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Authors: Hanlon, Cathleen A., Niezgoda, Michael, Hamir, Amir N., Schumacher, Carolin, Koprowski, Hilary, et al.

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FIRST NORTH AMERICAN FIELD RELEASE OF A VACCINIA-RABIES GLYCOPROTEIN RECOMBINANT VIRUS

Cathleen A. Hanlon,[1,2,7] Michael Niezgoda,[1,2] Amir N. Hamir,[4,5] Carolin Schumacher,[1,6] Hilary Koprowski,[1,3] and Charles E. Rupprecht[1,2]

[1] The Wistar Institute, 3601 Spruce Street, Philadelphia, Pennsylvania 19104 USA
[2] Current address: Centers for Disease Control and Prevention, Division of Viral and Rickettsial Diseases, Viral and Rickettsial Zoonoses Branch, 1600 Clifton Road, Atlanta, Georgia 30333 USA
[3] Current address: Thomas Jefferson University, Department of Microbiology and Immunology, Jefferson Alumni Hall, 1020 Locust Street, Philadelphia, Pennsylvania 19107 USA
[4] New Bolton Center, University of Pennsylvania, School of Veterinary Medicine, Kennett Square, Pennsylvania 19348 USA
[5] Current address: Oregon State University, Veterinary Diagnostic Laboratory, 142 Magruder Hall, Corvallis, Oregon 97331 USA
[6] Virbac Laboratories, BP 27, 06511 Carros Cedex, France
[7] Author to whom requests for reprints should be sent. (e-mail: cfh8@cdc.gov)

ABSTRACT: Following nearly 10 yr of extensive laboratory evaluation, a vaccinia-rabies glycoprotein (V-RG) vaccine was the first recombinant virus to undergo limited North American field release on 20 August 1990. The free-ranging raccoon population on Parramore Island (Virginia, USA) was exposed to a high density (10 baits/ha) of vaccine-laden baits distributed on a 300 ha vaccination area. An annual total of 887 raccoons were live-trapped for sedation, physical examination and blood collection for rabies antibody determination; there was no evidence of adverse effects or lesions due to the vaccine. Age and sex distributions, mean body weights, and live-capture histories of raccoons from the vaccination and non-baited control areas were compared. There were no statistically significant differences in survivorship between the baited and non-baited areas, nor between rabies antibody-positive and antibody-negative raccoons from the vaccination area. There was no trend in field mortality that suggested an association with either tetracycline or sulfadimethoxine, used as biomakers, or with vaccine contact determined by antibody status. No gross or histopathologic lesions due to the vaccine were demonstrated among a subsample of live-trapped raccoons collected for gross necropsy, biomarker analysis, histopathologic examination, and V-RG virus isolation attempts. Recovery of V-RG virus was limited to the tonsils of two biomarker-positive, clinically healthy raccoons collected from the vaccination area for postmortem examination on days 2 and 4 following bait distribution. These data reinforce the extensive body of safety data on the V-RG virus and extend it to include field evaluation where vaccine is offered free-choice in abundance, in baits designed to attract free-ranging raccoons, in a relatively simple ecosystem.

Key words: Field study, oral vaccination, *Procyon lotor*, rabies, raccoon, vaccine, vaccinia recombinant virus.

INTRODUCTION

The current rabies epidemic among raccoons (*Procyon lotor*) in the eastern USA has emerged as the most significant animal rabies outbreak recorded to date (Rupprecht and Smith, 1994; Rupprecht et al., 1995). Known human mortality due to this rabies variant has been averted in large part due to effective human post-exposure prophylaxis. Nevertheless, raccoon rabies is a notable public health concern in that it is a fatal, zoonotic disease, which incurs significant costs at the federal, state, and local level to meet the need for diagnosis, epizootiological information, public education, trained personnel to respond to calls involving potentially rabid wildlife, and the cost of pre- and post-exposure biologicals and their administration (Uhaa et al., 1992; Rupprecht at al., 1996).

Although the concept of oral rabies vaccination (ORV) originated in the USA (Baer et al., 1971), ORV was implemented for red fox (*vulpes vulpes*) rabies control in Europe and Canada via baits containing modified-live rabies virus vaccines (Steck et al., 1982; Johnson et al., 1988; Schneider et al., 1988; Wandeler et al., 1988; Wandeler, 1991). In contrast to red foxes, raccoons were not readily immunized by the oral route with modified live rabies vi-

