

VACCINATION OF EGYPTIAN FRUIT BATS (*ROUSETTUS AEGYPTIACUS*) WITH MONOVALENT INACTIVATED RABIES VACCINE

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Abstract: Twenty-six captive, adult Egyptian fruit bats (*Rousettus aegyptiacus*) were tested for the presence of rabies virus neutralizing antibodies (RVNA) using a rapid fluorescent focus inhibition test before and after vaccination. The bats were randomly assigned into three treatment groups: group A ($n = 10$) bats each received one 0.1-ml dose of monovalent inactivated rabies vaccine, group B ($n = 10$) bats each received two 0.1-ml doses of vaccine given 30 days apart, and group C ($n = 6$) bats remained unvaccinated. Plasma was collected from all bats before vaccination and on days 14, 30, 60, and 360. All bats were seronegative before vaccination, and all unvaccinated animals remained negative throughout the study. Rabies virus neutralization titers remained above 0.5 IU/ml from day 30 through day 360 for both vaccinated groups. Group B had significantly higher titers on day 60. This study demonstrated a measurable humoral immune response after vaccination with an inactivated rabies vaccine, with two doses producing a higher level of RVNA. This study confirms the feasibility of a rabies vaccination program for Egyptian fruit bats.

Key words: Egyptian fruit bat, *Rousettus aegyptiacus*, rabies, vaccination, rabies virus neutralizing antibodies.

INTRODUCTION

Vaccination of mammals in zoologic collections has been controversial because no parenteral vaccines are licensed for use in nondomestic mammals by the United States Department of Agriculture, and a few challenge studies have validated the efficacy of rabies vaccination in these species.^{23,29} Despite these concerns, killed rabies vaccines have been recommended for use in most mammals in zoologic collections^{17,23,26,29} based on the assumption that killed vaccines pose no risk of inducing rabies infection and may provide protection after rabies virus exposure. Most mammals in zoos are probably at low risk for rabies infection because they are typically managed with a strict quarantine program, are generally isolated from indigenous wildlife, are observed daily for abnormal behavior, and are monitored for rabies by routine necropsies. However, occasional exposures occur.¹⁸ Established captive populations of bats, including Egyptian fruit bats (*Rousettus aegyptiacus*), meet these criteria and are susceptible to rabies infection, yet few zoo-

logic parks have rabies vaccination protocols in place for bats.¹⁵

The order Chiroptera includes bats and consists of two suborders, the Megachiroptera and Microchiroptera.^{15,20} Unlike the New World insectivorous and hematophagous microchiropteran bats, the megachiropterans have not been recognized as significant reservoirs for classic rabies.^{10,20,31} However, rabies and other lyssaviruses have been documented in both wild and captive megachiropterans.^{1,3,6,10,11,13,15,20,21,25,27,29,33,34,37} Therefore, a rabies vaccination program for captive bats should be an important public health measure for zoo veterinarians to consider. Unfortunately, routine vaccination can be complicated by the fact that several species of bats have demonstrated variable rabies incubation periods and may survive infection.^{3,5,10,20,36} This has led some veterinarians to falsely believe that individual bats can maintain rabies within a captive population and that captive bats should not, therefore, be vaccinated for rabies. However, new information shows that the clinical course of rabies in bats and carnivores differs very little, and that bats are not more susceptible to rabies or able to maintain an infection compared with carnivores.^{24,31} In nonhibernating bats, the incubation period for rabies is neither longer nor more variable than in carnivores.²⁴ A productive rabies infection is usually fatal to bats, as it is in carnivores.³¹ As in other mammals, there is also little evidence of a carrier state, where clinically normal bats persistently shed virus.^{24,40} Rabies vaccination protocols for bats should not, therefore, differ from those for other mammalian species in zoologic collections.

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This study evaluated the humoral immune response of Egyptian fruit bats after a primary i.m. vaccination with a commercially available inactivated rabies vaccine and the anamnestic response to a single-dose booster vaccination 30 days later.

MATERIALS AND METHODS

Twenty-six captive, adult Egyptian fruit bats (13 males and 13 females), weighing 110–178 g, were studied. All bats were housed in indoor or outdoor enclosures at a private facility in north-central Florida and were in good health, based on physical examinations and daily observations. Individual bats were uniquely identified and segregated by sex into two separate enclosures for the duration of the study.

