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EFFICACY OF AN ORAL VACCINIA-RABIES GLYCOPROTEIN RECOMBINANT VACCINE IN CONTROLLING EPIDEMIC RACCOON RABIES IN NEW JERSEY

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ABSTRACT: A field trial to evaluate the efficacy of an oral vaccinia-rabies glycoprotein recombinant virus vaccine in controlling epidemic raccoon (*Procyon lotor*) rabies was conducted by distributing 180,816 doses ($10^{8.2}$ TCID₅₀/ml) of vaccine in wax ampules within fish-meal polymer baits at a rate of 64 doses/km²/treatment throughout a 552 km² area, forming an 18 km wide band across the northern Cape May Peninsula of New Jersey (USA). Vaccination treatments were conducted in the spring and fall between May 1992 and October 1994 from a helicopter along ecotones and from motor vehicles along roads. Vaccine-laden baits were removed by animals from tracking stations within 3 wk and 61% of the identifiable tracks were those of raccoons. Tetracycline incorporated in the baits as a biomarker was detected in 155 (73%) of the vaccination area raccoons following the fall 1993 and spring 1994 vaccinations. Eleven (61%) of the raccoons sampled in the same time period seroconverted (≥ 0.5 IU) in response to rabies virus glycoprotein. A raccoon diagnosed with rabies from the northern border of the vaccination area on 30 April 1993 provided the first evidence that the barrier was being challenged by the rabies epidemic. The prevalence of rabies in raccoons from the vaccination area for the first year (10%, $n = 96$) and second year (8%, $n = 61$) of challenge was reduced more than six-fold by vaccination compared to unvaccinated raccoons from northern adjacent surveillance areas during the corresponding first (65%, $n = 189$) and second years (53%, $n = 43$). Vaccination also effectively reduced by three-fold the rate at which the epidemic moved through the raccoon population (15 km/yr). The breach of the vaccination area resulted in a resumption of the high rate (43 km/yr) of epidemic movement and a significant nine-fold increase in rabies prevalence (77%, $n = 47$). The maximum linear movement (12.9 km) among five ear-tagged rabid raccoons in the study area was significantly greater than that of 19 normal radio-collared raccoons (2.58 km) in the area. These large movements of rabid raccoons, together with relocation of nuisance raccoons, spillover of raccoon rabies in skunks (*Mephitis mephitis*) and other species, insufficient funding and a decision to discontinue the program in 1994 (which could have resulted in insufficient population immunity among raccoons in the vaccination area) may have contributed to the eventual breach of the barrier.

Key words: Efficacy, epidemic, field trial, prevalence, *Procyon lotor*, rabies, raccoon, tetracycline biomarker, vaccinia-rabies glycoprotein recombinant oral vaccine.

INTRODUCTION

Raccoon (*Procyon lotor*) rabies in the United States, first reported in Florida in the 1950's (Scatterday et al., 1960), had spread to South Carolina by 1988 (Winkler and Jenkins, 1991). A mid-Atlantic states epidemic began on the West Virginia-Virginia border in 1977. It passed through Pennsylvania to enter northwestern New Jersey by the fall of 1989 (Centers for Disease Control, 1992). Rabies control in

wildlife populations has historically focused on population density reduction through trapping, hunting, and gassing (Bigler et al., 1973; Rosatte, 1987; Debbie, 1991). A vaccinia-rabies glycoprotein (V-RG) recombinant vaccine is effective for immunizing raccoons against rabies virus challenge in a laboratory setting (Rupprecht et al., 1986, 1988). Bait acceptance through placebo baiting trials in the mid-Atlantic states using fixed wing aircraft,

helicopter, and hand delivery demonstrated the feasibility of reaching a sufficient proportion of the raccoons in a population to potentially have an effect on a raccoon rabies epidemic (Johnston et al., 1988; Hanlon et al., 1989; Perry et al., 1989; Hadidian et al., 1989; Roscoe, 1991). A mathematical model of raccoon population dynamics suggested density reductions combined with vaccination may be the most economical method for raccoon rabies control (Coyne et al., 1989). A cost benefit analysis of using an oral rabies vaccine for controlling epidemic rabies in New Jersey suggested such a program would be economically beneficial (Uhaa et al., 1992). Laboratory exposures of 40 different species of animals to the V-RG vaccine and island and mainland field safety trials of the vaccine failed to detect any adverse effects on wildlife, domestic animals, or humans (Rupprecht et al., 1992; Hanlon et al., 1998).

Arrival of raccoon rabies in 1989 marked the end of three decades during which New Jersey had been free of terrestrial rabies. Raccoon rabies affected numerous domestic and wildlife species and prompted a field trial to evaluate the effectiveness of the V-RG vaccine as a potential wildlife rabies management tool. In 1991, an Interagency Oral Raccoon Rabies Vaccine Review Committee, partially composed of representatives from the state of New Jersey departments with regulatory authority in environmental protection, health and agriculture, facilitated United States Department of Agriculture (Washington D.C., USA) authorization of the study with what was then an unlicensed vaccine. The primary objective of the study was to create a barrier of immunized raccoons in the path of the advancing rabies epidemic and to compare the prevalence of rabies in raccoon populations within the vaccination area and unvaccinated adjacent populations for a period of at least 1 yr of rabies challenge. This paper reports the results of the first field efficacy

trial of an oral rabies vaccine for control of a raccoon rabies epidemic.

