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# VACCINATION OF VAMPIRE BATS USING RECOMBINANT VACCINIA-RABIES VIRUS

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ABSTRACT: Adult vampire bats (*Desmodus rotundus*) were vaccinated by intramuscular, scarification, oral, or aerosol routes ($n=8$ in each group) using a vaccinia-rabies glycoprotein recombinant virus. Sera were obtained before and 30 days after vaccination. All animals were then challenged intramuscularly with a lethal dose of rabies virus. Neutralizing antirabies antibodies were measured by rapid fluorescent focus inhibition test (RFFIT). Seroconversion was observed with each of the routes employed, but some aerosol and orally vaccinated animals failed to seroconvert. The highest antibody titers were observed in animals vaccinated by intramuscular and scarification routes. All animals vaccinated by intramuscular, scarification, and oral routes survived the viral challenge, but one of eight vampire bats receiving aerosol vaccination succumbed to the challenge. Of 31 surviving vaccinated and challenged animals, nine lacked detectable antirabies antibodies by RFFIT (five orally and four aerosol immunized animals). In contrast, nine of 10 non-vaccinated control bats succumbed to viral challenge. The surviving control bat had antiviral antibodies 90 days after viral challenge. These results suggest that the recombinant vaccine is an adequate and safe immunogen for bats by all routes tested.

*Key words:* Rabies, recombinant vaccinia-rabies, vaccination, vampire bats.

## INTRODUCTION

Rabies is a fatal viral encephalitis that affects wild and domestic homeothermic species, including humans (Soulebot et al., 1982). While rabies in domestic animals has been controlled in industrialized countries by parenteral vaccination (Acha and Arambulo, 1985), rabies in wildlife has been more difficult to control and has remained an important public health problem (Brochier et al., 1989). Even countries that have successfully controlled domestic animal rabies still face the threat of rabies virus reservoirs in wild animals such as red foxes (*Vulpes vulpes*) in Europe, or raccoons (*Procyon lotor*) and bats in North America (Toma and Andral, 1977; Rupprecht et al., 1988; Pastoret et al., 1995). Strategies for control of sylvatic rabies have been concentrated on development of practical methods of oral vaccination of wild rabies reservoir species. Oral bait vaccination of foxes led to virtual elimination of sylvatic rabies in Europe (Brochier et al., 1991).

Bats are frequent lyssavirus hosts, and insectivorous bats play an important role in the epidemiology of rabies and rabies-like lyssaviruses (Burns and Farinacci, 1955; Baer and Smith, 1991). Twenty-one of 36 cases of human rabies reported in the United States since 1980 were associated with nonhematophagous bats. Although *Eptesicus* spp. and *Myotis* spp. are the most common insectivorous bat species associated with rabies, 15 (71%) of 21 human cases were associated with silver-haired bats (*Lasionycteris noctivagans*; Morimoto et al., 1996; Noah et al., 1998; Haupt, 1999). In contrast, in Latin America, a single species of hematophagous bats, the common vampire (*Desmodus rotundus*), is the primary wild host and vector affecting human beings and domestic animals. Since 1975, 500 cases of human rabies associated with vampire bat species were reported in Latin America. These



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bats are associated with about 100,000 cases of bovine rabies each year (Acha and Arambulo, 1985; Flores Crespo, 1991; Pan-American Health Organization, 1991; Navarrete Navarro et al., 1999). Systematic elimination of vampire bats is unfeasible, as was demonstrated in a project using anticoagulants (Acha and Malaga Alba, 1988; Lord, 1988) and would also affect beneficial bats. An alternative strategy, such as vaccination (Lord, 1988), that would respect the ecologic role of bats, appears more attractive (Aguilar-Setién et al., 1998).

