

Results of vaccination of Asian elephants (*Elephas maximus*) with monovalent inactivated rabies vaccine

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Objective—To evaluate the humoral immune response of Asian elephants to a primary IM vaccination with either 1 or 2 doses of a commercially available inactivated rabies virus vaccine and evaluate the anamnestic response to a 1-dose booster vaccination.

Animals—16 captive Asian elephants.

Procedures—Elephants with no known prior rabies vaccinations were assigned into 2 treatment groups of 8 elephants; 1 group received 1 dose of vaccine, and the other group received 2 doses of vaccine 9 days apart. All elephants received one or two 4-mL IM injections of a monovalent inactivated rabies virus vaccine. Blood was collected prior to vaccination (day 0) and on days 9, 35, 112, and 344. All elephants received 1 booster dose of vaccine on day 344, and a final blood sample was taken 40 days later (day 384). Serum was tested for rabies virus–neutralizing antibodies by use of the rapid fluorescent focus inhibition test.

Results—All elephants were seronegative prior to vaccination. There were significant differences in the rabies geometric mean titers between the 2 elephant groups at days 35, 112, and 202. Both groups had a strong anamnestic response 40 days after the booster given at day 344.

Conclusions and Clinical Relevance—Results confirmed the ability of Asian elephants to develop a humoral immune response after vaccination with a commercially available monovalent inactivated rabies virus vaccine and the feasibility of instituting a rabies virus vaccination program for elephants that are in frequent contact with humans. A 2-dose series of rabies virus vaccine should provide an adequate antibody response in elephants, and annual boosters should maintain the antibody response in this species. (*Am J Vet Res* 2006;67:1934–1936)

Rabies vaccination of captive mammals in zoologic collections continues to be controversial because no parenteral vaccines are licensed for use in nondomestic mammals by the USDA and few rabies vaccination trials have been reported in nondomestic large

ABBREVIATIONS	
RFFIT	Rapid fluorescent focus inhibition test
RVNA	Rabies virus–neutralizing antibody
GMT	Geometric mean titer

mammal species.^{1–6} Despite these concerns, the use of killed-rabies virus vaccines has been recommended for many mammals in zoologic collections.^{1,6,7} These vaccination recommendations are based on the assumption that a killed virus vaccine has no risk of inducing an infection and may provide protection against rabies.^{1,5} Most captive mammals in zoos are considered to be at low risk for infection with rabies virus because they are typically managed with a strict quarantine program, are generally isolated from indigenous wildlife, and are observed daily for abnormal behavior. However, exposures in zoologic collections occasionally occur.⁸

Rabies has been occasionally observed in elephants, and 6 cases from Asia have been reported in Asian elephants (*Elephas maximus*).^{9–15} In those elephants, the paralytic form of rabies predominated; however, aggressive behavior has been observed in some cases.^{10,12–14} Most published accounts indicate that rabid domestic dogs were the source of infection and that the dogs had bitten the limbs or trunk of the elephants. In North America, captive elephants can potentially be bitten by rabid wild carnivores (eg, raccoons, skunks, and foxes) while unattended, especially during the night when husbandry practices of leaving food with elephants overnight may attract a potential rabies virus–reservoir species to the elephant-holding facilities. Insectivorous bats could also transmit rabies virus to elephants in North America. In Latin America, vampire bats (*Desmodus rotundus*) have been associated with cattle rabies, and these bats could potentially feed on captive elephants. Therefore, a rabies virus vaccination program for elephants maintained in captivity may prevent infection and provide protection for humans that come in contact with elephants through their vocation or recreational means.

The purpose of the study reported here was to evaluate the humoral immune response of Asian elephants after IM vaccination with either 1 or 2 doses of a commercially available inactivated rabies virus vaccine and evaluate the anamnestic response to a single-dose booster vaccination given on day 344.

Materials and Methods

Sixteen Asian elephants (14 females, 1 sexually intact male, and 1 castrated male) ranging in age from 6 to 48 years old and weighing 1,645 to 4,741 kg were included in this study. Although the rabies vaccination was initiated as part of

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the herd's routine preventative medicine program, the study was preapproved by an institutional animal care and use committee. All elephants were housed in indoor-outdoor enclosures at a private facility in central Florida. The elephants were all in good health determined on the basis of physical examinations and daily observations. Each vaccination dose consisted of 4 mL of a monovalent inactivated rabies virus vaccine⁸ given IM in the left hind limb semimembranosus muscle. The same lot of vaccine was used throughout the study. The dosage selected for elephants was twice the labeled dosage for sheep, cattle, and horses. The elephants were allocated into 2 study groups of 8 elephants each. In group A, each elephant received 1 dose of vaccine on day 0. In group B, each elephant received 1 dose of vaccine on day 0 and a second dose of vaccine on day 9. This interval was selected primarily because the owners requested that the group B elephants not receive the total planned dose at day 0. After approximately 1 year (344 days), an additional dose (4 mL) of vaccine was administered to both groups into the same left hind limb as the original series was given.

Approximately 10 mL of blood was collected with a vacuum tube from the ear vein of each elephant on days 0, 9, 35, 112, 202, 344, and 384. The blood was centrifuged and the serum removed and stored in 1.5-mL cryotubes at -70°C . Rabies virus-neutralizing antibodies were assayed at Kansas State University by use of an RFFIT as described.¹⁶ An endpoint assay was used to determine concentrations of RVNAs, and values were expressed as units per mL. Although an RVNA titer of 0.5 U/mL is considered to be indicative of an adequate immune response after rabies vaccination in humans as well as in dogs and cats exported to rabies-free regions of the world, there is no internationally recognized concentration of RVNAs that is considered acceptable or protective after vaccination for wild animals.¹⁷ For this study, it was assumed that any antibody titer > 0.5 U/mL was indicative of a response to vaccination.¹⁸⁻²¹

Samples with no measurable RVNAs (< 0.05 U/mL) were assigned a value of 0.05 for statistical analysis. Mean antibody titers were determined by use of the GMT. The SD of the GMT and ANOVA analyses were calculated from \log_{10} transformed RVNA titer data.²² A 2-way repeated-measures ANOVA analysis was performed to determine differences in neutralizing antibody titers between the treatment groups and changes in antibody response within groups, compared with baseline values.²³ A value of $P < 0.05$ was considered significant.

