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ORAL VACCINATION OF RACCOONS (*PROCYON LOTOR*) WITH AN ATTENUATED (SAD-B_{19}) RABIES VIRUS VACCINE

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ABSTRACT: Unlike previous reports to the contrary, raccoons (*Procyon lotor*) were successfully vaccinated against rabies with a liquid SAD-B_{19} attenuated virus vaccine administered per os and given in vaccine-laden baits. There was neither evidence of vaccine-induced rabies in raccoons nor in a limited safety trial with opossums (*Didelphis virginiana*) given SAD-B_{19}. Protection from lethal street rabies virus infection was not absolute: only three of nine raccoons given $1 \times 10^{6.0}$ TCID/ml were protected versus five of 10 raccoons given $1 \times 10^{7.0}$ TCID/ml of SAD-B_{19} and challenged 4 mo after consumption of vaccine-laden baits. Six of eight raccoons consuming $1 \times 10^{8.8}$ TCID/ml of SAD-B_{19} vaccine in baits survived street rabies virus challenge 2 mo postvaccination. Raccoon survivorship was not wholly dependent upon rabies virus-neutralizing antibody titer on the day of challenge. Vaccinated raccoons demonstrated a prominent anamnestic response within 1 wk following challenge. Surviving raccoons were observed for a minimum of 3 mo following street rabies virus challenge with neither clinical nor pathologic evidence of rabies. The SAD-B_{19} rabies vaccine administered within baits in captivity appears less effective for raccoons than for its demonstrated efficacy in the immunization of free-ranging foxes (*Vulpes vulpes*) in Europe.

Key words: Rabies, oral vaccination, raccoons, *Procyon lotor*, rabies vaccine, efficacy, experimental study.

INTRODUCTION

Over the past 25 yr, concomitant laboratory and field research in North America and Europe using the red fox (*Vulpes vulpes*) as a model has resulted in the development of orally-efficacious attenuated rabies vaccines. Deployment of these vaccines within carnivore-attractive baits has demonstrated that local rabies control of terrestrial wildlife is both feasible and economical (for recent reviews see Baer, 1988; Johnston et al., 1988; Wandeler, 1988). Rabies in other equally important reservoirs (e.g., feral domestic canids, mustelids, procyonids, etc.) has not been as easily controlled using conventional rabies vaccines (e.g., Flury HEP, ERA, etc.) per os (Baer, 1988).

The raccoon (*Procyon lotor*), once considered a major rabies reservoir only in the southeastern United States, is now at the forefront of a continuing epizootic in the mid-Atlantic region of North America (Centers for Disease Control, 1988). To date, effective, long-term oral vaccination of raccoons has been achieved only via recombinant rabies vaccine utilization, such as a vaccinia-rabies glycoprotein recombinant virus vaccine (Rupprecht et al., 1986). Although this particular bioengineered vaccine has been shown safe and effective in captivity by a variety of routes for numerous target and non-target species alike (Wiktor et al., 1985; Blancou et al., 1986; Tolson et al., 1987; Rupprecht et al., 1988; Rupprecht and Kieny, 1988) and has been released in limited field trials in France and Belgium with no untoward effects (Pastoret et al., 1988), no such field trials have yet commenced within North America. Pending the outcome of future safety field trials of these and other recombinant vaccines, and in order to expand the potential repertoire of available immunizing agents for the strategic control of wildlife rabies, other biologicals require equally careful consideration. This report details the safety and efficacy of an atten-

uated rabies virus vaccine, SAD-B_{19}, by the oral route for raccoons.

MATERIALS AND METHODS

Adult raccoons, originating from an animal supplier (Dude's Exotic Farm, New Carlisle, Ohio 45344, USA) or live-trapped in areas free of enzootic raccoon rabies in Pennsylvania, were maintained in captivity a minimum of 6 mo prior to study. Details concerning specific aspects of husbandry in captivity were previously described (Rupprecht et al., 1986). All raccoons were seronegative for rabies virus-neutralizing antibody (VNA) on day 0; this was determined using a modification of the fluorescent focus inhibition test (Reagan et al., 1983), entailing the addition of a constant concentration of rabies virus (CVS-11 strain, Wistar Institute Virus Collection) to serial dilutions of raccoon serum on baby hamster kidney (BHK) cells. Titers were expressed as the highest serum dilution capable of reducing the number of rabies virus-infected BHK cells by 50%, converted into international units (IU/ml) by comparison to U.S. Standard Rabies Immune Globulin (Office of Biologics Research and Review, FDA, Bethesda, Maryland 20205, USA) reference serum (lot R-3) as standard. At least a four-fold rise in titer of paired sera (e.g., >0.6 IU/ml) was used as indication of VNA induction. Routine sedation of raccoons consisted of the administration of ketamine hydrochloride (Ketaset, Bristol Laboratories, Syracuse, New York 13221, USA) at 10 mg/kg and xylazine hydrochloride (Rompun, Bayvet Division, Miles Laboratories, Inc., Shawnee, Kansas 66201, USA) at 0.4 mg/kg. In all trials, the vaccine consisted of a liquid preparation of cloned SAD-B_{19} virus passaged upon BHK cells, as previously described (Schneider and Cox, 1983), obtained from the Rabies Surveillance and Research Laboratory (Tubingen, Federal Republic of Germany).