rus vaccines (Rupprecht et al., 1989). Additionally, modified live vaccines possess a residual risk of vaccine-induced rabies, as demonstrated experimentally in at least one primate species (*Papio ursinus*) (Bingham et al., 1992), as well as in native North American fauna, such as the striped skunk (*Mephitis mephitis*) (Rupprecht et al., 1990). Furthermore, vaccine-induced rabies associated with oral baiting programs occurred in a red fox, domestic cat (*felis catus*), and a stone marten (*Martes foina*) in Switzerland (Wandeler, 1991), as well as in a striped skunk, raccoon, red fox, and calf (*Bos taurus*) in Canada (A. I. Wandeler, pers. commun.). However, a vaccinia-rabies glycoprotein (V-RG) virus (Kieny et al., 1984) has proven to be an orally efficacious vaccine for raccoons (Rupprecht et al., 1986; 1987; 1988). Moreover, it has been extensively evaluated in the laboratory for safety in over 50 vertebrate species (Blancou et al., 1986; Baltazar et al., 1987; Tolson et al., 1987; 1988; Blancou et al., 1989; Brochier et al., 1989; Artois et al., 1990; Rupprecht et al., 1992a; 1992b) with no adverse effects regardless of route or dose in immunocompetent hosts. A limited field release of the recombinant vaccine on a relatively biosecure island was a logical prerequisite to its intended widespread use on the mainland for control of raccoon rabies. A major objective of this recombinant vaccine field release was to evaluate the free-ranging raccoon population on Parramore Island (Virginia, USA) for adverse effects following high-density distribution of V-RG vaccine-laden baits.

MATERIALS AND METHODS

Study site description

As reviewed in preliminary background summaries (Hanlon et al., 1989; 1993), Parramore Island (USA: 37°11′N: 75°38′W) is the largest (3,440 ha) and most biologically diverse barrier island off the eastern shore of Virginia. It is 12.8 km long, 1.2 to 2.0 km wide, and 7.7 km from the mainland (Deuser et al., 1979). The island is bounded on its eastern shore by the Atlantic Ocean, and on its western edge by salt marsh and extensive bays or broad tidal channels. On the bayside of the southern third of Parramore Island is Revel's Island separated from Parramore by a tidal channel <0.3 km wide and 2.0 to 4.0 m deep at mean low tide.

Naturally-occurring carnivores on Parramore Island are limited to raccoons and red foxes; the only ungulates are white-tailed deer (*Odocoileus virginianus*). Four small mammal species are present: the rice rat (*Oryzomys palustris*), house mouse (*Mus musculus*), meadow vole (*Microtus pennsylvanicus*), and Norway rat (*Rattus norvegicus*) (Deuser et al., 1979). At the time of the study, raccoon rabies was not present on the Island nor on the nearby mainland.

Vaccination and control areas

A roughly rectangular (300 ha) vaccination area was designated on the central upland region of the island for the distribution of 3,120 vaccine-laden baits (10 baits/ha). In addition, four major control areas of approximately 50 ha each were established which did not receive vaccine-laden baits. The non-baited control areas consisted of (1) the northern, partially forested (pine and cedar), portion of Parramore Island, approximately 1.0 km north of the vaccination area; (2) an area 1.5 km south of the vaccination area which was an isolated upland hummock surrounded by marsh; (3) a beach, dune and adjacent marsh area approximately 3.0 km south of the vaccination area; and (4) an upland and adjacent marsh section of Revel's Island.

Vaccine, bait, and biomarker preparation

Approximately 1.0 ml of the V-RG recombinant virus vaccine (10^8 plaque forming units/ml; Rhone Merieux, Inc., Athens, Georgia, USA) was inserted into paraffin ampules (W. and F. Manufacturing Co., Inc., Buffalo, New York, USA). The sealed vaccine chambers were placed into fishmeal polymer cylindrical baits (length 4.0 cm, diameter 2.8 cm) (Hanlon et al., 1989). The fishmeal polymer baits consisted of fish oil, fish meal, a synthetic polymer binder and 100 mg tetracycline (E. I. Dupont Co., Orange, Texas, USA), as a calciphillic biomarker (Linhart and Kennelly, 1967). Fishmeal polymer plugs were inserted into both ends of the cylinder and sealed with melted paraffin.

Immediately prior to field distribution, each vaccine-laden bait was placed in an individual polyethylene bag carrying a descriptive label. Approximately 50 ml of a slurry, consisting of equal parts by volume of sucrose, whole chicken eggs, vegetable oil, and crushed shellfish, was added to the bag to enhance bait attractiveness to raccoons and repugnance to hu-

mans. In addition to tetracycline in the bait matrix, a commercial formulation of sulfadimethoxine (SDM) (ALBON®, Hoffmann-La Roche Inc., Nutley, New Jersey, USA) was included in the slurry at a dose of 250 mg per 50 ml of slurry, as a second biomarker (Hanlon et al., 1993).

