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Indirect oral immunization of captive vampires, *Desmodus rotundus*

Marilene F. Almeida [a,c,*], Luzia F.A. Martorelli [c], Caroline C. Aires [d], P.C. Sallum [e], Eduardo Massad [a,b]

[a] *School of Medicine, The University of São Paulo, LIM01 HCFMUSP, São Paulo, Brazil*
[b] *Department of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, London, UK*
[c] *Laboratory of Rabies, Zoonosis Control Center of São Paulo Municipality, São Paulo, Brazil*
[d] *Zoology Museum of São Paulo University, CP 42694, CEP 04299-970 São Paulo, Brazil*
[e] *Center of Control of Zoonosis of Guarulhos, São Paulo, Brazil*

Abstract

A vaccinia-rabies glycoprotein recombinant virus (V-RG) vaccine was tested in hematophagous bats (*Desmodus rotundus*) kept in captivity. The vaccine was applied in a neutral vehicle (Vaseline) spread on the back of one or two vector bats, which were then reintroduced into their groups. Our hypothesis was that, as in the case of vampire bat control by vampiricide paste, the administration of V-RG vaccine through paste to one bat could indirectly protect other bats from the same group. Eight groups were tested. The rabies virus strain used to challenge the bats was isolated from a naturally infected hematophagous bat (*Desmodus rotundus*). The survival proportion after the virus challenge ranged between 42.8 and 71.4%. The results are encouraging because a significant number of bats that did not receive the vaccine survived the challenge. The vaccine was shown to be safe and immunogenic to hematophagous bats. No adverse effects to vaccinia virus were observed.

Keywords: Rabies; Bats; *Desmodus rotundus*; Vaccine; Oral immunization

1. Introduction

The use of preventive vaccines against rabies largely contributed to the reduction or local eradication of rabies in domestic animals. Therefore, it would be natural the attempt to vaccinate wild animals. However, the control of rabies in wildlife population, which replaced dogs as reservoirs and vectors of rabies, required other strategies such as the successful oral vaccination of wild mammals in Europe.

Since 1978, several European countries conducted oral rabies vaccination programs for terrestrial wildlife. One of the vaccines used in the field is V-RG, a recombinant vaccinia virus, which expresses the glycoprotein of rabies virus (Kieny et al., 1984). Millions of baits containing V-RG vaccine were distributed in the last decade in Canada, United States, Switzerland, Germany, Belgium and France. Programs of oral vaccination of red foxes (*Vulpes vulpes*), raccoons (*Procyon lotor*) and coyotes (*Canis latrans*) were successful in eliminating rabies from large areas (Brochier et al., 1991; Pastoret et al., 1988; Hanlon et al., 1998; MacInnes et al., 2001).

In countries where progress has been made in terms of human rabies prevention, bats have been responsible by a significant proportion of human infections. In the USA, from 1990 to 2000, 30 of the 32 human rabies cases were associated with insectivorous bat rabies virus variants (CDC, 2000).

In Latin America, the hematophagous bat (*Desmodus rotundus*) is one of the main reservoirs of rabies virus and causing heavy economic losses in livestock annually. Currently, the control of rabies transmitted by *Desmodus rotundus* is performed by bat population reduction using anticoagulants applied on the backs of targeted vector bats (Linhart et al., 1972). Due to their habit of hygiene, the anticoagulant agent is spread among the colony, killing between 10 and 20 bats per vector bat (Greenhall, 1965). The use of anticoagulants is still very controversial. Some investigators have proved them to be successful (Arellano-Sota, 1988) while for others the anticoagulants have been shown to produce moderate success.

* Corresponding author. Tel.: +55 11 62245545; fax: +55 11 62512249.
E-mail address: mfalmeida@uol.com.br (M.F. Almeida).

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Yet, anticoagulants are not an effective method to stop the spread of disease (Jackson, 2002). Another control strategy, although much more expensive, is vaccination of cattle.

Analysis of animal rabies in Brazilian samples by monoclonal antibodies and molecular sequencing showed that dogs and vampire bats are the main vector reservoirs (Ito et al., 2001; Favoreto et al., 2002). The virus variant 3, characteristic of vampire bats, was isolated from 100% of cattle, horses and vampire bat samples (111) taken and in 58.3% of samples (35) of non-vampire bats, showing the importance of *Desmodus rotundus* as a reservoir of rabies in Brazil.