MATERIALS AND METHODS

The vaccination area consisted of 552 km² forming a band approximately 18 km deep (between 39°18'30"N and 39°06'00"N) spanning the northern Cape May Peninsula from the Atlantic Ocean on the east (74°35'00"W) to the Maurice River (75°01'30"W) and Delaware Bay on the west (74°51'30"W) (Fig. 1). The location was selected in part because of (1) the presence of water barriers, (2) its distance ahead of the advancing rabies epizootic to reduce the likelihood of rabies infection already existing in the population and to allow time for vaccination prior to rabies challenge, (3) the likelihood 80% or more of the raccoons would ingest vaccine from baits based on prior vaccine placebo uptake research conducted in the area (Roscoe, 1991), (4) availability of locally based helicopter support, and (5) large government land holdings including wildlife management areas, parks, refuges, and forests.

Included in the vaccination area were extensive tidal meadows (*Spartina* sp.) east of the narrow strip of sandy beach and dunes on the Delaware Bay shoreline, west of the heavily developed Atlantic coastal barrier islands and southwest of the Great Egg Harbor and River. Interspersed within the area were numerous tidal rivers, creeks, ditches, ponds, and guts. The biota included estuarine and freshwater communities, ranging from maple (*Acer* sp.)-gum (*Eucalyptus* sp.)-cedar (*Cedrus* sp.)-holly (*Ilex opaca*) swamp to characteristic upland vegetation, consisting of open agricultural and fallow fields, dominated by thickets and hedgerows, and woodlands composed of oak (*Quercus* sp.)-hickory (*Carya* sp.) or pine (*Pinus* sp.) communities. The human population was approximately 40,764 with the most heavily developed region along a corridor near the tidal meadows on the east side of the vaccination area. The undeveloped ground between dwellings, small businesses, malls, offices, and median strips consisted of upland vegetation, dominated by cedar and oak. There were 11 sparsely developed towns throughout the remainder of the vaccination area. Those portions of Atlantic and Cumberland Counties immediately north and west of the vaccination area and the portion of Cape May county south of the vaccination area were within the outer coastal plain physiographic region and have comparable raccoon habitat and developed areas. The raccoon populations in these areas were unvaccinated and were the source of sur-

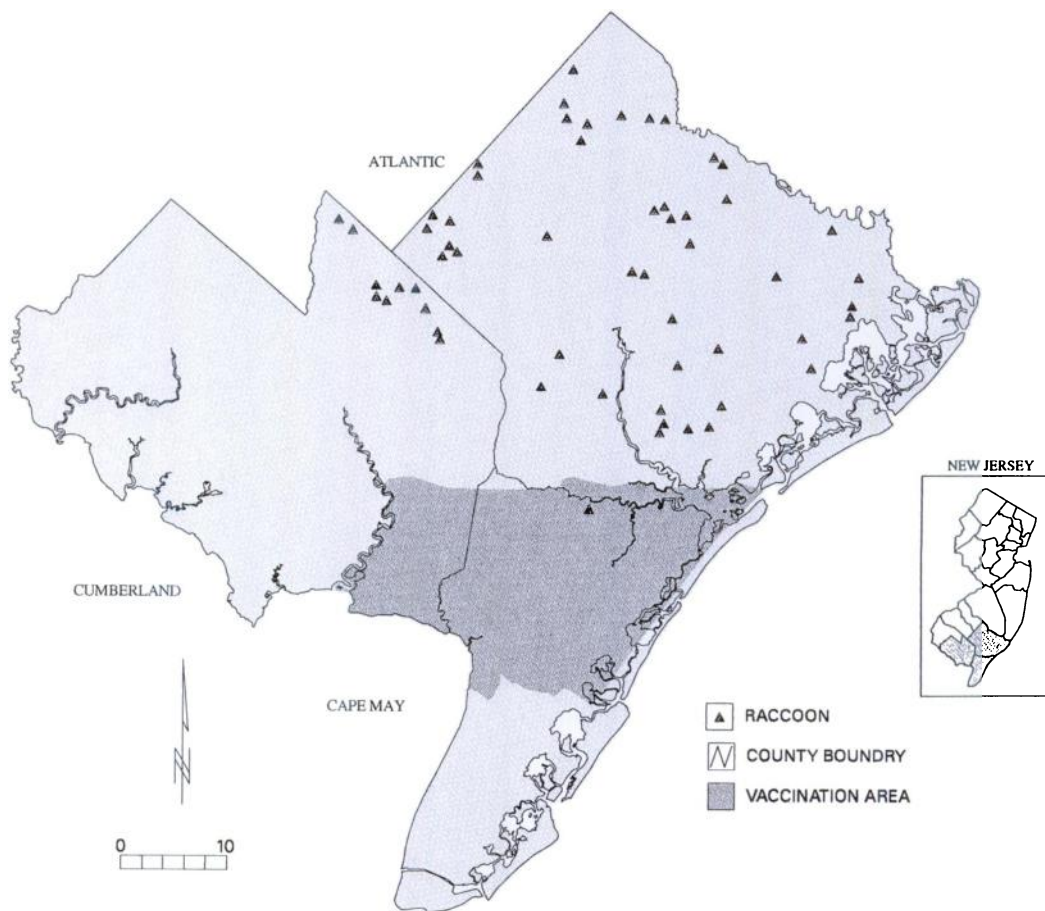


FIGURE 1. Arrival of the New Jersey raccoon rabies epidemic at the V-RG oral rabies vaccination area. Raccoon rabies cases between the first case in the Atlantic, Cumberland, and Cape May County study area on 5 July 1992 and the first case in the V-RG oral rabies vaccination area on 30 April 1993.

