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PREREQUISITES FOR ORAL IMMUNIZATION OF FREE-RANGING RACCOONS (*PROCYON LOTOR*) WITH A RECOMBINANT RABIES VIRUS VACCINE: STUDY SITE ECOLOGY AND BAIT SYSTEM DEVELOPMENT

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ABSTRACT: A model baiting system suitable for the delivery of an oral rabies vaccine to free-ranging raccoons (*Procyon lotor*) was developed and tested on barrier islands in South Carolina (USA). Features of barrier island physiography and ecology were studied relative to selective bait deployment and site biosecurity. Capture-mark-recapture data were obtained from 228 raccoons. Raccoon density estimates, using a modified census assessment technique, were one raccoon per 1.8 to 2.7 ha. Mean (±SE) and range home area estimates of radio-collared raccoons were 84 (±15.6) ha (27 to 176 ha) by a minimum convex polygon method and 138 (±22.8) ha (43 to 241 ha), by a harmonic mean transformation method. Habitat utilization determinations of radio-collared raccoons were conducted to identify study areas to potentially maximize selectivity of bait towards raccoons and to reduce the absolute number of baits deployed. Island raccoons showed a habitat preference for maritime forest, maritime shrub and marsh areas. Additionally, there was no evidence of inter-island or mainland exchange of ear-tagged or radio-collared raccoons. A disease and mortality survey was conducted to identify baseline pathology and incidental lesions in the target raccoon population, prior to actual vaccination initiation. Thirty-eight percent of 30 clinically suspect raccoons sampled had intracytoplasmic eosinophilic inclusions diagnostic of canine distemper; no other lesions suggestive of viral etiologies were found. Serological surveys for raccoon poxvirus and rabies virus antibodies were negative. Antibody titers to canine adenovirus 1 and 2 indicated a moderate level of exposure (approximately 10 to 16%) in the raccoon population. Overall, 93 to 100% of placebo baits were consistently disturbed by 7 days post-bait deployment, and bait acceptance rates by raccoons ranged from 49 to 85%, by using a modular systems approach to select the optimum combination of bait attractant, biomarker, matrix, density, and distribution. These results suggest that a large proportion (up to 85%) of a free-ranging island raccoon population can be selectively and safely targeted, marked and monitored utilizing a proposed oral bait delivery system for recombinant or other rabies vaccines.

Key words: Rabies, oral vaccination, raccoons, *Procyon lotor*, bait, field studies, recombinant vaccine, parasitologic survey.

INTRODUCTION

European wildlife rabies oral vaccination campaigns have been conducted in the field since the late 1970's when oral vaccines were first administered in attempts to control red fox (*Vulpes vulpes*) rabies (Steck et al., 1982; Schneider, 1985; Wandeler, 1988; Pastoret et al., 1988). To date, several million modified-live and recombinant rabies vaccine-laden baits have been deployed throughout Europe (Schneider et al., 1988). Vaccinated areas, in which fox rabies had been enzootic, are now reporting no new cases (Wandeler, 1988). Unfortunately, except for the red fox, no combined oral rabies vaccine and

baiting system has yet been thoroughly tested for safety and efficacy specifically towards actual field applications en masse for other important rabies vector species. These include free-ranging domestic dogs throughout the developing world and the raccoon (*Procyon lotor*) and striped skunk (*Mephitis mephitis*) in North America. Development of much-needed immunization programs for these latter species has until now been hindered by the lack of an efficacious oral vaccine (Baer, 1985), contributed to by overall low bait acceptance rates in the past. Before the actual deployment of any self-replicating viral vaccines (particularly genetically-modified agents developed through biotechnology), prospective field trial protocols require a combined molecular, pathological and ecological approach to provide sufficient baseline information to assess overall project biosafety.

In this study, a systems approach was applied towards the development and field testing of a bait delivery protocol preparatory to use in the oral vaccination of free-ranging raccoons with a vaccinia-rabies glycoprotein (V-RG) recombinant virus vaccine. The overall efficacy of this vaccine has been well established in the raccoon and other major wild and domestic animal species in captivity (Rupprecht et al., 1986; Blancou et al., 1986; Tolson et al., 1987; Rupprecht and Kieny, 1988), but no rabies control field trials with this or any other recombinant vaccine have taken place in North America; safety assessments are currently underway in Virginia and Pennsylvania. Major system components for anticipated vaccine deployment are complex and include the oral rabies vaccine-bait delivery system, the study area environment, and the targeted wildlife (e.g., raccoon) population (Fig. 1). We describe a field test site and bait deployment protocol for potential use with an oral V-RG or other recombinant virus vaccine, island study site biota, and related ecological facets pertinent to initiation of such a trial. The targeted raccoon population

