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Rabies Challenge of Captive Striped Skunks (*Mephitis mephitis*) following Oral Administration of a Live Vaccinia-Vectored Rabies Vaccine

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ABSTRACT: Twenty-four adult striped skunks (*Mephitis mephitis*) were administered the raccoon product formulation of Rabies Vaccine, Live Vaccinia-Vectored (Raboral V-RG®, Merial Limited, Athens, Georgia, USA), either by oral instillation or in vaccine-filled coated sachets either as single or multiple doses. A control group remained unvaccinated. Twenty-three of the skunks were challenged 116 days postvaccination with rabies virus (skunk isolate). Six of six naive skunks succumbed to challenge. Four of six skunks that received the vaccine by oral instillation survived challenge. The skunks that did not survive failed to seroconvert following vaccination. None of the skunks that accepted multiple doses of the vaccine offered in coated sachets survived challenge, nor were rabies virus-neutralizing antibodies (VNAs) detected in the sera. Likewise, none of the five skunks ingesting a single sachet developed VNA against rabies. However, in this group one skunk did survive rabies challenge. This preliminary study showed that the vaccinia-vectored oral rabies vaccine Raboral V-RG®, as formulated for use in raccoons, is capable of protecting a percentage of skunks against rabies. However, although the fishmeal-coated sachets were readily consumed, subsequent challenge of these animals revealed poor vaccine delivery efficiency.

Key words: Coated sachet, *Mephitis mephitis*, oral route, rabies vaccine, Raboral V-RG®, striped skunks.

Rabies is a fatal viral encephalitic infection that affects both wild and domestic mammals and is transmissible to humans. Striped skunk (*Mephitis mephitis*) and raccoon (*Procyon lotor*) populations are major wildlife rabies reservoirs in the eastern United States, possibly sharing epizootic cycles via spillover of species-specific variants (Guerra et al., 2003). In California and the central United States, three rabies variants are responsible for this disease in skunks (Krebs et al., 2004). In Europe an orally administered recombinant poxvirus, V-RG, has been shown to be an effective vaccine in controlling red fox (*Vulpes vulpes*) rabies (Brochier et al., 2001). This same vaccinia-based oral vaccine, contained inside fishmeal polymer baits, shows promise in controlling rabies in US raccoon populations (Hanlon et al., 1998). It has also contributed to the control of coyote (*Canis latrans*) rabies in southern Texas (Fearneyhough et al., 1998) and gray fox (*Urocyon cinereoargentus*) rabies in eastern Texas (Sidwa et al., 2003). Control of rabies in skunk populations, however, continues to be an elusive goal.

Modified-live rabies vaccines, historically used in Europe and Canada to control fox rabies, are ineffective and potentially pathogenic in skunks (Rupprecht et al., 1990; Tolson et al., 1990). The vaccinia-vectored rabies vaccine is safe in this species but has been suggested to be ineffective (Charlton et al., 1992). This statement is in contrast to a study in which efficacy was demonstrated in skunks given relatively high titers ($10^{9.0}$ pfu/dose) of virus by multiple routes (Tolson et al., 1987). Our study was conducted to determine if a commercial serial of the recombinant vaccinia virus, when given by direct instillation into the oral cavity, could protect caged skunks against a virulent rabies challenge. We chose the raccoon field dose of $10^{7.7}$ $TCID_{50}$/ml, contained in a 1.5–2.0 ml volume, given by direct instillation into the oral cavity and within a coated sachet. Caged skunks were used to allow direct observation of bait consump-

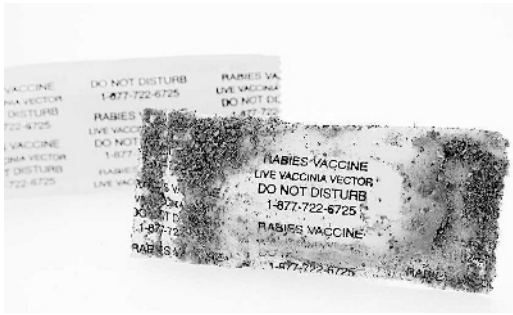

FIGURE 1. Coated sachet bait (2.0×3.5×3.5 cm, 26 g).

tion. The efficacy of direct oral instillation of the vaccine was compared to the efficacy of the vaccine delivered within a bait format.

