Efficacy of a Cold-Adapted, Modified-Live Virus Influenza Vaccine: A Double-Blind Challenge Trial

Hugh G. G. Townsend, DVM, MSc; Angela Cook, DVM; Trent C. Watts, DVM; J. Bogdan; Deborah M. Haines, DVM, PhD; Sidney Griffin, DVM; Thomas M. Chambers, PhD; Robert E. Holland, DVM; Patricia Whitaker Dowling, PhD; Julius S. Youngner, PhD; Steven J. Penner, PhD; and Randal W. Sebring, DVM

Protection against an experimental aerosol challenge with equine influenza virus was demonstrated at 5 weeks and 6 months after a single intranasal administration of a cold-adapted, modified-live equine influenza virus vaccine. Authors’ addresses: Dept. of Veterinary Internal Medicine (Townsend, Cook, and Watts) and Dept. of Veterinary Microbiology (Bogdan and Haines), University of Saskatchewan, Saskatoon, SK, Canada S7N 5B4; Carnduff Veterinary Clinic, Carnduff, SK, Canada S0C 0S0 (Griffin); Dept. of Veterinary Science, University of Kentucky, 108 Gluck Equine Research Center, Lexington, KY 40546-0099 (Chambers and Holland); Dept. of Molecular Genetics and Biochemistry, School of Medicine, University of Pittsburgh, Pittsburgh, PA 15261 (Dowling and Youngner); and Heska Corp., 1613 Prospect Pkwy., Fort Collins, CO 80525 (Penner and Sebring). © 1999 AAEP.

1. Introduction
Antibody stimulated by killed vaccines against equine influenza virus is short-lived, and disease among vaccinated animals is reported. The use of an intranasal, modified-live virus vaccine should approximate natural infection and result in immunity of greater efficacy and duration than can be achieved with killed virus or subunit vaccines. We are not aware of published reports on the experimental challenge of horses vaccinated with any of the equine influenza vaccines currently manufactured and marketed in North America. We report the results of a formally randomized, double-blind, experimental influenza challenge of a newly developed intranasal equine influenza vaccine containing a cold-adapted strain of influenza A/equine 2/Kentucky/91 (H3N8) virus.

2. Materials and Methods
This study was observed by the United States Department of Agriculture in partial fulfillment of the requirements for registration of the vaccine for sale. Sixty cross-bred fillies and colts younger than 1 year of age, with no detectable antibody to equine influenza, were selected and isolated on a large ranch. Forty animals were randomly assigned to
vaccination, and the other 20 served as controls. Each vaccinated animal received a single intranasal dose of vaccine containing a cold-adapted strain of influenza A/equine 2/Kentucky/91 (H3N8) virus, and nothing was administered to the controls. Five weeks later, 10 randomly selected controls and 20 vaccinates were challenged by nebulization of live influenza A/equine 2/Kentucky/91 (H3N8) virus into an aerosol chamber containing groups of six animals (4 vaccinates, 2 controls). The remaining animals were challenged 6 months after vaccination. Each challenge inoculum was 20 ml of fluid containing approximately $2 \times 10^6$ (5 weeks) to $1 \times 10^7$ (6 months) TCID$_{50}$ U/ml of virus. The challenge, clinical and laboratory investigators were blinded to the vaccination status of the individual horses.

Over the 10 days postchallenge, daily examinations of the horses were performed by the same veterinarian. Nasal swab specimens for virus isolation were collected daily for 8 days after challenge, and blood samples were collected from the animals on days 0, 7, 14 and 21 after challenge for serologic testing using the single radial hemolysis technique.

3. Results

Vaccination was easily achieved and well tolerated by the animals. There was no adverse response to vaccination in any animal. After both challenges, the vaccinates had significantly lower total clinical scores, experienced less increase in rectal temperature and shed less virus, for a shorter period, than did controls. Differences between the vaccine and control groups with respect to all parameters were less marked at 6 months than at 5 weeks. The clinical scores were significantly higher, and the amount and duration of virus shedding were significantly greater, among both control and vaccinated animals at 6 months than at 5 weeks. There was no evidence of serologic response to vaccination before challenge, but a marked increase in serum antibody was observed among the vaccinates by day 7 and among the control animals by day 14.

4. Discussion

These results demonstrate a significant protective effect 5 weeks and 6 months after administration of a single intranasal dose of vaccine. The experimental challenges resulted in clinical signs consistent with natural disease in terms of both duration and severity. The degree of protection achieved through vaccination, as measured by clinical data and the amount and duration of virus shedding, was less marked at 6 months than it was 5 weeks after vaccination. This result may have been caused by waning immunity over time and/or increased virulence of the second challenge. The serologic results suggest that immune mechanisms other than serum antibody were involved in protection of the vaccinated animals.

References