SEROLOGIC RESPONSE TO WEST NILE VIRUS VACCINATION IN THE GREATER ONE-HORNED RHINOCEROS (*RHINOCEROS UNICORNIS*)

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Abstract: Vaccination has been an important component of preventative health care programs of North American zoologic institutions in their protection of valuable species against West Nile virus (WNV) infection since its detection in 1999. Although approved only for horses, commercial WNV vaccine has been used for the purpose of protection of nondomestic species, including avian, equid, and rhinoceros species. Currently, there are two commercial equine vaccines available, a killed vaccine and a recombinant viral-vectored vaccine. Both products have been used for the vaccination of Greater One-horned rhinoceroses (*Rhinoceros unicornis*) held in North American zoologic institutions. In this study, the efficacy of these vaccines was evaluated in Greater One-horned rhinoceroses based on the humoral immune response stimulated by vaccine administration. Five rhinoceroses were vaccinated in 2005 by using the killed equine vaccine and four received boosters in 2006 by using the recombinant vaccine. Rhinoceroses were evaluated for differences in pre- and postvaccination neutralizing antibody titer and gamma and beta globulins on serum protein electrophoresis. No changes were observed after administration of the killed vaccine; however, antibody titers were observed in two of four rhinoceroses after administration of the recombinant vaccine. No significant changes were observed in the serum protein electrophoresis after either vaccine. Based on these findings, the WNV recombinant vaccine appeared to induce a more measurable humoral immune response than the killed product in the Greater One-horned rhinoceros. However, further investigation of both vaccines is warranted to evaluate whether changes in the frequency of administration, dosage, or adjuvant might stimulate an improved humoral response in these animals.

Key words: Greater One-horned rhinoceros, *Rhinoceros unicornis*, West Nile virus, vaccination, antibody titer, serum protein electrophoresis.

INTRODUCTION

Since the arrival of West Nile Virus (WNV) in North America in 1999, vaccination of susceptible species has been an important component of the preventative health care programs of North American zoological institutions. Currently, there are two vaccines available that are approved for use in the domestic horse, a killed vaccine (Innovator, Fort Dodge Animal Health, Fort Dodge, Iowa 50501 USA) and a newer recombinant vaccine (RECOMBITEK Equine WNV vaccine, Merial Limited, Duluth, Georgia 30096, USA). The killed equine vaccine has been used most commonly for the purpose of protecting a variety of nondomestic species, including avian, nondomestic hoof stock, and rhinoceroses.[7,8,15] Little information is available, however, regarding susceptibility to the virus or the efficacy of the two vaccines in stimulating an immunologic response in rhinoceroses and other nondomestic species.

Killed vaccines are considered relatively safe, because the virus cannot replicate and cause clinical disease. However, they tend to have a reduced capability to induce and sustain an effective and balanced immune response, thus requiring multiple and repeated doses to maintain protection.[10,11] Furthermore, killed vaccines tend to stimulate humoral immunity more so than cell-mediated immunity. Although this humoral stimulation results in a measurable antibody titer response, cell-mediated immunity likely plays a role in defense against WNV as well and is more difficult to quantify.[10,14] A safe alternative is a nonreplicating virus-vectored recombinant DNA vaccine that can elicit a more balanced, longer-lasting immune response.[10,14] This vaccine combines portions of the WNV genome that encodes the immunogenic M and E proteins with the genome of a canarypox virus vector. The effectiveness and safety of this vaccine are inherent in the fact that the viral vector infects but cannot replicate in mammalian cells, although the WNV genes are expressed to the immune system.[11] A disadvantage is that immunity can also develop to the vaccine vector, which prevents infection by the virus vector and foreign gene expression, thus reducing antigen-specific immune response.[13]

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The disease caused by WNV has never been definitively diagnosed in the Greater One-horned rhinoceros (*Rhinoceros unicornis*) (GOH) . During the initial outbreak of WNV in New York in 1999, two GOHs developed clinical signs, including anorexia, depression, and a lip droop, but they spontaneously recovered.[2,9] A blood sample from one of these rhinoceroses contained WNV-specific antibody, which indicated exposure to the virus. A literature search revealed no other reports of exposure to WNV or associated disease in the GOH or any other rhinoceros species. Despite this fact, a 2004 North American zoo-wide census conducted for the annual GOH Species Survival Program Veterinary Advisor report revealed that 10 of 21 institutions vaccinate their GOH collection for WNV by using the equine killed vaccine.[1] Pre- and postvaccine antibody titers were not measured in any of these animals. The objective of this study was to assess the serologic response of the GOH to vaccination with the killed equine WNV vaccine, as well as to an annual booster with the recombinant vaccine product.

