

Research Note—

Absence of Humoral Response in Flamingos and Red-Tailed Hawks to Experimental Vaccination with a Killed West Nile Virus Vaccine

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SUMMARY. Sixteen Chilean flamingos, *Phoenicopterus chilensis*, and 10 red-tailed hawks, *Buteo jamaicensis*, were vaccinated in the pectoral muscle with 0.2 ml of a commercially produced killed West Nile virus vaccine intended for use in horses. Half the birds of each species received a booster vaccination 3 weeks after the first injection. Three weeks after the booster vaccination, none of 13 birds surveyed had detectable antibody to West Nile virus.

RESUMEN. *Nota de Investigación*—Ausencia de respuesta humoral en flamingos y halcones de cola roja frente a la vacunación experimental con una vacuna muerta del virus del Nilo.

Diez y seis flamingos Chilenos, *Phoenicopterus chilensis*, y 10 halcones de cola roja, *Buteo jamaicensis*, fueron vacunados en el músculo pectoral con 0.2 ml de una vacuna comercial de uso en caballos que contiene el virus inactivado del Nilo. La mitad de las aves fueron revacunadas tres semanas después de la primera inyección. No se detectaron anticuerpos contra el virus del Nilo en ninguna de las 13 aves 3 semanas después de la revacunación.

Key words: West Nile virus, vaccination, flamingo, red-tailed hawk

Abbreviations: ADPH = Alabama Department of Public Health; ELISA = enzyme-linked immunosorbent assay; IM = intramuscular; PFU = plaque-forming units; SCWDS = Southeastern Cooperative Wildlife Disease Study; SERRC = Southeastern Raptor Rehabilitation Center; WNV = West Nile virus

Several epidemic features combined to initiate our investigation of the safety and efficacy of a commercially produced West Nile virus vaccine. In 2001, an adult male golden eagle at the Montgomery Zoo, AL, and a bald eagle from Mobile, AL, died from West Nile virus (WNV) infection as reported by the Alabama Department of Public Health (ADPH). In addition, 8% of birds diagnosed as having died of WNV infection in Alabama were raptors or birds of prey (ADPH). These deaths and the discovery of WNV as the cause of death in flamingos in the Bronx

Zoo in 1999 (5) initiated a group effort to test the safety and efficacy of the only commercially available WNV vaccine on Chilean flamingos and red-tailed hawks. The vaccine was a killed vaccine conditionally licensed by the United States Department of Agriculture for use in horses.

MATERIALS AND METHODS

Vaccine. Commercially produced West Nile virus vaccine was purchased from the Fort Dodge Animal

Health, Ft. Dodge, IA, and stored at 4 C. The killed vaccine was adjuvanted with MetaStim™ and was licensed conditionally for administration to horses twice intramuscularly (IM) at 3–6 week intervals in August 2001. The product was subsequently awarded full licensure in 2003.

Birds. Captive bred adult Chilean flamingos, *Phoenicopterus chilensis* ($n = 16$, four females, two males, 10 undetermined, 11–32 yrs of age) were housed at the Montgomery Zoo in an open exhibit and fed daily a commercially prepared diet (Flamingo Fare, Reliable Protein Products, Palm Desert, CA), a ground grain mixture (Fighting Scratch, The Feed Lot, Pike Road, AL), and chopped trout (Fur Breeders Agricultural Cooperative, Sandy, UT) *ad libitum*. Total weight of food was approximately 3% total body weight.

Nonreleaseable adult red-tailed hawks, *Buteo jamaicensis* ($n = 10$) were housed at the Southeastern Raptor Rehabilitation Center (SERRC), College of Veterinary Medicine, Auburn University, AL, and maintained in open front cages and fed whole rats weighing 120 g each (Gourmet Rodent, Archer, FL) 6 days per week.

Procedure. All birds were bled from the cubital or medial metatarsal vein and sera were stored at –20 C until determination of antibody titer. The dose recommended by the manufacturer for horses was 1.0 ml IM. Accordingly, birds were injected intramuscularly in the pectoral muscle, although the volume was reduced to 0.2 ml of vaccine (7). Birds were then observed by veterinarians for 20 min, and daily by keepers, for signs of adverse reactions to vaccination. Birds were subsequently bled for antibody evaluation every 3 weeks for five additional samples. At the first postvaccination bleeding, birds were assigned randomly into two groups through a random digits table, and one group received a booster vaccination of 0.2 ml in the pectoral muscle.

