RESPONSE TO VACCINATION WITH A COMMERCIAL INACTIVATED RABIES VACCINE IN A CAPTIVE COLONY OF BRAZILIAN FREE-TAILED BATS (TADARIDA BRASILIENSIS)

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Abstract: A captive colony of Brazilian free-tailed bats (Tadarida brasiliensis) was vaccinated with a commercial monovalent inactivated rabies virus (RABV) vaccine (RABVAC 1). Baseline rabies virus neutralizing antibodies (VNA) and the response to vaccination were measured in 50 bats. Rabies VNA was detected in the plasma of 64% (27/42) of bats that had been vaccinated 1 yr prior, but only 19% (8/42) had levels considered adequate. Rabies VNA was detected in the plasma of 63% (5/8) of bats with no record of previous vaccination, suggesting natural RABV exposure before captivity. All bats demonstrated a VNA response by 10 days postvaccination, and baseline titer significantly predicted humoral response to vaccination. No adverse reactions to vaccination or clinical signs of RABV infection were observed in the bats during a 6-mo observation period. Annual vaccination may maintain immunity against RABV infection in captive colonies of bats.

Key words: Bat, rabies virus, Tadarida brasiliensis, vaccination, virus neutralizing antibodies.

BRIEF COMMUNICATION

Bats are enzootic reservoirs of rabies and related lyssaviruses worldwide. The loss and degradation of suitable habitats for many bat species have led to increased occupancy of and reliance on artificial roosts, such as buildings and bridges.10,11 Occupancy of roosts in urban and suburban areas may lead to increased contact with humans and domestic animals, which can lead to intentional or unintentional injury of bats. Hundreds of bats are typically submitted to wildlife rehabilitators each year in the United States, and care may be provided for periods of weeks or months. However, in cases of serious injury, rehabilitation may last indefinitely. Long-term care of colonies of sick or injured (i.e., immune-compromised) animals is a serious concern for rehabilitators, because these animals may be more susceptible to various pathogens, including RABV,18,19 and many published reports document cases of bats in incubation phase of rabies infection upon arrival to a facility.3,6,7,15–17

Although pre-exposure prophylaxis against RABV is recommended for wildlife rehabilitation personnel,12 there are no currently licensed RABV vaccines for parenteral use in wildlife,4 and few reports on the safety or immunogenicity of monovalent commercial inactivated RABV vaccines for use in pre-exposure prophylaxis of exotic animals, e.g., bats in captivity.13 However, given the potential for RABV exposure,6,15,17 and in addition to strict quarantine guidelines, it is recommended that captive wildlife in exhibits, zoos, and rehabilitation or research facilities be vaccinated with a commercial licensed monovalent inactivated RABV vaccine.4 Vaccination of bats in captive colonies may provide immunity against RABV infection in large groups of potentially immune-compromised animals and can also limit transmission from infectious animals that enter a facility, because RABV-infected animals receiving prophylaxis may exhibit acute morbidity.14

This study evaluated the safety and efficacy of a commercial monovalent inactivated RABV vaccine in eliciting a rabies virus neutralizing antibody (VNA) response in a captive colony of Brazilian free-tailed bats (Tadarida brasiliensis) under rehabilitative care. All sample collection procedures were compliant with the University of Tennessee Institutional Animal Care and Use Committee. On July 9 and July 10 2005, baseline blood samples were taken from a captive colony of 50 Brazilian free-tailed bats under care at a coauthor’s (BAF) rehabilitation facility in Austin, Texas. A baseline sample (80–100 μl) of whole blood was collected in sterile heparinized
microcapillary tubes following aseptic preparation and puncture of a peripheral wing vein. Blood was immediately centrifuged, and plasma was separated and stored at −20°C. Immediately following baseline blood draw, all bats were vaccinated subcutaneously with 0.05 ml of a commercial monovalent inactivated RABV vaccine (RABVAC 1; Fort Dodge Animal Health, Overland Park, Kansas 66225, USA). Ten days after vaccination (July 19 and July 20 2005), blood samples were taken again from all bats. All bats were observed for 6 mo following vaccination and monitored for any adverse reactions or clinical signs of RABV infection.

A modified rapid fluorescent focus inhibition test (RFFIT),9,20 by using challenge virus standard (CVS-11, V399), was used to assay for rabies VNA in plasma of individual bats. Rabies VNA endpoint titers of individual bats were calculated, except where titers exceeded the upper limit of estimation in the assay (rabies VNA > 22.6 IU/ml). Titers at the upper limit of estimation were considered as endpoints for the purpose of conservative mean calculations. All titers were converted to international units (IU/ml) by comparison with standard rabies immune globulin control containing 2 IU/ml. Titers less than 0.06 IU/ml were considered negative for the presence of rabies VNA. Titers greater than 0.5 IU/ml were considered to be adequate humoral immune induction.23 The response to vaccination was estimated as the difference between baseline and postvaccination rabies VNA titers. Nonparametric analyses were performed using SAS version 9.1 (SAS Institute, Cary, North Carolina 27513, USA), as baseline and response rabies VNA titers among bats were not normally distributed (Shapiro–Wilk test, P < 0.01). Spearman’s rank-order correlation was used to test for association between baseline and response rabies VNA titers among all bats (n = 50). Mean rabies VNA titers (± SD) are reported.

