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# WHOOPING CRANE TITERS IN RESPONSE TO EASTERN EQUINE ENCEPHALITIS IMMUNIZATION

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Eastern equine encephalitis (EEE) is a viral disease caused by a vector-borne alphavirus. Various bird species, primarily native passerines in eastern North America, act as reservoir hosts without suffering clinical disease. When outbreaks occur, the disease is maintained and amplified through a mosquito-wild bird-mosquito cycle. EEE does not cause morbidity or mortality in North American passerines, but does cause mortality in some non-native birds such as pheasants (*Phasianus colchicus*) and emus (*Dromiceius novaehollandiae*) (Tengelsen et al. 2001) plus horses (Main 1979) and humans.

Between September and December 1984, EEE killed 7 of 39 captive whooping cranes (*Grus americana*) at the Patuxent Wildlife Research Center (PWRC), Laurel, Maryland (Dein et al. 1986). Following this epizootic, all captive whooping cranes at this facility received annual vaccinations with a human-licensed EEE vaccine (PE 6 WRAIR strain, The Salk Institute, Government Services Division, Stillwater, PA) supplied by the U.S. Army. This vaccination program proved efficacious, protecting whooping cranes when another outbreak of EEE was documented at the facility in 1989 (Olsen et al. 1997).

As a result of increased security measures in recent years, the source of the human EEE vaccine was no longer available for the captive whooping crane vaccination program. In the original testing of sandhill cranes (*Grus canadensis*) with EEE vaccines, an equine-licensed vaccine was also studied (Clark et al. 1987). Since 2000, 2 different equine EEE vaccines have been used with the captive whooping cranes. Results using a 1.0-ml intramuscular dose of Encephaloid M (Fort Dodge Laboratories, Fort Dodge, IA) were compared with antibody titers produced when the human EEE vaccine was last used in the late 1990s. That study demonstrated equal or superior antibody titers with the Encephaloid M vaccine (Olsen et al. 2005). Unfortunately, Encephaloid M is no longer marketed by the company. A new combination killed vaccine with EEE and Western Equine Encephalitis called Encevac with Havlogen (Encevac, Intervet Inc., Millsboro, DE) was chosen as an alternative, but dose recommendations ranged from the 1.0-ml equine dose down to 0.25 ml. The objective of this study was to test the high and low dose recommendations and measure the resulting antibody titers in whooping crane serum.

In the late summer of 2005, when whooping cranes normally receive their annual booster vaccination for EEE and prior to the onset of the fall mosquito season, blood samples were taken by jugular venipuncture from all adult whooping cranes in the flock at PWRC. Blood samples (4-5 ml) were collected in serum separator tubes (Vacutainer, Becton Dickinson, Franklin Lakes, NJ), centrifuged, and the serum collected and stored at -27°C until shipped to the National Veterinary Services Laboratory, Ames, Iowa, for EEE antibody titer determination using the hemaglutination-inhibition test. The lowest titer results reported were 1:10, which would be interpreted as no significant titer level.

Whooping cranes were randomly assigned to 1 of 2 groups, receiving a single intramuscular injection in the pectoral muscle of either 0.25 or 1.0 ml of Encevac. Additional blood samples from each group were collected 3 months after vaccination. In 2006 and 2007, blood samples were collected from each group during the late summer prior to whooping cranes receiving their booster EEE vaccination and again 3 months later. In 2006 and 2007 the whooping cranes received the same dose as they were given in 2005. The last blood samples were collected in the late summer of 2008, 1 year after the last vaccinations with Encevac were given. A total of 52 whooping cranes ranging in age from 3 months to 34 years (at the start of the study) completed the 3 years of the study; 29 of those were in the group that received the 1.0-ml dose of Encevac and 23 in the group that received the 0.25-ml dose. Statistical analysis of titers was performed using analysis of variance (Statistix 8, Analytical Software, Tallahassee, FL).

Antibody titers ranged from 1:10 to 1:1280 for both dose levels for prevaccination samples in summer 2005 (Table 1). This was because some cranes had been previously vaccinated with other products (Olsen et al. 2005). This previous vaccination and possible exposure to EEE confounds the interpretation of the results from this study. However, as the cranes were randomly

