Published in final edited form as:

Vaccine. 2007 October 16; 25(42): 7296–7300.

Oral vaccination of raccoons (*Procyon lotor*) with genetically modified rabies virus vaccines

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Abstract

Oral vaccination is an important tool currently in use to control the spread of rabies in wildlife populations in various programs around the world. Oral rabies vaccination (ORV) of raccoons represents the largest targeted program to control wildlife rabies in the United States. Currently, the vaccinia-rabies glycoprotein recombinant virus vaccine (V-RG) is the only licensed oral rabies vaccine in the US. In the current study, captive raccoons were used to evaluate two previously described constructs of a rabies virus vaccine developed by reverse genetics (SPBNGAS and SPBNGAS-GAS) for immunogenicity and efficacy compared to the V-RG vaccine. Four of five control animals succumbed to rabies virus after severe challenge, while three of five animals vaccinated orally with SPBNGAS succumbed. No mortality was observed for animals administered SPBNGAS-GAS or the V-RG vaccine. The results of this preliminary study suggest that SPBNGAS-GAS provides comparable efficacy to V-RG. Additional studies will be needed to determine the duration of immunity and optimal dosage of SPBNGAS-GAS and to examine its efficacy in other reservoir species.

Keywords

Rabies; Vaccine; Raccoons; Oral

1. Introduction

Raccoons (*Procyon lotor*) are native to a large geographic area extending throughout most of North America (Fig. 1) [1,2]. Rabies was first identified in raccoons beginning in the 1940s in Florida [3]. For the next 25 years the distribution of raccoon rabies remained largely confined to the southeastern United States. However, after the suspected translocation of rabid raccoons from the southeastern US into the mid-Atlantic region during the 1970s, raccoon rabies spread quickly throughout the largely naive raccoon population in the Northeastern states [4]. By 1995, the northern and southern foci of raccoon rabies had converged in North Carolina. Raccoon rabies has become enzootic throughout most of the eastern US and into Ontario, Canada. During 2005, 2534 rabid raccoons were reported to the Centers for Disease Control and Prevention (CDC), accounting for 39.5% of all rabid animals that year [2].

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Since 1990, raccoons have been the most reported species with rabies in the US. Raccoon rabies constitutes a significant public health concern in the US due to its overlap with large metropolitan areas, as well as the close relationship between raccoons and humans in suburban environments. Moreover, raccoon rabies presents a challenge to control due to the relatively high spillover rate of the raccoon rabies virus variant to other species. When spillover to non-reservoir species was included, this variant accounted for 76.3% of all terrestrial animal rabies cases in the US reported in 2005 [2]. To date, only one case of human rabies has been attributed to raccoon rabies [5], but the public health burden to manage potential human exposures is large. Post-exposure prophylaxis (PEP) for rabies consists of one dose of rabies immune globulin and five doses of vaccine given over a 28-day period. The costs for biologics alone average around US\$ 1646 for one complete course of rabies PEP [6].

Oral vaccination of foxes against rabies began in Europe during the 1970s. Through the strategic distribution of vaccine-laden baits, both Switzerland and France were declared free of terrestrial rabies by 2000 [2,7]. Such oral rabies vaccination (ORV) programs in Europe have largely relied on first generation attenuated rabies virus vaccines which have demonstrated a high level of efficacy in immunizing fox populations [8]. However, these first generation oral rabies virus vaccines have the potential of causing vaccine-induced rabies and appear to have limited efficacy in a broad spectrum of animal species [9]. During the late 1980s, a vaccinia-rabies glycoprotein recombinant virus vaccine (V-RG) was developed, creating a new generation of oral rabies biologics. A small field study of the V-RG vaccine was conducted in 1990 on Parramore Island, Virginia, and by 1995 the vaccine was conditionally licensed in the US for use in ORV programs [10]. The use of V-RG to control rabies in coyote populations along the US–Mexico border has recently been successful in eliminating that variant from the US [11]. To date, V-RG remains the only licensed oral rabies vaccine in the US.

Since 2001, a large scale ORV program has been coordinated by the US Department of Agriculture/Wildlife Services (USDA/WS) with the goal of preventing the westward spread of raccoon rabies from the eastern US. This ORV program, targeted at raccoons, consists of a combination of baiting programs and natural barriers stretching essentially from the Ohio–Pennsylvania border at Lake Erie to Alabama. However, despite the increased safety of the V-RG vaccine, it remains limited in its efficacy among multiple species, including skunks and mongoose [12,13]. The majority of spillover infections from raccoons is into skunks (predominantly *Mephitis mephitis*) and this species may have a role in maintaining the raccoon rabies virus variant within ORV zones [14].

