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INTRAMUSCULAR VACCINATION OF SKUNKS AND RACCOONS AGAINST RABIES

Richard C. Rosatte,[1] Dennis R. Howard,[2,3] James B. Campbell,[4] and Charles D. MacInnes[1]

[1] Ontario Ministry of Natural Resources, Wildlife Research Section, Rabies Unit, P.O. Box 5000, Maple, Ontario, Canada L6A 1S9
[2] Department of Veterinary Diagnosis, Veterinary Medical Center, Kansas State University, Manhattan, Kansas 66506, USA
[3] Deceased
[4] Department of Microbiology, University of Toronto, Fitzgerald Building, Toronto, Ontario, Canada M5S 1A8

ABSTRACT: Live-captured striped skunks (*Mephitis mephitis*) and raccoons (*Procyon lotor*) were immunized with inactivated rabies vaccine by intramuscular injection and released at the point of capture during a rabies control program in Metropolitan Toronto (Ontario, Canada). Serum samples collected prior to and following vaccination revealed that 100% of the skunks and 98% of the raccoons seroconverted. Rabies antibody was still detectable 314 to 757 days postvaccination. Five of six skunks vaccinated in the laboratory survived challenge with rabies virus 90 days postvaccination. To our knowledge, this is the first documentation of the successful seroconversion of skunks and raccoons vaccinated against rabies in the field.

Key words: Rabies, immunization, skunk, *Mephitis mephitis*, raccoon, *Procyon lotor*, inactivated rabies vaccine, serological test, field trial.

INTRODUCTION

The most feasible means to control rabies in carnivore populations is to vaccinate the species responsible for the spread of the disease. This is supported by the decrease of dog rabies in the United States and Canada following the development and utilization of rabies vaccines (Tierkel, 1975; Vaughn, 1975; Varughese, 1987; Tabel et al., 1974; Pacer et al., 1985). There are more than 20 rabies vaccines, both live and inactivated, available for use in domestic animals (Rhodes, 1981; Veterinary Biologics, 1986; National Association of State Public Health Veterinarians, 1986). These vaccines were, and continue to be, administered parenterally. During the period of extensive use (past 30 to 40 yr), enzootic dog rabies has been almost entirely eliminated; however, large areas are still affected by wildlife rabies. Trials to vaccinate foxes (using oral vaccine) are currently being carried out in Europe and North America (Schneider, 1985; Johnston and Voigt, 1982; Rosatte et al., 1987; MacInnes, 1987, 1988).

Testing of experimental oral rabies vaccines in wildlife species has been in progress since the early 1960's (Baer, 1975), and is continuing today (Kieny et al., 1984; Rupprecht et al., 1986; Tolson et al., 1987; Lawson et al., 1987; Rosatte et al., 1987). The two terrestrial wildlife species responsible for the spread of rabies in Ontario are red fox (*Vulpes vulpes*) and striped skunk (*Mephitis mephitis*) (MacInnes, 1987; Rosatte, 1988). Baits containing vaccine are currently being utilized in Ontario to control rabies in foxes (Johnston et al., 1988; MacInnes et al., 1988; R. C. Rosatte et al., unpubl. data). Unfortunately the available vaccine does not immunize skunks by mouth (Lawson et al., 1987). Therefore, when seeking means to reduce rabies among skunks in Metropolitan Toronto, Ontario, we required an alternative method for immunization, live-trapping and vaccination by injection (Rosatte et al., 1987).

No rabies vaccine is currently licensed for use in wildlife species in Canada or the United States (Veterinary Biologics, 1986). We report here the use of a commercial inactivated rabies vaccine in an attempt to immunize skunks (*Mephitis mephitis*) and raccoons (*Procyon lotor*) by intramuscular injection.

MATERIALS AND METHODS

During 1985, skunks and raccoons were live-trapped (#106 Tomahawk, Tomahawk, Wisconsin 54487, USA) in Metropolitan Toronto, Ontario (43°42'N, 79°25'W). Captured animals were immobilized and vaccinated against rabies with a 1 ml intramuscular (i.m.) injection of Imrab® (Rhone-Merieux) (MTC Pharmaceuticals, Mississauga, Ontario, Canada L4W 2S5) inactivated rabies vaccine (Rosatte et al., 1987). The relative potency of the vaccine was 13.5 International Units (IU/ml). Imrab® is a commercial vaccine available in Canada and the United States for use in dogs, cats, cattle and sheep.

