid, or both. Preliminary analyses suggested that animals treated with corticosteroids were less likely to die than were animals that were not treated with corticosteroids (Table 1), but this association was likely confounded by other variables, as this factor was not significant in multivariable analyses. Animals affected early in the epidemic were as likely to receive corticosteroids as were animals affected later, and there was no age or sex predilection associated with corticosteroid treatment (P = 0.08).

The location where veterinary care was provided was reported for 461 animals, of which 269 (58.4%) were treated exclusively at the facility where they had been housed and clinical signs had first been observed, 166 (36.0%) were hospitalized during treatment, and 26 (5.6%) were treated both where they were typically housed and at a veterinary hospital. Animals that were

hospitalized were significantly ($P < 0.001$) more likely to survive (141/166 [84.9%]) than were animals that were not (171/269 [63.6%]). This variable was not included in regression analysis, as it was considered likely to be confounded by other measured and unmeasured variables.

Outcome—Owners were able to report on clinical status for 339 of the 345 animals still alive at the time of the survey. Among these animals, 271 (79.9%) had reportedly fully recovered from disease, and 235 of these 271 (86.7%) had been returned to their previous uses. Mean ± SD duration of disease for these 271 animals was 22.3 ± 18.8 days (range, < 1 to 90 days). The remaining 68 animals (20.1%) that were still alive at the time of the survey reportedly were still clinically affected. Residual signs of infection were described for 67 of these 68 animals and included weight loss or loss of body condition (n = 14 [21%]), decreased stamina and lethargy (13 [19%]), ataxia (8 [12%]), stumbling (7 [10%]), and cranial nerve deficits manifesting as droopy ears, lips, and muzzles (5 [7%]).

Management—Owners of 463 of the 484 animals in the study were able to report on daily management of their animals. At the time these animals became affected, mean ± SD time spent outdoors each day was 22.8 ± 4.1 hours (range, 0 to 24 hours). Mean time spent outdoors each day did not differ significantly with sex or breed.

Methods of mosquito mitigation were reported by owners of 467 equids. Owners of 212 of these 467 (45.4%) equids indicated that no mosquito control measures were being used at the time of onset of clinical disease. Overall, an insecticide or insect repellent sprayed or wiped directly on the animal was the most common mode of protection (235 [50.3%]), followed by an insecticide sprayed on the premises (39 [8.4%]). Fans placed in stalls or barn doors, larvicidal bacteria in water sources, fly masks or sheets on the animal, and moving animals indoors during peak mosquito feeding times were each listed by owners of < 9 (2.0%) equids. A significantly ($P < 0.005$) higher percentage of Quarter Horse owners reported having no measures in place to protect against mosquitos (61.6%) than did owners of other equids (38.4%).

Owners of 473 of the 484 animals in the study were knowledgeable about transportation of their animals prior to disease onset. Thirteen animals had been transported out of their state of origin during the 2 weeks prior to disease onset; however, most of these animals were not transported to areas considered to be associated with a high risk of exposure to WNV. States to which these animals were transported included New Mexico (n = 1), Missouri (1), Wyoming (1), Iowa (2), South Dakota (1), and Kansas (1). In addition, 1 Nebraska horse was transported to Colorado, and 4 Colorado horses were transported to Nebraska. Eleven of the 13 animals were transported to a single state, while 1 animal was transported to 2 states, and 1 animal was transported to 3 states.

Additional equids affected or at risk—Owners of 439 of the 484 animals in the study reported on the presence of additional equids at the site where their animals were housed. Total number of equids housed on these premises (including affected animals) was 6,076 (6,002 horses, 57 donkeys, and 17 mules). In addition to affected equids included in the study, owners reported that 313 equids housed on these premises (311 horses and 2 mules) had clinical signs consistent with WNV infection, although infection was not confirmed through laboratory testing. An additional 30 equids with laboratory-confirmed WNV infection were owned by survey respondents but were not selected for inclusion in this study. In total, these data suggest that there were 782 equids with clinical WNV infection on these study premises. Thus, the attack rate for confirmed and unconfirmed clinical WNV infection among equids on these premises was 12.9% (782/6,076).

