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Abstracts
The serologic responses of naïve horses to tetanus, influenza, and equine herpesvirus (EHV) antigens varied markedly among vaccines registered in the United States. After live influenza virus challenge, variation in the degree of protection against the development of clinical signs and viral shedding was also observed among vaccine groups. All vaccine groups were protected against weight loss.

1. Introduction
Aside from the publications related to the blinded, randomized controlled challenge trials of a cold-adapted, modified-live virus influenza vaccine (Flu Avert), [1] no data have been published in peer-reviewed literature demonstrating efficacy of the equine vaccines marketed in North America. This, along with published reports of failure of vaccinated animals to mount an adequate immune response and disease in vaccinated animals, [2-4] has led to concerns regarding the efficacy of these vaccines. This report contains the results of a blinded, randomized controlled study of the serologic responses of 50 naïve horses to commercial killed vaccines against influenza, tetanus, and equine herpesvirus (EHV). We also report on the outcome of the live equine influenza virus challenge of these animals after a series of vaccinations administered 8, 7, and 4 mo before the challenge. The study involved the use of seven killed and one modified-live vaccine produced by the major manufacturers of equine vaccines in the United States (Boehringer Ingelheim, Intervet Inc., and Wyeth Ayerst/Fort Dodge).

2. Materials and Methods
The serologic study was conducted on a ranch in southeast Saskatchewan. Fifty naïve animals, approximately 9 mo of age, were used in the study. Horses were bled before vaccination and every 4 wk until challenged with equine influenza virus. Serum antibody against equine influenza was measured using single radial hemolysis (SRH) [5]. Tetanus and EHV titers were measured by enzyme linked immunosorbent assays (ELISA). Virus isolation and titration was performed on MDCK cells. The animals were randomly assigned to one of five treatment groups (10 animals per group) and vaccinated at 0, 4, and 16 wk after the initiation of the study. Groups 1, 2, 3, and 5 were maintained on one pasture and kept well separated from all other horses for the duration of the study. Animals in group 4 were moved to a separate site, more than 1 mi distant from all other horses and maintained at that site until 2 wk (14 days) after receiving a priming dose of the modified-live vaccine (Flu Avert). They were then reintroduced to the pasture holding groups 1, 2, 3, and 5. The vaccine groups are shown below, and horses were vaccinated according to the plan shown in Table 1. Fourteen weeks after receiving their final vaccination, the horses were transported to Goodale Farm, University of Saskatchewan, Saskatoon, where they were kept in one large pen. Two weeks later (16 wk after last vaccination) the horses were challenged with live equine influenza virus (Kentucky/99). Clinical signs, rectal temperatures, body weight, and viral shedding were monitored before and for 10 days after the challenge. Blood for serology was drawn on post-infection days -1, 7, and 14. Clinical scores, rectal temperatures, body weight, and viral shedding were determined daily on all animals after challenge. To detect differences among the vaccine groups, an analysis of these outcomes was performed by summing the values for the individual animals over time, ranking
the sums, and performing an analysis of variance on the ranked sums. Means of the ranked variables were compared using Tukey's test, and $P < 0.05$ was considered significant.

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<th>Table 1. Order of Vaccine Administration to Five Groups of Naïve Horses</th>
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1. Vaccine Combination 1 (Intervet) [a].
   - a. Encevac T (EEE, WEE, Tetanus)
   - b. Prestige II (EHV1&4, EIV)
2. Vaccine Combination 2 (Wyeth Ayerst/FortDodge) [b].
   - a. Equiloid (EEE, WEE, Tetanus)
   - b. Fluvac EHV - 4/1 Plus (EIV, EHV1&4)
3. Vaccine Combination 3 (Boehringer Ingelheim) [c].
   - a. Cephalovac EWT (EEE, WEE, Tetanus)
   - b. Calvenza EHV (EHV-1), Calvenza EIV (EIV)
4. Vaccine Combination 4 (Intervet) [a].
   - a. Flu Avert (EIV-MLV)
   - b. Encevac T (EEE, WEE, Tetanus)
   - c. Prestige II (EHV1&4, EIV)
5. Non-vaccinated controls