Animals were immobilized with an injection (in the hind limb) of ketamine hydrochloride (Rogar/STB Inc., London, Ontario, Canada N6A 4C6) (20–30 mg/kg body weight) and xylazine hydrochloride (Bayvet, Rexdale, Ontario, Canada M9W 1G6) (10:1 ketamine : xylazine). Blood samples were collected before vaccination from the jugular vein or by cardiac puncture using 10 ml Vacutainer evacuated blood collection tubes (Becton Dickinson, Mississauga, Ontario, Canada L5J 2M8), with 20 ga × 3.8 cm needles. Samples were centrifuged, and serum was withdrawn by a syringe and stored in 2 ml serum Provials® (Dynateck Laboratories, Chantilly, Virginia 22021, USA) at −20 C. Samples were also collected from animals recaptured more than 7 days postvaccination.

During 1988 and 1989, blood samples were collected from skunks and raccoons that had been vaccinated with Imrab® 1- to 2-yr previously in metropolitan Toronto. However, blood samples were not collected when those animals were initially vaccinated.

To determine whether vaccinated skunks were protected if they were infected with rabies, eight skunks (four male and four female), 6- to 12-mo-old, were anesthetized with ketamine hydrochloride (22 mg/kg), and acepromazine maleate (Ayerst Laboratories, Don Mills, Ontario, Canada M3B 1S3) (0.6 mg/kg) subcutaneously, and vaccinated intramuscularly with 1 ml of Imrab®. Serum samples were taken on the day of inoculation and on days 14, 30, 60 and 90 postvaccination. Three mo postvaccination, the vaccinated skunks and eight nonvaccinated controls were challenged with virus from salivary glands of naturally infected skunks from Ontario (Charlton and Casey, 1979). The titer of the challenge virus was $1 \times 10^{5.5}$ $MICLD_{50}$/0.03 g of tissue. Each skunk was inoculated with 0.3 ml of a 2×10^{-1} suspension inoculated in the *abductor digiti quinti* muscle of the right pelvic limb. Brains of all skunks were examined for rabies virus by the rabies fluorescent antibody test (FAT) (Dean and Abelseth, 1973), either immediately after onset of clinical signs of rabies, or when the animal was euthanized after 90 days of observation.

Serum was tested for rabies antibody using the rapid fluorescent focus inhibition test (RFFIT) (Smith et al., 1973), or by the fluorescent focus inhibition test (FIMT) (Zalan et al., 1979). In both tests, titers <0.12 IU were considered negative. Some of the field samples were tested by the enzyme-linked immunosorbent assay (ELISA) (Barton and Campbell, 1988). Generally, there was good compatibility among the 3 tests; however, that is the topic of another paper (J. B. Campbell, unpubl. data).

Mean antibody titers were determined using the geometric mean (Zar, 1974). The standard deviation of the geometric mean was calculated on $\log_{10}$ transformed data. Following calculation of an upper and lower deviation from the mean, the two values were then retransformed. That method accommodated the asymmetrical distribution of data in log form. Chi-square analyses were used to determine if there were statistical differences in seroconversion between ages, sexes or species (Zar, 1974).

RESULTS AND DISCUSSION

Some raccoons, but no skunks, had low prevaccination rabies antibody titers (Table 1). The significance of this is unknown, and there is the possibility that they were false positive values or were due to nonspecific reactions (J. B. Campbell, unpubl. data). There are problems with cytotoxicity of some serum samples collected from wild species such as foxes (Steck et al., 1982), raccoons (Barton, 1986), and skunks (Barton and Campbell, 1988) which will produce false positives.

In the 1985 field vaccination program, all animals except one raccoon seroconverted after vaccination (Table 2). Wild animals were recaptured during three periods. Five skunks and five raccoons were captured in more than one period. Their antibody titers are shown in Figures 1 and 2. Antibody values for the eight captive skunks are shown in Table 3.

Of the 14 skunks vaccinated in the field with Imrab® during 1985, seven were juvenile females, five were juvenile males, one was an adult male and one was an adult female. Five of the juvenile females

TABLE 1. Rabies-neutralizing antibody status of wild, free-ranging skunks and raccoons before vaccination.