Twenty-four adult striped skunks (*M. mephitis*) between the ages of 1 and 5 yr, obtained from a commercial source (Ruby Fur Farm, Inc., New Sharon, Iowa, USA), were housed individually in stainless steel cages, offered a commercial feline ration, and provided with water ad libitum. After an acclimation period of approximately 2 mo, during which time they were fed placebo baits, the animals were randomly assigned to one of four treatment groups of six skunks each. One group of skunks remained unvaccinated. Vaccinated skunks received 1.5–2.0 ml of a production serial of Rabies Vaccine, Live Vaccinia Vector (Raboral V-RG®, Merial Limited, Athens, Georgia, USA) containing $10^{7.7}$ $TCID_{50}$/1.5 ml dose. Two groups each were offered either a single coated sachet or a total of three sachets, given as individual doses, on three consecutive days. The coated sachets (Fig. 1) consisted of 0.5×2.0×6.0 cm polyethylene, heat-sealed, vaccine-filled sachets, surface-coated with wax and fish meal attractant (Linhart et al., 2002). Another group received a 1.5 ml dose of vaccine, equivalent to the contents of a single sachet, by oral instillation via a 3.0 ml, needle-free syringe while under light sedation by the intramuscular administration of 0.02 mg/kg medetomidine hydrochloride (Pfizer Animal Health, Inc., Westchester, Pennsylvania, USA) and 5 mg/kg ketamine hydrochloride (Fort Dodge Laboratories, Inc., Fort Dodge, Iowa, USA). Swallowing reflexes were observed in skunks receiving the vaccine by direct instillation. The skunks that were offered sachets were individually observed consuming the product. A positive result was recorded when the animal thoroughly chewed the sachet. One skunk, assigned to the multiple sachet group, refused the bait at all three offerings and was reassigned to the unvaccinated group, reducing the number in the multiple sachet group by one ($n=5$) and increasing the unvaccinated controls to seven ($n=7$). Another skunk that had been vaccinated via oral consumption of a single sachet was euthanatized between vaccination and challenge in response to the development of a necrotizing sterile abscess that developed as a result of an injection site reaction and was excluded from the data set for that group ($n=5$). Vaccine titer was confirmed postadministration by titration in cell culture. Blood samples were collected via the jugular vein of sedated skunks 3 days prior to vaccination, 32 days postvaccination, and on the day of challenge (116 days postvaccination). Sera were evaluated for rabies virus–neutralizing antibodies (VNAs) using the Rapid Fluorescent Focus Inhibition Test (RFFIT; Smith et al., 1996). For rabies challenge, each skunk was administered the diluted (1:25) rabies virus stock by two intramuscular injections of 0.5 ml, bilaterally, into each masseter muscle (1.0 ml total). The challenge virus stock was derived from a salivary gland homogenate from a naturally infected skunk from California (Skunk isolate, strain R98-0100), original titer $10^{6.3}$/ml mouse intracerebral lethal dose 50% ($MICLD_{50}$). Skunks were observed daily for 56 days postchallenge for clinical signs of rabies. Animals were humanely euthanized by the intracardiac injection of 300 mg/kg sodium pentobarbital (Fatal-Plus®, Vortech Pharmaceuti-

TABLE 1. Rabies virus–neutralizing antibodies and protection from rabies challenge in striped skunks (*Mephitis mephitis*) following uptake of V-RG vaccine by direct oral instillation and bait acceptance.

Group	Skunk ID	Rabies virus antibody titer[a]			Response to rabies challenge[b]
		Baseline	Postvaccination	Challenge	
Unvaccinated	S11	≤0.2	≤0.2	≤0.2	R (15)
	S12	≤0.2	≤0.2	≤0.2	R (15)
	S14	≤0.2	≤0.2	≤0.2	R (20)
	S15	≤0.2	≤0.2	≤0.2	R (15)
	S16	≤0.2	≤0.2	≤0.2	R (20)
	S2	≤0.2	≤0.2	≤0.2	R (18)
	S24	≤0.2	≤0.2	≤0.2	R (18)
Oral instillation	S19	≤0.2	≤0.2	≤0.2	R (15)
	S23	≤0.2	0.2	≤0.2	S
	S3	≤0.2	0.9	0.2	S
	S5	≤0.2	3.7	≤0.2	S
	S6	≤0.2	0.3	0.2	S
	S9	≤0.2	≤0.2	≤0.2	R (18)
Single sachet	S1	≤0.2	≤0.2	≤0.2	R (21)
	S13	≤0.2	≤0.2	≤0.2	R (16)
	S18	≤0.2	≤0.2	≤0.2	R (19)
	S4	≤0.2	≤0.2	≤0.2	R (22)
	S8	≤0.2	≤0.2	≤0.2	S
Three sachets	S10	≤0.2	≤0.2	≤0.2	R (25)
	S17	≤0.2	≤0.2	≤0.2	R (26)
	S20	≤0.2	≤0.2	≤0.2	R (15)
	S21	≤0.2	≤0.2	≤0.2	R (17)
	S22	≤0.2	≤0.2	≤0.2	R (20)

[a] Results are expressed in IU/ml. Baseline = 3 days prior to vaccination; Postvaccination = 32 days postvaccination; Challenge = 116 days postvaccination.

