2005

WHOOPING CRANE TITERS TO EASTERN EQUINE ENCEPHALITIS VACCINATIONS

Glenn H. Olsen
USGS Patuxent Wildlife Research Center

Erin Kolski
Eleanor Roosevelt High School

Jeff S. Hatfield
USGS Patuxent Wildlife Research Center, jhatfield@usgs.gov

Douglas E. Docherty
USGS National Wildlife Health Center

WHOOPING CRANE TITERS TO EASTERN EQUINE ENCEPHALITIS VACCINATIONS

GLENN H. OLSEN, USGS Patuxent Wildlife Research Center, 12302 Beech Forest Road, Laurel, MD 20708
ERIN KOLSKI, Eleanor Roosevelt High School, Greenbelt, MD
JEFF S. HATFIELD, USGS Patuxent Wildlife Research Center, 12100 Beech Forest Road, Laurel, MD 20708
DOUGLAS E. DOCHERTY, USGS National Wildlife Health Center, 6006 Schroeder Road, Madison, WI 53711

Abstract: In 1984 an epizootic of eastern equine encephalitis (EEE) virus killed 7 of 39 (18%) whooping cranes in captivity at the Patuxent Wildlife Research Center in Laurel, Maryland, USA. Since that time whooping cranes have been vaccinated with a human EEE vaccine. This vaccine was unavailable for several years, necessitating use of an equine vaccine in the cranes. This study compared the antibody titers measured for three years using the human vaccine with those measured for two years using the equine form. Whooping cranes developed similarly elevated titers in one year using the human vaccine and both years using the equine vaccine. However, in two years where the human vaccine was used, the whooping cranes developed significantly lower titers compared to other years.

PROCEEDINGS NORTH AMERICAN CRANE WORKSHOP 9:21-23

Key words: *Grus americana*, eastern equine encephalitis, titers, vaccine, virus, whooping crane

Eastern Equine Encephalitis (EEE) is a clinically important disease of horses, people, and some birds including whooping cranes (*Grus americana*). EEE is classified as a zoonotic vector-borne alpha virus. The virus is found in eastern North America and is maintained and amplified by a mosquito-wild bird cycle. Native wild passerines carry the virus and initiate the cycle, with viremic birds being found 37-51 days before virus is first detected in mosquitoes beginning in July or August and lasting through October (Crans et al. 1994). The virus is then spread by the mosquitoes and amplified in the hatch-year passerines. EEE does not typically produce morbidity or mortality in native passerines, but has been known to cause mortality in non-native birds (pheasants, emus; Tengelsen 2001), horses, bats (Main 1979), and humans.

Vaccination as a tool to control arbovirus infections in horses is a widespread practice that provides effective protection (American Association of Equine Practitioners, 1995; Tenglesen et al. 2001). Commercially available equine vaccines for EEE are used to protect emus from the disease (Tengelson et al. 2001). Between September 17 and December 5, 1984 an epizootic of EEE killed 7 of 39 captive whooping cranes at the Patuxent Wildlife Research Center, Laurel, Maryland USA (Dein et al. 1986, Carpenter et al. 1989). Following the 1984 EEE epizootic at Patuxent, all whooping cranes began receiving annual injections of EEE vaccine (Clark et al. 1987; Olsen et al. 1997). There were no challenge studies done with the endangered whooping cranes. Naturally occurring epizootics were documented in sentinel birds and in mosquitoes, with no morbidity or mortality in captive whooping cranes. This led to the conclusion that the vaccination program was efficacious (Olsen et al. 1997). In 2000 and 2001, the human EEE vaccine previously used was not available for the whooping cranes at Patuxent. As an alternative; the birds were vaccinated with a commercially available equine vaccine.

The objective of this study was to compare antibody titer levels observed in years when the human vaccine was used with the 2 years when the equine vaccine was used. When an equine vaccine was first tested in the 1980's, the titer levels were lower than those seen with the human vaccine (Clark et al. 1987), and we wanted to discover if this was still true with the currently available equine vaccine.