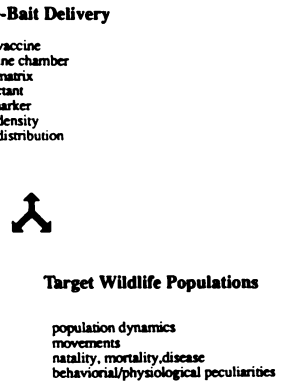

FIGURE 1. Diagrammatic representation of major components of a modular system protocol for oral immunization of wildlife against rabies.

structure, home range and movement patterns are discussed relative to site biosecurity, while serological and histopathological disease surveys relevant to a field trial with recombinant vaccine are described.

MATERIALS AND METHODS

Murphy, Cedar, and South Islands (33°20′N, 79°40′E) are coastal barrier islands in Georgetown and Charleston Counties, South Carolina (USA). They are located approximately 80 km north of the city of Charleston, as a part of the Santee Coastal Reserve System and the South Carolina Biosphere Reserve (Fig. 2). Island sites are separated from each other by the South and North Santee Rivers and Bays. They are separated from the mainland by the intracoastal waterway and 5 to 7 km of tidal marsh and creeks. Islands are bounded on their eastern shores by the Atlantic Ocean and on their western front across the intracoastal waterway and tidal marshes by 107,245 ha of the Francis Marion National Forest. The habitats on Cedar, Murphy, and South Islands are characteristic of the South Carolina maritime ecosystem, and highly influenced by salt spray and salinity. The islands are extremely irregular in shape, primarily due to large expanses of inland water from waterfowl management practices. Murphy Island is approximately 5 km by 10 km and encompasses a total of 3,189 ha. Two hundred seventy ha are upland forest, 486 ha are marsh, 2,227 ha are brackish water impoundments and the remainder is beach/dune habitat. Cedar Island is approximately 2.6 km by 7 km and encompasses a total of 1,576 ha. Approximately 116 ha are upland maritime forest, 283 ha are marsh, 1,098 ha are impoundments and the remainder is

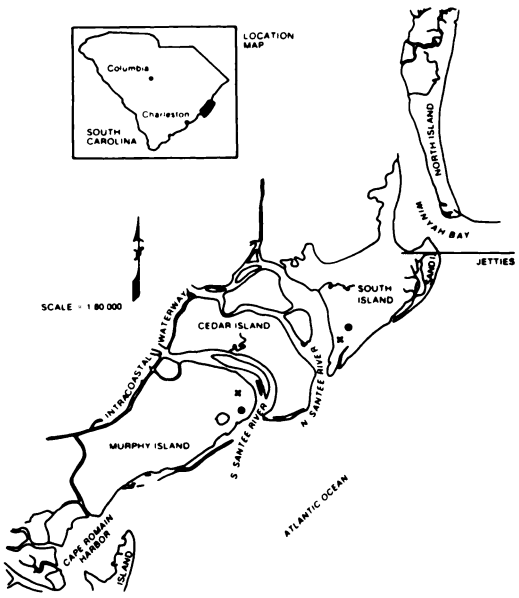

FIGURE 2. Diagram of the study area, South Carolina barrier islands (9 cm equals approximately 1.19 km). Study areas I and II on Murphy and South Islands are indicated by an X and O, respectively.

beach/dune habitat. South Island is approximately 4.2 km by 11.5 km and encompasses a total of 1,508 ha. Three hundred twelve ha are upland maritime forest, 487 ha are marsh, 703 ha are waterfowl impoundments and the remainder is beach/dune habitat. Murphy and Cedar Islands are uninhabited; permanent human residences occur only on South Island. Two study sites (0.5 and 2 km^2) were established in upland maritime forest habitat (Fig. 2), on both Murphy and South Island, separated by a distance of approximately 2.0 km, and one study site was selected in maritime forest on Cedar Island.