[b] S = survived; R = died or euthanatized following signs of rabies (day of death/euthanasia following challenge).

cals, Dearborn, Michigan, USA) following intramuscular administration of 2.2 mg/kg ketamine hydrochloride and 0.02 mg/kg acepromazine maleate (Fort Dodge Laboratories). The diagnosis of rabies was confirmed post mortem by subjecting brain tissue to direct immunofluorescent staining with a pool of fluorescein-labeled, antirabies virus monoclonal antibody conjugates (Velleca and Forrester, 1981). Collection of blood samples and administration of the challenge virus was performed under heavy sedation following intramuscular administration of 0.04 mg/kg medetomidine hydrochloride and 10 mg/kg ketamine hydrochloride. All animal care and experimental procedures were performed in compliance with Merial's established Institutional Animal Care and Use Guidelines.

The RFFIT data and protection against rabies challenge results are summarized in Table 1. Six of six (100%) naive skunks succumbed to challenge (mean survival time = 17 days postchallenge). Four of six (67%) skunks that received the vaccine by oral instillation survived challenge. In this group rabies VNA were present in the four surviving skunks at 32 days postvaccination (GMT=0.67) and declined to baseline or residual levels by the day of challenge (116 days postvaccination). The two skunks in this group that did not survive challenge (mean survival time = 16 days) failed to seroconvert following vaccination. One of those skunks was noted to have received less than the full dose of vaccine because of insufficient sedation. All 10 skunks that were offered the coated sachets readily accepted the

bait. Acceptance was scored as consumption of the entire sachet (i.e., no part remaining in the cage) or puncturing of the plastic material and absence of vaccine contents. Rabies VNAs were not detected in the sera of five skunks ingesting multiple doses of the vaccine offered in a coated sachet, nor did any of this group survive challenge (0% survival with a mean survival time of 21 days, range = 17–26 days). Likewise, none of the five skunks ingesting a single sachet developed VNA against rabies. However, in this group one of five skunks (20%) survived rabies challenge, suggesting that sufficient vaccine was consumed to elicit immunity. Brain tissues from 18 rabies-suspect skunks were positive for reactivity with rabies virus monoclonal antibodies by direct fluorescence. Brain tissue samples collected from the five surviving skunks at 56 days postchallenge were negative for detection of rabies virus antigens.

This preliminary study showed that direct oral instillation of 1.5 ml of Raboral V-RG® formulated at $10^{7.7}$ $TCID_{50}$/dose protected 67% of skunks against a virulent rabies virus challenge. The vaccine was immunogenic and efficacious in a small group of domestically raised skunks at a titer used for field application in raccoons. Although 10 skunks readily consumed the fishmeal-coated sachets, subsequent challenge of these animals revealed poor vaccine delivery efficiency whether one or three sachets were eaten (i.e., 90% and 100% mortality, respectively). In this study, as well as during the red fox vaccine field trials in Europe (Brochier et al., 1990), it was shown that vaccine delivery directly impacts the evaluation of oral vaccine efficacy. These results demonstrate the value of using direct instillation to evaluate oral vaccines in a target species. Evaluation of an oral vaccine in a chosen bait is critical for field efficacy, but postbaiting serology and rabies prevalence data remain indirect measures of vaccine efficacy. The dose used in this study was the same as that of the raccoon product currently in the field ($10^{7.7}$ $TCID_{50}$). We chose this dose to determine if the existing product, targeted primarily for the raccoon, would also be efficacious in skunks.

The vaccinia-vectored oral rabies vaccine Raboral V-RG®, as formulated for use in raccoons, is capable of protecting a percentage of skunks against rabies if given by direct instillation into the oral cavity. It remains to be seen if the vaccine can immunize wild skunk populations via current bait formats. The coated sachet was designed for raccoons, which are capable of picking up and easily manipulating a vaccine-filled bait. In this study skunks were observed dragging the sachet out of the feeder, holding it down with their front paws, and chewing the sachet from one end to the other. Since the eating habits of skunks are different from those of raccoons, there is a good chance that most of the coated sachet's contents are lost to the environment when consumed by skunks. While prebaiting skunks with placebo baits in this trial, leakage of sachet contents was detected by placing absorbent paper on the cage floor (unpubl. data). Absorbent paper was not placed in cages during the vaccine trial since doing so often interfered with bait acceptance.

Tolson et al. (1987) previously demonstrated oral vaccine efficacy in skunks immunized with 5.0 ml of vaccinia-vectored recombinant rabies vaccine given at 109 pfu/dose. Tolson's success, on an experimental scale, was due to a larger vaccine volume held within a polyurethane sponge. These authors were able to show seroconversion in six of seven skunks, five of which were protected from rabies challenge. If a method can be found to improve vaccine delivery, V-RG may prove to be an effective oral rabies vaccine for striped skunks. The logistic approach of distributing one vaccine into the environment to immunize both raccoons and skunks has obvious cost-saving advantages. Field studies in wild populations of

skunks using vaccine-filled coated sachets will provide additional data as to the suitability of this bait format for this species.

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