A new generation of oral rabies vaccines are currently under development using reverse genetics to provide targeted attenuation of the virus. Optimally, these vaccines should provide a high level of safety comparable to the V-RG vaccine while maintaining efficacy in a broad range of animal species. To this extent, we evaluated newly developed genetically modified rabies vaccines in raccoons to determine immunogenicity and efficacy compared to the V-RG vaccine [15].

2. Materials and methods

2.1. Vaccines

Construction of the SPBNGAS virus has been recently described [15]. The SPBNGAS-GAS, which contains an extra copy of the rabies virus glycoprotein gene from SPBN-GAS, was constructed similarly as previously described [16]. The V-RG vaccine (Raboral V-RG®, Rhone Merieux, Inc., Athens, GA, USA) was obtained from a commercial source.

2.2. Raccoons

Nineteen raccoons were obtained from a commercial breeder for the purposes of comparing the safety, immunogenicity, and efficacy of the SPBNGAS, SPBNGAS-GAS, and V-RG vaccines in raccoons. All animals were held in quarantine for at least 30 days according to CDC's Institutional Animal Care and Use Committee policies. Animals were divided into four groups. Five raccoons were used as a negative control and were given 1 ml of media (Eagle's Minimum Essential Medium supplemented with 10% fetal calf serum), five received SPBNGAS (1 ml, 1×10^8 TCID), five received SPBNGAS-GAS (1 ml, 1×10^8 TCID), and four were given V-RG (1 ml, 1×10^8 TCID). All viruses were administered per os while the animals were lightly sedated by intramuscular injection with 0.3 ml Tiletamine HCl and Zolazepam HCl (Telazol®, Fort Dodge Animal Health, Fort Dodge, IA, USA). All raccoons receiving either SPBNGAS or SPBNGAS-GAS were monitored daily following vaccination for development of any potential adverse reactions to either vaccine (e.g. loss of appetite, dramatic weight loss, or behavioral changes).

2.3. Serology

All 19 raccoons were bled before vaccination to obtain a baseline rabies virus neutralizing antibody (VNA) titer. After sedation, blood was collected from the jugular vein. Following vaccination, blood was collected on days 7, 14, 21, 28, 43 and 56. Blood was also collected 7 days post-challenge infection to monitor for a potential anamnestic response. Serum samples were evaluated for the presence of rabies VNA as determined by the rapid focus fluorescent inhibition test (RFFIT) as previously described [17].

2.4. Rabies virus challenge

Fifty-six days post-vaccination all animals were challenged with rabies virus (raccoon street virus; $1 \times 10^{4.9}$ MICLD50) by the inoculation of 0.5 ml each in the left and right masseter muscles. At the first clinical signs of rabies, the animals were sedated, blood was collected, and the animal was euthanized by the intravenous administration of 1.5 ml euthanasia solution (Beuthanasia®-D, Schering-Plough, Union, NJ, USA). The brainstem was collected at necropsy and tested by the direct fluorescent antibody (DFA) test for detection of any rabies virus antigens

[
<http://www.cdc.gov/ncidod/dvrd/rabies/professional/publications/DFAdiagnosis/DFAprotocol-b.htm>
]. All surviving animals were euthanized 90 days after the last detection of any clinical animals and tested by the DFA test.

3. Results

3.1. Safety

No adverse effects were observed among animals receiving either SPBNGAS or SPBNGAS-GAS during a combined 560 animal-days of observation (10 raccoons observed daily over 56 days after vaccination and before challenge with rabies virus).

3.2. Immunogenicity

All animals were seronegative for rabies VNA before vaccination. All control animals remained seronegative prior to inoculation with rabies virus (Table 1). Four of the five control animals demonstrated detectable rabies VNA 7 days after inoculation with rabies virus. Among the five animals vaccinated with SPBNGAS, one demonstrated detectable VNA 14 days following vaccination and another animal at 56 days post-vaccination. Four of five animals demonstrated VNA 7 days after challenge with rabies virus. Two animals vaccinated with SPBNGAS-GAS demonstrated detectable rabies VNA by day 14 post-vaccination. All four

animals vaccinated with the V-RG vaccine demonstrated detectable rabies VNA 14 days post-vaccination and demonstrated an anamnestic response following challenge with rabies virus.

3.3. Efficacy

Non-vaccinated animals began showing clinical signs of rabies approximately 15 days following rabies infection (mean 16.75 days, range 15–19 days). Four of the five (80%) control animals succumbed to infection with rabies virus. Three of five (60%) animals vaccinated with SPBNGAS were euthanized following the onset of signs of rabies and found positive for the detection of rabies virus antigen. Among the animals vaccinated with SPBNGAS-GAS or V-RG, no morbidity or mortality was observed and all animals were found negative for evidence of rabies virus by DFA testing at the conclusion of the study.

