The Anamnestic Serologic Response to Vaccination with a Canarypox Virus–Vectored Recombinant West Nile Virus (WNV) Vaccine in Horses Previously Vaccinated with an Inactivated WNV Vaccine*

D. A. Grosenbaugh, DVM, PhD*
C. S. Backus, DVM, PhD
K. Karaca, DVM, PhD
J. M. Minke, DVM, PhD
R. M. Nordgren, DVM, PhD

*aMerial Limited
115 Transtech Drive
Athens, GA 30601

*bCountryside Veterinary Service
2922 Black Fox Trail
Louisville, TN 37777

*cMerial SAS
254 rue Marcel Merieux
69007 Lyon, France

CLINICAL RELEVANCE

A new recombinant West Nile virus (WNV) vaccine has been licensed for use in horses. Before this vaccine became available in 2004, the only equine WNV vaccine on the market had been an inactivated vaccine. Since the recombinant vaccine only expresses selected viral genes, the present study investigated whether a single dose of the recombinant vaccine is effective in producing an anamnestic serologic response in horses previously vaccinated with an inactivated WNV vaccine. Vaccination of horses with a canarypox-vectored recombinant vaccine, under field conditions, results in a marked anamnestic response in horses previously vaccinated with an inactivated WNV vaccine.

INTRODUCTION

A new recombinant West Nile virus (WNV) vaccine, expressing the premembrane (prM) and envelope (E) genes of WNV, has been licensed for use in horses.¹ This vaccine has been shown to provide a rapid onset of protection from viremia following challenge with WNV-infected mosquitoes after a single administration of vaccine.² Immunization with a canarypox virus recombinant vaccine results in the presentation of antigens in a manner analogous to that of the wild-type infection, in the ab-
sence of productive viral replication. This method of antigen presentation by recombinant canarypox virus vaccines has been shown to be capable of providing protection after the decline of detectable serum antibody levels, at a time during which antigen-specific T-cell responses can be elicited. This authentic antigen presentation in the absence of viral infection removes the potential for transmission of the vaccine vector or the possibility of infectious disease resulting from incomplete inactivation of conventional vaccines, which has been implicated with respect to an inactivated Eastern equine encephalomyelitis virus vaccine.

Before the recombinant vaccine became available, the only equine WNV vaccine on the market had been an inactivated vaccine, which was introduced in 2001 in the face of a disease outbreak in the United States. Following a peak of reported equine WNV cases in 2002, the number of clinical cases in horses has declined, which has been attributed to effective vaccination programs, although subclinical exposure is likely to have played a major role. Because all horses vaccinated for WNV before 2004 would have received the inactivated vaccine, we investigated whether a single dose of the recombinant vaccine, expressing the prM and E genes of the WNV, could induce an anamnestic serologic response in horses previously vaccinated with the killed vaccine. Horses from both a WNV-endemic region and a region for which WNV had not yet been reported were included in the study.

### MATERIALS AND METHODS

Two trials were conducted independently. One was conducted in an area in which WNV disease in horses has been reported and the other in an area considered free from WNV at the time of the study.

#### Horses

Horses were identified from two regions of the United States: Blount County, Tennessee, where WNV is considered to be endemic, and Solano County, California, where WNV infection in horses had not been reported at the time of the trial. Each group of horses was housed on single premises under uniform management conditions. All horses were used under a protocol approved by Meri-al’s Institutional Animal Care and Use Committee.

In Trial 1, 27 horses ranging in age from 1.5 to 23 years were randomized into two groups with respect to date of last vaccination with an inactivated WNV vaccine. In Trial 2, 11 horses ranging in age from 1 to 16 years and likewise previously vaccinated with the inactivated vaccine but presumably unexposed to natural infection were included in the study. The age and gender of the two herds and their group assignment are listed in Table 1.

#### Vaccines

Recombitek Equine WNV vaccine (Meri-al) is an ALVAC (canarypox)–based recom-

---

### TABLE 1. Age and Gender of Horses from Trial 1 (Blount County, Tennessee) and Trial 2 (Solano County, California)

<table>
<thead>
<tr>
<th>Location</th>
<th>Vaccination Group</th>
<th>Age (yr ± SE)</th>
<th>Gender (M/G/S)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blount County, Tennessee</td>
<td>Inactivated</td>
<td>4.4 ± 1.3 (1.5–19 yr)</td>
<td>10/2/1</td>
</tr>
<tr>
<td>Blount County, Tennessee</td>
<td>Recombinant</td>
<td>7.5 ± 2.2 (1.5–23 yr)</td>
<td>8/4/2</td>
</tr>
<tr>
<td>Solano County, California</td>
<td>Recombinant</td>
<td>4.0 ± 1.4 (1–16 yr)</td>
<td>6/4/1</td>
</tr>
</tbody>
</table>

G = gelding; M = mare; S = stallion.
Vaccination

Two trials were conducted in which all horses had been previously vaccinated with the inactivated WNV vaccine as per label recommendation.

**Trial 1 (Blount County, Tennessee)**

In Trial 1, the primary vaccination protocol (two doses) was completed between 18 and 25 weeks (24 ± 2 weeks; mean ± SD) before the initiation of the study in September of the same year. Horses received a single dose (1 ml IM) of either the inactivated WNV vaccine or the recombinant WNV vaccine.

**Trial 2 (Solano County, California)**

In Trial 2, horses in the WNV-free area had received either a single “booster” between 32 and 42 weeks before the start of the study (n = 8) or the three-dose protocol of the inactivated vaccine 21 weeks previously (n = 3). The mean duration to revaccination was 35 ± 9 weeks (mean ± SD). All horses received a single dose (1 ml IM) of the recombinant WNV vaccine in March of the following year.