Bait distribution and disturbance documentation

Approximately 3,120 vaccine-laden baits were hand-placed on linear transects to achieve a baiting density of 10 baits/ha (1,000 baits/km^2; four baits/acre). Each bait location was marked with a numbered flag to facilitate bait recovery and for recording purposes. To augment identification of animal species contacting baits, 100 tracking stations were established at randomly chosen bait sites. These were checked daily for bait disturbance. In addition, photographic and written records were taken on discernable animal tracks that could be characterized in origin as carnivore, small mammal, ungulate or avian.

Biomarker analysis

A rapid commercial card test (Environmental Diagnostics, Inc., Burlington, North Carolina, 27215, USA) was used to screen raccoon sera collected during the first 6 days after baits were placed in the field for the presence of SDM as an indication of slurry ingestion and thus, potential exposure to vaccine (Hanlon et al., 1993).

Bait acceptance was assessed from a subsample of raccoons routinely collected for postmortem evaluation, by examination of mandibular bone samples under a Leitz ultraviolet illumination microscope for tetracycline deposition within alveolar bone and cementum and dentine of adjacent teeth (Linhart and Kennelly, 1967). Additionally, bone samples were collected for tetracycline analysis from carcasses found in the vaccination or control areas.

Rabies antibody seroprevalence

Blood samples were collected with a 5 cc syringe and a 20 ga needle via the jugular or anterior vena cava from all live-trapped raccoons while they were sedated for physical examination and ear-tagging. Serum samples were removed from clotted blood samples and frozen at −20 C for subsequent determination of rabies virus neutralizing antibody titers by a modification of the rapid fluorescent focus inhibition test (Reagan et al., 1983), with values reported as the reciprocal of the dilution with 50% virus neutralization.

Raccoon live-trapping

Tomahawk live traps (Tomahawk Live Trap Co., Tomahawk, Wisconsin, USA) were placed in pairs at permanent stations 100 m apart on three parallel transects throughout the vaccination area and on a single transect through each of the four control areas. The traps were baited with fresh fish, crabs, or commercial canned dog food, set for 4 continuous nights, and checked daily, shortly after sunrise.

The trapping schedule throughout the vaccination area was determined on a weekly basis by a random number lottery. Access to the control areas was restricted by tides and weather. Hence, during a week with favorable tides and weather, all traps along a single transect within a particular control area were set and checked daily.

Live-trapped raccoons were sedated with a mixture of 10 mg/kg ketamine hydrochloride (Veterinary Products, Bristol Laboratories, Division of Bristol-Meyers Co., Syracuse, New York, USA) and 0.4 mg/kg xylazine (Haver, Bayvet Division, Miles Laboratory, Inc., Shawnee, Kansas, USA) administered intramuscularly. All animals were examined for lesions consistent with orthopoxviruses, such as vaccinia or cowpox (Gaskill et al., 1983; Fenner et al., 1988), including papules, macules, vesicles or ulcerations of epidermis and mucous membranes. After recording the sex, age (Sanderson, 1950), and weight, the animals were eartagged (National Band and Tag Co., Newport, Kentucky, USA), blood samples were collected, as described previously, and, if clinically normal, the animals were then released.

Definition of resident and adjunct resident raccoons

Raccoons captured during the first 2 wk following vaccine-laden bait distribution (20 August through 3 September 1990) were designated "residents" of the area in which they were captured. The vaccination area residents were considered at highest probability of vaccine contact because they were spatio-temporally associated with the presence of vaccine-laden baits. After 3 September 1990, first-time-captured raccoons were designated "adjunct residents" of the area in which they were trapped.

Mean body weight, capture frequency

Given the hypothesis that potential adverse vaccine effects may result in a loss of body condition, mean body weights of vaccination and control area residents at first capture and at the first recapture during each subsequent season

were evaluated for statistically significant differences by analysis of variance (Zar, 1974). Also, because theoretically possible morbidity associated with vaccine contact may have resulted in a reduced number of recaptures per individual raccoon, the capture histories (number of times captured) of individual raccoons from the vaccination area were compared to those of control area raccoons.

Radiotelemetry

Thirty-two subadult and adult raccoons of both sexes from the vaccination area and two raccoons from control areas were radio-collared during the first 2 wk and monitored for movements on a regular basis during the 12 mo study period. Radio-collars were equipped with a mortality signal, to allow monitoring for deaths that may have occurred during the study, and recovery of carcasses for post-mortem examination. Animals were monitored for mortality and general location at least once weekly from August 1990 through August 1991 with a telemetry receiver (Lotek Engineering, Inc., Aurora, Ontario, Canada). Additionally, as in the pre-vaccine phase of this research (Hanlon et al., 1989), radio-collars were used to document the extent of raccoon movements between the study area and nearby control areas.