veillance specimens for comparison with the raccoon population in the vaccination zone.

A total of 180,816 doses ($10^{8.2}$ TCID₅₀/ml V-RG) of vaccine (Rhone Merieux, Inc., Athens, Georgia, USA), in wax ampules within fishmeal polymer bait (38 g) cylinders containing 100 mg of tetracycline as a calciphillic biomarker, were distributed during six vaccinations in the spring and/or fall between 1992 and 1994 (Table 1). Rather than randomly distributing vaccine throughout the entire vaccination area, heavily utilized edge type raccoon habitats or ecotones were targeted. These consisted primarily of tidal meadow-upland, tidal meadow-dune, marsh-upland, lake-upland, forest-field edge, hedge rows, sand pit-forest edge, stream-forest, wetlands-forest, sand roads, powerlines, railroads, stands of large trees, road edges, median strips, and dumpsters. Iophenoxic acid, a vaccine placebo, distributed in baits during pla-

cebo baiting trials from a helicopter along ecotones at a rate of 200 doses/km², or two baits every 160 m, was taken up in the serum from 80% of 59 raccoons subsequently sampled (Roscoe, 1991). Therefore, a vaccine distribution rate of 250 doses/km² or 2.5 doses every 160 m of edge traveled was estimated to be sufficient to vaccinate all the raccoons. The vaccination rate based on the entire vaccination area is approximately 64 doses/km². The V-RG vaccine-laden baits were distributed at that rate by hand from the open door of a helicopter (Hiller UH12E, Hiller Aviation, Inc., Porterville, California, USA) and from the open window of automobiles. The doses of vaccine distributed in the spring 1993 and fall 1994 vaccinations were fewer than the targeted >30,000 due to inadequate funds. The shortfall in 1993 prevented vaccination of raccoons in central, eastern and southern regions or one third of

TABLE 1. Doses of V-RG vaccine distributed in the New Jersey field trial for oral vaccination of raccoons.

Target area (method)	Date of vaccination						Total
	MAY 1992	OCT 1992	MAR 1992	NOV 1993	MAR 1994	OCT 1994	
Ecotones (helicopter)	23,393	31,059	16,600	28,266	28,266	17,950	145,534
Roadside (automobile)	6,856	7,344	3,850	6,634	7,148	0	31,832
Tracking stations (hand)	100	100	0	100	100	50	450
Hot spots ¹ (helicopter)	0	0	1,000 ²	1,000 ³	1,000 ⁴	0	3,000
Total	30,349	38,503	21,450	36,000	36,514	18,000	180,816

¹ Vaccine distributions in an area surrounding the location of a rabid animal.

² Two vaccinations of 500 doses each (7 May 1993, 5 August 1993).

³ One vaccination (20 December 1993).

⁴ One vaccination (7 April 1994).

the vaccination area that spring. The shortfall in 1994 resulted in no fall roadside distribution of vaccine and a generalized reduction in helicopter distribution. In addition to the scheduled vaccine distributions, four "hotspot" vaccinations were conducted within a 2.5 km radius of raccoon rabies cases in the vaccination area.

One hundred tracking stations, consisting of a 1 m² raked dirt area with a vaccine laden bait centrally placed, were located throughout the vaccination area preceding most vaccinations. The pits were examined on a daily basis for evidence of bait disturbance and animal tracks during 1 mo following the first day of vaccine distribution. These data were used to estimate bait disturbance and identify species taking the bait.

Thin (8–10 µm) sections of mandibles collected from raccoons which died in the vaccination area were cut at the root of the canine tooth with a low speed Isomet saw (Buehler, Ltd., Lake Bluff, Illinois, USA) equipped with two diamond wafering blades. These mandible sections were mounted (Gel/Mount, Biomedica Corp., Foster City, California, USA) and examined under fluorescence microscopy (excitation filter 380–425 nm, barrier 460 nm) for the tetracycline biomarker (Johnston et al., 1987). The tetracycline fluoresced gold/green along the margins of dental cementum. Biomarker uptake served as an index of bait ingestion by the raccoon population. Temporal distribution of tetracycline positive and negative specimens was determined for 145 raccoons collected after the fall 1993 and spring 1994 vaccinations.