Trial One

In a preliminary study of the efficacy of vaccine by oral infusion, six raccoons were sedated and were given 1.0 ml of SAD-B_{19} rabies virus vaccine per os ($1 \times 10^{7.5}$ TCID/ml). Six control raccoons received an equal volume of phosphate buffered saline. Raccoons were bled by venipuncture for the development of rabies VNA on days 0, 14, and 28. Raccoons were challenged on day 30 by the inoculation of 0.3 ml ($1 \times 10^{5.4}$ $MICLD_{50}$/ml) of street rabies virus strain MD5951 (originally obtained from G. Baer, Centers for Disease Control, Atlanta, Georgia 30333, USA) in the cervical musculature, as described (Rupprecht et al., 1986). Raccoons were observed daily for clinical signs suggestive of rabies virus infection, and were euthanized by the intravenous administration of a barbiturate solution (Euthanasia-6 Solution, Vet Labs Limited, Lenexa, Kansas 66215, USA) at the first definitive clinical signs of rabies (e.g., in extremis), and all survivors on day 90. Brainstem and salivary gland samples were obtained for rabies virus diagnosis by the fluorescent antibody (FA) technique (Goldwasser and Kissling, 1958).

In a limited vaccine safety test using opossums (*Didelphis virginiana*), a common non-target species in North America that may be a major ecological competitor with raccoons (Kirkland and Gillman, 1984) and hence may contact vaccine-laden baits in the field, five oppossums obtained from an animal supplier (Dude's Exotic Farm, New Carlisle, Ohio 45344, USA) were maintained in captivity for 6 mo prior to use. All were seronegative for rabies VNA. On day 0 they were sedated and bled as above and were given 2.0 ml ($1 \times 10^{7.5}$ TCID/ml) of SAD-B_{19} virus per os. Opossums were observed daily for signs suggestive of vaccine-induced rabies, bled on days 30 and 120, and were euthanized at 4 mo, as above. Opossums were not inoculated with street rabies virus.

Trial Two

To test the efficacy of self-administered vaccine by the oral route, 20 individually-caged raccoons were each given a vaccine-laden bait containing SAD-B_{19} virus. Half of the raccoons received a bait containing 1.8 ml ($1 \times 10^{6.0}$ TCID/ml) of vaccine. The remainder received a bait containing 1.8 ml ($1 \times 10^{7.0}$ TCID/ml) of vaccine. Six control raccoons received bait containing cell culture medium (Eagle's minimal essential medium, 10% fetal calf serum). Baits consisted of tallow-covered polyurethane sponge cubes (Johnston et al., 1988) or fishmeal-covered polystyrene sachets (Schneider et al., 1988). Any untouched baits were removed after 48 hr and the vaccine contents were then administered per os to that raccoon under sedation, as above. Raccoons were bled for rabies VNA determination on days 0, 14, 21, 28, and 120. The geometric mean titers (GMT) and standard errors (SE) were calculated from the reciprocal VNA titers in each group for comparison. On day 120, raccoons were challenged by the intra-masseter inoculation of 0.3 ml ($1 \times 10^{5.6}$ $MICLD_{50}$/ml) of a 10% suspension of pooled salivary glands obtained from naturally-infected rabid raccoons from Pennsylvania, prepared as described by Winkler et al. (1985). Raccoons were observed daily and treated thereafter as described in Trial One.