Mortality and intentionally removed raccoons

All observed raccoon carcasses were collected for post-mortem examination, as were any live-trapped animals exhibiting abnormal clinical signs or gross lesions at any time throughout the study. Additionally, throughout the year following bait distribution, subsamples of raccoons from the vaccination and control areas were routinely collected for further laboratory study or euthanatized for post-mortem examination, histopathology, virus isolation studies, and biomarker determination.

Gross necropsy, histopathologic evaluation, and virus isolation

Field post-mortem examination, histopathologic evaluation, and virus isolation attempts were conducted on subsamples of live-trapped raccoons and on any suitable tissues from raccoons found dead in the field during the 12 mo study. Subsamples of live-trapped animals were euthanized by intravenous administration of sodium pentobarbital (Euthanasia-6 Solution, Vet Labs Limited, Lenexa, Kansas, USA). Tissue samples were collected for routine histopathological evaluation for lesions compatible with an orthopox-viral related etiology during field necropsy. These included representative samples of: heart (right and left ventricle); diaphragm; tongue; masseter muscle; lung; liver; gall bladder; kidney; pancreas; spleen; skin (body and paw); thymus (when available); eye; mesenteric lymph node (three sections); stomach; intestines (minimum four sections); salivary glands; urinary bladder; prostate; testicle (ovary; uterus); trachea; thyroid; esophagus (two sections); aorta; brain (cerebrum, cerebellum, and brainstem); and cervical spinal cord. These were processed for microscopic examinations as previously described (Rupprecht et al., 1986). Additionally, portions of brainstem, lung, liver, spleen, kidney and tonsil were collected with sterile technique from euthanized and freshly dead animals for V-RG vaccine virus isolation attempts and immediately placed on dry ice for transit, and then stored at −70 C until analysis.

Samples for virus isolation which had been stored frozen were individually homogenized in Eagle's minimal essential media (MEM-10, supplemented with 10% fetal calf serum, penicillin and streptomycin) with a mortar and pestle to achieve a 20% w/v homogenate. The supernatants of organ homogenates were incubated in suspension with BHK-21 cells and were examined daily for cytopathic effects, as previously described (Rupprecht et al., 1988).

Survival analysis

Resident raccoons trapped in the vaccination and control areas were categorized by subsequent capture history (1) recaptured from September through January 1991, (2) recaptured from February through August 1991, (3) "lost-to-follow-up" or not recaptured from September 1990 through August 1991, and (4) removed from the population during the first 2 weeks following bait distribution. The distributions of resident vaccination and control area raccoons among follow-up categories were compared by Chi Square analysis (Zar, 1974).

Actuarial life tables were constructed comparing survivorship of (1) all vaccination and control area raccoons including relatively fresh carcasses of raccoons presumed to have been alive at the beginning of the study, (2) vaccination and control area raccoons with serological results (both antibody-positive and antibody-negative raccoons from the vaccination area versus raccoons from control areas which were all antibody-negative) from captures between months 1 and 7 of the study, and (3) antibody-positive and antibody-negative raccoons from the vaccination area. Results were compared for statistical significance with the Mantel-Haenszel test (Miller, 1981).

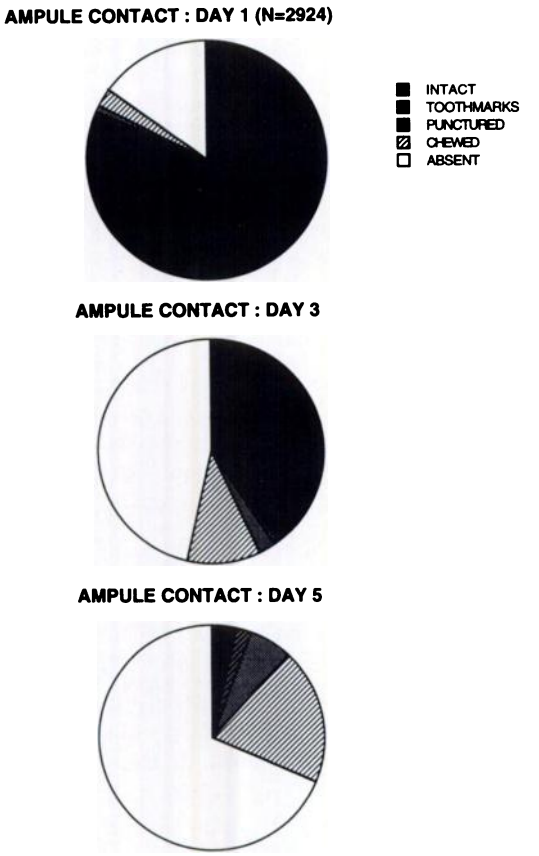

FIGURE 1. Vaccinia-rabies glycoprotein recombinant virus vaccine-bait disturbance over time at Parramore Island, Virginia.

