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SHORT COMMUNICATIONS

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Vaccination of Free-Ranging Pennsylvania Raccoons (*Procyon lotor*) with Inactivated Rabies Vaccine

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ABSTRACT: Thirty-nine free-ranging raccoons (*Procyon lotor*) in an endemic rabies area of Pennsylvania (USA) were vaccinated with a single intramuscular inoculation of commercial inactivated rabies virus vaccine, 17 June to 23 August 1987. Paired serum samples, pre- and postvaccination, were obtained from eight raccoons and were analyzed in vitro for rabies virus neutralizing antibody using a modified rapid fluorescent focus inhibition test. Seven of eight (88%) recaptured raccoons demonstrated seroconversion within 15 to 26 days of vaccination. At 1 yr postvaccination, three vaccinated raccoons were recaptured and challenged in captivity with street rabies virus, resulting in the death of two of three vaccinates and five of five unvaccinated control raccoons.

Key words: Raccoon, *Procyon lotor*, rabies, hand immunization, oral immunization, rabies control, experimental study.

Oral vaccination of wild and feral, terrestrial carnivores is the only practical, cost-effective, long-term strategy for the control of sylvatic rabies (Baer, 1988; Schneider et al., 1988; Wandeler, 1988). Although widely practiced in Europe, field trials of rabies vaccine for wildlife have not progressed in the USA to date. Prior to licensure and availability of safe, efficacious oral wildlife vaccines in the USA, individual parenteral animal vaccination may provide limited initial but nonetheless useful information concerning the ideal conceptual role of vaccination in sylvatic rabies control.

Mammalian hosts are naturally subject to environmental stress (e.g., unpredictable resources, infectious diseases, etc.) not necessarily experienced by captive animals used in laboratory trials of vaccine efficacy. In addition, natural variants of street rabies virus are antigenically diverse (Dietzschold et al., 1988), in contrast to the few fixed rabies strains used for vaccine production. Therefore, the antigenicity of different commercial inactivated rabies vaccines, and the feasibility of producing relative immune barriers to a rabies epizootic by vaccination of individual free-ranging animals, may be evaluated by this method. For example, the Delmarva Rabies Initiative was undertaken to prevent epizootic raccoon (*Procyon lotor*) rabies from spreading southward into the eastern shore of the Delmarva Peninsula (USA) by an experimental trap-vaccinate-release program of wild raccoons (Anonymous, 1987a). Also, in Toronto, Ontario (Canada), a trap-vaccinate-release program was initiated in an attempt to control the spread of endemic urban rabies among skunks (*Mephitis mephitis*) (Rosatte et al., 1987). Both programs have apparently been successful in creating relative barriers to sylvatic rabies spread by induction of herd immunity, albeit at a level not applicable to large scale control in free-ranging animals. While vaccination of free-ranging raccoons may theoretically result in initial seroconversion of a large proportion of the population, unless vaccinated raccoons are actually challenged at some point with street rabies virus (either naturally or in captivity), then effective protection or duration of immunity conferred by a given vaccine cannot be definitively determined; this is an important consideration in any type of vaccination program. We report

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here baseline titers for free-ranging raccoons in Pennsylvania, seroconversion after intramuscular (i.m.) administration of a commercial inactivated rabies virus vaccine, and protection tests of a small sample of such seroconverted raccoons recaptured approximately 1 yr postvaccination.

As part of an ongoing investigation of the epizootiology and control of wildlife rabies in Pennsylvania, during 17 June to 23 August 1987, raccoons were live-trapped using Tomahawk traps (Model #207, Tomahawk Live Trap Company, Tomahawk, Wisconsin 54487, USA) baited with fresh fish on State Game Lands (SGL) 176 (40°50'N, 77°53'W) and 92 (40°59'N, 77°46'W), Centre County, Pennsylvania (USA). Characteristics of these study sites were previously described (Brown, 1989). Based upon diagnostic reports of the distribution of positive rabies cases, both SGL's were determined to be on the leading edge of the mid-Atlantic raccoon rabies epizootic during 1987 (Centers for Disease Control, 1988). Captured raccoons were immobilized with a combination of 10 mg/kg ketamine hydrochloride (Ketaset, Bristol Laboratories, Syracuse, New York 13220, USA) and 1 mg/kg xylazine (Rompun, Haver-Lockhart Laboratories, Shawnee, Kansas 66203, USA) administered i.m. After sedation a 3 to 5 ml blood sample was obtained via a peripheral vein or by cardiac puncture; the sex and approximate age (Sanderson, 1950) of the raccoons were recorded, and metal ear tags were inserted (National Band and Tag Company, Newport, Kentucky 41072, USA). On SGL 92 (but not on SGL 176), raccoons were vaccinated i.m. with 1.0 ml of commercial inactivated rabies virus vaccine (Rabguard-TC®, Norden Laboratories, Lincoln, Nebraska 68521, USA). The specific potency value of this lot of commercial vaccine was not precisely available, but the relative vaccine potency in general has been reported as 0.04 per dose in mice (Sharpee et al., 1985). Blood samples were allowed to clot overnight at 4 C; serum was frozen at −20 C prior to analysis for rabies virus neutralizing antibody (VNA). Rabies VNA titers were determined by a modification of the rapid fluorescent focus inhibition test (Reagan et al., 1983) and a geometric mean titer (GMT) was computed for each SGL. Rabies VNA titers were considered negative if they were ≤0.07 IU/ml (≤1:5). For computation of a GMT, 0.07 IU/ml was used in calculations as a baseline titer. Subsequent to the initial blood collection, additional blood samples were drawn if raccoons were recaptured at least 10 days after the last blood collection. A minimum four-fold increase in titer levels of paired sera was considered indicative of seroconversion. Generally, a mean serum titer of 1:68 corresponded to approximately 1.0 IU/ml.