Discussion

Circumstances of the epidemic that prompted the present study were somewhat unique, as equids in this region were unlikely to have been naturally exposed to the virus previously and represented a population that was mostly immunologically naïve. Many local equids that were vaccinated against WNV in 2002 did not have adequate time for optimal immunity to develop following vaccination. In addition, environmental conditions in Nebraska and Colorado preceding this epidemic were optimal for growth of mosquito populations, providing increased opportunity for viral exposure in resident animals. For these reasons, it was useful to evaluate whether disease occurrence in this equid population was different from reported WNV occurrence in other regions of the country.

Reported case fatality rates for epidemics of WNV infection in populations in the Western Hemisphere vary from 30% to 44%,[13-17] and the estimated case fatality rate in the present study (28.4%) was comparable. Results of the present study suggested that older animals were more likely to die or be euthanatized. Although age of animals that died in the present study was not significantly different from age of animals that were euthanatized, age was known for only 32 animals that died. Thus, a significant difference may have been found if a larger population had been studied. It is possible that some owners were less likely to pursue treatment in older animals on the basis of a perceived poor likelihood of recovery or lower monetary value, but examination of such factors was beyond the scope of our study. A previous study[18] suggested that older animals were more likely to manifest severe disease when exposed to WNV and more likely to die as a consequence of disease. In contrast, 2 other studies[1,13] reported no significant difference in age between horses that died and horses that survived, but these studies involved small numbers of horses (23 and 14 horses).

The present study was not designed to determine whether vaccination against WNV would protect against infection. However, all 11 equids in the study that were properly vaccinated (ie, 2 doses 3 to 6 weeks apart) and had had sufficient time to develop immunity before exposure survived. Despite the small number of animals, these results still suggest that vaccination

may have had a protective effect. Concerns that administration of a single vaccine dose prior to exposure to the virus would cause an increase in the severity of infection were not supported by results of the present study. In fact, animals that were vaccinated at least once prior to the onset of disease were significantly more likely to survive than were animals that had not been vaccinated, although factors other than partial protection resulting from vaccination could have been responsible for this observation (eg, owners that vaccinated may have been more likely to detect abnormalities in their animals sooner or may have been more likely to pursue treatment).

Several risk factors for death in affected animals were identified in the present study. Recumbency with an inability to rise was the strongest negative predictor of survival, with 80.3% of equids that were unable to rise dying or being euthanatized. Similarly, in a previous study,[18] > 60% of recumbent horses admitted to the University of Florida Equine Teaching Hospital for management of signs related to WNV infection during 2001 died or were euthanatized. Although admission of an animal to a referral hospital often represents a willingness on the part of the owners to pursue intensive treatment, prolonged recumbency without resolution of clinical signs still appears to be a significant factor contributing to euthanasia in these animals. In the present study, hospitalized animals had a case fatality rate of 15.1%, but this value may have been biased, as the decision to take an animal to a referral facility could have depended on the veterinarian's or owner's assessment as to the likelihood of the animals' recovery. Therefore, some severely affected animals that were more likely to die may not have been taken to a veterinary hospital at all.

Male equids were overrepresented in the present study, which was consistent with findings of other studies.[15,17] However, to our knowledge, this study was the first to determine that affected females were more likely to die than affected males. There was no significant difference between male and female equids in regard to the amount of time animals spent outdoors, nor were males less likely to have insect protection. However, additional measures of exposure were not tested. A previous study[19] found no significant sex difference in regard to the percentage of subclinically infected equids with detectable WNV antibody. The effects of sex on susceptibility to and survivability of WNV infection are unknown, and further research in this area is indicated.