Results

Serum samples from day 0 (prevaccination) and day 9 yielded negative results (RVNAs < 0.05 U/mL). By day 35, all elephants in both groups had measurable titers > 0.05 U/mL that ranged from 0.2 to 7.0 U/mL. At day 35, group A had a GMT of 0.44 ± 0.62 U/mL, whereas group B had a significantly greater GMT of 1.11 ± 2.96 U/mL. By day 112, all elephants had a decrease in RVNA titer; titers ranged from 0.05 to 1.5 U/mL. At day 112, group A had a GMT of 0.12 ± 0.36 U/mL, whereas group B had a significantly greater GMT of 0.27 ± 0.28 U/mL. By day 202, all elephants had RVNA titers that were either decreased or remained unchanged and ranged from 0.05 to 0.5 U/mL. At day 202, group A had a GMT of 0.08 ± 0.38 U/mL, whereas group B had a significantly greater GMT of 0.13 ± 0.34 U/mL. Both groups had similar RVNA titers at 344 days, ranging from 0.05 to 0.8 U/mL. At day 384, 40 days after the 1-year booster, group A had a GMT of 2.33 ± 6.10 U/mL and group B

had a GMT of 2.70 ± 2.96 U/mL. Within group A, there was a significant increase from baseline (day 0) only at day 35. In contrast, group B had significant increases from baseline at days 35, 112, and 202. There were no observed injection site reactions or obvious signs of pain from the administration of the vaccine.

Discussion

In this study, all the captive adult Asian elephants produced detectable concentrations of RVNAs by day 35 after an initial vaccination with 1 or 2 doses of inactivated rabies virus vaccine, indicating that a rabies prevention program that includes vaccination is a feasible option to help protect captive elephants from rabies infection. In addition, all elephants that received a booster vaccination on day 344 responded with a strong anamnestic response, similar to the response seen in cattle.²⁴⁻²⁷ Although the vaccinated elephants were not challenged with a virulent rabies virus, detection of an anamnestic response provided an indication of how vaccinated elephants might respond to exposure from a rabid animal by increasing the production of RVNAs and likely increasing their chances of survival.

At day 9, none of the elephants had detectable RVNA titers, suggesting that production of measurable RVNAs within 9 days was limited. In humans, RVNA titers are not detectable until days 10 to 14.²⁸ Group B elephants received a second dose of vaccine at day 9 and therefore received a total of twice the volume of rabies virus vaccine. At day 35, all elephants had detectable RVNAs, with 4 of 8 of the elephants in group A and 7 of 8 in group B having titers ≥ 0.5 U/mL. There were significant differences in GMTs between groups at days 35, 112, and 202. Group B elephants generally had greater RVNA titers, and more elephants maintained titers > 0.5 U/mL for a longer period. Interestingly, the RVNA titers decreased to < 0.5 U/mL in many of the elephants in both groups by day 112. The low and relatively short duration of GMTs > 0.5 U/mL was similar to results reported for other vaccine studies in camels, reindeer, and cattle, in which such titers are nonetheless considered to represent an adequate response to the vaccine.^{3,4,25-27} Unfortunately, variables such as the type of vaccine and initial doses used in those studies make direct comparisons with this study difficult. The low titers in the present study were interesting despite the fact that twice (group A) and 4 times (group B) the labeled dose of vaccine was given. All elephants in this study responded to the vaccine, and the larger dose given to group B generally yielded greater titers than those of group A. Perhaps delaying the second dose in group B until day 35 would have resulted in greater or longer lasting RVNA titers.

The RFFIT is a sensitive and reliable assay that has been used for several decades to measure rabies virus neutralizing antibodies in several species.^{2-4,17,24,25,27-29} Although not antibody class specific, this test primarily detects IgG because it is the principle neutralizing antibody in most species. An RVNA titer cannot be used as direct evidence of the protective effect of a vaccine.³⁰ Only by challenging vaccinated animals with a live virulent rabies virus can the efficacy of a rabies virus vaccine be assessed. Despite the seroconversion

detected in the present study, the possibility of vaccinated elephants contracting and dying of rabies should always be considered.

Captive elephants in North America are common and popular in zoos and circuses. Under typical husbandry practices, many elephants may come in contact with rabid indigenous carnivores or bats, providing an opportunity for exposure and infection. The rationale for vaccinating captive Asian elephants is not simply to prevent the introduction of rabies, but to also protect zookeepers and the public from the dangers of managing these large animals in the event of a neurologic disease. Clinical signs in an infected elephant and in other large herbivores may include abnormal behavior, ataxia, overt aggression, or progressive paralysis that leads to recumbency.^{10,12-14,31-33} Given the rarity of rabies in elephants, the potential for misdiagnosis of rabies is high and may lead to human exposure via infectious secretions during the treatment of affected animals.

No adverse reactions to the vaccine were observed in the elephants included in this study, suggesting that the vaccine is safe in this species. Results indicated that a 2-dose series of a monovalent inactivated rabies virus vaccine should provide an adequate antibody response in elephants, and annual boosters should maintain the antibody response in this species.

a. Imrab Bovine Plus, Merial Inc, Athens, Ga.

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