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Efficacy of Eastern Equine Encephalitis Immunization in Whooping Cranes

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ABSTRACT: An epizootic of eastern equine encephalitis (EEE) at the Patuxent Wildlife Research Center (PWRC), Laurel, Maryland (USA), in 1989 provided an opportunity to determine if EEE immunization protected whooping cranes (*Grus americana*). Based on seroconversion of 31% of sympatric hatch-year sandhill cranes, *Grus canadensis*, and a previous 35% case fatality rate in whooping cranes, 17 (37%) of the 46 susceptible whooping cranes should have been exposed to virus and six should have died. As there were no deaths in these birds, the EEE vaccination program appeared to be efficacious in this whooping crane population.

Key words: Eastern equine encephalitis virus, *Grus americana*, *Grus canadensis*, whooping cranes, sandhill cranes, vaccine.

Eastern equine encephalitis (EEE) virus occurs in the eastern and north-central sections of North America and at scattered locations in Central and South America, including some of the Caribbean islands (Morris, 1988; Scott and Weaver, 1989). While not generally fatal to birds native to enzootic areas, infection with EEE virus can kill a high percentage of exotic birds (Morris, 1988). The mosquito, *Culiseta melanura*, is the primary enzootic vector of this virus (Morris, 1988; Scott and Weaver, 1989) and is found at the Patuxent Wildlife Research Center (PWRC), Laurel, Maryland (USA). Between 17 September and 5 December 1984, eastern equine encephalitis (EEE) killed seven of 39 captive endangered whooping cranes (*Grus americana*) housed at PWRC (Dein et al., 1986; Carpenter et al., 1989).

Because of the deaths associated with EEE viral infection in 1984, an immunization program of all whooping cranes at the PWRC was initiated in 1985 (Clark et al., 1987). Because these birds were endangered, it was not possible to challenge them with virulent virus to determine if the immunization was protective. However, an epizootic of EEE virus at the PWRC in 1989 (Pagac et al., 1992) provided a natural challenge experiment to determine if the immunization program initiated at the PWRC would protect whooping cranes from natural infection.

After the EEE epizootic in 1984, all whooping cranes received an intramuscular injection of 0.5 ml of inactivated human EEE vaccine (PE 6 WRAIR strain, The Salk Institute, Government Service Division, Swiftwater, Pennsylvania, USA), followed by a second injection of the vaccine 6 mo later. Beginning in 1986, all newly hatched whooping cranes received 0.5 ml of vaccine in July, with a second injection 30 days later. All whooping cranes received a 1.0 ml intramuscular booster of the vaccine annually. In the years since the vaccination program was initiated, there has not been a detectable adverse response to the vaccine. Because EEE virus did not produce a lethal infection in the sandhill crane, *Grus canadensis*, (Dein et al., 1986), these birds were not included in the immunization program.

In September 1989, we observed an increase in the numbers of *C. melanura* collected at the PWRC (Pagac et al., 1992). For the first time since 1984, active transmission of EEE virus was confirmed by isolation of EEE virus from *C. melanura* and seroconversion in sentinel bobwhite quail (*Colinius virginianus*) (Pagac et al., 1992).

Because sandhill cranes were maintained in pens adjacent to whooping cranes, and both species had similar prevalence rates during the 1984 epizootic:

34% (n = 38) in sandhill cranes, 44% (n = 32) in whooping cranes (Dein et al., 1986), virus-exposure rates in the sandhill cranes during the 1989 epizootic should have approximated those in the sympatric whooping crane population. However, sandhill cranes infected in previous years might have had circulating residual EEE-specific antibodies. Thus, only birds hatched in 1989 were sampled. At the time of exposure to the mosquitoes and virus (September to November 1989; Pagac et al., 1992), these sandhill cranes were kept outside in pens under similar conditions to the whooping cranes. Serum samples were obtained from 13 hatch-year sandhill cranes in October 1989 and again in March 1990 as part of their routine health monitoring program. These sera were tested for EEE virus-specific antibodies in a plaque-reduction neutralization test (Earley et al., 1967). The antibody prevalence in these birds was used to estimate the exposure rate in the 56 whooping cranes maintained at the PWRC in 1989.

The number of expected whooping crane deaths in 1989 was based on the seven deaths that had occurred during the 1984 EEE epizootic. Among the 32 surviving whooping cranes, 14 (44%) had neutralizing antibody to EEE virus (Dein et al., 1986). However, one of these birds had been a resident at the PWRC for 17 yr, and its antibody response was typical of a prior EEE infection. If this bird is not counted, 20 (53%) of the 38 potentially susceptible whooping cranes had evidence of infection with EEE virus. Based on the seven deaths, we estimated a case fatality rate of 35% in this species.