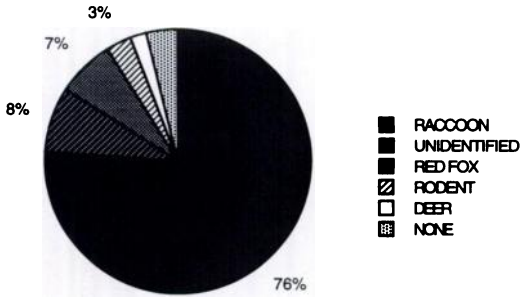

FIGURE 2. Results from tracking stations with vaccinia-rabies glycoprotein recombinant virus vaccine-baits at Parramore Island, Virginia.

RESULTS

Bait disturbance

Approximately 50% of baits had evidence of animal contact within 48 hr of distribution. Bait contact evidence ranged from complete disappearance of the bait and bag from the bait station to finding only a torn or chewed bag, remnants of the fishmeal polymer bait or chewed or punctured remains of the wax ampule. By day 5, more than 90% of the vaccine-laden wax ampules within the baits had been disturbed (Fig. 1).

Carnivores were the major species implicated in the disturbance and consumption of vaccine-laden fishmeal polymer baits; >75% were raccoons (Fig. 2).

Biomarker analysis

As reported elsewhere (Hanlon et al., 1993), SDM was detectable in 38 (77%) of 49 sera from 49 individual raccoons from the vaccination area during the first 6 days after bait distribution. Conversely, none of the sera (n = 12) from control area raccoons were SDM-positive.

Evidence of tetracycline ingestion was found in 47 (84%) of 56 bone samples from vaccination area raccoons (Hanlon et al., 1993). Conversely, all 34 bone samples from control area raccoons were tetracycline-negative.

Serology

Prevalence of rabies antibody among resident raccoons from the vaccination area, reflecting apparent V-RG vaccine contact and sero-conversion, was 57% (30 of 53). Seroprevalence among adjunct residents from the vaccination area was 47% (27 of 57). Overall seroprevalence among resident and adjunct resident raccoons live trapped in the vaccination area was 52% (57 of 110). The geometric mean titer (GMT) of antibody-positive raccoons live-trapped sequentially throughout the study was highest (3.54 ± 0.08 IU/ml) during September, 4–6 wk following bait distribution. The GMT remained high (1.24 ± 0.06 IU/ml) during October, but then declined rapidly throughout the remainder of the year to around or below the cut-off level for an adequate titer in humans of 0.5 IU/ml (Fig. 3). All serum samples from

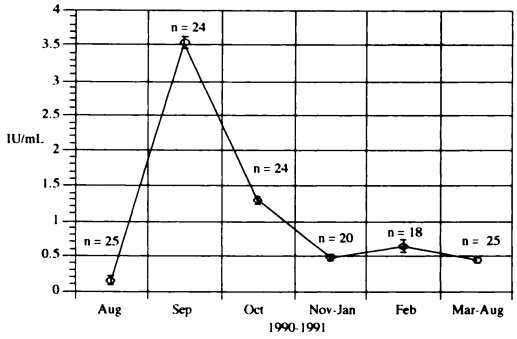

FIGURE 3. Geometric mean titers of antibody positive raccoons at Parramore Island, Virginia. Vaccine-laden baits were distributed on 20 August 1990. N is the number of samples from antibody-positive raccoons obtained during the period. Rabies virus neutralizing antibody titers were determined with the rapid fluorescent focus inhibition test (RFFIT). Geometric mean titers were calculated using log transformed values of the reciprocal of the dilution with 50% neutralization of virus.

control area raccoons (n = 89) were negative.

Raccoon live-trapping

From August 1990 through August 1991, 14,180 trapnights yielded 887 raccoon captures in the vaccination and control areas. With the application of 10,158 trapnights in the vaccination and 4,022 trapnights in the control areas, there were 210 and 126 total captures and 409 and 142 recaptures, respectively. There were no significant differences between the overall trap success in the vaccination area of 6.1% (619 total captures per 10,158 trapnights) and 6.7% (268 total captures per 4,022 trapnights) in the control areas.

There was no evidence of adverse effects or lesions suggestive of an orthopoxvirus etiology in the 887 raccoons clinically evaluated at the time of live-capture.

The age and sex distribution of live-trapped antibody-positive resident raccoons (n = 27; 10 adult males, eight adult females, four immature (subadult and juvenile) males, and five immature females) in the vaccination area was not significantly different from that of antibody-negative raccoons captured from both the vaccination and control areas (n = 42; 11 adult males, 12 adult females, 10 immature males and nine immature females) ($P > 0.05$). There were no significant differences between the age and sex distributions of all first time captures, regardless of antibody status, within each season from the vaccination and control areas (data not shown).