**Table 1. Descriptive statistics for eastern equine encephalitis antibody titers for whooping cranes vaccinated with Encevac at 0.25 ml or 1.0 ml intramuscularly at the Patuxent Wildlife Research Center, Laurel, Maryland. Blood (serum) for titer determination was taken each summer just prior to vaccination and again 3 months later in the fall of each year. The final samples were collected in the summer of 2008, 1 year after the last vaccination.**

| Date | Dose (ml) | *n* | Mean ± SD | Min | Max |
|---|---|---|---|---|---|
| Summer 2005 | 0.25 | 23 | 304.4 ± 356.8 | 10.0 | 1280.0 |
| Summer 2005 | 1.0 | 29 | 401.7 ± 356.5 | 10.0 | 1280.0 |
| Fall 2005 | 0.25 | 23 | 310.9 ± 410.4 | 10.0 | 1280.0 |
| Fall 2005 | 1.0 | 29 | 386.9 ± 322.4 | 10.0 | 1280.0 |
| Summer 2006 | 0.25 | 23 | 120.9 ± 172.4 | 10.0 | 640.0 |
| Summer 2006 | 1.0 | 29 | 145.5 ± 231.9 | 10.0 | 1280.0 |
| Fall 2006 | 0.25 | 23 | 152.6 ± 263.4 | 10.0 | 1280.0 |
| Fall 2006 | 1.0 | 29 | 149.7 ± 229.8 | 20.0 | 1280.0 |
| Summer 2007 | 0.25 | 23 | 123.5 ± 149.7 | 10.0 | 640.0 |
| Summer 2007 | 1.0 | 29 | 160.7 ± 235.0 | 10.0 | 1280.0 |
| Fall 2007 | 0.25 | 23 | 153.0 ± 147.4 | 10.0 | 640.0 |
| Fall 2007 | 1.0 | 29 | 204.8 ± 254.5 | 20.0 | 1280.0 |
| Summer 2008 | 0.25 | 23 | 109.6 ± 104.2 | 10.0 | 320.0 |
| Summer 2008 | 1.0 | 29 | 167.6 ± 236.7 | 20.0 | 1280.0 |

assigned to the dose levels tested, any difference due to dose levels given in the 3 years of this study should have been detected.

No statistical differences in titers were found between the 2 dose levels at each time blood samples were taken. There was a decline in measured antibody titer for both dose schedules from the levels seen in summer and fall 2005 to subsequent sampling periods in 2006, 2007, and 2008. The cause of this is unknown but might be related to higher titers persisting from the Encephaloid M vaccine used in the previous year (2004).

When comparing antibody titers by sex over the 3 years of the study, there were no statistically significant differences. Initially there were no differences based on age (summer 2005 pre-vaccination titers, $F = 1.52$, $P = 0.14$), but starting 3 months post initial vaccination and continuing for the remainder of the study, there were significant differences based on age of the cranes vaccinated (fall 2005, $F = 2.27$, $P = 0.02$; summer 2006, $F = 5.55$, $P < 0.01$; fall 2006, $F = 4.47$, $P < 0.01$; summer 2007, $F = 4.78$, $P < 0.00$; fall 2007, $F = 4.80$, $P < 0.01$; summer 2008, $F = 9.17$, $P < 0.01$). This is attributed to the persistently high titers of a handful of older cranes that had been exposed to natural infections in 1984 and 1989, whereas the general trend among younger cranes, as has been discussed, was for the titers to become lower over time.

Seven cranes were added to the flock, 4 in 2005 and 3 in 2006. All these new cranes received 0.25-ml doses of the vaccine. Of the 4 cranes in 2005, by the fall of 2005, 2 had no detectable titers (1:10), while 1 had a titer of 1:40 and the last had a titer of 1:80. In the summer of 2006, all 3 cranes vaccinated with 0.25 ml of the vaccine failed to have titers (1:10) when tested 3 months later in the fall. These cranes with the 1:10 titers continued to have the same titer when retested in the fall of 2007. No naïve cranes were given the 1.0-ml dose.

The general conclusion is that a dose of 1.0 ml or 0.25 ml Encevac gives similar results measured by antibody titer. During 2005-2007 EEE was not detected in either mosquitoes or in young-of-the-year sandhill cranes whose titers were tested, so natural exposure of the test birds in those years was unlikely. In the summer of 2008, mosquitoes carrying EEE were detected at PWRC. None of the whooping cranes on the study died from this potential exposure to EEE, but 1 whooping crane juvenile not on the study did die in 2008. Based on the previous EEE epizootic in 1984, mortality in the whooping crane population should have been 18% (7 of 39 died in 1984), or 9 whooping crane deaths among the 52 whooping cranes on this study. This was thus an opportunity to test the efficacy of the new vaccine, similar to what was done in 1989 (Olsen et al. 1997), and indicated that the new Encevac product continued to protect whooping cranes from EEE and that a vaccination program for whooping cranes in EEE

endemic areas, such as Maryland, is still necessary.

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