MATERIALS AND METHODS

Study subjects

The study group in 2005 was composed of five adult GOHs (3 males, 2 females; age range 9–27 years) at three separate institutions. The 2006 study group was made up of four adult GOH from two institutions (3 males, 1 female; age range 7–27 years). Naïve individuals were chosen for the 2005 study and were qualified by having a negative WNV antibody titer (negative at 1:20) and no history of a prior WNV vaccination. In 2006, individuals had previously been vaccinated against WNV with the killed vaccine product in preceding years and had a negative WNV titer (1:20) preceding the 2006 booster vaccination. Three of these animals had been part of the 2005 study group, and the fourth animal had been vaccinated with the initial series in 2002 and boosters on an annual basis thereafter. Subjects had no signs of illness based on visual examination, clinical history, and pre- and postvaccination complete blood cell (CBC) counts and biochemistry profiles.

Vaccination and serologic protocol

A CBC count and a serum biochemistry profile were performed for each animal before and after vaccination to ensure that all subjects were in good health throughout the study in 2005 and 2006. Antibody titers for WNV were measured opportunistically within 6 mo before vaccination as part of the screening process for the initial vaccination series in 2005. All animals were then vaccinated in January and February of 2005 by using the commercial equine killed vaccine, according to manufacturer's recommendations for domestic horses. Two 1-ml doses of the killed vaccine were intramuscularly administered to each animal 4 wk apart. This was accomplished either by hand injection or pole syringe in the neck musculature. A second antibody titer was measured 3 to 4 wk after vaccination.

In 2006, prevaccination antibody titers were measured from blood collected on the day of booster vaccination. A single 1-ml dose of the recombinant DNA vaccine was administered intramuscularly to each animal according to manufacturer's instructions for domestic horses. A second antibody titer was measured 4 wk after vaccination. Immune response was evaluated based on comparisons of pre- and postvaccination 1) antibody titers and 2) gamma and beta globulin fractions on serum protein electrophoresis (SPE), which reflect immunoglobulin (Ig) G and acute-phase protein production, respectively.[6]

Sample handling and testing

Blood was collected from each animal via an ear vein or radial vein. Chemical sedation was used as necessary in some animals for this procedure. Serum was stored frozen until shipped overnight on ice to the Animal Health Diagnostic Laboratory of Cornell University School of Veterinary Medicine for neutralizing antibody testing. WNV-specific antibody titers were measured by using the plaque-reduction neutralization test.[12] Fresh serum and whole blood were shipped directly to Antech Diagnostic Laboratory after each collection for CBC count and biochemical profile. Serum protein electrophoresis was measured by the Antech Diagnostic Laboratory in 2005 and the University of Miami Comparative Pathology Laboratory in 2006.

Data analysis

Changes in the percentage of beta and gamma globulins before and after vaccination were compared for four GOH individuals in 2005 and 2006 separately by using a paired *t*-test. Percentages were arcsin transformed before being subjected to the paired *t*-test. The Statistical Package for the Social Sciences program (Chicago, Illinois 60606 USA) was used for these analyses.

RESULTS

All five animals in 2005 had a negative antibody titer before and after vaccination against WNV when administered the killed equine product (Table

Table 1. 2005 and 2006 pre- and postvaccination West Nile virus antibody titers.

Yr	Rhinoceros	Prevaccination[a]	Postvaccination[a]
2005	1	neg 1:40	neg 1:20
	2	neg 1:20	neg 1:20
	3	neg 1:20	neg 1:20
	4	neg 1:20	neg 1:20
	5	neg 1:20	neg 1:20
2006	3	neg 1:20	neg 1:20
	4	neg 1:20	neg 1:20
	5	neg 1:20	pos ≥1:640
	6	neg 1:20	pos 1:160

[a] neg, negative; pos, positive.

1). A mild increase in the percentage of gamma globulins occurred in one animal after vaccination, as did a mild increase in the percentage of beta globulins in another animal, however, no significant response was detected in either gamma ($t = 0.711$, df = 3, $P \geq 0.52$) or beta globulins ($t = 0.676$, df = 3, $P \geq 0.54$) in the four GOH for which pre- and postvaccination SPE values were available (Fig. 1a, b).