Insertion of a glycoprotein rabies virus gene in vaccinia virus enhances vaccine efficacy (Brochier et al., 1989, 1991; Pastoret et al., 1995). Rabies virus glycoprotein gene insertion removes the thymidin kinase gene from the vaccinia virus vector rendering it less pathogenic than its predecessor (Kieny et al., 1984; Boulanger et al., 1995). Recombinant vaccinia–rabies glycoprotein (V-RG) vaccine is immunogenic, stable, and safe, although an incidental, non-fatal case of human infection due to exposure to recombinant vaccinia-rabies glycoprotein virus was recently reported (Rupprecht et al., 2001). Vaccination with V-RG, if effective, could help control rabies and reduce human and animal mortality. We tested immunogenicity and safety of V-RG vaccine when delivered by different routes to common vampire bats.

## MATERIALS AND METHODS

### Diagnosis of rabies

Diagnosis of rabies was established in brain smears by fluorescent antibody testing (FAT) as recommended by the World Health Organization (Dean and Abelseth, 1973).

### Animals

Common vampire bats (weight 30–45 g) were captured from shelters located in Tehuacán (18°33′00″N, 97°52′38″W) and Taxco (18°34′05″N, 99°37′23″W), Mexico. Bats were confined in biosafety level II security housing at constant temperature (23±2.5 C) and 70% relative humidity. All bats went through a 30-day captivity-habituation period prior to the study. Of 90 seronegative bats, 20 died within the first 11 days of captivity. None of these animals had rabies. The rest adapted well. Eleven pregnant bats were excluded from the study. Four gave birth and their offspring were used to test vaccine safety. Bats were fed defibrinated blood from healthy cattle and pigs. Fifteen-20 ml of blood (with multivitamin, Clusivol®, Wyeth, S. A. de C. V., Mexico), 1.5 ml/l added) in 200 ml bottles were provided daily to each bat.

### Vaccine

V-RG vaccine (VVTGgRAB-26D3), the result of combining vaccinia modified-virus (Copenhagen strain) with rabies virus (ERA strain) glycoprotein, was employed (Pincali and Paoletti, 1982; Kieny et al., 1984). Vaccine (lot 5L24, manufactured on June 15, 1987, containing $10^8$ cell culture infecting doses/ml), was reconstituted as previously described (Aguilar-Setién et al., 1998).

### Vaccination protocol

Four groups comprising eight adult vampires each were vaccinated as follows: group A, intramuscular (IM); group B, scarification; group C, oral; and group D, aerosol. Group E consisted of 10 control animals (PBS injected). For groups A and B, lyophilized V-RG vaccine was suspended in 1 ml of distilled water. Group A animals were injected IM once in the dorsal muscle at the level of the scapular cartilage with 0.25 ml of V-RG vaccine. In group B, one drop (0.1 ml) of vaccine was applied once to a 1.5×1.5 cm scarified area in the ventral skin of the right wing. In group C, lyophilized V-RG vaccine ($10^8$ $CCID_{50}$) was resuspended in one ml of defibrinated bovine blood lacking rabies antibodies (Smith et al., 1973), and after 48 hr fasting, 1 ml blood was delivered once directly into the bat's mouth with a needle-free syringe. In group D, lyophilized V-RG vaccine ($10^8$ $CCID_{50}$) was first resuspended in 1 ml of distilled water and then diluted in 20 ml of phosphate buffered saline (PBS, pH 7.4). This material was placed in a nebulizer (DeVilbiss R, Ohio, USA, particle size 3–5 μm) connected to a hermetic plastic chamber (30×40×15 cm) with a hose, the latter connected to a chlorine trap. Bats were placed in the chamber and exposed to a 15 min nebulization, during which 5 ml of diluted V-RG vaccine was consumed by the nebulizer. The adult control bats received 0.25 ml PBS IM 30 days prior to viral challenge.

