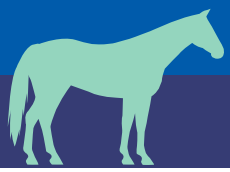
PreveNile™ West Nile Virus Vaccine Full Year Duration of Immunity

PreveNile™ Advanced Science Powerful Protection



Summary Points

- PreveNile™ vaccine utilizes a live attenuated human Yellow Fever chimera virus with certain structural genes exchanged by ChimeriVax™ technology for those of West Nile virus.
- The PreveNile™ duration of immunity to West Nile virus infection was tested by a severe intrathecal (injection into the cerebrospinal fluid) challenge that caused moderate or severe signs of West Nile virus disease in 80% of control horses.
- Only 1 of 9 vaccinated horses developed signs of West Nile virus disease when rigorously challenged a year after a single vaccination.
- PreveNile™ is the first West Nile vaccine licensed with claims to prevent viremia and aid in the prevention of diseases such as encephalitis caused by West Nile virus infection.
- PreveNile™ is an excellent tool for equine practitioners to use in their fight against West Nile virus disease in horses.

Background

West Nile (WN) virus was discovered in 1937 in the blood of a feverish Ugandan woman in the West Nile region and caused sporadic outbreaks in Africa, the Middle East and Europe. These outbreaks were of erratic clinical significance over the next 60 years. In the last decade, the outbreaks began increasing in both frequency and severity with an increased proportion of neurologic disease in birds, horses, and humans.[1,2] WN virus infection in horses did not usually cause clinical signs prior to 1999, the year it somehow crossed the Atlantic and was first detected in New York. By 2001, the outbreak spread westward and reached epizootic proportions, infecting a population of horses that was immunologically naïve to WN virus. Equine disease in the United States due to WN virus peaked in 2002 with the report of 15,257 cases that year.[3] By 2003 WN virus had swept the nation.

The 1999 outbreak was thought to cause disease in about 10% of horses and 1% of humans infected.[4,5] The low rate of clinical disease among infected horses complicates evaluation of prevention methods. This was demonstrated by a study intended to evaluate the onset of immunity of another WN virus vaccine, a study that utilized mosquitoes infected with WN virus as the challenge.[6] This method successfully caused WN viremia, but failed to cause significant signs of WN virus disease in the unvaccinated control horses, thus resulting in vaccine evaluation based solely on the prevention of viremia.

The clinical features of WN virus infection include fever, neurologic signs and death. The neurologic signs include ataxia (stumbling), excitability, somnolence (sleepiness), depression, paresis (weakness), paralysis (especially hindquarter), skin fasciculations (twitching), muscle tremors, muscle rigidity (inflexible stiffness), dysphagia (difficulty swallowing) and recumbency. These signs reflect central nervous system pathology. Mortality rate is highest for recumbent horses, but the overall mortality of horses presenting with signs of WN virus infection is approximately 25%-60%.[2,4]

WN virus is one of over 70 viruses in the Flavivirus genus of the family *Flaviviridae*, many of which are important veterinary and human pathogens. The prototype of this genus, Yellow Fever (YF), the original viral hemorrhagic fever, is a deadly human disease. In 1936, a live attenuated vaccine, Yellow Fever Vaccine 17D (YFV17D), was developed for human use. The safety of YFV17D was proven by over 60 years of use in approximately 400 million people.[7] ChimeriVax™ technology was used to produce PreveNile™ utilizing a live attenuated human YF virus chimera with certain structural genes exchanged for those of WN virus.[7,8]

Objective

The objective of this study was to determine if the administration of a single dose of PreveNile™ vaccine would protect horses from WN virus disease one year later.