Raccoons cage-trapped in the vaccination area were sedated with a mixture of ketamine hydrochloride (10 mg/kg; Bristol Laboratories, Syracuse, New York, USA) and xylazine hydrochloride (0.4 mg/kg; Lloyd Laboratories, Shenandoah, Iowa, USA) and 3 ml blood samples

were collected from the jugular vein in silicon coated vacutainer tubes (Becton Dickinson, Rutherford, New Jersey, USA). The animals were ear tagged (National Band and Tag Company, New Port, Kentucky, USA) and released at the site of capture after a recovery period. The serum was separated under refrigeration (4 °C) and centrifugation. Sera were frozen (–20 °C) and stored pending analysis. Rabies virus neutralizing antibody titers in the sera were determined using a modification of the rapid fluorescent focus inhibition test (Reagan et al., 1983) and the seroconversion (≥0.5 IU/ml) rate was estimated.

To test the stability of the vaccine in the delivery system, vaccine filled wax ampules and vaccine filled wax ampules within fishmeal bait cylinders were placed in a mammalian and avian scavenger-proof wire mesh cage exposed to sunlight and elements at a site within the vaccination area in May of 1992. Unprotected wax ampules were monitored because domestic cats would occasionally eat the fishmeal bait cylinders leaving the wax vaccine ampules undamaged at tracking stations. Extremes in monthly air temperatures were obtained from the Federal Aviation Administration Weather Bureau (Pomona, New Jersey, USA). Samples were collected and viral vaccine titers were measured (Rupprecht et al., 1988) at once weekly intervals (1 bait/wk), from May 1992 through February 1993.

Surveillance for rabies in Atlantic, Cumberland and Cape May counties included examination of raccoons, striped skunks (*Mephitis mephitis*), red foxes (*Vulpes vulpes*), gray foxes (*Urocyon cinereoargenteus*), woodchucks (*Marmota monax*) and other wildlife found dead of unknown causes, killed during trapping seasons, or submitted because of clinical signs of disease. Passive surveillance involved contacting, directly and through news releases, hunters, trappers, wildlife rehabilitators, wildlife bi-

TABLE 2. Tetracycline biomarker detection from raccoon jaws in relation to the time of V-RG vaccination in New Jersey.

Period of sampling	Number positive/ Number tested	Percent positive
Pre-fall 1992 vaccination	2/24	8
Post-fall 1992 vaccination	27/68	40
Post-spring 1993 vaccination	31/43	72
Pre-fall 1993 vaccination	17/39	44
Post-fall 1993 vaccination	133/180	74
Post-spring 1994 vaccination	22/33	67
Pre-fall 1994 vaccination	1/10	10
Post-fall 1994 vaccination	5/17	29
Total	238/414	57

ologists, conservation officers, park rangers, police, veterinarians, animal control officers, health officers, and the general public for submission of specimens to County Health Departments for rabies examination. Active surveillance involved weekly collection of raccoons and other animals found dead along roads transecting the vaccination area and portions of adjacent unvaccinated surveillance areas. Brain or spinal cord, when brain specimens were unsuitable because the skull was crushed, were tested at the New Jersey Department of Health Laboratory (Trenton, New Jersey, USA) or The Thomas Jefferson University, Centre for Neurovirology (Philadelphia, Pennsylvania, USA) using the fluorescent antibody technique (Goldwasser and Kiseling, 1958). Rabies virus typing using the monoclonal antibody technique (Rupprecht and Dietzschold, 1989) was performed on two skunks and one river otter (*Lutra canadensis*) to identify the strain of field virus.

The species, date and location of raccoon rabies cases were entered into a data base using ARC INFO GIS software (ESRI, Redlands, CA) to generate three rabies case distribution maps. The rate of movement of the raccoon rabies epidemic in New Jersey, Atlantic County, the vaccination area, and that portion of Cape May County south of the vaccination area was estimated by measuring the distance from the first case in the area of interest to the most distant case and dividing by the time interval in days.

Rabies prevalence was estimated for surveillance raccoon populations by dividing the number of rabid raccoons by the total number tested. A comparison of rabies prevalence between raccoons collected through submissions from the public and those collected primarily as road-kills in the treatment area for a one year

period (August 1993–July 1994) was made to determine if these surveillance methods biased the rabies prevalence estimates. A similar comparison was made for raccoons collected from the vaccination area by a fur-trapper using snares from December 1993 to March 1994. Comparisons of rabies prevalence also were made between unvaccinated and vaccinated populations for the same time periods in different areas and different time periods in the same area. A comparison of the proportion of rabies cases comprised of species other than raccoons in vaccinated versus unvaccinated populations was performed. The chi-square test or Fisher's exact test in the event that some of the cells had expected counts of <5 (SAS Institute, Inc., 1987) were utilized for these comparisons. The null hypothesis assumed no difference in rabies prevalence between collection methods, areas or time periods.

Twenty raccoons trapped for serum sampling were fitted with a radio-collar (Lotek Engineering Inc, Newmarket, Ontario, Canada) and monitored at least three times weekly and at different times of day for a mean of 23 observations per raccoon during 1 to 6 mo between September 1993 and July 1994 to determine the maximum linear movement from the site of capture. Capture locations were selected to represent various raccoon habitats distributed throughout the vaccination area. Differences in log transformed travel distances between healthy and rabid raccoons were analyzed using a one-way analysis of variance (PROC GLM); (SAS Institute Inc., 1987).