Two of four nursing 1 wk old vampire bats were inoculated IM with one V-RG vaccine

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TABLE 1. Seroconversion (antibodies detected in adult vampire bats by rapid fluorescent focus inhibition test) 30 days after vaccination with recombinant V-RG vaccinia rabies virus vaccine.

| Vaccination route (group) | Antibody titer (IU)[a] in individual animals | | | | | | | | | | Mean ±SE | *P* vs group E |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | | |
| Intramuscular (A) | ≥10 | 4 | 4 | 4 | 4 | 3 | 2 | 2 | — | — | 4.12±2.37 | <0.0001 |
| Scarification (B) | ≥10 | ≥10 | 4 | 4 | 4 | 3 | 2 | 2 | — | — | 4.87±3.1 | <0.0002 |
| Oral (C) | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | — | — | 0.37±0.48 | NS[b] |
| Aerosol (D) | 3 | 1 | 0.5 | 0.5 | 0 | 0 | 0 | 0 | — | — | 0.63±0.96 | NS |
| Control (E) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | — |

[a] International units.
[b] Not significant.

dose ($10^4$ $CCID_{50}$). These bats were not challenged with rabies virus.

### Serology

Blood was drawn from the marginal vein of the forearm membrane and serum obtained by centrifugation. All animals were bled the day before and 30 days after vaccination (Aguilar-Setién et al., 1998). A surviving bat from each group was also bled at the end of the experiment. Neutralizing antibody titers against rabies virus were measured by the rapid fluorescent focus inhibition test (RFFIT, sensitivity 0.25 IU/ml; Smith et al., 1973) and were expressed as international units per ml (IU/ml) when compared with a standard reference serum. An antibody concentration of 0.5 IU ml is considered protective in humans (Smith et al., 1973).

### Virus challenge

Animals were challenged 31 days after vaccination or PBS administration. The rabies virus variant CASS88, isolated in 1988 from a rabid vampire bat (Instituto Nacional de Investigaciones Forestales y Agropecuarias, Mexico City, Mexico), was employed (Cuevas Romero et al., 1989). The genome of this variant virus was characterized as a vampire bat strain, and its lethality by the IM route for adult vampire bats established in the same work (Aguilar-Setién et al., 1998). Virus challenge consisted of a single $10^6$ mouse intracerebral 50% lethal dose (MICLD50, 21-day old BALB/C mice) 4 mm deep IM injection in the muscle at the site of the scapular cartilage (Aguilar-Setién et al., 1998). Animals were observed daily to assess their health (depression, incoordination, tremor, and blood consumption) for 90 days after viral challenge, and those surviving this period were euthanized with ether and autopsied (Commission of Life Sciences, 1996).

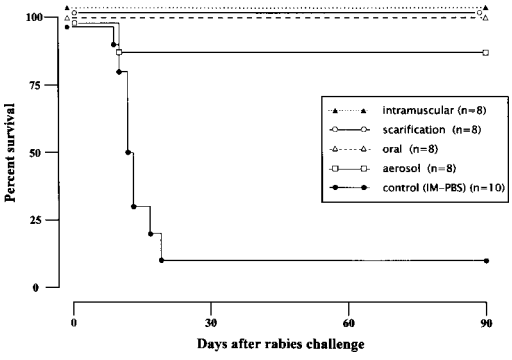



FIGURE 1. Kaplan-Meier survival rates of vaccinated and non-vaccinated (control) vampire bats.

### Statistical analysis of results

Survival rates between the control group E, and the vaccinated groups were analyzed by Kaplan-Meier test and differences in antibody levels between groups were statistically compared using the non-parametric Mann–Whitney test (Dawson-Saunders and Trapp, 1994).

## RESULTS

Seroconversion rates 31 days after vaccination are shown in Table 1. Seroconversion rates were significant ($P<0.0002$) only in groups A (IM) and B (scarification), when compared to control group E. Orally and aerosol-vaccinated animals seroconverted less vigorously than animals vaccinated IM or by scarification (Table 1).

Protection against viral challenge is shown in Figure 1. All vaccinated animals survived the rabies virus challenge ($P<0.0001$), except for a single aerosol-vaccinated bat that lacked detectable an-

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tibodies. Nine of 31 surviving vaccinated bats lacked detectable antirabies antibodies by RFFIT (five orally and four aerosol immunized) when tested 30 days post-vaccination. Nine (90%) of 10 control bats succumbed to viral challenge. Sera obtained from one bat in each group, 90 days after viral challenge had ≥10 IU of antirabies antibodies.

