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Original Research

Serologic Responses of West Nile Virus Seronegative Mature Horses to West Nile Virus Vaccines

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ABSTRACT

A 42-day study was conducted to assess the impact of three West Nile virus vaccines given either as separate injections or incorporated with their counterpart equine encephalitis and tetanus vaccines on serological responses under field use conditions. Two hundred forty mature, West Nile virus seronegative (<4) horses were followed serologically pre- and postprimary and secondary vaccination with six different vaccination programs, all including West Nile virus antigens. Forty horses were unvaccinated sentinel horses. All vaccines stimulated both a primary and secondary (booster) response to vaccination that was significantly higher than that of seronegative controls. However, inclusion of West Nile virus with equine encephalitis viruses and tetanus toxoid in vaccines had a significant detrimental impact on West Nile virus serum neutralization antibody production to both the primary and secondary vaccinations.

1. Introduction

West Nile virus (WNV) is a member of the *Flaviviridae* family and belongs to the Japanese encephalitis serogroup of the genus *Flavivirus* [4]. WNV infections in horses were first observed in the United States in 1999 [1]. Since then, over 25,000 cases of WNV-induced encephalitis have been reported. Infections in horses represent 96.9% of all reported non-human mammalian cases of WNV disease [2,3].

The virus is transmitted from avian reservoir hosts by mosquitoes (and infrequently by other bloodsucking insects) to horses, humans, and a number of other mammals. WNV is transmitted by many different mosquito species, and this varies geographically. The virus and mosquito host interactions result in regional change in virulence of the virus, and no prediction can be made regarding future trends in local activity of the viruses. Horses and humans are considered dead-end hosts for WNV; the virus is not directly contagious from horse to horse or from horse to human. Indirect transmission via mosquitoes from infected horses is highly unlikely, as these horses do not circulate a significant amount of virus in their blood.

The case fatality rate for horses exhibiting clinical signs of WNV infection is approximately 33%. Data have supported the fact that 40% of horses that survive the acute illness caused by WNV still exhibit residual effects such as gait and behavioral abnormalities 6 months postdiagnosis. The first vaccine against WNV was licensed as a monovalent viral inactivated vaccine. Since then, incorporation of the WNV antigen in multivalent vaccines has become common. Currently, WNV vaccine is recommended by the American Association of Equine Practitioners as the core vaccine for all horses in North America.

All authors above have no conflicts of interest.

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2. Materials and Methods

2.1. Study Animals

Three hundred thirty-seven horses, 2-17 years old, were initially screened for enrollment in the study. All horses had individual identification. The horses were all grade Quarter Horse-cross animals housed on the same ranch in two locations. There was a mixture of mares, stallions, and gelding in the study group. Of the 337 horses, 280 horses were selected for inclusion in the study. These horses were then blocked by site, age, and sex and then randomly assigned to one of the seven treatment groups, using a random number generated within SAS software.

During the study, all groups commingled within a site. The horses were maintained on native pasture grazing with supplemental hay feed during the winter. The primary water sources were natural water (creeks, lakes, and other sources) and wells.

2.2. Vaccines Evaluated

Three serials, with different expiration dates and lot numbers, of each of six US Department of Agriculture (USDA)-licensed WNV vaccines were evaluated (Table 1). Immune responses to WNV vaccine (groups 1, 4, and 6) that also included encephalitis/tetanus/WNV combinations were compared to WNV vaccine as a single antigen from the same manufacturer (groups 3, 5, 7). group 2 was left as a nonvaccinated sentinel group (Table 1).

2.3. Study Design

On study day −30, 25 randomly selected horses from the potential study herds had blood drawn for serological examination of existing antibody titers for WNV. If the selected horses were seronegative for WNV (<1:4), then the herd moved onto the next phase of the study. On study day −15, blood was drawn from 337 horses, and the 280 horses that were determined to be WNV serum neutralization (SN) antibody negative were selected for inclusion in the study.

On Day 0, each horse was treated as per the assignment (Table 1). All products were administered intramuscularly on the same side of the neck. Three serials, with different expiration dates and lot numbers, of each product were used for primary and secondary (booster) vaccinations. Following processing, all horses were commingled until the next processing. On days 0, 7, 21, 28, and 42 all study horses had blood drawn for serologic analysis. On day 21, all horses were revaccinated with their assigned vaccines (Table 2).

Table 1
Vaccine use by group assignment

No. Treatments	Treatment Description	Approximate Number of Horses per Treatment
T1	West Nile Innovator (Zoetis Animal Health-Fort Dodge, Iowa, USA) EWT (contains West Nile, Eastern and Western equine encephalitis, and tetanus antigens and Metastim adjuvant) and Fluvac Innovator EHV 1/4 (contains equine influenza and equine herpes 1/4 antigens and Metastim adjuvant)	40
T2	Sterile saline (Saline Control)	40
T3	Fluvac Innovator 5 (Zoetis Animal Health- Fort Dodge, Iowa, USA) (contains equine influenza, equine herpes 1/4, Eastern and Western equine encephalitis antigens, and Metastim adjuvant) and West Nile Innovator (Zoetis Animal Health-Fort Dodge) (contains West Nile antigen and Metastim adjuvant)	40
T4	VeteraGold (Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO) (contains equine influenza, equine herpes 1, Eastern and Western equine encephalitis, West Nile, and Tetanus antigens and Carbimmune adjuvant)	40
T5	Vetera EWT + EIV/EHV (Vetera WNV-Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO) (contains Eastern and Western equine encephalitis, tetanus, equine influenza and equine herpes 1 antigens and Carbimmune adjuvant) and Vetera WNV (contains West Nile antigen and Carbimmune adjuvant)	40
T6	Prestige V + WNV (Intervet Inc., Summit NJ, US) (contains Eastern and Western equine encephalitis, equine influenza, equine herpes 1/4, tetanus and West Nile antigens and Havlogen adjuvant)	40
T7	Prestige V (Intervet Inc., Summit, NJ) with Havlogen (contains Eastern and Western equine encephalitis, equine influenza, equine herpes 1/4 and tetanus antigens and Havlogen adjuvant) and EquiNile (contains West Nile antigen and Havlogen adjuvant)	40

EIV, equine influenza virus; EHV, equine herpes virus; EWT, E (EEE, eastern equine encephalitis) W (WEE, western equine encephalitis) T (Tetanus); T, Treatment; WNV, west Nile virus.

2.4. Sample Preparation and Handling and Laboratory Evaluation

Blood samples were stored on ice during each process and then transported directly to the Colorado State University veterinary diagnostic lab for serum preparation. All samples collected on days 0, 7, 14, 21, and 28 had the serum separated and were stored in cryovials at −20°C, held frozen until the completion of the study and run at one time. The serum samples that were prepared at the Colorado State University veterinary diagnostic lab were shipped to the New York State Animal Health Diagnostic Laboratory for analysis. A microneutralization assay was used to assess the antibody status of the animals in this study. The test as conducted is essentially the same procedure as that proscribed for equine arteritis virus by