4. Discussion

The results of this preliminary study suggest that the SPBNGAS-GAS rabies virus vaccine may be an acceptable alternative to the V-RG vaccine for ORV programs. The lack of any overt adverse reactions in raccoons used in this study, in conjunction with previous studies in other species, suggests this vaccine is safe at the administered dose [13,15]. Additional studies will be needed to determine the optimal dose and duration of immunity, as well as efficacy via administration in a bait matrix. Despite showing equivalent efficacy compared to V-RG, the SPBNGAS-GAS vaccine did not demonstrate a strong VNA response as detected by the RFFIT. This finding demonstrates that rabies VNA titers, while roughly correlated with protection, are not an absolute indicator of survival against rabies virus challenge. Furthermore, the increased efficacy of the SPBNGAS-GAS vaccine over the SPBNGAS vaccine is consistent with previous studies, suggesting the induction of superior rabies VNA from recombinant rabies viruses expressing two copies of the glycoprotein compared to viruses containing only one copy [16].

Ideally, the SPBNGAS-GAS vaccine may provide a distinct advantage over the V-RG vaccine in its ability to vaccinate a broader range of mammalian species. The SPB-NGAS vaccine has already demonstrated efficacy under experimental conditions in mongoose, a species in which the V-RG vaccine showed no experimental efficacy. The ability to vaccinate multiple species with a single vaccine would be optimal, especially in ORV programs targeted at raccoons where considerable spill over to non-reservoir species occurs. Clearly, additional testing will be required to determine the efficacy of SPBNGAS-GAS in other species. Skunks (such as, *M. mephitis*) represent another key reservoir species with which the V-RG vaccine has historically demonstrated inconsistent efficacy [18,19]. Further studies to examine the efficacy of SPBNGAS-GAS in skunks are needed to determine if this vaccine could provide protection against rabies in both species.

Acknowledgements

The authors thank the personnel in the Animal Resources Branch at CDC for their assistance in maintaining the animals used in this experiment and to Kayla Bean in the rabies laboratory for her assistance with serology samples. This research was supported in part by NIH grants AI45097 and AI060686. The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.

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Fig. 1.
Distribution of raccoons (*Procyon lotor*) and the raccoon rabies virus variant in the United States.

Table 1

Raccoon response to oral rabies vaccination and challenge

Group	Raccoon no.	Rabies VNA (IU/ml) post vaccination (day) ^a					VNA day 7 PI ^b		Died/survived ^c
		7	14	21	28	43	56		
SPBNGAS	SG1	<0.06	<0.06	<0.06	<0.05	<0.04	<0.05	0.35	D
	SG2	<0.06	<0.06	<0.06	<0.05	<0.04	<0.05	0.25	D
	SG3	<0.06	0.07	0.08	0.1	0.07	0.1	47	S
	SG4	<0.06	<0.06	<0.06	<0.05	<0.04	<0.05	<0.04	D
	SG5	<0.06	<0.06	<0.06	<0.05	<0.04	<0.05	7.8	S
SPBNGASGAS	SGG1	<0.06	0.1	0.08	0.08	0.1	0.8	13	S
	SGG2	<0.06	<0.06	<0.06	<0.05	<0.04	<0.05	0.1	S
	SGG3	<0.06	0.1	0.07	0.07	0.08	0.1	12.2	S
	SGG4	<0.06	<0.06	<0.06	<0.05	<0.04	<0.05	<0.05	S
	SGG5	<0.06	<0.06	<0.06	<0.05	<0.04	<0.05	0.1	S
V-RG	V1	0.08	0.6	0.6	1.25	2.35	1.74	215.2	S
	V2	<0.04	0.12	0.1	0.1	0.08	0.06	59.1	S
	V3	<0.04	0.14	0.5	1.9	0.43	0.48	219.2	S
	V4	0.08	0.56	3.3	3.9	6.09	7.6	104.3	S
Control	C1	<0.06	<0.06	<0.06	<0.05	<0.04	<0.05	<0.04	D
	C2	<0.06	<0.06	<0.06	<0.05	<0.04	<0.05	0.44	S
	C3	<0.06	<0.06	<0.06	<0.05	<0.04	<0.05	0.07	D
	C4	<0.06	<0.06	<0.06	<0.05	<0.04	<0.05	0.04	D
	C5	<0.06	<0.06	<0.06	<0.05	<0.04	<0.05	0.09	D

^a Rabies virus neutralizing antibodies; International Units per ml (IU/ml).

^b Post rabies virus inoculation.

^c All raccoons that died tested positive for rabies virus antigen in their brain by the direct fluorescent antibody test (DFA); survivors were DFA test negative.