

Equine Influenza A2 Cross-reactive Antibody Studies

Following the Administration of CALVENZA™ EIV/EHV Vaccine

Study Location

Animal Health Trust (AHT), Newmarket, Suffolk CB8 7UU

Study Sponsor

Boehringer Ingelheim Vetmedica, Inc., St. Joseph, Missouri USA

Study Objectives

To demonstrate the cross-reactive antibody produced by the EIV A2 fractions of an equine rhinopneumonitis-influenza, killed virus vaccine, CALVENZA™ EIV/EHV, administered by both intramuscular (IM) regimens and combined intramuscular/intranasal (IN) vaccination regimens.

CALVENZA EIV/EHV Vaccine

CALVENZA EIV/EHV is a killed virus, equine influenza and equine herpes virus vaccine incorporating a carbomer adjuvant. The EIV fraction of the vaccine is composed of subgroup A1 (Newmarket/77) and A2 subgroups (Kentucky/95 and Newmarket/2/93). Kentucky/95 is representative of current North American isolates of EIV and Newmarket/2/93 is representative of current European isolates of EIV. The vaccine for this trial was formulated at a minimum EIV antigen load. The complete vaccine combination product was used in these studies to demonstrate that EHV fractions do not compromise the efficacy of the EIV fraction.

Test animals

All animals were approximately six-month-old male or female Welsh Mountain ponies. Initially all ponies, with no history of exposure to EIV, were seronegative. Throughout the vaccination series all ponies were held on pasture.

Study design

Forty-nine (49) ponies were randomly assigned to one of five groups. Seven (7) were vaccinated IM-IM, seven (7) were vaccinated IM-IN, seven (7) were vaccinated IM-IM-IM, seven (7) were vaccinated IM-IN-IN, and twenty-one (21) ponies were assigned to 3 unvaccinated control groups. In this study, designed along European vaccination conventions, administration of the first two doses of vaccine were separated by 5 weeks. Two groups received a third dose six months following the second administration. Serum samples were collected pre-vaccination, at regular intervals during and following completion of the vaccination series, as well as following challenge.

Samples selected for cross-protective assay

Serum samples collected at 2 weeks following administration of two doses by both IM-IM and IM-IN regimens, 6 months following a three-dose IM-IN-IN regimen, and 12 months following a three-dose IM-IM-IM regimen of CALVENZA EIV/EHV were evaluated from the four vaccinate groups.

Serological assay

Initially, a single radial hemolysis (SRH) test for determination of antibody to EIV A2/Newmarket/1/93 (*analogous to North American EIV-A2/Kentucky/95*), and A2/Newmarket/2/93 was performed by AHT standardized procedures.

In a subsequent assay to determine cross-reactive antibody, a single radial hemolysis (SRH) test for determination of antibody to EIV A2/Saskatoon/90, A2/Florida/94, A2/Kentucky/98, and A2/Kentucky/99 was performed by AHT standardized procedures.

Serological results

Serological evaluation post-vaccination was determined by an SRH assay. This assay procedure was chosen because antibody levels as determined by this assay have been shown to correlate with protection of horses after challenge with virulent EIV. All ponies were seronegative to the A1 and A2 equine influenza viruses prior to initial vaccination. Non-vaccinated control horses remained seronegative throughout the sampling period outlined above.

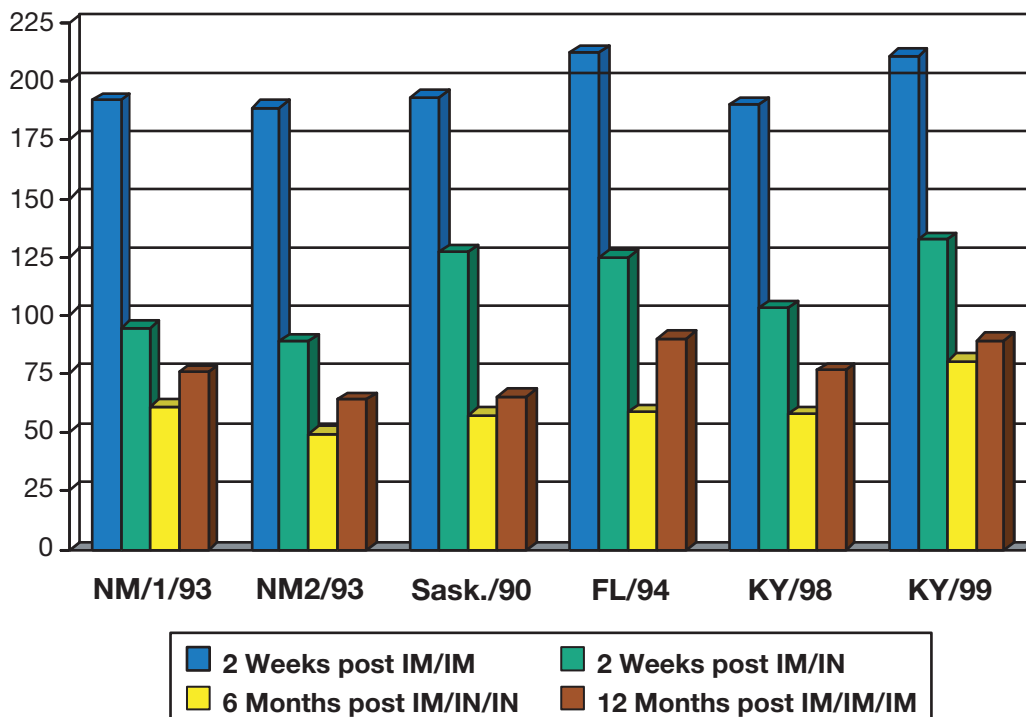
Both vaccination regimens generated high levels of serum antibody to each of the A2 North American and European EIV strains native to the vaccine as well as cross-protective antibody to other relevant North American and European EIV A2 strains.

Summary of the EIV A2 Cross-reactive Antibody Titer Results

Vaccinate Group	SRH antibody (mm^2) levels to equine influenza A2 strains					
	NM/1/93*	NM/2/93*	Saskatoon 90	Florida 94	Kentucky 98	Kentucky 99
2-dose Efficacy						
IM/IM	191.9 \pm 14.1	188.6 \pm 15.6	193.1 \pm 30.9	212.5 \pm 16.2	190.3 \pm 17.2	211.0 \pm 16.5
IM/IN	94.6 \pm 46.5	89.1 \pm 38.5	127.0 \pm 10.9	124.7 \pm 25.6	103.2 \pm 27.2	132.6 \pm 22.4
6 month DOI						
IM/IN/IN	60.8 \pm 30.1	49.4 \pm 39.9	57.4 \pm 25.9	58.7 \pm 28.9	57.8 \pm 22.9	80.6 \pm 31.2
12 month DOI						
IM/IM/IM	75.8 \pm 20.1	64.1 \pm 20.7	65.2 \pm 17.7	90.1 \pm 18.2	76.5 \pm 14.0	89.2 \pm 22.5

* EIV A2 Strains incorporated in Calvenza™ EIV/EHV vaccine

Mean Cross-reactive SRH (mm^2) Antibody Levels for Specific Relevant Strains of Equine Influenza A2 Viruses



Serum samples were collected at 2 weeks following administration of two doses by both IM-IM and IM-IN regimens, 6 months following a three-dose IM-IN-IN regimen, and 12 months following a three-dose IM-IM-IM regimen of Calvenza™ EIV/EHV from 4 groups of ponies that were seronegative to influenza prior to initial vaccination. The first and second doses were administered five weeks apart. In the two groups receiving the three-dose regimen, the third dose was administered six months following the second dose.