Species	Number positive[a]/ total	GMT (SD)[b]	Range
Skunk	0/12	<0.12	all <0.12
Raccoon	6/43	0.25[c] (0.12–0.49)	0.14–0.67

[a] Titers in IU/ml.
[b] GMT = Geometric mean titer; SD = Standard Deviation.
[c] Mean, SD and range given only for those titers >0.12 IU.

and two of the juvenile males were vaccinated on 16 to 18 July 1985. The estimated age of those animals based on parturition dates for skunks in Ontario was 2- to 3-mo-old (Rosatte, 1987). All animals seroconverted.

Of the 44 raccoons with postvaccination blood samples, there were 12 adult males, eight adult females, 15 juvenile males and nine juvenile females. Fourteen juvenile males and eight juvenile females were vaccinated between 19 June and 15 August 1985. The estimated age was 2- to 4-mo-old (Sanderson, 1987). All seroconverted. No statistical differences in antibody titers were noted with age, sex or species.

All young animals responded well serologically following vaccination with antibody detected as early as 8 days postvaccination. Black and Lawson (1980) noted that fox pups (4-mo-old) did not respond as well to rabies vaccination as adults (ERA® orally in baits).

Although the prevaccination serological status of skunks and raccoons collected during 1988 and 1989 was unknown, all of the skunks and 10 of 11 raccoon serum samples had detectable rabies antibody (Table 2). Those animals were sampled 300 to 757 days postvaccination (Table 2).

All skunks vaccinated for the challenge experiment eventually developed antibody, although only six of eight showed antibody on day 14 (Table 3). Two individuals maintained only low levels of antibody (0.35 and 0.17 IU/ml). One skunk died of rabies on day 23 after challenge. Two others died on day 22 of causes not related to rabies. Therefore, effective survival after challenge was five of six animals. None of the survivors were FAT-positive when euthanized 90 days postchallenge. All controls developed rabies (confirmed by FAT) 18 to 35 days after challenge.

These results clearly show that Imrab® was effective in stimulating antibody production in almost all skunks and raccoons, and that a high proportion of individuals are probably protected against rabies. The data in Figures 1 and 2 indicate that Imrab® induced immunity in both skunks and raccoons which should last for more than 1 yr. However, we recommend annual revaccination of skunks and raccoons. A 1 ml injection of Imrab® is licensed in Canada to protect dogs, cats and sheep for 3

TABLE 2. Antibody titers of wild skunks and raccoons after vaccination.

Species	Days after vaccination	Number positive/ total	GMT (SD)[a]	Range
Skunk[b]	5–20	2/2	0.14 (0.14)	0.14
	35–55	11/11	2.92 (0.87–9.85)	0.18–20.0
	80–130	3/3	2.75 (1.02–7.37)	0.78–8.90
	314–373	3/3	1.09 (1.09)	1.09
Raccoon[b]	5–20	6/6	1.02 (0.31–3.37)	0.18–6.94
	35–55	36/37	4.93 (2.08–11.95)	0.21–63.3
	80–130	15/15	2.40 (0.91–6.27)	0.72–15.6
	300–757	10/11	1.56 (0.60–4.04)	0.46–6.09

[a] GMT, Geometric mean titer; SD, Standard Deviation shown as GMT + 1 SD; titers in IU/ml.
[b] The skunk and raccoon samples taken ≥300 days postvaccination were collected during 1988–1989. No prevaccination samples were taken for those animals.

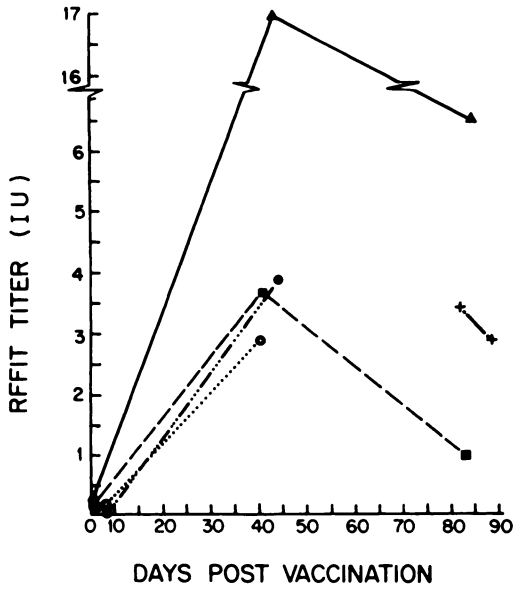

FIGURE 1. Antibody responses of five wild skunks following vaccination against rabies with Imrab®.