TABLE 1. Rabies VNA titers (IU/ml) and response to rabies challenge in raccoons (*Procyon lotor*) given SAD-B_{19} vaccine ($1 \times 10^{7.5}$ TCID/ml) per os under sedation.[a]

Raccoon number	Day 0	Day 14	Day 28	Survivorship
1	<0.1	7.2	6.0	survived
2	<0.1	3.0	3.2	survived
3	<0.1	0.3	0.4	died
4	<0.1	0.7	0.8	survived
5	<0.1	0.7	0.7	survived
Controls (6)	<0.1	<0.1	<0.1	6/6 died

[a] Raccoons were inoculated i.m. on day 30 with 0.3 ml ($1 \times 10^{8.4}$ $MICLD_{50}$/ml) of street rabies virus strain MD5951.

Trial Three

In order to test the safety and effectiveness of concentrated vaccine, 10 raccoons were given vaccine-laden bait, as described for Trial Two, containing 1.0 ml of SAD-B_{19} virus at a concentration of $1 \times 10^{8.8}$ TCID/ml. Five controls were given cell culture media in baits. Raccoons were bled weekly for rabies VNA development from days 0 to 68. On day 60 raccoons were sedated and inoculated intra-masseter with 0.5 ml of street rabies virus strain MD5951 ($1 \times 10^{6.0}$ $MICLD_{50}$/ml), and were treated thereafter as described in the two previous trials.

RESULTS

In the first experiment, a raccoon in the vaccinated group died on day 5 postvaccination of apparent respiratory arrest following ketamine-xylazine administration. No serum rabies VNA nor rabies virus antigens in brainstem or salivary glands were detected from postmortem specimens. Four of the remaining five vaccinates had detectable rabies VNA by day 14 (Table 1). No raccoons had clinical signs suggestive of vaccine-induced rabies prior to street rabies virus challenge. All controls and one of five vaccinates succumbed within 25 days of challenge and were FA-positive for rabies virus in brain samples (but negative for virus in salivary glands, as were all sampled rabid raccoons from subsequent trials) taken at necropsy. All survivors remained healthy until euthanasia at day 90; no rabies virus antigen was detected in tissues taken from survivors.

Also in a preliminary safety test, all vaccinated opossums remained clinically normal after SAD-B_{19} vaccination. Despite a two-fold increase in vaccine dosage over that given raccoons per os, only one of five opossums demonstrated evidence of seroconversion by day 30 (2.0 IU/ml). No postmortem evidence suggestive of vaccine-induced rabies was found in any animal at the end of the 4 mo observation period. At that time, four opossums had no detectable rabies VNA (levels $\leq$ 0.07 IU/ml); only the single previously seropositive opossum had remaining evidence of rabies VNA (0.6 IU/ml).

In the second trial with raccoons, all vaccine-laden baits were either totally consumed or chewed within 48 hr except for one animal in the $1 \times 10^{6.0}$ TCID/ml group. This raccoon (#218) was sedated, and the vaccine contents of the bait were harvested and administered per os. Raccoons did not show a detectable rabies VNA response until at least day 28 postvaccination. Titers at that time ranged from 0.2 to 1.8 IU/ml and 0.4 to 2.2 IU/ml for the $1 \times 10^{6.0}$ and $1 \times 10^{7.0}$ TCID/ml vaccine groups, respectively (Table 2). Except for one animal that succumbed to apparent cardiac tamponade associated with bleeding on day 28, all raccoons remained healthy until the time of challenge. By day 120, all rabies VNA titers had decreased to prevaccination levels. Challenged raccoons demonstrated typical clinical signs of rabies from days 20 to 41 postchallenge; survivorship ranged from 33 to 50% in the vaccinated groups, versus <20% survivorship in controls (Table 2). All survivors (including raccoon #218) were FA-negative for rabies virus at euthanasia, 3 mo postchallenge.

In the third trial, eight of 10 raccoons had totally consumed or chewed concentrated vaccine-laden baits containing $1 \times 10^{8.8}$ TCID/ml of SAD-B_{19} within 48 hr; the remaining two raccoons that did not consume baits were placed in the control group. By day 30, four of eight raccoons had seroconverted and by day 60, five of eight raccoons had evidence of circulating

TABLE 2. Serological and protective responses of raccoons (*Procyon lotor*) to SAD-B_{19} rabies virus in vaccine-laden baits.[a]

Vaccine group (TCID/ml)	VNA (GMT ± SE, IU/ml): Day 0	14	21	28	120	Survivorship
$1 \times 10^{6.0}$	<0.1 ± 0.01	0.2 ± 0.04	0.1 ± 0.04	0.5 ± 0.17	<0.1 ± 0.01	3/9 survived
$1 \times 10^{7.0}$	<0.1 ± 0.01	0.3 ± 0.05	0.2 ± 0.05	1.1 ± 0.18	<0.1 ± 0.01	5/10 survived
Controls (6)	<0.1 ± 0.01	<0.1 ± 0.01	<0.1 ± 0.01	<0.1 ± 0.01	<0.1 ± 0.01	1/6 survived