The animals were randomly divided into three study groups. In group A, five male and five female bats each received 0.1 ml of a monovalent inactivated rabies vaccine (Defensor 3, Pfizer Inc., Animal Health, Exton, Pennsylvania 19341, USA) i.m. on day 0. In group B, five male and five female bats received 0.1 ml of the same vaccine i.m. on day 0 and then again on day 30. Group C, containing three males and three females, included unvaccinated controls. To facilitate handling, bats from all three groups were induced and maintained on oxygen and isoflurane gas (IsoFlo®, Abbott Laboratories, North Chicago, Illinois 60064, USA) administered by mask during vaccination and blood sampling. Bats in groups A and B received i.m. injections in the right biceps brachii muscle.

One milliliter of heparinized blood was collected from the medial saphenous vein of each bat on days 0, 14, 30, 60, and 360 after vaccination. The blood was centrifuged, and the plasma samples were removed and stored at -20°C . Rabies virus neutralizing antibodies (RVNA) were assayed at Kansas State University (Mosier Hall, Manhattan, Kansas 66506), using a rapid fluorescent focus inhibition test (RFFIT) as previously described.³⁵ An endpoint assay was used to determine RVNA titers expressed as international units per milliliter. Although an RVNA titer of 0.5 IU/ml or greater indicates an adequate immune response after rabies vaccination in humans as well as in dogs and cats being exported to rabies-free regions of the world, there is no internationally acceptable protective level of RVNA after vaccination for wild animals.³ Therefore, we assumed that any antibody titer equal to or above 0.5 IU/ml in our bats indicated a response to vaccination, and any titer less than 0.5 IU/ml indicated a negative response.^{2,7,9,41}

Samples with no measurable RVNA (<0.05 IU/ml) were assigned a value of 0.05, and samples

with titers >60.9 were assigned a value of 60.9 for statistical analysis. Mean antibody titers were determined using the geometric mean. The standard deviation of the geometric mean was calculated from \log_{10} -transformed RVNA titer data.³⁰ Differences in neutralizing antibody titers between the vaccinated treatment groups were compared using an unpaired, two-tailed *t*-test, assuming unequal variances at each time interval after day 0. To maintain an overall experiment-wise *P* value of 0.05, Bonferroni's approach was used to obtain a pairwise comparison *P* value of 0.0125 for statistical significance.³²

RESULTS

Plasma samples from day 0 (prevaccination) were all negative for RVNA as were all plasma samples from the bats that remained unvaccinated throughout the study (group C). By day 14, all but one of the vaccinated bats had detectable antibody titers that ranged from 0.5 to 2.4 IU/ml. On day 14, group A had geometric mean titer (GMT) of 0.72 ± 0.30 IU/ml, whereas group B had GMT of 1.76 ± 1.86 IU/ml. By day 30, all vaccinated bats' titers increased to 2.4–36.5 IU/ml. On day 30, group A had GMT of 4.48 ± 2.00 IU/ml, whereas group B had GMT of 7.74 ± 2.17 IU/ml. The bats in group B were given their second vaccination after the day 30 samples were collected. On day 60, group A (one vaccination) had RVNA titers that increased only slightly to 2.2–12.2 IU/ml. All bats in group B on day 60 had increased RVNA titers by at least twofold as compared with day 30, to 12.2 to >60.9 IU/ml, and were significantly higher than in group A ($P < 0.0125$). On day 60, group A had a GMT of 5.05 ± 2.29 IU/ml, whereas group B had a GMT of 47.58 ± 1.16 IU/ml. All bats in groups A and B maintained a measurable titer through day 360 (2.6–30 IU/ml). On day 360, group A had a GMT of 7.27 ± 2.14 IU/ml, whereas group B had a GMT of 8.83 ± 2.13 IU/ml (Fig. 1). Throughout the study, there was no observed morbidity or mortality produced from the vaccine.

DISCUSSION

The adult Egyptian fruit bats produced high levels of RVNA when injected with inactivated rabies vaccine, indicating that a rabies vaccination program including parenteral vaccination is a feasible option for megachiropteran bats housed in zoologic parks. In addition, all bats that received a booster vaccination on day 30 responded with a classic anamnestic response, suggesting that previously vaccinated bats have the ability to respond immunologically in the event that a rabies exposure should