No clinical signs attributable to vaccination were observed except few small crusts at the scarification site found in group B. None of the rabid vampire bats exhibited aggressive behavior (i.e., threatening attitudes, biting between infected animals, etc.). Clinical signs of anxiety, altered reflexes, tremor, and paralysis were observed 72–24 hr before death occurred. All animals alive 90 days after challenge appeared healthy and were feeding normally (15–20 ml/day). No evidence of rabies was found by FAT at autopsy 90 days after challenge.

## DISCUSSION

With the exception of the single aerosol vaccinated bat, all vaccinated animals survived the rabies virus challenge. Not all surviving animals had detectable anti-rabies antibodies by RFFIT 30 days post-vaccination. As expected, the IM and the scarification routes were more effective in leading to seroconversion than the more practical oral and aerosol routes. Thus, the recombinant V-RG vaccine appears to protect most vampire bats against experimental rabies irrespective of the immunization route employed.

Conventional rabies vaccines may be pathogenic for small mammals (Artois et al., 1992). Our results confirm efficacy and safety of V-RG vaccine when applied to adult and nursing hematophagous bats (Brochier et al., 1989).

An antibody titer of 0.5 IU ml is indicative of successful rabies immunization in humans (Smith et al., 1973). Protective levels may be lower in vampire bats when using V-RG vaccine, however, because protection was found even in bats with undetectable antibody levels. Cellular immunity and/or natural immune mechanisms may be important in rabies virus infection (Medzhitov and Janeway, 2000) and must therefore be studied in these animals. Absence of antibodies prior to viral challenge appears to be dependable evidence of lack of exposure to rabies virus, but does not constitute absolute proof that an animal had no prior exposure to rabies virus (Prabhakar and Nathanson, 1981). We speculate that the single surviving bat in the control group developed protective antirabies antibody as a result of an unexpected booster effect. On the other hand, protection in the absence of antibody has been reported in foxes vaccinated orally with V-RG vaccine (Pastoret et al., 1992).

Because some bats without antibodies were protected against rabies virus challenge, evaluation of vaccine efficacy in the field will have to rely on epidemiologic and demographic rather than serologic evidence.

Our data support previous reports that bats are more resistant to rabies (and other lyssaviruses) than species such as dogs and foxes; $10^3$ MICLD50 of the "vulpine" homologous rabies virus variant is capable of killing 80% of red foxes (Blancou et al., 1979) whereas $10^6$ MICLD50 of CASS88 "vampire bat" homologous variant is required in order to kill 80% of vampire bats (Moreno and Baer, 1980; Aguilar-Setién et al., 1998; McColl et al., 2000).

A preliminary study reported V-RG vaccine in bats produced a short-term seroconversion around 18–30 days post-vaccination (Aguilar-Setién, 1998). This measured antibodies 30 days after vaccination. In the future, antibody levels and protection beyond 30 days after vaccination should be studied, in order to establish duration of immunity, as well as to evaluate the need for boosters.

Vaccination of hematophagous bats could increase the epizootic threshold for rabies propagation (Anderson, 1982), and result in the need for removal of fewer an-

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imals in traditional rabies control campaigns based on reduction of species populations. Lord (1988) recommended vampire vaccination as a suitable method for the control of rabies because: "*. . . an immunized animal is doubly valuable because it not only cannot maintain the epizootic, but also because it continues to occupy its habitat niche, defending it against invaders . . .*". Even if vampire rabies is controlled, the possibility remains that the virus will find other susceptible bat species. Therefore, attractive as vampire bat vaccination appears to be, questions such as bat population dynamics, intra/interspecies rabies dissemination within Chiroptera, and rabies strain variation that may limit vaccination efficacy, remain unanswered.

Aerosol vaccination in shelters shared by various bat species may be a convenient strategy for reducing transmission of rabies to the other species. A combined strategy of vaccination and limited population reductions, such as was applied to foxes by Bogel et al. (1981), may lead to control of bat-transmitted rabies.

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