METHODS

All adult whooping cranes housed at Patuxent were kept in 15 x 20 m outdoor pens. Very young whooping crane chicks being costume-reared for release programs spent the first 7-10 days confined indoors in 3 x 3 m pens, but afterwards were in indoor/outdoor pens until 40-50 days of age, after which time they were housed totally outside.

All whooping crane chicks received their first injection of EEE vaccine in July. For 1989-1999 this was 0.5 ml intramuscular injection of the PE 6 WRAIR strain human EEE vaccine (The Salk Institute, Government Service Division, Swiftwater, Pennsylvania, USA). This was followed 1 month later by an intramuscular injection of 1.0 ml. Yearly revaccination using the same 1.0 ml dose of the PE 6 WRAIR EEE vaccine occurred in late August or early September, to coincide with the seasonal peak of activity (August-October) for the EEE carrying mosquito (*Culiseta melanura*) at Patuxent (Pagac et al, 1992).

In 2000 and 2001, all adult whooping cranes received a 1.0 ml intramuscular dose of Encephaloid M (EEE and western equine encephalitis vaccine, Fort Dodge Laboratories, Fort Dodge, Iowa USA) in August of each year. All whooping crane chicks received the same Encephaloid M as 0.5 ml intramuscularly in July and 1.0 ml intramuscularly in August of their hatch year.

Blood samples to obtain serum for titers were collected

in late October or early November of each year during routine health examinations of the whooping cranes. Serum was shipped frozen to the USGS National Wildlife Health Center, Madison, Wisconsin, USA for analysis by hemaglutination-inhibition testing (HI, 1994-1997) or by serum neutralization testing (SN, 2000-2001) at the National Veterinary Services Laboratory, Ames, Iowa, USA. We analyzed the EEE titers for 1994, 1996 and 1997, years in which the human vaccine was used, and the most recent years (2000, 2001) when the equine vaccine was used. Due to budget constraints, no titers had been tested in 1995, 1998 or 1999.

Data were analyzed with a repeated measure analysis of variance (ANOVA) using a compound symmetry model in the mixed-models procedure of SAS (Statistical Analysis System, Version 6.12; 1997). The subject factor was crane identification number nested with gender. Prior to analysis, titers were transformed with a log 10 transformation to help achieve normality and homoscedasticity of residuals. Pairwise comparisons were performed on the least square means using a Tukey multiple comparison procedure ($P < 0.05$).

RESULTS AND DISCUSSION

Antibody titers were available from a total of 52 whooping cranes for the years 1994-1997 (human vaccine) and 2000-2001 (equine vaccine). For the 3 years when the human vaccine was used, the titers were highly variable (Table 1). The titers for 1994 were very low, while the 1996 titers were high, equivalent to those found with the equine vaccine. The 1997 titers were higher than 1994 but lower than 1996. Table 1 shows the untransformed means, sample sizes, and standard deviations, as well as pairwise comparison results of the log-transformed least square means. The mean titers for 1996, 2000, and 2001 were not significantly different ($P > 0.05$) from each other. The titer means for 1994 and 1997 were significantly different from each other and were different from 1996, 2000, 2001 EEE mean titers ($P < 0.05$). We used two different tests to determine the titers (HI and SN) but saw no differences in titer levels in 1996 (HI), 2000 or 2001 (SN). Titers seen with the human vaccine were considered protective due to the absence of morbidity or mortality seen in whooping cranes in years since 1984 when EEE was detected in mosquitoes and sentinel birds (Olsen et al. 1997). The equine vaccine tested produced titers equal to or higher than those produced by the human vaccine, which would suggest adequate protection.