Study Materials and Methods

The study utilized 20 yearling horses without previous WN exposure, as indicated by West Nile virus neutralization (WNVN) titers of ≤ 2. Ten (10) horses were randomly selected for the vaccination treatment group, and 10 were assigned to the unvaccinated control group. Treatment horses were vaccinated intramuscularly (IM) in the neck with a single 1 mL dose of vaccine, and control horses were injected IM in the neck with 1 mL of sterile diluent. Control and vaccinate horses were housed together in an insect- and rodent-proof facility during the vaccination portion of the study and were moved to a Bio-Safety Level-3 facility for the challenge portion. Treatment and control groups were mixed, split into subgroups and processed sequentially because of facility size limitations. Horses were randomized, using a random number generator, into 3 subgroups (4 vaccinates/4 controls, 4 vaccinates/4 controls,

2 vaccinates/2 controls) to facilitate this process.

Monitors, who were blinded as to whether the horses were in the vaccination or control group, reported all abnormal signs both before and after challenge. The injection sites were observed Day 1 through Day 10 for reactions and rectal body temperature was taken daily from Day -1 through Day 10 postvaccination. Blood drawn for determination of WNVN titer on Day 0 and Day 28 postvaccination and this test was repeated monthly until challenge.

The challenge for this study was rigorous and severe to achieve the best possible evaluation of the vaccine. Intrathecal challenge involves injecting the challenge virus into the cerebrospinal fluid. The challenge virus was a virulent WNV strain. Using this severe challenge model was necessary since only about 10% of horses naturally exposed to WN-virus-laden mosquitoes will develop signs of WN disease.

Body temperature was monitored daily from Day -1 to Day 21 postchallenge. Horses were also monitored for other signs of WN virus disease (abnormal mentation, ataxia, fasciculations, and paresis) and graded as indicated in Table 1. Not included were signs observed for less than two consecutive days that were mild and could go unnoticed.

Blood samples for virus isolation were taken daily from Day -1 through Day 10 postchallenge. Blood samples for serology were taken at the time of challenge and 21 days postchallenge or earlier (at necropsy if euthanasia before Day 21 postchallenge was necessary). Neurologic tissue samples for histopathology were taken at necropsy. Histopathological lesions were scored as indicated in Table 2.

Study Results – Prechallenge

Although all horses were in good health at the start of the study, one of the vaccinated horses developed laminitis, unrelated to vaccination, and was euthanized at 7 months postvaccination. No injection site or systemic reactions were noted in the vaccinated horses. None of the vaccinated horses developed fever (≥102.5°F or 39.2°C)).

All study horses were seronegative for WN virus at the time of vaccination, and the control horses remained seronegative until intrathecal

Table 1 – Abnormal Mentation, Ataxia, Fasciculation, and Paresis Grading Scale

Grade	Description
0	Normal
1	Very mild and could go unnoticed
2	Moderate
3	Severe

Table 2 - Histopathology Grading Scale

Grade	Description
0	None
1	Very mild/Mild
2	Moderate
3	Severe

challenge. The WNVN antibody geometric mean titer (GMT) of the vaccinated horses was 17 one month after vaccination. The vaccinated GMT value oscillated mildly through the rest of the prechallenge period, dropping to a low of 9 at 12-months postvaccination (see Figure 1).

The WNVN antibody result for every control horse was <2 at 12 months postvaccination illustrating no exposure to WNVN for the entire year. The VN antibody response to vaccination was excellent in most of the horses with titers at 12 months postvaccination ranging from 8 to 32 except for one horse, which did not respond as well as the others and had a titer of 4 at 11 months and <2 at 12 months postvaccination.

