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Oral Vaccination of Skunks with Raccoon Poxvirus Recombinants Expressing the Rabies Glycoprotein or the Nucleoprotein

Makonnen Fekadu,[1] John H. Shaddock,[1] John W. Sumner,[1] Dane W. Sanderlin,[1] Janice C. Knight,[2] Joseph J. Esposito,[2] and George M. Baer,[1] [1] Viral and Rickettsial Zoonoses Branch and [2] Viral Exanthems and Herpes viruses Branch, Division of Viral and Rickettsial Diseases, Center for Infectious Diseases, Centers for Disease Control, Public Health Services, U.S. Department of Health and Human Services, Atlanta, Geogria 30333, USA

ABSTRACT: Twenty nine skunks (*Mephitis mephitis*) were vaccinated orally with raccoon poxvirus (RCN) recombinants: 10 with a recombinant expressing the rabies virus glycoprotein (RCNRG), 10 with RCNRG mixed with a recombinant expressing the rabies virus nucleoprotein (RCNRN) and nine with RCN alone. Rabies virus neutralizing antibodies were detected in six of the 20 skunks; five skunks (three given RCNRG, two given a mixture of recombinants) survived a rabies challenge that was lethal for nine skunks vaccinated with RCN alone.

Key words: Raccoon poxvirus, recombinant vaccine, rabies, skunk, *Mephitis mephitis*, vaccination.

The striped skunk is the most commonly reported rabid wild animal in the United States. Between 1979 and 1989, the number of reported rabid skunks ranged between 1,657 and 4,096 and constituted 36% to 64% of the total number of rabid animals in the country. The midwest area of the USA extending from Minnesota and Michigan to southern Texas is most affected by skunk rabies; two additional enzootic areas are in California (Reid-Sandin et al., 1990). For many years attempts were made to control rabies by reducing the population of rabies reservoir animals; these attempts were based on the hypothesis that a reduction of animal-to-animal contact would eliminate the disease from limited areas. Animals were usually poisoned or trapped. In the early 1960's, attempts were initiated to immunize wild animals (Baer et al., 1963) with killed vaccine thereby reducing transmission by creating an immune barrier. The attempts eventually led to the development of an attenuated oral rabies virus vaccine that was effective in foxes (*Vulpes vulpes*) (Baer et al., 1971; Winkler and Baer, 1976; Black et al., 1973; Follmann et al., 1988). Attenuated virus was first field-tested in Switzerland in 1978 (Steck et al., 1982; Wandler et al., 1988). After successful results in Switzerland, oral rabies vaccination of foxes was incorporated in most western European rabies control efforts (Schneider et al., 1988), and it is now being extended to Canada and eastern European countries (Schneider et al., 1989). European countries have set as their goal to be free of terrestrial animal rabies within a decade.

The attenuated rabies vaccine being used to immunize foxes in Europe and Canada has not been effective for immunizing other rabies reservoir species, including the skunk. However, groups of raccoons (*Procyon lotor*) and skunks have recently been experimentally protected against rabies with a vaccinia virus recombinant (VRG) expressing the ERA (Evelyn-Rokitnicki-Abelseth) rabies virus glycoprotein (Tolson et al., 1987; Rupprecht et al., 1986). Five of seven striped skunks fed VRG in spongebaits have been protected (Tolson et al., 1987). More recently, a raccoon poxvirus (RCN) recombinant has been developed at the Centers of Disease Control (CDC) that expresses the challenge virus standard (CVS) rabies virus glycoprotein (Esposito et al., 1988). The raccoon poxvirus rabies virus glycoprotein recombinant (RCNRG) has been shown to induce rabies virus-neutralizing antibodies in raccoons, dogs, cotton rats (*Sigmodon hispidus*), rabbits (*Oryctolagus cuniculus*), bobcats (*Lynx rufus*) and foxes. To date raccoons, dogs and cotton rats have been protected against

lethal rabies challenge (Esposito et al., 1989). In addition, a vaccinia virus (Copenhagen strain) recombinant expressing the rabies virus nucleocapsid protein (VRN) has been made at CDC that protects mice immunized by scarification against lethal street-rabies challenge (J. W. Sumner, pers. comm.). In the present study we measured the immune response in striped skunks and their protection against a lethal skunk street rabies virus challenge when they were syringe-fed RCNRG, or a mixture of raccoon poxvirus rabies virus nucleocapsid protein recombinant (RCNRN) and RCNRG.

Twenty-nine one-year-old striped skunks (R. Zoo, Inc., Neshkoro, Wisconsin 54960, USA) were divided into three groups: 10 received RCNRG, 10 received RCNRG mixed with RCNRN, and nine received RCN alone. Animals were kept separately in stainless steel cages. Skunks were anesthetized with a single intramuscular 50-mg dose of ketaset (Aveco, Inc., Fort Dodge, Iowa, 50501 USA) prior to oral administration of the recombinants.

Stocks of RCNRG and RCNRN recombinants each contained 3×10^8 PFU/ml; vaccine had been stored at −20 C until use. After rapidly thawing the virus stocks, appropriate dilutions of the viruses were made in phosphate-buffered saline (PBS) containing 10% fetal bovine serum. Virus was administered with a syringe by slowly dripping liquid onto the tongue of the animal. In one group, skunks were each given 1 ml of RCNRG (1.5×10^8 PFU). Each of 10 skunks in a second group was given 1 ml of a mixture of RCNRG and RCNRN (1.5×10^8 PFU of each virus). Each of nine skunks in the third group was given 1 ml of RCN (1.5×10^8 PFU).