**Serology**

Blood was collected from all horses before they were vaccinated and at 7 and 14 days post-vaccination in Trial 1 or 16 and 29 days post-vaccination in Trial 2. The serum was separated and maintained at −20°C until analysis for the presence of serum neutralization antibodies against WNV by the plaque reduction neutralization test (PRNT) at Colorado State University, Animal Disease Lab, Foothills Campus, Fort Collins, Colorado. Personnel doing the laboratory analysis were blind to group assignment.

Briefly, sera were heat-inactivated at 56°C for 30 minutes and serial twofold dilutions of those sera were mixed with an equal volume (0.10 ml) containing 200 to 300 plaque-forming units of the NY99-4132 strain of WNV. Following overnight incubation at 4°C, 0.1 ml of each mixture was applied to Vero cell monolayers in six-well plates. Neutralization endpoints were recorded as the highest dilution of serum result-
Statistical Analysis

The serologic data were related as powers of 2; thus, the base 2 logarithm titers were analyzed. Titors reported as “<10” were recoded as “5” for analysis. Geometric mean titers (GMTs) were calculated. The transformed data and the difference between the log baseline titer and the 7- and 14-day postvaccination log titers were analyzed using one-way analysis of variance. Statistical significance was based on two-tailed tests of the null hypothesis with $P \leq 0.05$.

**RESULTS**

**Serum Neutralization Antibody (IgG)**

**Trial 1 (Blount County, Tennessee)**

The distribution of initial WNV serum antibody titers between the vaccination groups is tabulated in Table 2, and the results of the statistical analysis of the data are presented in Table 3. There was no significant difference ($P > 0.10$) between the vaccine groups for baseline GMT, although a wide range of titers was observed. All horses showed an anamnestic response within 7 days of vaccination, with no significant differences between the two groups.

By 14 days postvaccination, the difference in titers between horses vaccinated with the recombinant (GMT = 1,400) and the inactivated (GMT = 545) vaccine approached significance ($P = 0.06$) in favor of the recombinant vaccine. The magnitude of the change of log titer from day of vaccination to 14 days postvaccination was significantly higher for horses that received the recombinant vaccine than for horses vaccinated with the inactivated product ($P = 0.02$).

**Trial 2 (Solano County, California)**

The distribution of initial WNV serum antibody titers is presented in Table 4. The starting GMT for the horses from the non-WNV endemic region was comparable to that of those in the endemic area (GMT = 20). By 16 days postvaccination, the GMT in this group of horses was 3,981.

**IgM Antibody**

Samples from all of the horses in Trial 1, at all time points, were negative when assayed for WNV-specific IgM (data not shown).

**DISCUSSION**

Recombinant and inactivated vaccines induced an anamnestic, humoral response in horses vaccinated previously with the inactivated WNV vaccine. Although the role of serum-neutralizing antibodies in protecting horses from WNV disease has not been established,
the level of antibodies induced following revaccination was much higher than that of previous studies in which horses vaccinated with the recombinant vaccine were protected from WNV challenge. Increases in GMT were observed as early as 7 days postvaccination; by 14 days, horses in the WNV endemic groups receiving the recombinant vaccine approached a significantly higher GMT than those revaccinated with the inactivated product. Statistical comparison of the data from the endemic versus nonendemic area was not made because of differences in primary vaccination schedule and sampling times.

Not all of the horses in this study were seronegative for WNV antibody before vaccination. The effects of the previous vaccination can satisfactorily explain low levels of serum antibody in approximately half of the horses in Trial 1. The location of the herd used in this study was Blount County, Tennessee, for which no positive mosquitoes were reported during the time of the study, but six equine

<table>
<thead>
<tr>
<th>Day</th>
<th>Recombinant Vaccine (n = 14)</th>
<th>Inactivated Vaccine (n = 13)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>19 (≤10–640)</td>
<td>26 (≤10–320)</td>
<td>&gt;.10</td>
</tr>
<tr>
<td>7</td>
<td>820 (80–2,560)</td>
<td>465 (40–1,280)</td>
<td>&gt;.10</td>
</tr>
<tr>
<td>14</td>
<td>1,400 (320–5,120)</td>
<td>545 (40–2,560)</td>
<td>.06</td>
</tr>
<tr>
<td>0–7 (change)</td>
<td>5.43</td>
<td>4.15</td>
<td>.09</td>
</tr>
<tr>
<td>0–14 (change)</td>
<td>6.21</td>
<td>4.38</td>
<td>.02</td>
</tr>
</tbody>
</table>

Horses were vaccinated on day 0 with a canarypox-based recombinant WNV vaccine or an inactivated WNV vaccine; all horses had been previously vaccinated with the inactivated WNV vaccine.

The results of this study clearly demonstrate that vaccination of horses with a recombinant WNV vaccine following a primary course of killed vaccine elicits a relevant serologic response that is greater in magnitude than revaccination with the homologous, killed vaccine.
different vaccine modalities will be of great value. This is the first study that addresses concerns regarding the ability of a recombinant WNV vaccine to elicit an anamnestic immune response to WNV in horses previously vaccinated with an inactivated vaccine.

**CONCLUSION**

Vaccination of horses with a canarypox-vectored recombinant vaccine expressing the prM and E genes of WNV, under field conditions, results in a marked anamnestic response in horses previously vaccinated with an inactivated WNV vaccine.

**ACKNOWLEDGMENTS**

We thank R. Bowen, DVM, PhD, for valuable discussions and his review of the manuscript and for performing the serum neutralization WNV serum antibody titrations. We also thank the Center for Equine Health at the University of California, Davis, for providing equine sera from the Solano County site.

**REFERENCES**