RESULTS

Seventy-four percent ($n = 450$) (range = 50 to 87%) of the vaccine filled baits at tracking stations were gone within the first week of distribution and all were gone by the end of the second or third week. Raccoon tracks were present in 165 (61%) of the tracking stations that had one or more of nine species (domestic cat, opossum [*Didelphis virginianus*], domestic dog, fox [red or gray], striped skunk, white-tailed deer [*Odocoileus virginianus*], gray squirrel [*Sciurus carolinensis*], human) of identifiable animal tracks associated with bait removal.

The highest indices of bait and vaccine uptake followed the fall 1993 and spring 1994 vaccinations. The tetracycline biomarker was detected in 155 (73%) raccoons in the vaccination area (Table 2).

TABLE 3. Rabies virus neutralizing antibodies (≥ 0.5 IU) from raccoons sampled in the V-RG vaccination area in New Jersey relative to the time of vaccination.

Period of sampling	Number seropositive/ Number sampled	Percent- age sero- positive
Post-spring 1992 vaccination	39/109	36
Pre-fall 1992 vaccination	1/14	7
Post-fall 1992 vaccination	24/41	59
Pre-fall 1993 vaccination	5/14	36
Post-fall 1993 vaccination	6/11	55
Post-spring 1994 vaccination	5/7	71
Total	80/196	41

The biomarker positive specimens were widely distributed throughout the vaccination area with no apparent voids. Eleven (61%) of 18 raccoons sampled during the same period in the vaccination area had seroconverted in response to rabies glycoprotein (Table 3). The rates of seroconversion were usually less than for biomarker positivity in the same interval. Both biomarker positivity and seroconversion rates were lower in the pre-fall vaccination periods. Opossums, domestic cats, and skunks were major bait competitors based on biomarker detection in non-target species (Table 4).

In the environmental vaccine stability study, vaccine virus titer rapidly declined and was undetectable after 3 mo exposure to sunlight and warm temperatures in the wax ampule (Table 5). The titer of vaccine in ampules protected from the environment by the bait cylinder was maintained

TABLE 5. V-RG vaccine virus stability measured in the New Jersey V-RG vaccination area during 1992–1993.

Month	Temperature (C)		V-RG virus titer (log TCID ₅₀ /ml)	
	High	Low	Ampules ^a	Baits
May	27	8	7.4–8.0	8.2–8.5
June	30	4	5.1–7.3	7.8–8.0
July	36	13	3.2–4.2	7.2–8.2
August	27	11	0	6.7–7.5
September	23	4	0	6.3–6.9
October	21	–4	0	6.3–7.0
November	18	–7	0	6.6–6.8
December	18	–8	0	6.4–6.7
January	20	–11	0	0–5.4
February	16	–14	0	0–2.6

^a Unprotected from light and heat by a bait cylinder.

near the initial titer during the first 3 mo and did not substantially decline until mid-winter, 8 mo later.

Figure 1 depicts cases diagnosed between the date the first rabies positive animal was detected in the tri-county area (5 July 1992) and the date the first rabid animal was found in the vaccination area (30 April 1993). The other two maps (Figs. 2, 3) include additional cases from two successive 1 yr intervals (1 May 1993 to 30 April 1994 and 1 May 1994 to 30 April 1995).

The front of the raccoon rabies epidemic moving along watersheds from north-western New Jersey at approximately 50 km/yr was first detected in northern Atlantic County on 5 July 1992. It moved south through Atlantic County at approximately

TABLE 4. Tetracycline biomarker detection in non-target mammals from the New Jersey raccoon V-RG vaccination area.

Period of sampling	Opossum	Cat	Skunk	Red fox	River otter
Pre-fall 1992 vaccination	11/25 (44) ^a	2/12 (17)	1/12 (8)	0/1	0/0
Post-fall 1992 vaccination	21/31 (68)	3/4	2/2	1/3	0/0
Spring 1993 vaccination	18/22 (82)	1/4	9/15 (60)	1/5	1/5
Post-spring 1994 vaccination	4/5	6/8	5/9	1/3	0/0
Pre-fall 1994 vaccination	0/0	0/0	0/1	0/1	0/0
Post-fall 1994 vaccination	0/0	0/0	0/0	1/1	0/0
Total	54/83 (65)	12/16 (75)	17/27 (63)	4/14 (29)	1/5

^a Number positive/number tested (%).