Eight bats from the wild had no previous record of vaccination while being housed at the rehabilitation facility, but 63% (5/8) had detectable rabies VNA before vaccination. Twenty-four male and 18 female free-tailed bats were vaccinated using a similar protocol at the same facility in the previous year (July 2004). Of these, 64% (27/42) had detectable rabies VNA in circulation immediately before the July 2005 vaccination (i.e. >0.06 IU/ml), but only 19% (8/42) had titers considered adequate (i.e., >0.5 IU/ml).23 The mean baseline titer for rabies VNA among the 42 previously vaccinated bats was 0.20 IU/ml (±0.24; range, 0.02–1.00 IU/ml). Regardless of prior vaccination status, all of the bats in this study (50/50) demonstrated a VNA response within 10 days postvaccination; and 90% (45/50) had titers that were considered adequate humoral induction (i.e., >0.5 IU/ml).22 Baseline VNA titer was a highly significant predictor of response to vaccination among all bats (50/50) (Spearman ρ = 0.71, P < 0.0001; Fig. 1). No adverse reactions or clinical signs of RABV infection were observed in the vaccinated bats during the 6-mo observation period following vaccination. The mean VNA response at 10 days postvaccination was 7.53 IU/ml (±9.33; range, 0.21–22.6 IU/ml) in previously vaccinated bats, although 26% (11/42) of these bats had postvaccination titers that were at the upper limit of estimation (i.e., >22.6 IU/ml). For the eight bats with no previous record of vaccination, the mean VNA response at 10 days postvaccination was 9.42 IU/ml (±10.81; range, 0.16–22.5 IU/ml), although the three bats without previous vaccination or detectable VNA before vaccination demonstrated a mean VNA response of 1.04 IU/ml (±0.92; range, 0.16–1.99 IU/ml).

This study demonstrates that subcutaneous vaccination of Brazilian free-tailed bats with a commercial monovalent inactivated RABV vaccine is safe, and effective in eliciting a VNA response. None of the bats demonstrated adverse reactions to vaccination, and none developed clinical RABV infection throughout the course of the study. Although vaccine efficacy was not tested, postvaccination VNA titers in the majority of bats (90%) exceeded the level that is accepted as evidence of immune induction (i.e., >0.5 IU/ml).22 These results agree with a previous RABV vaccination study in Egyptian fruit bats (Rousettus aegyptiacus),13 where no animals developed visible adverse reactions or clinical signs of RABV infection, and all bats demonstrated a humoral VNA response. A strong correlation between baseline VNA titer and response to vaccination suggests that immunologic memory may play an important role in the response to RABV exposure. Evidence of natural exposure to RABV in the captive bats without record of vaccination is consistent with previous accounts of moderate to high rabies VNA seroprevalence in natural colonies of Brazilian free-tailed bats despite observed low (<1%) prevalence of clinical infection.2,21 However, given the dynamic nature of rabies VNA titers, the absence of rabies VNA in bats captured from the
Although it has been demonstrated that Egyptian fruit bats maintain adequate titers for up to 1 yr following intramuscular vaccination,\textsuperscript{13} the results of this study suggest that humoral immune response following subcutaneous administration may differ qualitatively from responses induced by intramuscular vaccination. Alternatively, anamnestic VNA response may be different compared with the response of naïve animals. Experimental infection studies are necessary to compare the efficacy of different routes of vaccine delivery in bats and to investigate the relationship between VNA titer and protection against RABV infection. Oral administration of recombinant RABV vaccines in vampire bats (\textit{Desmodus rotundus})\textsuperscript{1,2} has suggested a correlation between short-term vaccine immunogenicity and protection against RABV infection. However, as VNA titers of bats are dynamic through time,\textsuperscript{9,13,17} it is unclear whether bats with previous exposure to live or inactivated RABV would be susceptible to subsequent infection in the absence of detectable VNA.

Similar to recommendations for domestic animals,\textsuperscript{4} the results of this study suggest that it would be prudent to administer yearly rabies vaccine boosters to maintain immunity against RABV infection in captive colonies of bats. Such practice may limit the spread of infection among bats in captivity, thereby also reducing the risk of transmission to personnel that have close contact with captive animals. However, pre-exposure prophylaxis for such personnel would be recommended without regard to the vaccination status of the animals in such colonies, as would relevant postexposure recommendations. Such recommendations may be of limited safeguard in other parts of the world (e.g., Asia and Africa) where RABV-specific VNA offers little or no cross-reactivity against bat lyssaviruses, such as Lagos Bat Virus and West Caucasian Bat Virus.\textsuperscript{8}

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