The five major habitat types described for the islands follow Swiderek (1982) and Sandifer et al. (1980), as follows: maritime forest, maritime shrub thicket, marsh, impoundment dikes and beach/dune. Maritime forest is a broad-leafed (*Quercus* sp.) mixed-evergreen forest, including loblolly pine (*Pinus taeda*) and cedar (*Juniperus virginiana* and *J. silicicola*) species. Maritime shrub thicket is an ecotonal habitat found between maritime forest and dune ridge or marsh communities. Shrub thickets occur on many earthen dikes and represent much of the habitat on small hummocks or marsh islands. This ecotone can have little or no ground cover and reaches a height of approximately 4 m. Dominant species include salt myrtle (*Baccharis halimifolia*), wax myrtle (*Myrica cerifera*), yaupon (*Ilex vomitoria*) and red cedar (*J. virginiana*). Impoundment dikes on the South Carolina islands date back to early 18th century rice culture. Three different dike habitat cover types have been described: wooded dikes, shrubby dikes and grassy dikes (Swiderek, 1982). Wooded and shrubby dikes have vegetative cover similar to maritime forest, with the addition of hackberry (*Celtis laevigata*) and Chinese tallowtree (*Sapium sebiferum*). Grassy dikes are dominated by various grasses approximately 1 m in height. Two major types of marsh, salt and brackish-water, are classified as either open tidal or managed impoundments. Vegetation of the impoundments differs depending on management, salinity and water level. The type of ground cover on beach/dune ridges differs with the undulations of the ridges and the rough topography. Some dry ridges have canopy, shrub and ground layers. Shrub layers consist of a mixed-deciduous, scrub oak layer, while ground cover is generally sparse.

Mean annual surface air temperatures approximate 17.8 C for the region. Mean annual surface water temperatures for the Georgetown area are approximately 16.0 C. The first freezing air temperatures in autumn occur near the end of November and the last freezing air temperatures in the spring occur near mid-March. Precipitation for the region generally ranges 120 to 130 cm annually.

Data concerning mammalian species occurrence for Cedar, Murphy and South Islands were compiled directly from live-trapping and field observations (e.g., scat, tracks), and by personal communication with state wildlife biologists familiar with these areas. In each baiting trial, live-trapping for mammals was initiated 10 days post-placebo bait deployment, along established linear transects in each study area. Trapping was continued for 20 nights per study site. The first 10 nights were devoted to marking and releasing. The second 10 nights of trapping were devoted to recapture. Three nights separated capture-mark and recapture trapping. Forty to 50 #207 Tomahawk live traps (Tomahawk Live Trap Co., Tomahawk, Wisconsin 54487, USA) were baited with whiting (*Menticiurrus* sp). All traps were checked and rebaited the next morning. During small mammal surveys, 50 Sherman live traps (H. B. Sherman Traps, Inc., Tallahassee, Florida 32304, USA) were baited with a peanut butter and oatmeal mixture and placed on parallel transect lines near Tomahawk live traps to simultaneously monitor small mammal diversity, bait contact and acceptance. Live-captured furbearers were sedated intramuscularly with a combination of ketamine hydrochloride (Veterinary Products, Bristol Laboratories, Division of Bristol-Meyers Co., Syracuse, New York 13220, USA), 10 mg/kg and xylazine hydrochloride (Haver, Bayvet Di-

vision, Miles Laboratory, Inc., Shawnee, Kansas 66203, USA), 1 mg/kg, (Fowler, 1978). Once sedated, animals were bilaterally tagged with #893 ear-tags (National Band and Tag Co., Newport, Kentucky 41072, USA), bled via jugular vein or by cardiac puncture and a record was made of each animal's weight, sex and age (following Sanderson, 1950). From a physical examination, the following data were recorded: teat or baculum length, reproductive status and any gross lesions.

A modified census assessment technique was used to derive raccoon density and population estimates, as described by Kennedy et al., 1986 for trap placement and animal density calculations. This technique allowed collection of population density data simultaneously with mark and recapture efforts for calculation of bait acceptance. Trapping concentrated in upland areas because preliminary observations (tracks and scat) indicated raccoon use of marsh and beach areas was relatively infrequent. Additionally, intensive marsh live-trapping was impractical because of certain logistical difficulties in marsh habitats, such as traversing long distances by foot and the high probability of animals drowning with unpredictably high tidal water flow.