Forty-three blood samples were collected from raccoons on SGL 176; 53 blood samples were collected from raccoons on SGL 92. Due to significant hemolysis in some samples, only 41 and 50 serum samples, for each respective SGL, were analyzed for rabies VNA. The GMT for rabies VNA from initial pre-vaccination serum samples from all raccoons were 0.08 ± 0.01 and 0.11 ± 0.08 IU/ml, for SGL 176 and SGL 92, respectively, indicating no or low previous rabies activity in these populations as a whole. Some baseline virus activity, as suggested by pre-existing VNA (e.g., titers of 1.3 and 9.0 IU/ml on SGL 92), is not entirely surprising and may be due either to current rabies infection or past exposure to sub-lethal virus (McLean, 1975); previous vaccination and release of animals; or non-specific antibody detected by the test. Multiple paired blood samples were collected from 11 raccoons on SGL 92 and from six raccoons on SGL 176. Suitable paired sera were only available for eight raccoons on SGL 92 and four raccoons on SGL 176 (Table 1). Seven of eight raccoons from SGL 92 demonstrated seroconversion, as indicated by at least a four-fold increase in titer levels of paired sera. If only titers from these seven raccoons were used to compute a GMT, then the GMT for subsequent serum samples from

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SGL 92 was 2.37 ± 0.12 IU/ml suggesting primary seroconversion from vaccination, versus no significant change in baseline levels from unvaccinated raccoons on SGL 176 (Table 1). Caution should be exercised in direct interpretation of these rabies VNA levels because blood samples were collected at various intervals, 15 to 38 days apart. Testing of sequential serum samples for three juvenile littermates indicated an initial rise in titer on days 15, 20 and 21. However, rabies VNA titers of two juvenile females decreased by 38 days following vaccination, while one juvenile male exhibited a further increase in titer 37 days following vaccination (data not shown).

Three raccoons vaccinated the previous summer (2411, 2419, 2421) were recaptured during June to August 1988. Two of these three raccoons had seroconverted within 4 wk of vaccination; the third (2421) had not. The titer for both 2411 and 2419 was 0.66 IU/ml approximately 1 yr after initial vaccination; the titer for 2421 was 0.2 IU/ml. These three previously vaccinated raccoons and five unvaccinated controls were maintained in captivity for a quarantine period from 30 to 90 days, before laboratory challenge by the inoculation of 0.5 ml in the right masseter muscle with street rabies virus strain MD5951 ($1.0 \times 10^{5.5}$ $MICLD_{50}$ ml), as described (Rupprecht et al., 1986). One wk following challenge, all raccoons were sedated and bled. Only the three previously vaccinated raccoons had detectable rabies VNA; 2411, 2419 and 2421 had levels of 54.0, 2.0 and 18.0 IU/ml, respectively. Two of the vaccinated raccoons (2411, 2419) challenged with street rabies virus died, as did the five controls 10 to 21 days following rabies virus inoculation. Death of these two vaccinates may have been a result of inadequate long-term protection by the vaccine, regardless of the short-term induction of VNA within 30 days of vaccination or the mounting of an apparent anamnestic response. Obviously, the induction of VNA is one potential mechanism in rabies immunoprophylaxis. Previous workers had shown that a single i.m. inoculation of this commercial vaccine was at least antigenic for captive raccoons and was protective for greater than 90% of dogs and cats vaccinated i.m. more than 3 years before (Sharpee et al., 1985). Nevertheless, protection against virulent virus infection is complex, especially in regards to rabies (Rupprecht and Dietzschold, 1987). In general, relatively "high" VNA levels suggest immu-

TABLE 1. Titer levels (IU/ml) of rabies virus neutralizing antibody (VNA) in paired sera or raccoons (*Procyon lotor*) live-trapped on State Game Lands 92 and 176 in Centre County, Pennsylvania, 18 June to 23 August 1987.