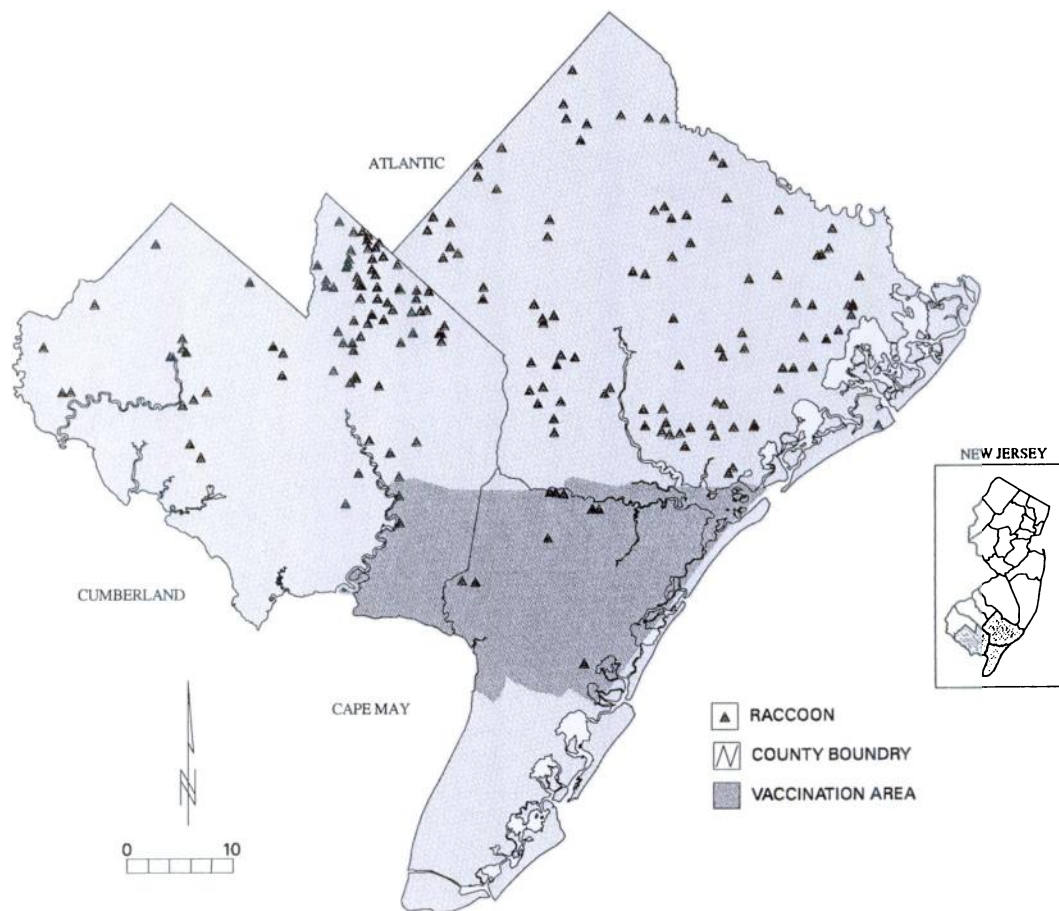


FIGURE 2. Raccoon rabies cases following 1 yr of challenge to the V-RG oral rabies vaccinated population within the Atlantic, Cumberland, and Cape May counties between 5 July 1992 and 30 April 1994.

47 km/yr reaching the northern border of the vaccination area in late April 1993. This was documented by the first case of rabies being diagnosed in the vaccination area 30 April 1993 (Fig. 1). One year later 11 rabid raccoons were detected in the vaccination area (Fig. 2). The epidemic moved through the vaccination area at an estimated rate of 15 km/yr with the first case detected south of the vaccination area on 13 November 1994. The breach of the barrier was characterized by a proliferation of cases immediately below the border of the treatment area followed by cases progressively farther south through this unvaccinated area. The epidemic progressed at a rate of 43 km/yr and reached the

southern end of the peninsula by 30 April 1995 (Fig. 3).

The prevalence of rabies (10%) in 51 raccoons submitted by the public and 41 raccoons collected by investigators along roads in the vaccination area during the same 1 yr period was identical (Fisher's 2-tail test, $P = 1.000$). Therefore, raccoons collected by these two methods were grouped for prevalence calculations and comparisons. The prevalence of rabies (<1%) in the snared population (172) of raccoons from the vaccination area during the 4 mo of the trapping season was significantly lower (Fisher's 2-tail test, $P = 0.027$) than the 15% rabies prevalence in 20 unsnared raccoons. Therefore, the sam-