Sampling. Because of seasonal pressure on Southern Cooperative Wildlife Disease Study (SCWDS) diagnostic facilities at Athens, GA, a limited number of samples could be run. In order to maximize the likelihood of discovering an immune response, only sera from boosted flamingos ($n = 8$, two females, one male, five undetermined) and boosted red-tailed hawks ($n = 5$) were submitted, along with prevaccination serum samples.

Plaque reduction neutralization test. The 13 selected sera were sent to the SCWDS for antibody titration by plaque reduction neutralization test as described by Lindsey *et al.* (4). Briefly, serum samples were screened at a 1:5 dilution in BA-1 media (1× Medium-199 with Hanks balanced salt solution, 0.05 M Tris buffer [pH 7.6], 0.35g/L sodium bicarbonate (NaHCO₃), 1% bovine serum albumin) supplemented with 1× antibiotic/antimycotic solution (100 units penicillin/mL, 100 µg streptomycin/mL, 250 ng amphotericin B/mL). Samples were heat inactivated at 56 C for 30 min prior to being further diluted in an

equal volume of working virus solution containing 100 plaque-forming units (PFU) of WNV (Georgia isolate DES 160/01-02). Virus diluent was supplemented BA-1 containing 8% human serum. Dilutions were then incubated at 37 C for 1 hr. Following the neutralization step, each virus/serum mixture was inoculated onto 4-day-old Vero Middle America Research Unit (MARU) cells and allowed to adsorb at 37 C in a humidified atmosphere containing 5% CO₂ for 1 hr with periodic rocking. Infected cultures were then overlaid with 1% gum tragacanth/1× minimum essential media supplemented with 3% heat inactivated fetal bovine serum and 2× antibiotics (1). On day 5 postadsorption, cultures were inactivated with 10% buffered formalin. Overlay/formalin solutions were then decanted, and wells were stained with crystal violet. Plates were then washed with tap water for plaque visualization. Test serum samples that exhibited a 90% reduction in plaque levels, relative to the negative serum control, were considered positive for WNV neutralizing antibodies.

RESULTS

No evidence of adverse systemic reactions was noted in any of the 24 birds vaccinated. No evidence of virus neutralizing activity was detected in any of the 13 sera in prevaccination samples, in samples 3 weeks postvaccination, or in samples collected 3 weeks after the booster vaccination. Because of the demand for plaque neutralization testing and the preponderance of negative data, no further testing of these sera is planned.

DISCUSSION

More valuable birds continued to die of West Nile virus infection at the Montgomery Zoo and surrounding community, confirming that the virus has become endemic in the area since 2001 (ADPH). In addition, reports of recommendations for vaccinating raptors, psittacines, and other rather rare or valuable birds with killed virus vaccine have been telephoned in or reported on the internet. Evidence from this study and anecdotal evidence from other investigators (A. Allison, unpubl. data) indicates that an adequate vaccine for prevention of West Nile virus infection of birds is not currently available.

In retrospect, the failure of this vaccine is not entirely surprising: inactivation of the virus has altered the surface antigens of the virus to the point where the vaccine no longer functions as a hemagglutinin (Nusbaum, unpubl. data); therefore, its

ability to serve as an antigen and initiate a protective immune response must be questioned (6). An additional possibility may be that nonneutralizing antibody is formed against a large envelope protein, protein E (9). Further assay of sera for anti-WNV activity by enzyme-linked immunosorbent assay (ELISA) was prohibited by the absence of specific antibodies against redtail or Chilean flamingo antibodies.

Clinicians and researchers always take a chance when they adjust a dose of vaccines between species of greatly different sizes (2). Indeed, if we assume that a 1.0-ml vaccine dose is efficacious in a small horse of approximately 370 kg or 800 pounds, a bird would have to weigh about 160 pounds or 73 kg for 0.2 ml to be a proportionate dose. Injection site and antigenic mass cannot be dismissed as noncritical variables when conducting vaccine research, and further investigation of this vaccine may be warranted based on use of a greater immunogenic mass.

When emu were vaccinated two or three times with killed Eastern and Western equine encephalomyelitis vaccine at the recommended equine dose, similar titers were found in both groups 5 weeks after the last immunization (3,8).

It is possible that sufficient cellular immunity was elicited to protect the birds; however, in the absence of intracellular replication, this outcome is unlikely. Virulent challenge of vaccinated birds was not feasible due to insufficiently secure facilities, but, based on the absence of evidence of humoral immunity and the unlikely stimulation of cellular immunity, we are confident that our evaluation of the vaccine as used in this trial reflects a clinical reality.

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