Before release, 20 raccoons (seven adult females, three sub-adult males and ten adult males) from the South Island Study Area were equipped with radio transmitter collars (Lotek Engineering, Inc., Aurora, Ontario L4G 4J9, Canada) with movement and mortality functions. Raccoons were located at least once weekly for 11 mo. The exact time of animal monitoring varied within each week, but over the life of the transmitter, locations were obtained for each animal at least once weekly during each quarter of a 24 hr day: morning, afternoon, sunset, sunrise. Two or more compass bearings were used to triangulate the location of these animals on U.S. Geological Survey topographical maps (Franklin Maps, King of Prussia, Pennsylvania 19406, USA), 1:660, using a 0.1 ha grid coordinates-system overlay. Data were analyzed by McPaal (Stuwe and Blohowlak, 1986), which used the following module codes to calculate home range: Minimum Convex Polygon (Eddy, 1977) and Harmonic Mean Transformation (Dixon and Chapman, 1980). Only raccoons with a minimum of 20 reliable radiolocations were included in the home range analysis. Radiolocations were also used to determine raccoon habitat use, because previous studies have suggested a correlation between specific habitats and relative raccoon abundance (Leberg and Kennedy, 1988). The predominant habitat type (following Sandifer et al., 1980) within a given radiolocation 0.1 ha grid was classified as marsh, maritime forest, maritime shrub, beach/dune ridge or impoundment. However, because changes in habitat type occurred over relatively short distances, and locations of beach/dune were often within several meters of the maritime shrub habitat, these two areas were considered together for estimates of telemetry accuracy and raccoon habitat occurrence.

A crude baseline disease survey was conducted to identify potentially significant factors of mortality and disease affecting the study raccoon populations. Techniques included field necropsy collection for histopathologic and parasitologic analysis and serological surveys. A pilot study of raccoon histopathology was conducted on 30 raccoons collected on South Island during March 1987. Tissues, including brain and samples of all representative organ systems, were collected and processed as described previously (Rupprecht et al., 1986, 1987). Blood samples were obtained from all captured furbearers. Serum was harvested from clotted blood and was stored at −20 C prior to analysis. Virus-neutralizing antibody titers to rabies virus were obtained by a modification of the rapid fluorescent focus inhibition test (Reagan et al., 1983). Serum survey techniques for raccoon poxvirus and canine adenoviruses based upon virus neutralization and inhibition of cytopathic effect, followed Alexander et al. (1972) and Wiktor et al. (1984).

Four different baits for raccoons were tested on South Carolina barrier islands: two different self-contained polystyrene blister-pack baits used successfully for fox rabies control in Europe (Schneider et al., 1988) and Canada (Bachmann et al., 1990); a polyurethane sponge and tallow cube bait (Johnston et al., 1988); and a fishmeal polymer bait cylinder (Hanlon et al., 1989) consisting from between 0.5 to 10% ethylene copolymer, 75 to 95% fishmeal, and 5 to 20% fish oil, by weight, containing a gelatin capsule (Bell-Tex Laboratories, Little River, Texas 76554, USA). Specifics of bait types, biomarker and baiting densities used for two areas each on Murphy and South Islands are summarized in Table 1. Four placebo baiting trials were conducted, two in autumn and two during spring. Only Murphy and South Islands were used in these baiting trials and each had a separate 0.5 km^2 and 2 km^2 study site. Bait test sites on the two islands were assumed to be roughly equivalent with regard to habitat and relative raccoon population densities. Sites were located at least one estimated raccoon home range apart and were separated from one another on each island by large bodies of inland water.

Different attractants (e.g., synthetic fermented egg, and commercial essences of persimmon, raccoon urine, sweet corn, shellfish, and shrimp) were originally tested using scent post tracking pits (Linhart and Knowlton, 1975), deployed at 0.1 km intervals along island causeways. The

TABLE 1. Placebo baiting trials designed for the oral vaccination of free-ranging raccoons with a vaccinia-rabies glycoprotein recombinant virus vaccine, South Carolina Barrier Islands.

Study site	Baiting area (ha)	Bait type	Biomarker	Placebo vaccine chamber	Bait density[a]	Raccoon bait acceptance[b]
Murphy Island I	200	German fishmeal Canadian chicken	Tetracycline	Polystyrene/foil sachets	2	71% (12/17)
Murphy Island II	50	Fishmeal polymer	Tetracycline	Gelatin capsule	10	85% (17/20)
South Island I	200	Tallow/sponge German fishmeal	Tetracycline	Polyurethane cube; polystyrene/foil sachet	3	49% (19/39)
South Island II	50	Fishmeal polymer	Rhodamine B	Gelatin capsule	5	46% (11/24)

[a] Number of baits distributed per ha.