Initial testing of a commercially available equine EEE vaccine and the Salk human EEE vaccine in the 1980's had shown superior titer levels when using the human vaccine (Clark et. al, 1987). Therefore, the decision was made to vaccinate the captive whooping cranes with the human vaccine. Unavailability of this vaccine starting in 2000 prompted the switch to an equine form of EEE vaccine. These results show that the titers obtained from this new equine EEE vaccine equal or exceed the titers seen with the human EEE vaccine used in the past. The human vaccine continues to be unavailable today, and, given current world events, it is unlikely to be released by the military any time soon. Therefore, continued use of an equine EEE vaccine appears to be an appropriate management tool to prevent this disease in captive whooping cranes.

ACKNOWLEDGMENTS

The authors thank the Crane Crew at the Patuxent Wildlife Research Refuge Center for their help in obtaining blood samples, veterinary technicians Lorie Shaul, Alex Lewis and Jennifer Kodak at Patuxent, the laboratory technicians at the

Table 1. Geometric mean, sample size, standard error, and Tukey's multiple comparison test of eastern equine encephalitis virus antibody titers measured in whooping cranes (Grus americana) at Patuxent Wildlife Research Center, Laurel, Maryland USA, 1994-2001.

Year	Sample Size (n)	Geometric Mean	Standard Error	Tukey's test[a]
1994	33	6.08	1.22	A
1996	39	88.13	1.21	B
1997	37	15.07	1.21	C
2000	52	81.23	1.19	B
2001	51	82.64	1.19	B

[a] **Means with the same letter are not significantly different ($P > 0.05$), based on Tukey's multiple comparison procedure of the least square means generated from the log-transformed titer levels in the repeated measures ANOVA.**

NWHC, especially Renee Long and Kristina Egstad. We also thank the USAMRIID, Fort Detrick, Fredrick Maryland, USA for supplying us with the SALK human EEE vaccine used at Patuxent for the protection of the whooping cranes throughout the 1990's.

LITERATURE CITED

Carpenter, J. W, G. G. Clark, and D. M. Watts. 1989. The impact of eastern equine encephalitis virus on efforts to recover the endangered whooping crane. Technical Publication No. 10, International Council for Bird Preservation, Cambridge, UK. Pages 115-120

Clark, G. G., F. J. Dein, C. L. Crabbs, J. W. Carpenter, and D. M. Watts. 1987. Antibody response of sandhill and whooping cranes to an eastern equine encephalitis virus vaccine. Journal of Wildlife Diseases 23:539-544.

Council on Biology and Therapeutic Agents. 1995. Guidelines for vaccination of horses. Journal of the American Veterinary Medical Association, 207:426-430.

Crans, W. J, D. F. Caccamise, and J. R. McNelly. 1994. Eastern equine encephalomyelitis virus in relation to the avian community of a coastal cedar swamp. Journal of Medical Entomology 31(5):711-728

Dein, F. J, J. W Carpenter, G. G. Clark, R. J. Montali, C. L. Crabbs, T. F. Tsai, and D. E. Docherty. 1986. Mortality of captive whooping cranes caused by eastern equine encephalitis virus. Journal of the American Veterinary Medical Association, 189:1006-1010.

Main, A. J. 1979. Eastern equine encephalomyelitis virus in experimentally infected bats. Journal of Wildlife Diseases, 15(3):467-477

Olsen, G. H., M. J. Turell, and B. B. Pagac. 1997. Efficacy of eastern equine encephalitis immunization in whooping cranes. Journal of Wildlife Diseases 33(2):312-315.

Pagac, B. B., M. J. Turell, and G. H. Olsen. 1992. Eastern equine encephalomyelitis virus and *Culiseta melanura* activity at the Patuxent Wildlife Research Center, 1985-90. Journal of the American Mosquito Control Association, 8(3):328-330.

Tengelsen, L. A., R. A. Bowen, M. A. Royals, G. L. Campbell, N. Komar, and R. B. Craven. 2001. Response to and efficacy of vaccination against eastern equine encephalomyelitis virus in emus. Journal of the American Veterinary Medical Association, 218:1469-1473.