Administration of the annual booster vaccination in 2006 by using the recombinant DNA product resulted in seroconversion of two of the four GOH (Table 1) previously vaccinated by using the killed vaccine product. An increase in the gamma globulin ratio was observed in one GOH that seroconverted; however, no significant change was observed in either gamma globulins ($t = 0.054$, df = 3, $P \geq 0.96$) or beta globulins ($t = 0.239$, df = 3, $P \geq 0.82$) in the four GOHs tested (Fig. 2a, b).

Serum biochemical profile and CBC values for all animals were within normal limits compared with the species reference ranges of the International Species Information System.[5] None of the animals developed an adverse clinical reaction to vaccination; however, a single animal developed a draining lesion in 2005 at the location of the booster vaccine site; the lesion healed uneventfully.

DISCUSSION

The response observed in these GOHs differs from similar studies conducted in domestic horses and camelids. Approximately two thirds of horses vaccinated by using the same killed vaccine protocol develop a neutralizing antibody titer ≥ 1:100 at 4–6 wk after vaccination.[3] In a similar study of llamas and alpacas, more than 90% seroconverted by 3 wk after the second vaccination, and more than 97% by 3 wk after a third vaccination.[7] Possible factors that contribute to the lack of detectable
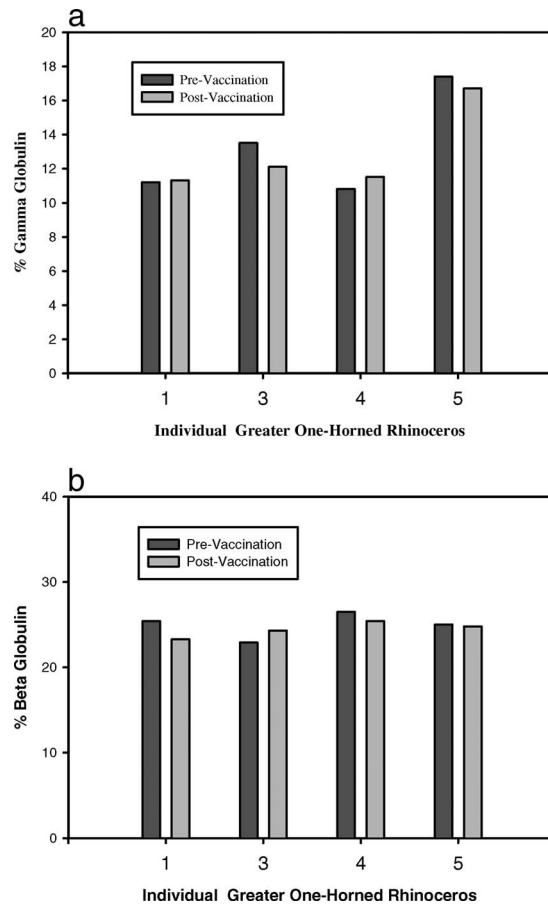
Figure 1. **a.** Changes in gamma in serum protein electrophoresis associated with West Nile virus vaccination by using the commercial equine killed vaccine product. **b.** Beta globulin fractions in serum protein electrophoresis associated with West Nile virus vaccination by using the commercial equine killed vaccine product.

immune response in the GOHs may include ineffectiveness of the killed product, species-specific response to adjuvant, inadequate dosage, and/or frequency of administration. In addition, a delayed immune response might have been missed by the timing of the postvaccination titer measurement.