Each of the 29 animals was observed daily for 3 mo. To determine the level of rabies virus-neutralizing antibody, blood samples were collected at 1, 2, 4 and 8 wk post-vaccination and again at 13 wk just prior to rabies challenge. Serum rabies virus-neutralizing antibodies were measured by the rapid fluorescent focus inhibition test (Smith et al., 1973).

All skunks were challenged by injection into the masseter muscle with 1 ml of street rabies, a 10% suspension of PBS of salivary-gland tissue from naturally infected skunks. The titer of the S-329 isolate was $10^{5.5}$ mouse intracerebral lethal dose 50% per ml ($MICLD_{50}$/ml). Survivors were euthanized 6 mo after challenge. All skunks appeared healthy until challenge. As shown in Table 1, three of the skunks showed neutralizing antibodies at 2 wk post vaccination (two from the group given RCNRG and one from the group given the mixture). At 4 wk post vaccination, seroconversion was noted in two other skunks of the RCNRG group and one other of the group given the mixture. Rabies neutralizing antibodies persisted in the six animals until 90 days post vaccination, when all animals were challenged. Five of the six animals with detectable rabies virus-neutralizing antibodies survived the challenge, three in the RCNRG group and two in the group fed RCNRG mixed with RCNRN. One skunk (#4) developed rabies in spite of having a high rabies antibody titer, and skunk #22, vaccinated with the mixture of RCNRN plus RCNRG, showed no detectable antibody yet survived challenge. All the other animals (those given RCN, RCNRG, or the mixture) developed rabies 12 to 14 days after challenge. Rabies virus antigen was confirmed by fluorescent antibody staining (FA) in all animals that died of challenge. At the end of the study period, the six surviving animals were euthanized with phenytoin sodium (Shering Corp. Kenilworth, New Jersey 07033 USA); rabies virus antigen was not detected by (FA) in the brain of any of these animals.

In a previous report in which VRG (10^9 PFU) was given experimentally in sponge-baits to skunks, five of seven skunks were protected (Tolson et al., 1987). In that study skunks vaccinated with rather high doses of VRG by various other routes (six of six intradermally, three of four intramuscularly, and four of eight by intestinal tubes)

TABLE 1. Rabies virus neutralizing antibodies and resistance to challenge of skunks fed raccoon pox (RCN), RCN expressing rabies virus glycoprotein (RCNRG), or a mixture of RCNRG and RCN expressing rabies nucleoprotein (RCNRN).

Skunk number	Vaccine	Virus neutralizing titers				Mortality
		7	14	28	90	
1	RCNRG		—	(56)	(56)	0
2	RCNRG	—	—	—	—	+
3	RCNRG	—	—	—	—	+
4	RCNRG	—	—	(125)	(125)	+
13	RCNRG	—	—		—	+
15	RCNRG	—	—	—	—	+
16	RCNRG	—	—	—	—	+
17	RCNRG	—	—	—	—	+
18	RCNRG	—	(210)	(85)	(56)	0
19	RCNRG	—	(56)	(110)	(280)	0
20	RCNRG + RCNRN	—	—	—	—	+
21	RCNRG + RCNRN	—	—	—	—	+
22	RCNRG + RCNRN	—	—	—	—	0
23	RCNRG + RCNRN	—	—	—	—	+
24	RCNRG + RCNRN	—	—	(280)	(280)	0
25	RCNRG + RCNRN	—	(540)	(800)	(800)	0
26	RCNRG + RCNRN	—	—	—	—	+
27	RCNRG + RCNRN	—	—	—	—	+
28	RCNRG + RCNRN	—	—	—	—	+
29	RCNRG + RCNRN	—	—	—	—	+
			—	—	—	
5	RCN	—	—	—	—	+
6	RCN	—	—	—	—	+
7	RCN	—	—	—	—	+
8	RCN	—	—	—	—	+
9	RCN	—	—	—	—	+
10	RCN	—	—	—	—	+
11	RCN	—	—	—	—	+
12	RCN	—	—	—	—	+
13	RCN	—	—	—	—	+

were protected against skunk street rabies virus challenge. In our study, in which syringe-feeding of vaccine resulted in three of ten skunks being immunized after oral vaccination with RCNRG, and three of ten given RCNRG and RCNRN were immunized and protected against skunk street rabies virus. The RCNRG vaccine seemed to be the major component for protection; the addition of RCNRN did not appear to have improved protection rates. Our rationale for trying the RCNRN was that in an earlier study (Dietzschold et al., 1988) mice inoculated intraperitoneally with a purified rabies virion ribonucleoprotein (RNP) preparation plus complete Freund's adjuvant were protected against intramuscular challenge with CVS or Duvenhage (DUV 6) virus. Also, we recently have protected mice by scarification with a vaccinia recombinant expressing the rabies nucleoprotein (VRN) (J. W. Sumner, pers. comm.). Further, we were able to protect dogs immunized intradermally with VRN against im challenge with a street rabies virus (M. Fekadu, unpubl. data). Thus, in our studies in skunks, RCNRN was mixed with RCNRG in hopes that an enhancement of protection would be noted; no such effect was seen.

We surmise that although a number of animal species, including raccoons, dogs, foxes, bobcats, and cotton rats, can be immunized orally (baited-vaccine or syringe-

fed vaccine) with raccoon poxvirus glycoprotein recombinants, it is difficult to establish significant protection in skunks syringe-fed the current raccoon poxvirus recombinants at the 10^8 PFU doses level. More promising results were reported (Tolson et al., 1987) for skunks fed VRG 10^9 PFU in sponge-baits. Thus, bait-enhancement studies of vaccine take may be a worthwhile pursuit for immunizing skunks with various candidate oral vaccines in the future.

In this paper the use of trade names is for identification purposes only and does not imply endorsement by the Public Health Service or the U.S. Department of Health and Human Services.

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