Mean body weight

There were no significant differences in the mean (±SD) body weights of antibody-positive raccoons (3.2 kg ± 1.2, n = 27) versus antibody-negative raccoons (3.7 kg ± 1.6, n = 42) from the vaccination and control areas. At first capture, there were significant differences in mean body weights of resident raccoons; these differences were among age classes rather than related to residence area or serological status, with mean adult body weights consistently higher than immature raccoon mean body weights. The mean body weights of seropositive vaccination area raccoons, grouped by age and sex, were not significantly lower during subsequent seasons nor were they lower than the mean weights from seronegative counterparts in the vaccination or control areas (data not shown).

Individual raccoon capture frequency

In the vaccination area, individual raccoons (n = 210) were captured a median of 5.14 times (mean = 2.95; range 1–27). Comparatively, control area raccoons (n = 126) were captured a median of 2.03 times (mean = 2.13; range 1–17). The distribution of individual capture histories was not significantly different between raccoons from the vaccination and control areas.

Radiotelemetry

Thirty two raccoons from the vaccination area and two raccoons from control areas were radio-collared while live-captured before and during the first 14 days of the field trial. Radio-collars facilitated retrieval of four raccoons that died in the field from 2 to 8 mo after placement of vaccine-laden

baits. One antibody-positive, adult male raccoon from the vaccination area died as a result of traumatic blood sampling in October. Adverse environmental conditions during a northeastern storm appeared to be a contributing factor in a second death of an antibody-negative, adult female raccoon in October. During a week of temperatures consistently at or below 0 C in January, a large antibody-positive adult male raccoon was found in the vaccination area with no gross or histopathological lesions suggestive of an orthopox-viral infection; emaciation was noted. A fourth adult male, antibody-status-undetermined, raccoon died in late spring 1991 during warm environmental temperatures that resulted in rapid tissue decomposition which precluded histopathologic examination.

Mortality and intentionally removed raccoons

Nine (including four previously described radio-collared raccoons) and six raccoon carcasses, were collected from the vaccination and control areas, respectively. Six (75%) of eight bone samples from raccoons recovered from the vaccination area were positive for tetracycline; none of the bone samples from control area raccoons were tetracycline-positive. Using trapnight effort as an index of person-hours spent in the area, with nine carcasses per 10,158 trapnights in the vaccination area and six per 4,022 trapnights in the control areas, the number of raccoon carcasses was not significantly different between the two areas ($\chi^2 = 0.32$; $P > 0.05$).

Throughout the year, 52 and 32 live-trapped raccoons were collected from the vaccination and control areas, respectively, for either euthanasia and necropsy (30 from the vaccination area; 21 from control areas) or further study in captivity (22 from the vaccination area; 11 from control areas) (Rupprecht et al., 1993). The ratio of the number of animals removed to the number of individuals live-trapped in the vaccination area (52/210) and the control areas (32/126) was the same (25%) ($\chi^2 = 0.92$; $P > 0.05$).

Gross necropsy, histopathology and virus isolation

Thirty raccoons from the vaccination area and 21 raccoons from control areas were collected for euthanasia and gross and histopathologic examination, virus isolation, and tetracycline analysis. All raccoons were clinically normal upon physical examination. Additionally, histopathologic examination was conducted on tissues collected from (1) two raccoon carcasses from Revel's Island in December, (2) one radio-collared raccoon carcass recovered in the vaccination area in January, and (3) two raccoons that died during handling in February. Upon postmortem examination, only one gross lesion which could be compatible with an infectious viral etiology was observed; this resulted in the first described case of papillomatosis in a raccoon (Hamir et al., 1995). Multiple incidental skin lesions, primarily due to ectoparasites and trauma, were found in 13 (62%) of 21 raccoons from control areas and in 20 (67%) of 30 raccoons from the vaccination area. Other incidental lesions included multifocal mineralization of blood vessels in the meninges of the brain (A. N. Hamir, unpublished data), endogenous lipid pneumonia (Hamir et al. 1996), and *Phagicola* sp. induced-enteritis and lymphadenitis (Hamir et al. 1993). The V-RG virus was isolated from tonsils of two biomarker-positive, clinically healthy raccoons on days 2 and 4 post-bait distribution from the vaccination area; no histopathological lesions were observed in the tonsils of these two raccoons. All other tissues from these raccoons and 14 other raccoons collected during the first 2 wk of the vaccine trial were virus isolation negative.

