Abstract

Significant differences exist between the immunogenicity of different commercial vaccines, and these differences should be considered in product selection. It is difficult to identify vaccines that generate significant immune responses to respiratory viruses. Anti-equine herpes virus type 1 (EHV-1) abortion vaccines outperform EHV-1 respiratory vaccines and could be considered for prevention of all forms of EHV-1 infection.

1. Introduction

Infectious disease in horses is an important problem, causing extensive suffering and economic loss. Consequently, vaccination is widely practiced, but there is limited efficacy data available for current commercial vaccines. The aim of this study was to compare immune responses to commercially available vaccines that are in common use in the United States. The study was conducted in two independent experiments. In one experiment, seroconversion to vaccination against a common combination of agents was compared using products from three different suppliers. The antigens selected included equine influenza virus (EIV), equine herpes virus type 1 (EHV-1), Eastern equine encephalomyelitis (EEE), and tetanus. A two-dose vaccination regimen, such as might be employed in a horse with an uncertain vaccination history, was used. In a second experiment, immune responses to two EHV-1 anti-abortion vaccines were compared using a conventional three-dose regimen. In addition to seroconversion, interferon gamma (IFN-\(\gamma\)) mRNA responses to EHV-1 stimulation were measured. These cytokine responses can be a useful surrogate for quantifying cytotoxic lymphocyte responses [1], which have been shown to be a measure of protective immunity to EHV-1 infection [2].

2. Materials and Methods

Experimental Animals - Fifty-five female, aged, and non-pregnant small breed horses (average 750 lb body weight) were maintained in a single group for the duration of the trial. Full vaccination histories for these horses were not available, although it was anticipated that the majority would have received annual spring vaccination for EIV, EEE, Western equine encephalomyelitis (WEE), and tetanus on more than one occasion. All horses were identified by implantation of a microchip.

Conventional Vaccine Seroconversion Study - Forty horses were randomly assigned to four groups of 10 horses. Three horse groups were administered two doses of one vaccine product (A, B, or C) at a 4-wk interval. Group A received the Intervet [a] vaccines Encevac T (EEE, WEE, tetanus) and Prestige II (EHV-1, EHV-4, EIV). Group B received the Fort Dodge [b] vaccines Equiloid (EEE, WEE, tetanus) and Fluvac EHV-4/1 Plus (EIV, EHV-1, EHV-4) [b]. Group C received the Boehringer Ingelheim [c] vaccines Cephalovac EWT (EEE, WEE, tetanus) and Calvenza EIV/EHV (EHV-1, EIV) [c]. Group D served as a control and was administered a placebo. All vaccinations were given in the neck. Horses were bled before each vaccination and at monthly intervals for 6 mo after the second vaccination by jugular venipuncture into serum separator tubes. Subsequently, the serum was separated and stored at -80\(^\circ\)C pending analyses.

EIV antibody response was measured by single radial hemolysis [3,4]. EHV-1 antibody response was determined by serum virus neutralization (VN) assay at the Wisconsin Veterinary Diagnostic Laboratory. EEE antibody response was determined...
using a plaque reduction neutralization test at the National Veterinary Services Laboratory. Tetanus antibody responses were determined using an enzyme-linked immunosorbent assay (ELISA) system as previously described [5].

**EHV-1 Abortion Vaccine Study** - Fifteen horses were randomly assigned to three groups of five horses. Two groups were administered three IM doses of a vaccine product (E or F) at 2-mo intervals. Horses in group E were administered the Intervet Prodigy EHV-1 vaccine and horses in group F were administered the Fort Dodge Pneumabort K EHV-1 vaccine. Horses in group G served as a control and were administered a placebo. All vaccinations were administered in the neck. Horses were bled for serum collection before the first vaccination and at monthly intervals until 1 mo after the final vaccination. Horses were bled for collection of lymphocytes before administration of the first vaccine and 1 mo after the administration of both the second and third (final) vaccines.

EHV-1 antibody responses were measured as described above. Interferon-gamma production was measured by determination of IFN-γ mRNA production using a real-time polymerase chain reaction (PCR) assay after in vitro stimulation of lymphocytes with EHV-1 antigen and a 48-h incubation. Cytokine mRNA assays were performed on freshly collected lymphocytes.

**Statistical Analyses** - Because the data were not normally distributed and to deal with repeated measures, data values for each animal were summed over time, and ranked data were analyzed by one-way analysis of variance (ANOVA). Means were compared using Tukey's test. Data for single time points was similarly analyzed for time points of biological interest. Significant differences were reported when P < 0.05.

### 3. Results

**Conventional Vaccine Seroconversion Results** - There were no systemic or local adverse reactions to vaccination. Antibody responses to EIV were limited, but over the time period of the experiment, EIV titers in group C were significantly higher than controls (P < 0.05). EIV antibody levels in group A and group B were not significantly different from group C or from the control group. Over the entire course of the experiment, EHV-1 antibody responses were not elevated in any vaccine group when compared with controls.

Vaccination against EEE resulted in antibody titers in group A and group B that were significantly greater than the control group over the time course of the experiment (P < 0.05). Group C was not significantly different from the control or other vaccination groups.

Antibody responses to tetanus demonstrated the greatest ability to distinguish between vaccines. Group A horses had higher tetanus antibody levels than group B and group C. Additionally, in all vaccination groups (A, B, and C), anti-tetanus titers were significantly higher than those in the control group over the time course of the experiment (P < 0.05). When individual time points were examined, group A had significantly higher anti-tetanus titers than all other groups at all time points after the first vaccination, including 6 mo after the last vaccination.

**EHV-1 Abortion Vaccine Results** - Both vaccination groups (E and F) demonstrated an antibody response to the first vaccine administration with no further increase after the second dose. There was a response to the third dose of vaccine in Group E. Statistical analysis showed group E antibody levels to be significantly different from the control group's levels over the entire course of the experiment (P < 0.05); group F was not different from the control group or group E. Interestingly, the VN titers achieved by these vaccines were substantially higher that the responses seen in the respiratory products tested in the conventional vaccine seroconversion study. One mo after the third vaccination, IFN-γ response to EHV-1 was increased in both vaccination groups, although this increase was statistically different from the control group in the horses in group E only; there was no significant difference between group E and group F.

### 4. Discussion

Immune responses to individual vaccine antigens varied widely between the products of different companies with no single supplier consistently outperforming the others. Antibody responses to respiratory viruses in the conventional vaccine seroconversion study were generally unimpressive, although anti-EIV responses generated by the product C vaccine were statistically significant. For EEE, both the product A vaccine and the product B vaccine generated significant immune responses. Finally, in the case of tetanus, the product A vaccine generated the highest and most long-lived vaccine responses when compared with the placebo, vaccine B, and vaccine C.

In the EHV-1 abortion vaccine study, antibody responses to product E were statistically significant when compared with controls. The titers achieved by product E were twice those seen after the peak response to respiratory vaccines studied in the seroconversion experiment. Of greatest interest, vaccination with three doses of product E generated a significant IFN-γ response. This parameter is a good correlate for the protective immunity to many viral infections, and it is also a useful surrogate measure of cytotoxic T-lymphocyte (CTL) precursor activity [1]. To our knowledge, this is the first demonstration
of such a response to EHV-1 in horses. Given the greater immune responses to vaccination, it may be wise to consider using the anti-abortion EHV-1 formulations for control of other forms of EHV-1 infection as well as abortion. Results of these experiments demonstrate that significant differences exist between the products of different manufacturers; this information should be considered during product selection. Availability of comparative data about immunogenicity and, ideally, about protection from challenge infection is critical for such decisions.

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References


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