Tag numbers	Capture dates	Age[a]	Sex[b]	VNA titer (IU/ml)
	State game lands 92[c]			
2411	21 June	A	F	<0.07
	17 July	A	F	2.00
2415	11 July	J	F	<0.07
	01 August	J	F	1.33
	18 August	J	F	0.18
2417	11 July	J	F	<0.07
	26 July	J	F	2.00
	18 August	J	F	0.55
2419	11 July	J	M	<0.07
	31 July	J	M	0.66
	17 August	J	M	6.00
2421	11 July	A	M	<0.07
	03 August	A	M	0.07
2431	16 July	J	M	0.07
	11 August	J	M	4.00
2445	27 July	A	F	0.07
	18 August	A	F	5.00
2449	28 July	A	F	0.15
	13 August	A	F	6.00
	State game lands 176[d]			
1611	30 June	A	F	<0.07
	30 July	A	F	<0.07
1613	30 June	A	M	<0.07
	31 July	A	M	<0.07
1639	27 July	J	M	<0.07
	11 August	J	M	<0.07
1033	20 June	A	F	<0.07
	04 August	A	F	<0.07

[a] A, adult; J, juvenile.
[b] M, male; F, female.
[c] Raccoons vaccinated i.m. on the first day of capture with commercial inactivated rabies vaccine.
[d] Control population of free-ranging, unvaccinated raccoons.

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nogenicity, but absolute correlation to levels of "protective" antibody are lacking. Raccoons may survive or succumb to rabies virus challenge, irrespective of absolute VNA level (Rupprecht et al., 1989). Additionally, one may question the relatively high concentration of rabies challenge virus (>300,000 $MICLD_{50}$) given raccoons in this experiment and others as unrealistic, and which might "overwhelm" the immune response of the study population. But truly potent vaccines will easily protect raccoons even in excess of this virulent challenge (Rupprecht et al., 1986) and the concentration of rabies virus in salivary glands of naturally-infected raccoons can exceed those used for laboratory challenge (Winkler et al., 1985).

When challenge studies with rabies virus indicated inadequate protection from rabies for dogs and cats after subcutaneous administration, the U.S. Department of Agriculture (USDA) withdrew approval for subcutaneous administration of Rabguard-TC®, despite comparable rabies VNA responses for both i.m. and subcutaneous administration (Anonymous, 1987b). The results of our study, initiated before withdrawal of USDA approval by the subcutaneous route, indicated that vaccination of free-ranging raccoons using a commercial inactivated rabies virus vaccine i.m. results in seroconversion of a majority (seven of eight, 88%) of sampled vaccinates. However, the efficacy and duration of immunity under field conditions is questionable with this particular product, even by the i.m. route. We are currently investigating the potential of alternative inactivated, parenterally-administered, commercial vaccines for the control of raccoon rabies in Philadelphia (USA) (Sharrar et al., 1989).

The use of other rabies vaccines and vaccination protocols (Rosatte et al., 1987) suggests that a trap-vaccinate-release program would not be effective if 2 wk or more were required to capture the majority of animals in an area, due to time and economic constraints. In a small area this may be feasible; however, capture of a majority of animals in a 2 wk period on a large-scale basis would require excessive effort. For example, during any 14-day period of this study, a maximum of 46% and a minimum of 13% of the total raccoons captured on the study area were captured (Brown, 1989).

Considering the strategic limitations of trap-vaccinate-release programs, and the success of oral vaccination programs for red foxes (*Vulpes vulpes*) in Europe (Wandeler, 1988), oral immunization appears to be the only effective long-term strategy for the wide-spread control of sylvatic rabies. Limited i.m. vaccination may be useful in suburban parks and particular urban settings, in conjunction with barriers to animal movements (e.g., rivers, highways, etc.), provided that vaccine efficacy and safety can be established beforehand. Depending upon budgetary and logistical constraints, annual revaccination of trappable segments of local populations may be necessary if long-term protection cannot be achieved. Until such time that safe, efficacious, inexpensive, oral vaccines are widely available, the use of individual parenteral vaccination may provide limited but valuable information concerning the overall role of vaccination in the control of raccoon rabies under various epidemiological circumstances.

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