Duration of passive immunity to West Nile virus in foals and response to vaccination

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Research Summary:
Pregnant mares were appropriately vaccinated against West Nile virus and antigen titers monitored in gestating mare and foal serum and in colostrum to assess the immune response in mares and the passive transfer of immunity to newborn foals. If additional funding can be secured, the effect of age at vaccination in young growing foals will also be assessed to determine an optimal time to initiate an initial vaccination regimen.

Introduction:
We hypothesize that: (I) booster vaccination of previously vaccinated pregnant mares in late gestation will increase serum titers of immunoglobulins against West Nile Virus (WNV), that levels of specific anti-WNV immunoglobulins in colostrum will be proportional to serum titers within a mare, and that mares may experience a decrease in serum titer during colostrogenesis; (II) the level of passively acquired immunity to WNV in foals suckling fully vaccinated dams will be proportional to the serum titer of the dam and will decay at an individually variable rate; and (III) passively acquired maternal antibodies will interfere with the development of an active immunoglobulin response to vaccination in foals and may lead to antigenic tolerance.

Since its introduction to New York in 1999, WNV has spread rapidly to cover most of the contiguous United States. The impact of WNV on horse owners includes the devastating emotional effects of a clinically affected animal, many of which will not survive, and the financial burden incurred.

A killed vaccine for WNV has now been granted full licensure (West Nile – Innovator®, Fort Dodge Animal Health, Fort Dodge, Iowa). The manufacturer recommends an initial course of 2 doses given 3-6 weeks apart to previously unvaccinated animals, followed by annual boosters given prior to the onset of the mosquito season. However, no specific guidelines have been provided for WNV vaccination of broodmares or foals and there are no published studies available on which to base recommendations. General recommendations for broodmare vaccinations are that boosters be given 4-6 weeks prior to foaling to ensure maximal transfer of protective antibodies to the foal via colostrum (Wilson, 1999). Subsequently, foals are vaccinated to acquire their own active immunity, with the primary series generally commencing at 3-4 months of age (Wilson et al., 1995b). Recently, the timing of vaccinations administered to foals, especially the age at which vaccination is commenced, has been questioned (Wilson, 1999). Evidence is accumulating that foals at 3-4 months may not respond to some vaccines due to interference from passively acquired maternal antibodies, rendering the vaccination ineffective. This phenomenon has been demonstrated for several diseases including tetanus (Wilson et al., 2001), influenza (Cullinane et al., 2001; Holland et al., 1999; Wilson et al., 2001) and rhinopneumonitis (Breathnach et al., 1999; Wilson and Rossdale, 1999). Of potentially greater concern that such early vaccinations may induce a state of tolerance whereby horses vaccinated as young foals are unable to mount and immune response to vaccination given later in life (Wilson, 1999). This has been shown to occur with influenza (Cullinane et al., 2001) and Eastern Equine Encephalitis (Wilson et al., 1995). These findings have lead to the recommendation that primary vaccinations for some of these diseases be delayed until 6 months of age and the number of doses in the primary series be increased (Wilson, 1999).

While a preliminary report demonstrated passive transfer of anti-WNV antibodies to a single foal born to a seropositive mare (Ostlund et al., 2001), there is no data on colostral antibody transfer from vaccinated mares, the persistence of these antibodies in foals or their effect on the response of foals to vaccination. The half-life of passively acquired antibodies to other equine diseases ranges from 27-39 days (Breathnach et al., 1999; Gibbs et al., 1988; Hullinger et al., 1998; van Maanen et al., 1994; Wilson et al., 2001) but is highly variable. Passively acquired antibodies to equine viral arteritis became undetectable in individual foals anywhere from 76-230 days of age in one study (Hullinger et al., 1998).

There is an urgent need to obtain and publish independent data on the duration of passive immunity to WNV afforded to foals through ingestion of colostrum, and to determine the age at which vaccination becomes effective. Such studies are needed

for the development of vaccination schedules that will effectively protect foals and weanlings from WNV.

Materials and Methods:
The Dickinson Research Extension Center maintains a herd of 27-30 Quarter Horse or Paint Horse broodmares that are pasture bred to a Quarter Horse stallion. Twelve mares are scheduled to foal in 2004 and this number will be increased in 2005 and 2006. The majority of mares in this herd foal in June and July.