Survivorship

During the first 2 wk of the field trial, 100 resident raccoons were live trapped in the vaccination area. Similarly, 26 raccoons were identified as residents in control areas during this same time period. The follow-up on these populations was not significantly different ($\chi^2 = 2.45$; df = 7, P

> 0.05), with 53% (53/100) versus 50% (13/26) recaptured from September through January 1991. An additional 10% (10/100) versus 8% (2/26) were recaptured from February through August 1991. Twenty five (25/100) versus 31% (8/26) "lost-to-follow-up" or not recaptured from September 1990 through August 1991, in the vaccination and control areas, respectively. The remaining animals, consisting of 12 vaccination area residents and four control residents, were removed from the populations during the first 2 wk of the vaccine trial for necropsy and virus isolation.

There were no statistical differences in 1 yr survival between all raccoons identified from the vaccination ($\bar{X} \pm SE = 91.6\% \pm 0.1$) and control areas ($88.0\% \pm 0.2$; $P > 0.05$). Moreover, there was no significant difference in survivorship of rabies antibody-positive ($94.5\% \pm 0.2$) versus antibody-negative raccoons ($96.7\% \pm 0.1$; $P > 0.05$) from the vaccination area.

DISCUSSION

The first environmental release of a self-replicating genetically engineered organism should necessarily be conducted conservatively, in relative biocontainment, such as on an island. The geographic isolation of the Parramore Island raccoon population was conducive for intensive study of the raccoons at risk of vaccine contact. Moreover, field activities had been conducted on the island preceeding vaccine release to describe baseline findings such as background gross and histopathologic lesions (Hanlon et al., 1989). Although no V-RG viral-associated gross lesions, regardless of route or dose, had been observed during extensive laboratory evaluation in over 50 mammalian and avian species (Rupprecht et al., 1992a; 1992b), all live-trapped raccoons were examined carefully for overt morbidity or gross lesions compatible with an orthopoxvirus-related etiology. Among the 210 and 126 individuals recaptured 409 and 142 times in the vaccination and control areas, respectively, only one had evidence of localized skin lesions which were grossly suggestive of a possible orthopox-viral etiology. Further detailed histopathogical and immunohistopathological examination resulted in the first description of a papilloma-virus in raccoons (Hamir et al., 1995). There were no adverse effects or lesions due to an orthopoxvirus.

Among any free-ranging population of animals, variations in health or immune status among individuals may be expected due to biological and environmental conditions, as well as varying levels of exposure to and infection with a wide variety of naturally occurring parasites and pathogens. In contrast, laboratory-born animals are typically maintained under minimal to no pressure from parasites, pathogens, competitors, and predators, and in a constant, sheltered environment with predictable food and water sources. Thus, the response to a self-replicating biological agent may be more variable among individuals of free-ranging populations, particularly when the vaccine is self-administered through ingestion of baits offered free choice and in abundance. However, no adverse effects in raccoons were found in this study following environmental broadcasting of V-RG virus in baits. Yet concurrent with findings during previous placebo baiting trials (Hanlon et al., 1989; Hable et al., 1992), bait disturbance and tracking station data documented that the probability of raccoons contacting a majority of the baits was high.

Tetracycline, one of the biomarkers included in the vaccine-bait package, was useful to determine the proportion of raccoons which had consumed the fishmeal polymer bait, albeit retrospectively, since assessment was based upon postmortem bone samples. Additionally, it was useful in documenting the limited spatial impact of bait distribution, with all tetracycline-positive raccoons originating in the vaccination area and no positive samples from control areas. In contrast to tetracycline, SDM, a short-term seromarker (Hanlon et

al., 1993), was useful to generate immediate information from ante-mortem samples processed with card tests on site, regarding bait contact status of specific individuals, and for preliminary determination of the proportion of raccoons contacting vaccine-laden bait packages. From a biocontainment perspective, there was no biomarker evidence of bait consumption among animals from the surrounding control areas either by animals physically carrying baits out of the vaccination area or by movement of vaccinated animals from the vaccination area to control areas.

In addition to the presence of biomarker(s), rabies seroprevalence was indicative of V-RG vaccine contact. The identification of a rabies antibody-positive cohort allowed the comparison of raccoons with direct evidence of vaccine contact (and thus at highest risk of manifesting potential adverse vaccine effects) to antibody-negative, or antibody status-undetermined, vaccination area cohorts, as well as to the control area cohort. In addition to enhancing the detection of potentially vaccine-exposed raccoon movement out of the vaccination area, the control areas also served to monitor for inappropriate, unexplained seroporevalence, at distant sites and lacking association with biomarker, as an indicator of potential biological V-RG vaccine transmission, for which there was no evidence.