Study Results – Postchallenge

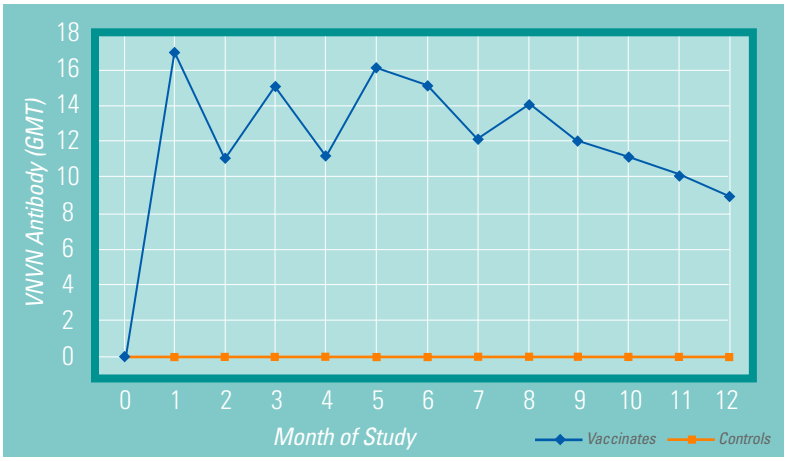
Intrathecal challenge of control horses with a virulent strain of WN virus resulted in clinical signs consistent with those observed in naturally infected horses, but at a much higher rate of infection than when horses are naturally exposed to infectious mosquitoes. There was a significant difference ($P<0.05$) between control and vaccinate groups regarding clinical signs of WN virus infection, with a higher proportion of control horses exhibiting paresis, fasciculations, and ataxia when compared to vaccinates.

After the challenge, 70% of the control horses were febrile, 80% had moderate or severe signs of WN virus disease and 80% were euthanized for humane reasons after demonstration of changes in any of the clinical signs in the categories of mentation, paresis, fasciculations, ataxia or overall severe health condition as a result of WNV infection. In sharp contrast, 1 of the vaccinates had moderate changes in mentation (grade 2) for 1 day and moderate or severe fasciculations (grade 2 and 3) for 3 consecutive days after challenge, but none of the vaccinates had signs necessitating postchallenge euthanasia prior to the end of the study. In addition, none of the vaccinated horses was febrile.

There was a significant difference ($P<0.05$) between the groups with 1 of 9 vaccinates and 8 of 10 control horses classified as experiencing failure of disease protection due to exhibiting signs of WN virus disease after the rigorous intrathecal challenge.

Virus-neutralizing antibody to WN virus was detected in all control horses postchallenge, but antibody titers were lower in controls compared to vaccinates. The WNVN antibody GMT of the vaccinated horses was 9 just prior to challenge and 1,024 at 21 days postchallenge. In contrast, the GMT of unvaccinated horses was <2 just prior to challenge and 42 at time of humane euthanasia due to exhibition of severe WN virus clinical signs or at 21 days postchallenge, whichever came first.

Figure 1 - West Nile Virus Antibody* Postvaccination



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WN viremia was not detected in any vaccinated horse, but was recovered from 9 of 10 control horses from 1 to 5 days after the challenge.

Neurologic tissue samples from every horse in the study were examined in the following 4 locations: pons, medulla, cervical cord and lumbar cord. Histopathology consistent with WN disease at the conclusion of the study was noted in only 1 of 9 vaccinated horses compared to 8 of 10 control horses.

Discussion

The PreveNile™ vaccinated group responded remarkably well to the severe challenge administered, although there was 1 horse in the vaccinated group that did not respond as well as the others and after challenge displayed abnormal mentation, had significant fasciculations, and had histopathological evidence of WN virus encephalitis. Even with those signs, this horse was not viremic and fared better than 80% of the horses in the unvaccinated group. Thus the PreveNile™ vaccinated group was well protected from this unnaturally intense WN virus challenge made a full year after administration of a single vaccination.

This fact, coupled with the fact that control horses developed moderate or severe clinical signs and demonstrated histopathologic lesions indicative of WNV encephalomyelitis, provides authenticity to the PreveNile™ product claims of prevention of viremia and as an aid in the prevention of disease and encephalitis caused by WN virus infection.

Conclusion

PreveNile™ is an extremely important weapon for equine practitioners to have in their armamentarium and use in their fight against the devastation that WN virus disease routinely causes to unprotected horses. The level of confidence created when using a vaccine evaluated in this manner is obviously going to be very high. And so it should be. PreveNile™ is the first WN vaccine licensed with a claim to prevent viremia and aid in the prevention of disease and encephalitis caused by West Nile virus infection. These label claims were made possible only through the judicious use of the intrathecal challenge model.