[a] Raccoons were inoculated on day 120 with 0.3 ml ($1 \times 10^{5.6}$ $MICLD_{50}$/ml) of a 10% suspension of pooled salivary glands obtained from naturally-infected rabid raccoons. The minimum detectable VNA titer was 0.07 IU/ml.

rabies VNA (Table 3); all eight had seroconverted in the 1 wk following challenge. Until the time of street rabies virus inoculation, all raccoons remained healthy. Challenged raccoons displayed clinical signs of rabies from days 11 to 30 poststreet virus inoculation. In the control group, only 20% survivorship was observed, versus >70% survivorship in the vaccinated group. The detection of specific rabies VNA was not an absolute predictor of survivorship; a raccoon with the highest VNA titer at challenge succumbed, while two raccoons without a detectable response survived. Survival of raccoons against lethal rabies virus infection was thus independent of rabies VNA level at the time of challenge ($\chi^2 = 0.178, 0.50 < P < 0.75$). All survivors were FA-negative at euthanasia.

DISCUSSION

This report details the safety and efficacy of the SAD-B_{19} vaccine given orally to captive raccoons. Previous research (Rupprecht et al., 1986; Baer, 1988) had documented the inadequacy of several different attenuated rabies virus strains (including the fixed rabies virus ERA, a derivative of SAD virus) administered per os to raccoons, despite obvious potency of these attenuated vaccines when tested by the oral route in foxes. Overall, approximately 60% (19 of 32) of the SAD-B_{19} vaccinates survived rabies challenge in which only 12% (two of 17) of control raccoons survived. Considering the occurrence of mortality among controls and vaccinates alike and the inverse relationship between rabies virus inoculum and incubation period, it is highly unlikely that the 3 mo postchallenge termination of each experiment would have significantly missed potentially lethal infections among surviving raccoons, all negative by the FA test at euthanasia. While maximum natural incubation periods of some rabies viral subtypes particularly adapted to raccoons may be in excess of 90 days (McLean, 1975), the majority of our raccoons inoculated with the MD5951 rabies virus strain experienced mortality within 30 days and none have ever produced incubation periods greater than 2 mo following experimental exposure with virulent virus concentrations similar to those described here.

The differences in relative survivorship between various doses of SAD-B_{19} vaccine used in Trials Two and Three in this study, while limited in number, lend support to a general dose–response relationship of raccoons to SAD-B_{19} vaccine, only effective at concentrations several fold in excess of those previously reported as minimally protective for foxes (Schneider et al., 1988). The particular thermostability of SAD-B_{19} virus, retaining its efficacy even when deployed in baits under the vagaries of actual field conditions (Schneider, 1985), may be a critical factor in the oral vaccination of free-ranging carnivores, compared to other attenuated rabies vaccines. However, the explanation for inherent mammalian species differences in response to oral vaccination under controlled conditions in captivity with the same attenuated virus,

TABLE 3. Oral vaccination and protection of raccoons (*Procyon lotor*) with SAD-B_{19} rabies vaccine.[a]

Treatment	VNA (IU/ml): Day 0	30	60	68	Survivorship
Vaccine	<0.1	6.0	6.0	54.0	survived
	0.2	2.0	2.0	18.0	survived
	<0.1	0.6	2.0	54.0	survived
	<0.1	0.2	0.2	54.0	died
	0.2	0.2	2.0	2.2	survived
	0.2	0.2	0.2	6.0	survived
	0.2	0.2	0.2	6.0	survived
	<0.1	18.0	18.0	486.0	died
Controls (5)	<0.1	<0.1	<0.1	<0.1	4/5 died

[a] Raccoons were given 1.0 ml of SAD-B_{19} vaccine ($1 \times 10^{8.8}$ TCID/ml) in baits on day 0 and were inoculated i.m. on day 60 with 0.5 ml of street rabies virus strain MD5951 ($1 \times 10^{6.0}$ $MICLD_{50}$/ml). The minimum detectable titer was 0.07 IU/ml.

such as between raccoons and opossums in this study, is not readily apparent and certainly not easily explainable as a simple body mass to vaccine concentration.