Due to the theoretical potential for morbidity or mortality associated with the risk of vaccine contact, raccoon trap success was evaluated as a gross comparative index of the relative density of "live-trapable" raccoons in the vaccination and control areas (Moore and Kennedy, 1985; Kennedy et al., 1986). The control areas were established primarily for biosecurity, to enhance the probability of detecting biomarker-positive or sero-positive raccoons at sites removed from the immediate vaccination area, and as such, were extensive narrow strips of marsh and upland hummocks, on the beach or bayside of Parramore or Revel's Island, with a single trapline in a linear fashion to maximize sample sizes which may have biased toward new captures. In contrast, the vaccination area was rectangular, extending from beach to bay across the widest upland area of Parramore Island, with three permanent parallel traplines to maximize recaptures of individual raccoons. Moreover, the vaccination area was chosen because of its optimal habitat to maximize the number of raccoons at risk of vaccine contact, whereas, the control areas consisted of marginal habitat with fewer potential den sites and no permanent fresh water sources. Nevertheless, despite higher trap effort applied to the vaccination area, overall trap success in the vaccination area was not significantly different from the control area.

As a result of antagonistic interactions with conspecifics or varying reproductive or growth status (Kaufman, 1982), a particular subgroup of a free-ranging population, due to age or gender, may experience more severe environmental or biological stresses resulting in a lower nutritional plane, higher parasite burdens, greater environmental exposure due to inferior shelter, etc., which may predispose the subgroup to potential adverse vaccine effects. Thus it was particularly relevant to examine the vaccination area population for changes in demographics following vaccine distribution. However, as reported, the age and sex distribution of first time captures from the vaccination area was not significantly different from control areas during the study.

Survival analysis was applied to evaluate potential differences between vaccination and control area raccoons. The inequity of applied trapping pressure (more in the vaccination area and less in control areas), does not affect the data used for survival analysis. For example, survivorship could be determined and compared from intensive trapping yielding multiple captures of an individual throughout a specific period or from intermittent trapping with only an initial capture at the begining of the period

and a final capture at the end. The lack of statistically significant differences in survivorship indicated that there was no detectable increase in mortality that could be associated with the overall vaccination area population, nor specifically with rabies antibody-positive raccoons, which had clearly come into contact with the experimental vaccine.

Although vaccine efficacy was not the primary objective of this field evaluation, the substantial live-trapping conducted during this study generated extensive serological data. Based upon GMT results from this study, the optimal time for detection of maximal antibody levels as a result of vaccine-laden bait consumption appears to be approximately 4 to 6 wk following bait distribution, although most antibody-positive animals were reliably detected between 2 and 12 wk following bait distribution.

In parallel with laboratory findings, V-RG virus isolation was limited to the tonsils of two biomarker-positive, clinically healthy raccoons on days two and four post-bait distribution from the vaccination area providing no suggestion of an abnormal or prolonged viral infection nor evidence for biological transmission. When considered collectively, these data add to the extensive body of knowledge regarding V-RG vaccine safety and extend it to include evaluation where vaccine is offered free-choice in abundance, in baits designed to attract raccoons, in a relatively simple ecosystem.

The development and field testing of the V-RG vaccine provides a potential adjunct method of rabies control to complement traditional efforts, such as public education, domestic animal vaccination, and human post-exposure prophylaxis. The ultimate utility of this adjunct method will be dependent in part upon further demonstration of efficacy. As an outcome of these investigations, more than 80% of a subsample (n = 22) of raccoons removed from Parramore Island in the seventh month of the field evaluation survived rabies virus challenge in the laboratory, whereas more than 90% of control area raccoons (n = 11) succumbed (Rupprecht et al., 1993).

Since this study, the annual number of vaccine-laden baits distributed for raccoon rabies control in the USA has risen nearly exponentially to a total of over 800,000 in 1997. Eleven subsequent field projects have been conducted or are in progress in Pennsylvania (1991–1992), New Jersey (1992–1994), Massachusetts (1994–present), Florida (1995–present), New York (1994–present; five projects), Vermont (1997–present), and Ohio (1997–present). Although the development of oral rabies vaccination for raccoon rabies control has progressed rapidly, definitive values for basic parameters, such as bait density, distribution pattern, frequency of distribution, etc., have not been fully elucidated. Rigorous review of current projects coupled with responsible design and intensive follow-up during new initiatives will greatly advance the current methods of oral wildlife vaccination, which is still in its relative infancy for control of raccoon rabies. Nonetheless, successful implementation of oral vaccination for this fatal zoonotic disease may serve as a model for other wildlife management strategies. In view of the ever-increasing close association of humans and wildlife, there is a need for increased investigation of free-ranging animal populations and their relationship to wildlife diseases, particularly zoonoses. The eventual goal of these investigations is judicious, environmentally- and ecologically-sound management of wildlife populations in a humane manner which would concurrently increase the quality of human and animal life.

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