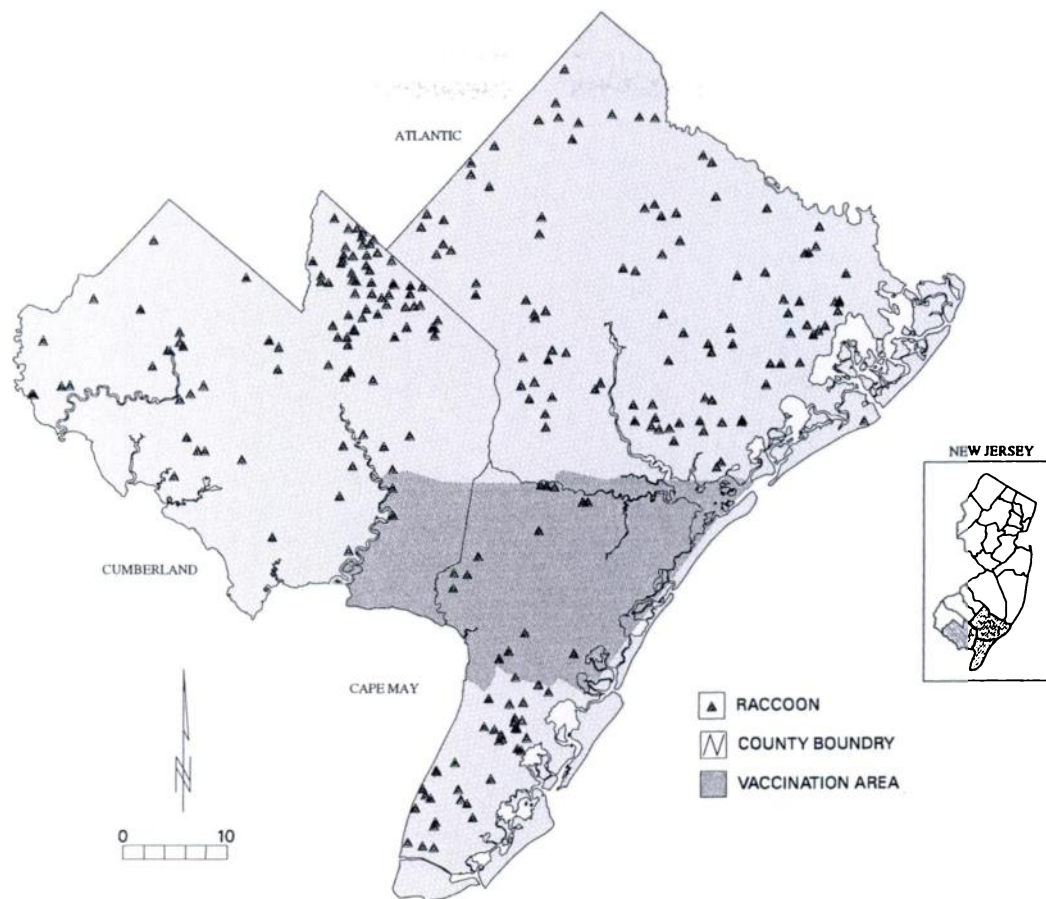


FIGURE 3. Raccoon rabies cases following 2 yr of challenge to the V-RG oral rabies vaccinated population within the Atlantic, Cumberland, and Cape May counties between 5 July 1992 and 30 April 1995.

ple of snared raccoons was not included in calculations or comparisons of rabies prevalence, since a snare sampling was not conducted in the unvaccinated areas.

The prevalence of rabies in 96 raccoons from the vaccination area collected during the first year of challenge (30 April 1993 to 30 April 1994) by the advancing epidemic was 10%. This was significantly lower ($\chi^2 = 76.43$, 1 df, $P = 0.001$) than the 65% prevalence among 189 unvaccinated raccoons to the north and west of the vaccination area (Atlantic and Cumberland counties). The 8% rabies prevalence among 61 vaccination area raccoons during the second year of rabies challenge (1 May 1994 to 30 April 1995) was six-fold lower ($\chi^2 = 26.30$, 1 df, $P = 0.001$) than

the 53% prevalence among 43 raccoons submitted from the unvaccinated area to the north and west. The apparent slight decline in rabies prevalence in raccoons from both the vaccination area and the unvaccinated area between the first and second year of rabies challenge was not significant ($\chi^2 = 0.21$, 1 df, $P = 0.65$ and $\chi^2 = 2.02$, 1 df, $P = 0.16$, respectively).

The 77% prevalence of rabies in 47 unvaccinated raccoons south of the vaccination area between 1 May 1994 and 30 April 1995 was nine-fold higher ($\chi^2 = 52.73$, 1 df, $P = 0.001$) than that of the 61 raccoons (8%) in the vaccination area. It also was significantly higher ($\chi^2 = 5.31$, 1 df, $P = 0.021$) than the prevalence (53%)

among 43 unvaccinated raccoons north and west of the vaccination area.

Five skunks, a gray fox, a river otter, and a house cat also were diagnosed as rabid between 30 April 1993 and 30 April 1995 in the vaccination area. They were found along the same north to south river drainages thought to be the route by which the vaccination area was breached. Thirteen of 16 rabid raccoons from the vaccination area were found along this route (Fig. 3). Species other than raccoons comprised 33% of the 24 rabies cases in the vaccination area. This was significantly greater (Fisher's 2-tail test, $P = 0.013$) than in the unvaccinated areas, where 13% of the 308 rabies cases were skunks ($n = 30$), foxes ($n = 5$) and cats ($n = 5$). The raccoon strain of rabies virus from skunks and an otter was confirmed by monoclonal antibody typing.

Nineteen non-rabid radio-collared raccoons moved a mean (\pm SD) maximum straight line distance of 1.5 ± 0.5 km during the monitoring period. These movements ranged from 0.80 km for a juvenile female, to 2.58 km for an adult female. Five raccoons trapped in the vaccination area without displaying clinical signs of rabies, including one radio-collared raccoon, were ear-tagged and subsequently diagnosed as rabid from recovery sites at a mean distance of 8.4 ± 4.3 km. The maximum linear movement of the rabid raccoons was significantly greater ($P = 0.0001$) than that of the non-rabid radio-collared raccoons in the area. The recovery sites for these rabid raccoons were 1.9, 7.6, 8.0, 11.6, and 12.9 km from the original trapping location after 1, 10, 4, 3, and 2 mo, respectively. One homeowner in Atlantic County north of the vaccination area was relocating live-trapped nuisance raccoons 53 km to the tip of the Cape May Peninsula in the spring of 1992. Another homeowner in Atlantic County was relocating raccoons 18 km to a wildlife management area on the southern border of the vaccination area during the summer in