3. Results

Serology
All control animals maintained serum antibody concentrations below detectable limits of the assay, consistent with naïve animals not exposed to the virus during the study. All vaccine groups experienced a significant increase in serum antibody concentration against equine influenza virus ($P < 0.0001$). Variation in response was observed among both the vaccine groups and the individual animals. This difference among the vaccine groups was most evident after the third vaccination. The serum antibody concentrations achieved by the group receiving Calvenza was significantly greater than that of the other groups. The second highest concentrations were achieved by the Prestige II group. The response of the Fluvac group was significantly lower than that of the Prestige II group ($P < 0.05$). Compared with the control group, all vaccine groups responded to tetanus toxoid ($P < 0.0001$). The antibody response was marked, and it improved after each vaccination. The Encevac T group achieved the highest concentrations of antibody, significantly greater than the Equiloid group ($P < 0.05$), but not significantly greater than the Cephalovac group. Serum tetanus antibody concentrations declined steeply over the 16 wk after the last vaccination. Compared with the controls, the Calvenza, Fluvac, and Prestige II groups experienced a significant increase in serum antibody against equine herpesvirus ($P < 0.0003$). This was most evident after the third vaccination.

Influenza Challenge
The challenge was successful. All controls developed a fever on day 2, and this persisted for at least 6 days in 9 of 10 of these animals. They all developed severe clinical signs typical of the disease, and they all lost body weight. Rectal temperatures among vaccinates were significantly lower than among the controls ($P < 0.0001$). Three of 10 horses in the Calvenza group experienced a 1-day fever after challenge. The majority (24 of 30) of the horses in the other vaccine groups experienced a fever after challenge, but the median rectal temperature for these groups had returned to baseline by post-challenge day 3. The Calvenza and the Flu Avert (prime)-Prestige II (boost) groups coughed less, had less nasal discharge, and shed less virus than did the controls ($P < 0.0001$). On average, vaccinated animals gained 11 lb over the 10 days after challenge, whereas the controls lost 54 lb ($P < 0.0001$).

4. Discussion
In addition to showing that vaccination protected against the weight loss caused by equine influenza, this study provides the
first published evidence that killed influenza vaccines marketed in North America are capable of reducing clinical signs and viral shedding in experimentally challenged horses. Horses achieving the highest serum antibody concentrations after the third vaccination were the least likely to develop clinical signs and shed virus. This occurred despite the fact that the serum antibody concentrations declined to relatively low concentrations just before challenge (4 mo after the last vaccination). The group of animals vaccinated with Calvenza developed significantly higher serum antibody concentrations and experienced less fever, nasal discharge, and viral shedding than those animals in the Prestige II and Flucvax groups, whereas animals vaccinated with Calvenza did not have significantly less fever, nasal discharge, coughing, or viral shedding than those animals in the Flu Avert (prime)- Prestige II (boost) group. This apparent effect of priming with Flu Avert, 32 wk before challenge, is consistent with our previous data showing excellent clinical protection against live virus challenge 3 and 6 mo after a single intranasal vaccination of naïve animals [1,6], and less fever and viral shedding 12 mo post-vaccination [1]. The influenza challenge employed in this study was severe. All horses in this study were exposed to a concentration of virus during the challenge that would logically exceed that normally experienced in nature. As well, all vaccinates were kept in immediate contact with control animals that were shedding large quantities of virus during the post-challenge phase. Under natural field conditions, the killed vaccines used in this study may provide sufficient herd immunity to yield better protection than we observed after our experimental challenge protocol. All the vaccine groups experienced a marked serum antibody response to tetanus. This response improved after each additional dose of vaccine. The marked decline in serum antibody after the last vaccination in all groups is notable. The relationship between serum antibody, as measured in this study, and protection against a natural challenge with Clostridium tetani is not known. There is some suggestion in the literature to indicate that the duration of immunity after successful tetanus vaccination in horses may extend well beyond 1 yr. The results of this study suggest that this may not be true and that this issue deserves further investigation. There are no published studies demonstrating a relationship between serum antibody concentration and EHV-related respiratory disease. The antibody response to the EHV vaccines examined in our study was significant but of low magnitude. The results of comparative respiratory challenges are required to make a complete assessment of the efficacy of these vaccines.

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Footnotes
[a] Intervet Inc., Millsboro, DE 19966-0318, USA.
[b] Wyeth/Fort Dodge Animal Health, Fort Dodge, IA 50501, USA.
[c] Boehringer Ingelheim Pharmaceuticals Inc., Ridgefield, CT 06877, USA.

References

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