In Brazil, the number of human rabies cases has been 20–30 per year since 1994. Bats are the second most frequently identified vector-related to human rabies cases. Bat bites on humans have been reported in Brazil always associate to enviromental modifications in bats habitats (Batista da Costa et al., 1993; Scheneider et al., 2001). Recently, in two Brazilian villages of the North country, 21 people bitten by a vampire bat died of rabies. The antigenic analysis of samples from this outbreak identified the variant 3 virus. As for animal rabies in the period between 1994 and 2003, 20,590 cases of rabies in cattle, 2314 in horses and 676 in hematophagous bats and non-hematophagous bats were reported (Health Ministry from Brazil, 2004).

The objective of this study is to assess the immunogenicity of a recombinant rabies vaccine (V-RG) applied in a neutral vehicle spread on the back of hematogphagous vector bats (*Desmodus rotundus*), kept in captivity. Our hypothesis is that the behavior of the vampire bat (self-hygiene and intense corporal contact to each other in the colony) allows the paste mixed with an oral vaccine applied on the back of one bat to indirectly protect other bats from the same colony. This strategy may represent an alternative control method to the anticoagulant, vampiricide paste, which is decimating the population of *Desmodus rotundus*.

To test this we carried out a set of experiments in which we applied an oral vaccine mixed with neutral Vaseline paste on the back of one bat that was then allowed to live with 6–9 other non-pasted bats in groups distributed into special cages designed to this end. All the bats of all groups were then challenged with intramuscular injections of live rabies virus and the proportion of survivals was annotated.

2. Materials and methods

2.1. Captivity

The bats (*Desmodus rotundus*) used in this experiment were adults (males and females), maintained in cages designed specially for rabies experimental purposes, at the School of Medicine of University of São Paulo. They were collected in caves of Salto do Pirapora and Salto de Itu Cities (State of São Paulo), Southeastern Brazil.

The air circulation in the interior of the cages occurred through an inlet and an outlet system with biosafety filters. The system was designed with consideration for the possibility of rabies virus aerosol formation, as previously suggested (Constantine, 1962, 1967). The cages were maintained in an air-conditioned room, with temperatures between 19 and 23 °C.

The cages of a cylindrical form, 40 cm in diameter and 50 cm in height were made of polycarbonate (resistant and transparent) allowing total visualization of bats for observation, photos and monitoring by filming. The cages offered suitable conditions for longevity to bats and minimized the stress of captivity to the animals.

The bats were fed with defibrinated swine blood, 20–30 ml a day per animal. The weight of animals was monitored once a week and the consumption of blood was monitored daily. Once the amount of blood consumed and body weight was stabilized, the animals were considered adapted and ready for the experiments. The adaptation period ranged from 30 to 45 days.

2.2. Serology

Sera of all animals were tested for rabies virus neutralizing antibody (VNA) by rapid fluorescent focus inhibition test (RFFIT) (Smith et al., 1973). Four samples were collected from each animal, before the vaccination, 20 days after the vaccination, 25 days after the challenge and one at the end of the observation period or at the moment of the euthanasia of a sick bat. Blood was collected from a cephalic vein in the wing (0.2–0.3 ml) using a hematocrit tube (Baer and McLean, 1971). The last sample of blood was collected by cardiac puncture. The conventionally defined VNA level of 0.5 IU/ml for humans was considered as a cut-off for rabies immunization.

2.3. Rabies diagnostic

A diagnosis of rabies in bats was made from their brain by fluorescent antibody test (FAT) (Dean et al., 1996). The test was performed at the Rabies Laboratory of Zoonosis Control Center of São Paulo Municipality using antirabies conjugate, Sanofi (72114 lot 1B006).

2.4. Vaccine

The vaccine used in this experiment was the vaccinia-rabies glycoprotein recombinant vaccine (RABORAL V-RG), manufactured by Merial Inc. (France, lot 00H472). This oral vaccine is routinely used to immunize wild animals in Europe and North America. No one ever used it routinely to protect bats and, therefore, the standard dose for this animal is not known. The initial volume and concentration of the vaccine was reduced to small volumes in dialysis bags (Inlab 132, cut–off of molecular weight 12,000–16,000 and porosity of 25 Å) using a super saturated solution of sacarose.

For the “oral vaccination” experiment, the vaccine was concentrated from 3.5 to 0.7 ml. For the “indirect vaccination” experiment the volume of nine vaccine sachets (22.5 ml)