Potential associations between breed and risk of clinical disease could not be evaluated in the present study because unaffected horses were not included in the study. Most (63.1%) equids in the present study were Quarter Horses, but this is the predominant horse breed in the western United States (45.0%)[10] and Colorado (48.3%).[20] Quarter Horses may have been more likely to be exposed to the virus than were other breeds in that a higher percentage of Quarter Horse owners reported having no measures in place to protect against mosquitoes than did owners of other equids. Whether Quarter Horses have a genetic predisposition to developing signs of clinical disease when infected with WNV has not been explored, but researchers have demonstrated a genetic determinant for severity of WNV infection in laboratory mice.[21]

Although Quarter Horses appear to be somewhat overrepresented in the present study, donkeys and mules appeared to be underrepresented, compared with their prevalence in the equine population in the United States.[10] Only 3 mules were identified as part of this study. Two donkeys were selected for enrollment, but the owners could not be contacted, and the animals were subsequently excluded from analysis. The apparent discrepancy between the percentage of clinically affected donkeys and mules versus the percentage of donkeys and mules in the population may be attributable to several factors. Laboratory-confirmed cases were randomly selected for enrollment in the study, and because the mule and donkey population is small, compared with the horse population, omission of even a few nonhorse equids could have affected our results. Alternatively, donkeys and mules may be more resistant to WNV infection than horses or may develop milder signs following exposure. The 3 mules in the present study all survived, and none became recumbent during the course of disease. However, laboratory submission forms did reveal that 1 donkey whose owner was not contacted had been unable to rise and was subsequently euthanatized, suggesting that equids other than horses can also display severe signs of disease. Finally, it is possible that donkeys and mules were not common in the present study because they are less likely to be exposed to the virus or less likely to be examined by a veterinarian and tested for disease on the basis of management practices or animal location.

Mean age of affected equids in this study was 9.5 years. While this was similar to mean age in a previous report,[22] it contrasts with the mean age of 14 years reported during the 2001 epidemic.[15] According to a previous study,[11] most equids in the United States are between 5 and 20 years old, so the fact that mean age of equids in the present study was 9.5 years may simply reflect equine demographics in the United States.

Clinical signs reported in the present study were similar to those reported in previous studies,[15,17,23] and ataxia was the most common clinical sign reported. Fever was uncommonly reported in this study and previous studies,[1,15,17] although it remains one of the most common early signs of WNV infection in humans. However, 95% of all treated animals in the present study received some type of anti-inflammatory drug, and in at least some of these animals, such drugs were likely administered before rectal temperature was measured. Experimental infection of horses with WNV results in a characteristic transient, low-grade, biphasic fever beginning 3 to 6 days after inoculation, with the second febrile phase occurring in horses that develop clinical signs of infection.[24] Because the initial fever can precede other clinical signs, it is conceivable that some of the equids in this study did initially have mild pyrexia.

Nonsteroidal anti-inflammatory drugs were the most common treatment in the present study. Animals that became recumbent were just as likely to have been treated with corticosteroids as not; thus, treatment

with corticosteroids did not appear to affect disease severity either positively or negatively.

Finally, results of our study suggest that the magnitude of the epidemic in these states was potentially underreported in that owners described additional equids with clinical signs characteristic of WNV infection on many of the premises on which equids with laboratory-confirmed infection resided. Obviously, other neurologic conditions could have been responsible for the clinical signs in some of these equids, but it appears likely that a substantial portion of equids had WNV infection.

[a]WebCT Inc, Lynnefield, Mass.
[b]PROC GLM, SAS Institute Inc, Cary, NC.
[c]PROC GENMOD, SAS Institute Inc, Cary, NC.

References

1. Trock SC, Meade BJ, Glaser AL, et al. West Nile virus outbreak among horses in New York state, 1999 and 2000. *Emerg Infect Dis* 2001; 7:745–747.

2. USDA Animal and Plant Health Inspection Services Web site. Available at: www.aphis.usda/gov/lpa/issues/wnv/wnvstats/html. Accessed Jan 1, 2003.

3. Rueda LM, Patel KJ, Axtell RC, et al. Temperature-dependent development and survival rates of *Culex quinquefasciatus* and *Aedes aegypti* (Diptera; Culicidae). *J Med Entomol* 1990;27:892–898.