A sixth GOH not included in the 2005 study was administered the same killed vaccine protocol in 2002 and received boosters with this vaccine annually for 3 yr. At the time of the third booster, pre- and postvaccine neutralizing antibody titers were measured. Although the prevaccination titer was negative (1:20), the postvaccination titer (measured 4 wk after vaccination) was positive at 1:80. This case lends further support to the notion that the killed product does not induce a sustained humoral immune response in this species.
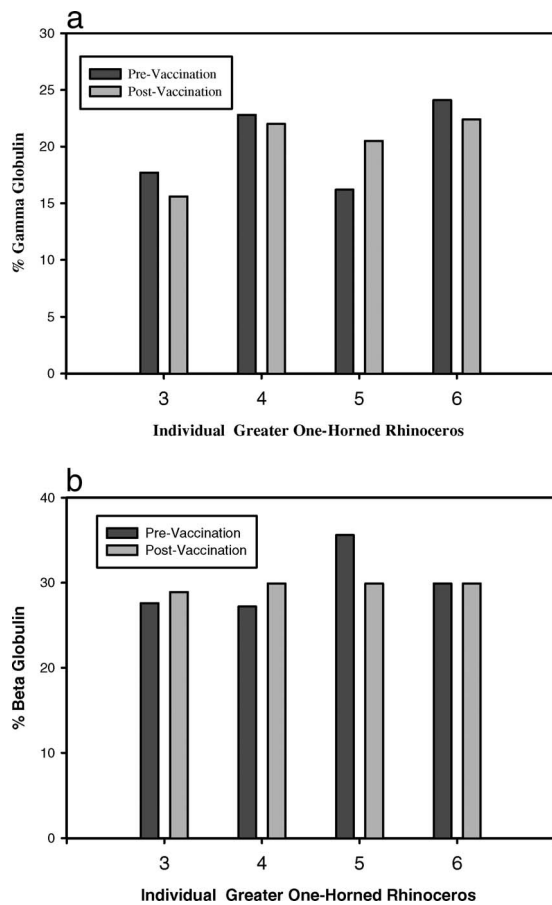
Figure 2. **a.** Changes in gamma in serum protein electrophoresis associated with West Nile virus vaccination by using the commercial recombinant DNA vaccine product. **b.** Beta globulin fractions in serum protein electrophoresis associated with West Nile virus vaccination by using the commercial recombinant DNA vaccine product.

The GOH (rhinoceros no. 5, Table 1) with the strongest response to the recombinant vaccine booster was among the study animals in 2005 and had no antibody response to vaccination with the killed product. The other GOH (rhinoceros no. 6, Table 1) to respond was the rhinoceros previously mentioned, which showed a mild response to its third annual booster vaccination with the killed vaccine product in the previous year. These findings are similar to reports in domestic horses in which booster vaccination by the recombinant vaccine product elicited a stronger anamnestic response as observed by higher antibody titers.[4] Furthermore, the majority of horses vaccinated with the recombinant product produced neutralizing antibody against WNV; however, even horses that did not produce an acceptable antibody titer after vaccina-

tion were protected from the development of viremia when challenged, which may be a result of cell-mediated immunity induced by the vaccine.[10,14] This ability to successfully booster the killed vaccine product with a recombinant product offers a good option for zoologic institutions seeking to change their vaccine strategy.

Determination of antibody production in response to vaccination is a common method for the assessment of vaccine efficacy in nondomestic species, although it does not measure the full response of the immune system and its protection against the virus. Measurement of cellular immunity is another option available for assessing immune response to these vaccines, particularly in nondomestic species in which challenge studies are not an option.[16] Additional measurement of the cellular immune response in this study would have allowed for a more complete assessment of vaccine response and particularly valuable with regard to the recombinant vaccine, which is expected to induce a more balanced immune response. Further investigation of both vaccines is warranted to evaluate the cellular response and whether changes in frequency of administration, dosage, or adjuvant might stimulate an improved humoral response in these animals.

An increase in gamma globulins and beta globulins might be observed on SPE with the production of IgG and the development of acute inflammation respectively, both responses expected with vaccination.[6] The lack of change observed in beta globulins in this study may be because of timing. If an increase in beta globulins were to occur as a result of acute phase protein production, then measurement on SPE shortly after vaccination might have revealed such an increase. Measurement at 4 to 5 wk after vaccination may have allowed any increase in beta globulins to subside to prevaccination levels. Although a mild increase in the percentage of gamma globulins was observed on SPE in one of the GOHs that seroconverted, it is possible that such a change was not observed in the other rhinoceros if the antibody titer was not at a high enough level to reflect the increase in IgG on SPE.

In this trial, vaccination of the GOH against WNV with the killed equine product at manufacturer's recommendations did not incite a measurable humoral immune response. Without the ability to conduct challenge studies in this species to fully assess vaccine effectiveness, there is uncertainty of the protection afforded this species through the use of this vaccine product. Vaccination with the recombinant DNA vaccine product shows some promise in its ability to elicit a measurable humoral

immune response in the GOH. Veterinarians and animal health care staff should consider the overall susceptibility of this species to WNV, the intended effect of the vaccination protocol, and the risks associated with vaccination when determining whether to include vaccination against WNV in the GOH preventative health program.

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