Objective 1. *To determine the serum and colostral response of IgG and IgM titers to a booster WNV vaccination given to previously vaccinated mares 6 weeks prior to parturition to determine if serum titers decline during colostrogenesis.* Twelve mares confirmed in foal and previously given a primary course of WNV vaccine will be given a booster vaccination 4-6 weeks prior to foaling. Timing of this booster will be determined using analysis of pregnancy examination records. Since mares are pasture bred, routine management of this herd includes routinely performed early ultrasound pregnancy diagnoses. Results of repeated examinations are compared to known standards of equine embryonic development to estimate the day of ovulation that resulted in the conception. The pre-foaling booster vaccination will be performed at approximately 300 days of gestation based on these records.

Seven serum samples will be taken from each mare into 10 ml tubes (Vacutainer™, Becton Dickinson, Franklin Lakes, NJ) on the following schedule: immediately prior to booster administration, 2-3 weeks post vaccination, prior to parturition (udder distended with colostrum), within 1 day after parturition, and 1, 2 and 4 weeks post-partum. Serum will be allowed to clot for at least 20 minutes at room temperature prior to transportation to St. Joesph Hospital for processing. Samples will be stored at –20°C. When all samples have been collected they will be packaged on dry ice in insulated containers and shipped by commercial overnight parcel service to the Virology Laboratory at the University of Minnesota where they will remain frozen until assayed.

Data will be expressed as inverse titers, examined for normality and transformed as needed (based on published vaccination response studies for other diseases, log transformation is anticipated). Analysis for the effect of booster vaccination, and changes during late pregnancy and early lactation, will be performed using repeated measures analysis of variance procedures (Proc GLM, SAS). The level of significance will be set at $p<0.05$ but actual p statistics will be reported.

Objective 2. *Monitor the titers of anti-WNV IgG and IgM passively acquired by foals from colostrum ingestion, to determine the half-life of these acquired immunoglobulins and their decay to undetectable levels.* Twelve foals born to dams given a booster of WNV vaccine in late pregnancy will have blood samples taken on the following schedule:
Days 1 and 2 and weeks 1, 2, 4, 6, 8, 12, 16, 20, 24, 28, 32 and 36 of age (total of 14 samples per foal). Where possible an additional pre-suck sample will be taken, however since mares foal on pasture this will not always be possible. Additional serum will be obtained at the day 1 sampling (and 2 if needed) for assessment of adequacy of passive transfer. Samples will be obtained, handled and stored as described in objective 1 prior to assay for WNV specific IgM and IgG.

Antibody half-life will be determined by regression analysis and time to disappearance by survival analysis.

These foals will not be vaccinated in the first year of the study. Since they will be born in June and July it is anticipated that passively acquired antibodies will provide adequate protection against WNV infection during the late summer and early fall. They will be vaccinated in the spring of the second study year (approximately 9 months old) as part of Objective 3.

Objective 3. *Determine the change in serum titers of foals in response to a primary course of vaccination against WNV commencing at 3, 6 or 9 months of age and to examine whether tolerance to the vaccine occurs when vaccination commences prior to the decline in passively acquired anti-WNV immunoglobulins.* Two groups of 10 foals born in the second year of the study will be assigned to begin the primary course of vaccination against WNV at either 12 (group 1) or 24 (group 2) weeks of age. Mares will be fully vaccinated and be given a booster vaccination in the last 4-6 weeks of gestation. Following their initial vaccination (12 or 24 weeks) a booster dose will be given to foals 4 weeks after the initial administration. Blood samples will be taken at 24 hours after birth (to assess passive transfer), immediately before initial and booster vaccinations and at 4 weeks after the booster vaccination. Nine-month-old foals from the previous year (group 3) will also be vaccinated with 2 doses 4 weeks apart and sampled on a corresponding schedule.

Responses to vaccination between the different age groups will be compared using repeated measures

analysis of variance procedures on suitably transformed inverse titers.

The vaccine used in these experiments will be a killed WNV vaccine (WNV-Innovator™, Fort Dodge Animal Health, Fort Dodge Iowa) purchased from commercial sources. The vaccine has full FDA licensure and is readily available. No support (financial or product donation) has been or will be sought from the manufacturer.

Results and Discussion:

Samples for objectives 1 and 2 were obtained in the first year of the study. We began collecting samples for these objectives in the spring and summer of 2004 to facilitate timely completion. Sampling and sample storage can be performed at relatively small cost compared to antibody assays. If funding is not secured, samples will be stored until such time as a source is obtained. Samples for objective 3 will not commence until additional funding is obtained. To date, no additional funding has been obtained and other potential sources are being explored.

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