1993. Both individuals were instructed to cease illegally relocating raccoons.

DISCUSSION

Comparisons of tracking station, bio-marker, and serologic data demonstrate that raccoons were a primary target species for the vaccine. Differences between raccoon seroconversion rates and tetracycline positivity rates may be due to several factors, including different sample populations, ingestion of the bait and not the vaccine in the ampule, rapid but transient seroconversion (Chappuis, 1995), and failure to incorporate tetracycline in the canine portion of the mandible (Hable et al., 1992). Low pre-fall tetracycline positivity and seropositivity appear primarily attributable to recruitment of juvenile raccoons unexposed in the spring vaccinations. Since vaccine virus titers remained relatively unchanged in the bait ampule system for 3 mo, the vaccine probably remained at full potency during the 3 wk period of bait uptake. Therefore, it is unlikely that low vaccine potency was a factor in seroconversion rates in raccoons ingesting baits.

The methods utilized for sample collection and calculation of rabies prevalence were biased. Rabies prevalence in the raccoon population sampled in trail set snares may more closely approximate the actual prevalence in the wild population, since rabies associated behaviors would not enhance detection. In contrast, aggressive behavior may enhance the likelihood of being struck by a car or observed and destroyed by a pet or homeowner. These biases may explain the 15-fold difference in rabies prevalence between samples collected by snaring (<1%) versus other surveillance methods (15%). The non-snare methods utilized in the vaccination and non-vaccination areas were comparable. The intensity of the surveillance effort in the vaccination area may have been indirectly enhanced by the large amount of time spent by the investigators interacting with residents, trapping, radiotracking,

driving road-kill routes and performing vaccine distributions. In spite of this increased effort, only 16 rabid raccoons were detected in 2 yr. This contrasts with 36 rabid raccoons detected in the smaller non-vaccination area south of the barrier within 5 mo of the first detected case. While the prevalence estimates do not express the actual occurrence of rabies in the raccoon population they are similarly biased throughout the study areas and when combined with mapping of cases are useful for comparisons and detecting effects of rabies vaccination.

Studies of raccoon movement provided useful insights into propagation of an outbreak into the vaccination area. The mean (\pm SD) maximum linear distance traveled by raccoons in the vaccination area during rabies challenge (1.5 ± 0.5 km, $n = 19$) was similar to the mean linear distance (1.2 ± 2.2 km, $n = 36$) for raccoons in the same area in the year prior to the arrival of rabies (C. E. Rupprecht, unpubl. data). It was identical to the 1.5 km maximum distance traveled by raccoons on Paramore Island, Virginia during vaccine safety trials (Hanlon et al., 1998). The increased linear movements of rabid raccoons are similar to those (8 to 23 km) of relocated adult raccoons seeking a new home range (R. C. Rosatte, pers. comm.). This raccoon behavioral change may be advantageous to rabies virus perpetuation through increased likelihood of encountering naive hosts.

Breach of the vaccination barrier may have been facilitated by the increase in the maximum linear movement of rabid raccoons, shortfalls in vaccination coverage resulting in lower population immunity, relocation of nuisance raccoons by homeowners, and, less likely, the occurrence of raccoon rabies in species other than raccoons. The failure of a proportional decline in rabies for skunks and other non-raccoon species may be an artifact of small sample sizes, lower bait-vaccine uptake, vaccine failure in non-target species, higher densities of these non-target species in

the vaccination area or the greater likelihood the skunks would be sampled by investigators as opposed to those involved in passive surveillance.

Reduced rabies prevalence in a raccoon population having previously experienced 2 yr of epidemic rabies may reflect reduction of population density due to rabies and "natural" post-exposure immunization (Anthony et al., 1990). It is likely, these factors contributed to the lower rabies prevalence among the unvaccinated raccoons north of the vaccination area relative to those south of the vaccination area in 1995.

Oral vaccination with the V-RG vaccine was effective in reducing the prevalence of rabies in the raccoons from the vaccination area by six to nine-fold below that of the unimmunized population. The rate of movement for raccoon rabies in unvaccinated areas of this study (43–50 km/yr) was within the range (25–60 km/yr) reported by Jenkins and Winkler (1987). The rates they reported were greater than the rate (15 km/yr) in the vaccination area raccoons. Immunization reduced by three-fold the rate at which the New Jersey rabies epidemic had been moving. However, the more rapid rate of movement resumed as soon as the vaccination barrier was breached. Thus, this field research demonstrated the utility and feasibility of using an oral rabies vaccine for slowing transmission of rabies among free-ranging raccoons.

Future efforts to improve on raccoon rabies immunization should consider utilizing greater bait densities ($>64/\text{km}^2$), wider barriers based on multiples of rabid raccoon travel distances (13 km), and identification of raccoon concentrations possibly using aerial infrared sensing for subsequent targeting during non-random vaccine distributions. In addition, funding initiatives should encompass a minimum of 4 to 5 yr for program initiation, monitoring and analysis if wildlife vaccination and disease control are to become a reality.

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