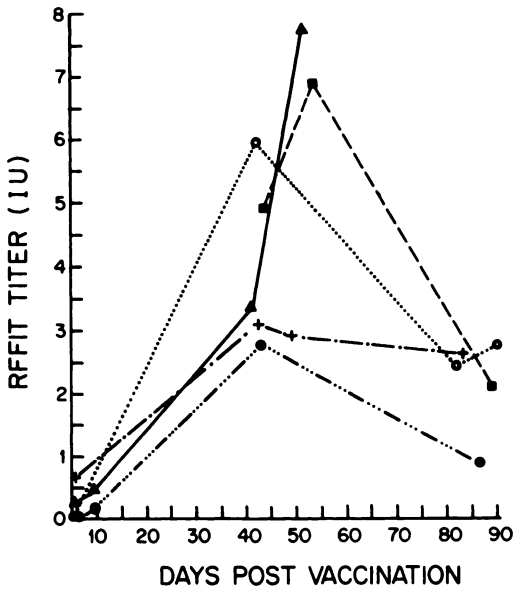

FIGURE 2. Antibody responses of five wild raccoons following vaccination against rabies with Imrab®.

yr. Two ml of vaccine provides immunity in cattle for 1 yr and in horses for 6 mo.

Other rabies vaccines have proved effective in these species. Tolson et al. (1988) showed that ERA/BHK^{-21} (Connaught Laboratories, Willowdale, Ontario, Canada M2N 5T8) was highly effective in producing rabies antibody when injected i.m. into skunks. Wiktor et al. (1985) showed that a vaccinia recombinant preparation induced high levels of rabies antibody in raccoons after i.m. administration; thus, other vaccines may be effective for field use.

Animals such as skunks and raccoons can be captured easily with live-traps (Rosatte, 1987). Vaccination by intramuscular injection in urban areas where rabies is a problem could be a very appealing alternative to oral vaccination as currently no oral rabies vaccine has proven effective in skunks and raccoons over the long term. In fact, that approach to rabies control has been in use in metropolitan Toronto since

TABLE 3. Serum rabies-neutralizing antibody titers[a] of captive skunks vaccinated with Imrab®.

Skunk number	Day 0	Day 14	Day 30	Day 60	Day 90	Challenge
1	0	2.78	2.78	0.70	0.35	S[b]
2	0	0	0.35	0.35	0	D (22)
3	0	2.78	2.78	0.70	0.35	D (22)
4	0	2.78	1.39	0.35	0	S
5	0	2.78	2.78	0.35	0.35	S
6	0	0	0.17	0	0	R (23)
7	0	2.78	2.78	0.70	0.35	S
8	0	1.39	2.78	1.39	0.35	S

[a] International Units/ml.

[b] S, Survived; D, Died, day of death in parentheses; skunks 2 and 3 were FAT negative; R, Rabies FAT positive.

1984 (Rosatte and MacInnes, 1987; Rosatte et al., 1987) and in Maryland (USA) since 1987. Live-trapping and parenteral vaccination also could be very applicable to areas such as Washington, D.C. (USA) where rabies is present in raccoons (Jenkins and Winkler, 1987). As well as for application in the field, i.m. vaccination with Imrab® could be used in skunks and raccoons as part of a rabies prevention program in zoos, game farms, commercial fur ranches and in wildlife parks. Use of an inactivated vaccine such as Imrab® obviates the risk of vaccine-induced rabies which is a potential hazard when using a modified live-virus vaccine (Debbie, 1979).

This project was initiated because at the time, available vaccines would not immunize skunks or raccoons by the oral route with sufficient reliability to reduce the disease in wild populations. However, we prefer oral vaccination because Trap-Vaccinate-Release is labour and cost intensive (Rosatte et al., 1987). Until a safe and effective oral vaccine becomes available Imrab® will be an effective vaccine for wildlife rabies control.

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