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Table 3 - WN Virus Challenge Results

	Neurologic Signs†	Feverδ	Viremia	Encephalitis
Vaccinate Group (9 total)*	1	0	0	1
Control Group (10 total)	8	7	9	8

* One vaccinated horse removed from study prior to challenge

† Change in mentation, paresis, fasciculation and ataxia/recumbency observed for two or more consecutive days. Not included were signs observed for less than two consecutive days that were very mild and transient and could go unnoticed

δ Body temperature ≥ 102.5° F

PreveNile research studies were conducted by Maureen Long, D.V.M, Ph.D., Dipl. ACVIM at the University of Florida, Gainesville, Fla.

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WEST NILE VIRUS VACCINE
Live Flavivirus Chimera

DESCRIPTION: PreveNile™ contains a lyophilized Yellow Fever West Nile (YF-WN) chimera virus vaccine. The human vaccine yellow fever virus, YF-17D, was used as the backbone. The YF-17D strain is a highly effective, well-tolerated live, attenuated vaccine that has been used for over 60 years to immunize approximataely 400 million people[1]. The YF-17D virus was modified using molecular technology to form a YF-WN chimera virus that expresses the PreMembrane and Envelope proteins of WNV. Following injection, limited replication of the YF-WN chimera virus allows expression of these proteins that stimulate a protective immune response in the horse.

INDICATIONS: For the vaccination of healthy horses to prevent viremia and as an aid in the prevention of disease and encephalitis caused by West Nile virus infection.

SAFETY DATA: Studies have shown that the YF-WN chimera virus in **PreveNile™** has limited to no replication in cells of mosquitoes or in the mosquito vectors of West Nile Virus or Yellow Fever Virus[2]. Studies of the YF-WN chimera virus in non-target animal species also show a lack of pathogenicity[3]. Safety studies have demonstrated that the vaccine virus is stable and does not revert to virulence, does not disseminate into the tissues of vaccinated horses, and is not shed by vaccinated horses following administrations. An overdose study, 10X the standard vaccine dose was administered to horses, no clinical signs were seen in any horses, nor was vaccine virus recovered from the plasma, nasal, oral, and rectal swabs or from tissues from these vaccinated horses. Transmission of vaccine virus to sentinel horses kept in contact with these vaccinated horses did not occur. In a large field safety study of 919 horses, **PreveNile™** was demonstrated to be >99% reaction-free when administered to horses of various ages, breeds, and sex. Of the 919 horses vaccinated, 229 were 4 months of age or younger, and 302 were pregnant mares, including 17 mares in the first trimester of gestation, 11 mares in the second trimester of gestation and 274 mares in the third trimester of gestation. No post vaccinal adverse events were observed in any of the foals or mares. Studies to continue to evaluate safety in these mares through foaling are ongoing. The results of this study show the vaccine to be safe when used to vaccinate horses 4 months of age or older by the intramuscular route.

EFFICACY, ONSET OF IMMUNITY AND DURATION OF IMMUNITY DATA: For complete efficacy data, see the PreveNile™ package insert.

ADMINISTRATION AND DOSAGE: Aseptically reconstitute the desiccated vaccine with the sterile diluent provided and administer 1 mL by the intramuscular route. Initial vaccination of healthy horses may be given as early as 5 months-of-age. For primary vaccination, administer a single dose only. Re-vaccinate annually with a single dose. For horses previously vaccinated against West Nile, administer a single dose only.

SUPPLIED:
Code: 034955 10 vials (1 dose each) of lyophilized vaccine, 10 vials (1 mL) of sterile diluent.
Code: 042149 2 vials (5 doses each) of lyophilized vaccine, 2 vials (5 mL) of sterile diluent.
U. S. Patent Nos: 6,878,372 and 6,962,708

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