Sera from whooping cranes in 1984 had a higher percentage of antibody response in birds 16 to 18 yr old, 80% (n = 5) than in younger birds, 40% (n = 27) (Dein et al., 1986). Serum samples stored at −70 C from 1974 had an antibody response of 8% (n = 12) for whooping cranes and 25% (n = 8) for sandhill cranes. Based on these tests, there was another EEE enzootic in between 1968 and 1974. Unfortunately, there is little material available today to further substantiate this earlier disease incident.

Of the 56 resident whooping cranes during the 1989 epizootic, 10 (18%) had survived infection in 1984. Thus, they might have been protected in 1989 from their natural exposure to EEE virus, rather than by subsequent immunization. This left 46 whooping cranes that were potentially susceptible to EEE virus. It is unlikely that any whooping cranes were infected by mosquitoes between 1985 and 1988. Populations of *C. melanura* were low during these years, and none of the sentinel quail seroconverted (Pagac et al., 1992). Furthermore, if any whooping cranes were bitten by infected mosquitoes, there was no evidence of virus infection in any of the cranes necropsied during these years. If, in the unlikely case, given the low numbers of the mosquito and no detection of the virus, a whooping crane was infected but failed to develop the disease, this can be attributed to the protection provided by the vaccination program.

Four (31%) of the 13 hatch-year sandhill cranes had circulating antibodies to EEE virus (>1:10,000, n = 2; 1:1,280, n = 1; 1:40, n = 1; <1:10, n = 9). Assuming the same exposure rate in the sympatric whooping cranes, we estimated that 14 (31%) of the 46 potentially susceptible resident whooping cranes would have been exposed to EEE virus during the 1989 epizootic. Based on the 35% case fatality rate during the 1984 epizootic, we would have expected five deaths among the 14 exposed whooping cranes.

Although two whooping cranes died during the fall of 1989, both died from complications of trauma, and neither bird had signs consistent with EEE viral infection (Dein et al., 1986). Based on a prevalence of infection of 31% and a case fatality rate of 35%, the probability of a crane dying would be 0.11, and the probability of survival is $1 - (0.31)(0.35) = 0.8915$, with the standard error of this

probability being ±0.3110. The *P*-value for testing the null hypothesis that the probability of survival = 0.8915, versus the alternative hypothesis that the probability of survival is larger than 0.8915, is $P = 0.0064$ (Steel and Torrie, 1960). Thus, the highly significant absence of virus-related deaths in this population suggests that the EEE immunization program is efficacious in whooping cranes.

Every year some of the whooping cranes immunized at the PWRC failed to develop a detectable titer (>1:10) against EEE virus when tested 2 to 3 mo after immunization; as an example, among whooping cranes in 1989, seroconversion was >1:640, $n = 3$; 1:40, $n = 4$; 1:10, $n = 2$; <1:10, $n = 1$. There has been concern that these cranes lacked sufficient protection against naturally acquired EEE infection. The use of inactivated EEE vaccines has had inconclusive results in ring-necked pheasants (*Phasianus colchicus torquatus*) (Snoeyenbos et al., 1978; Eisner and Nusbaum, 1983) and in emus (*Dromaius novahollandiae*) (Tulley, 1996). However, in mammals, vaccine-induced protection can occur in the absence of detectable neutralizing antibody in the serum (Schmaljohn et al., 1982). This may also be the case with whooping cranes, as evidenced by the absence of deaths among the immunized whooping cranes in our study.

Currently efforts are underway to establish a non-migratory flock of whooping cranes in an area south of St. Cloud, Florida (USA). Whooping cranes are being reared at Patuxent and the International Crane Foundation in Baraboo, Wisconsin (USA) and are being released in Florida. The area in Florida where the releases occur is known to harbor EEE. In addition, plans are being formulated to establish a migratory whooping crane flock, with a breeding site in Manitoba, Canada, and a wintering site in the southeastern USA.

Because the eventual goal of any whooping crane release program is to establish a self-perpetuating flock, the role of EEE as a limiting factor for whooping crane populations needs to be thoroughly assessed. The population dynamics of a new flock sustaining substantial mortality from EEE needs to be determined before the viability of a release program can be assessed, if no effective immunization program is available for released cranes and their subsequent offspring. If population dynamics studies contain evidence for a low chance of success for establishing a viable, self-sustaining whooping crane flock in areas enzootic for EEE virus, then the release program may need to be reevaluated.

The absence of virus-related deaths and adverse vaccination reactions is evidenced that the EEE immunization program is both safe and efficacious in the endangered whooping cranes. Given the low total number of whooping cranes in North America (approximately 350 in 1996), evaluating the efficacy of a vaccine for a potentially lethal disease by traditional scientific methods of vaccinated and control animals being challenged with the virus is not practical. Therefore, natural exposure of vaccinated whooping cranes may be the only way to assess whether the vaccine is effective in this species.

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