4. Epstein PR, Defilippo C. West Nile virus and drought. *Global Change Hum Health* 2001;2:105–107.

5. Shaman J, Day JF, Stieglitz M. Drought-induced amplification of Saint Louis encephalitis virus, Florida 2002. *Emerg Infect Dis* 2002;8:575–580.

6. Sardelis MR, Turell MJ, Dohm DJ, et al. Vector competence of selected North American *Culex* and *Coquillettidia* mosquitoes for West Nile virus. *Emerg Infect Dis* 2001;7:1018–1022.

7. Turrell MJ, O'Guinn ML, Dohm DJ, et al. Vector competence of North American mosquitoes (Diptera: Culicidae) for West Nile virus. *J Med Entomol* 2001;38:130–134.

8. Goddard LB, Roth AE, Reisen WK, et al. Vector competence of California mosquitoes for West Nile virus. *Emerg Infect Dis* 2002; 8:1385–1391.

9. Fleiss JL. *Statistical methods for rates and proportions*. 2nd ed. New York: John Wiley & Sons, 1981;33–49.

10. USDA. *Lameness and laminitis in US horses*. Publication #N318-0400. Fort Collins, Colo: USDA, Animal and Plant Health Inspection Service, Veterinary Service, National Animal Health Monitoring System, 2000;4–5.

11. USDA. *Part I: baseline reference of 1998 equine health and management*. Publication #N280.898. Fort Collins, Colo: USDA, Animal and Plant Health Inspection Service, Veterinary Service, National Animal Health Monitoring System, 1998.

12. Hosmer DW, Lemeshow S. Model-building strategies and methods for logistic regression. In: *Applied logistic regression*. New York: John Wiley & Sons, 1989;82–126.

13. Autorino GL, Battisti A, Deubel V, et al. West Nile virus epidemic in horses, Tuscany region, Italy. *Emerg Infect Dis* 2002;8: 1372–1378.

14. Murgue B, Murri S, Zientara S, et al. West Nile outbreak in horses in southern France, 2000: the return after 35 years. *Emerg Infect Dis* 2001;7:692–696.

15. Ostlund EN, Crom RL, Pedersen DD, et al. Equine West Nile encephalitis, United States. *Emerg Infect Dis* 2001;7:665–669.

16. Tber AA. West Nile fever in horses in Morocco. *Bull Off Int Epizoot* 1996;108:867–869.

17. Porter MB, Long MT, Getman LM, et al. West Nile virus encephalomyelitis in horses: 46 cases (2001). *J Am Vet Med Assoc* 2003;222:1241–1247.

18. Long MT, Ostlund EN, Porter MB, et al. Equine West Nile encephalitis: epidemiological and clinical review for practitioners, in *Proceedings*. 48th Annu Meet Am Assoc Equine Pract 2002;1–6.

19. Durand B, Chevalier V, Pouillot R, et al. West Nile virus outbreak in horses, southern France, 2000: results of a serosurvey. *Emerg Infect Dis* 2002;8:777–782.

20. USDA. *National Agricultural Statistics Service equine population estimates for the US*. Washington, DC: USDA, 1999.

21. Mashimo T, Lucas M, Simon-Chazottes D, et al. A nonsense mutation in the gene encoding 2′-5′-oligoadenylate synthetase/L1 isoform is associated with West Nile virus susceptibility in laboratory mice. *Proc Natl Acad Sci U S A* 2002;99:11311–11316.

22. Snook CS, Hyman SS, Del Piero F, et al. West Nile virus encephalomyelitis in eight horses. *J Am Vet Med Assoc* 2001;218: 1576–1579.

23. Cantile C, DiGuardo G, Eleni C, et al. Clinical and neuropathological features of West Nile virus equine encephalomyelitis in Italy. *Equine Vet J* 2000;32:31–35.

24. Oudar J, Joubert L, Hannoun C, et al. Reproduction expérimentale de la méningo-encéphalomyelité du cheval par l'abrovirus West Nile. II. Etude anatomo-clinique. *Bull Acad Vét Fr* 1971;44: 147–158.