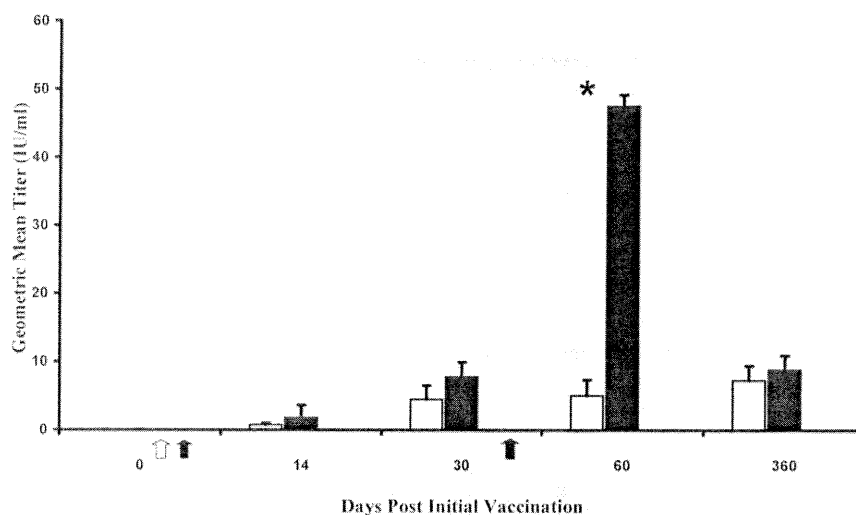


Figure 1. GMT of RFFIT (IU/ml) response to 0.1 ml intramuscular injection of a monovalent inactivated rabies vaccine (Defensor 3, Pfizer Inc.) in group A (one vaccination, white bars) and group B (two vaccinations, black bars) in captive Egyptian fruit bats (*Rousettus aegyptiacus*). The white arrow indicates time (day 0) vaccine was given to group A. The black arrows indicate time (days 0 and 30) vaccines were given to group B. *indicates GMT that are significantly ($P < 0.0125$) different between treatment groups on day 60.

occur. Although the vaccinated bats included in this study were not challenged with a virulent rabies virus, the demonstration of an anamnestic response in this study provides a model of how vaccinated bats might respond to an exposure from a rabid animal by increasing the production of RVNA.

By day 30 after vaccination, all vaccinated bats had detectable antibody levels above 0.5 IU/ml and maintained a high level of RVNA for at least 1 yr after primary vaccination. The production of RVNA and evidence of prolonged detectable titers present in the bats included in this study are similar to those in previously published reports in other species.^{8,12,16,28,30}

The study population had no detectable rabies antibody titers on day 0, indicating that they were probably not exposed to rabies. The control bats had no detectable antibody titers throughout the study, suggesting that rabies virus had not entered the population during the study period. If a zoologic facility decides to establish a rabies vaccination program for megachiropteran bats, blood samples should be obtained before implementation to ensure that no bat has a preexisting titer that could identify prior exposure to rabies lyssavirus in the population.

The RFFIT is sensitive and reliable and has been used for several decades to measure the presence of rabies antibody in several species.^{3,14,19} Although a good predictor, the presence of RVNA cannot be used as direct evidence of a protective effect of a

vaccine.⁴ Only by challenging vaccinated animals with a live virulent rabies virus can the efficacy of a rabies vaccine be assessed in animals. Efficacy studies have been conducted in vampire bats (*Desmodus rotundus*), skunks (*Mephitis mephitis*), and raccoons (*Procyon lotor*).^{2,28} These studies showed that RVNA titers of >0.5 IU/ml were generally associated with higher rates of survival after exposure and that vaccination is a feasible method to prevent rabies.

The rationale for vaccinating animals in an established rabies-free zoologic collection is to help prevent the introduction of rabies. Even in "closed" collections, megachiropteran bats may come in contact with indigenous bats and thus be exposed to rabies. In addition, rabid native wildlife have been reported to have entered "secure" enclosures,¹⁸ and there has been at least one instance of European bat lyssavirus 1 transmission into a captive population of Egyptian fruit bats, probably from a wild European microchiropteran species.^{27,39}

Vaccination may serve as a management tool to decrease the risk of rabies introduction into an established collection, but it cannot definitively prevent infection,³⁰ and vaccinated animals might contract rabies and die. All dead bats should be necropsied and tissues specifically examined for evidence of rabies virus. Bats and carnivore species should not have direct physical contact with the public.²² All personnel with captive mammal contact should receive preexposure prophylaxis as rec-

ommended for those with frequent potential rabies exposure.³⁸ In addition, animal inflicted injuries from vaccinated captive mammals should be reported to the responsible health office immediately, and each exposure should be investigated individually.⁹

Egyptian fruit bats developed an immunologic response to the commercially available rabies vaccine, which we studied without adverse effects. A two dose series with rabies vaccine should provide an antibody response in this bat species similar to that presumed to occur for carnivores currently being vaccinated in zoologic collections.^{17,26}

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