The differences in protective activity of SAD-B_{19} vaccine, given per os to sedated raccoons in Trial One, versus those actively consuming vaccine-laden baits in Trials Two and Three, demonstrate an obvious discrepancy when attempting to directly extrapolate experimental results in protocols where immobilized animals are force-fed vaccine as opposed to auto-inoculation or bait consumption. The predilection of live rabies vaccines for the buccal mucosa and tonsils (Rupprecht et al., 1986), reduced transit times and relative antigenic mass of virus available from direct deposition of liquid vaccine per os may be key elements in differentiating these somewhat conflicting results. Whereas, it is unlikely that animals which fail to respond to rabies vaccine via direct oral infusion under sedation will respond well when allowed to consume vaccine-laden baits, little else can be assumed concerning vaccine safety or efficacy if only vaccine infusion is utilized. Physiological or behavioral traits peculiar to any species in question while undertaking normal foraging functions are additional facets for experimental consideration.

Additionally, because native rabies virus glycoprotein alone is responsible for the induction of rabies-specific VNA, it seems plausible that comparative assays of the relative glycoprotein content of rabies vaccines would yield evidence of potential protective capability; yet the demonstration of the efficacy of a glycoprotein-free rabies vaccine administered parenterally to raccoons (Dietzschold et al., 1987b) questions this basic assumption. The absolute efficacy of any given rabies vaccine can only be shown by direct challenge of the immunity of a relevant host species, not merely by a vaccine's ability to induce VNA. While the induction of rabies VNA may give some general indication as to the relative antigenicity of a vaccine, no firm correlation exists between any absolute titer level of VNA resulting from oral vaccination and protection per se, especially when considering antigenically-diverse street rabies virus challenge and highly variable interspecific host susceptibility. As evident in Trial Two, no surviving raccoons had evidence of residual systemic VNA when challenged and in Trial Three, some raccoons without a detectable serological response survived lethal rabies challenge, whereas others having an apparently high titer succumbed, even after an appropriate humoral anamnestic response 1 wk following challenge virus inoculum. This specific discrepancy between

VNA titer and rabies protection was previously documented as well for laboratory rodents (Dietzschold et al., 1987a) and is not easily dismissed regardless of the repeated emphasis on VNA alone in the past.

The SAD-B_{19} vaccine is highly efficacious for foxes even at $1 \times 10^{6.0}$ TCID/ml (Schneider et al., 1988), but much less so for raccoons. While >70% efficacy in captive raccoons was obtained with the SAD-B_{19} vaccine at $1 \times 10^{8.8}$ TCID/ml, it is extremely doubtful if VNA alone were responsible for the prevention of lethal infection. Similar to other viral-induced diseases, rabies prophalaxis is probably mediated by a complex interdependence of several host species effector mechanisms, including VNA, interferon, T-helper and cytotoxic T lymphocytes. Clearly, conclusions concerning the field utility of any one vaccine protocol for all free-ranging furbearers are limited, when neither the exact effector mechanisms underlying the efficacy of oral rabies immunization nor the putative importance of immunological epiphenomena temporally associated with vaccination (e.g., VNA) are well established for any mammalian species. Rabies-specific VNA are unquestionably significant in the overall scheme of viral clearance, but sole concentration upon a single effector mechanism of the immune system for historical reasons or because of technical ease in measurement may produce fallacious conclusions.

In the European fox rabies campaign, more than 2.4×10^6 doses of SAD-B_{19} vaccine have been placed in the field to date without a single documented case of vaccine-induced rabies. By the end of 1986, a total of 19,319 km^2 of the Federal Republic of Germany that had been baited were rabies-free (Schneider et al., 1988), prompting the extension of SAD-B_{19} vaccine studies to other rabies reservoirs. Given these initial encouraging results with SAD-B_{19} vaccine in raccoons, additional research is needed to critically determine: (1) the limiting field dose and duration of immunity in raccoons resulting from oral attenuated rabies virus vaccination; (2) any residual pathogenicity in raccoons resulting from oral consumption of multiple concentrated vaccine doses over an abbreviated time schedule; (3) the most attractive baits to maximize buccal contact with attenuated vaccine, especially for animals such as raccoons possessing considerable manual dexterity; (4) the cost-effectiveness and comparative safety of attenuated rabies vaccines versus recombinant-derived products (e.g., vaccinia-vectored vaccines) for intended target species; and (5) the safety and efficacy of these proposed biologicals for other important rabies reservoirs, such as the striped skunk (*Mephitis mephitis*), and for endemic non-target fauna in North America.

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