[b] Bait acceptance determined by microscopic examination of bones and teeth for biomarker incorporation.

pits were checked daily over all seasons for animal tracks and markings. Shellfish essence, consisting of 10.0% fresh blue crab (*Callinectes sapidus*) offal, 0.1% synthetic shellfish essence oil, 9.9% sucrose, 70.0% vegetable oil, and 10.0% whole eggs, was a readily accepted attractant of coastal raccoons in these pilot scent post trials, and was the attractant used in all subsequent baiting trials. Ten to 20 ml of this homogenate was placed into individually perforated polyethylene bags to coat each bait in all large scale bait trials, regardless of bait type. All baits were frozen at −20 C for 48 hr before distribution. Tetracycline hydrochloride (Sigma Chemical, St. Louis, Missouri 63178, USA; 150–200 mg/bait) incorporated into the bait was the standard matrix biomarker used in bait trials. Upon ingestion, tetracycline is preferentially bound by the calciphilic tissues of bait-consuming animals (Johnston et al., 1987). The industrial dye rhodamine B (Sigma Chemical) was also tested as a potential biomarker on South Island. It had been used successfully to mark free-ranging mountain beaver (*Aplodontia rufa*) (Lindsey, 1983) and feral dogs (Perry et al., 1989). When used, 300 mg of rhodamine B powder was incorporated into the shellfish essence homogenate of each bait.

All baits and the attractant were packaged in individually perforated polyethylene bags on which a public identification notice was stamped. Bags aided handling, storage and deployment, potentially acted as visual attractants to raccoons and improved post-deployment surveillance for bait contact determination. Baits were distributed by hand at flagged sites along transect lines to facilitate monitoring of bait contact and non-target species activity. To potentially increase bait consumption by raccoons, baits were placed near known den sites and along waterways or trails to known foraging areas. Bait densities ranged from two baits/ha to 10 baits/ha (Table 1). All bait stations on transect lines were checked on day 2 and day 7 post-deployment to evaluate bait contact and non-target species activity. Additionally, along bait transects on Murphy Island, 80 tracking pits, 1 m in diameter, were constructed with a sifted sand base and lime overlay (Linhart and Knowlton, 1975). Data on species activity were recorded from the tracking pits on day 2 and day 7 post-bait deployment. Bait contact was defined as consumption of ≥50% of the outer bait matrix, inner vaccine chamber penetration, or baits missing from a bait station.

Subsamples of raccoons and selected non-target species were euthanatized following recapture for determination of bait acceptance rates. Furbearers were sedated with an intramuscular xylazine/ketamine mixture as above, and euthanatized with an intravenous barbiturate solution (Euthanasia-6 Solution, Vet Labs Limited, Lenexa, Kansas 66125, USA). Rodents were killed by cervical dislocation. Preliminary cage trials with wild raccoons demonstrated that a preferred calciphilic tissue to assay for detection of tetracycline incorporation was mandibular bone, especially osteocytes in haversian systems. Mandibles from dead furbearers were collected in the field using heavy shears to fracture the mandibular symphysis and to disarticulate the temporo-mandibular joint. One mandible was kept for age analysis using cementum annuli (Johnston et al., 1987); the other was used for tetracycline analysis. To reduce potential loss of fluorescence, excess tissue was removed and the mandibles were stored frozen at −20 C until examined. Non-decalcified mandibles were mounted and cut on a double-set, diamond-bladed saw (Isomet, Bueler Ltd., Malvern, Pennsylvania 19355, USA). Samples 60 to 150 μm thick were mounted on a glass slide, following Johnston and Watt (1981), and were viewed under a Leitz-ultraviolet light microscope at 450

nm. Tetracycline incorporation in bone and teeth produces a characteristic yellow-gold fluorescence; rhodamine B produces a characteristic orange-red fluorescence. All samples were read blind by at least two observers. Bait acceptance rates were calculated by study area and species, as the percent of tetracycline or rhodamine B-positive animals from the total number examined. Acceptance rates generated from placebo bait field trials were based on tetracycline (except the South Island Study Site II, where rhodamine B was used).

RESULTS

Mammalian species inventory

A qualitative mammalian species inventory for the three South Carolina barrier islands compiled from this study included: white-tailed deer (*Odocoileus virginianus*), locally abundant (commonly sighted on each study area) in maritime forest and maritime shrub habitat; bobcat (*Lynx rufus*), present in beach/dune ridge habitats and impoundments, based upon tracks and scat; river otter (*Luttra canadensis*), present in tidal creeks based upon tracks and scat; opossum (*Didelphis virginiana*), trapped in maritime forest, but for which actual densities were not obtained; raccoon, abundant in maritime forest, in marsh and to a lesser degree in impoundment habitats; and marsh rabbits (*Sylviligus palustris*), observed and occasionally live-trapped on Cedar and Murphy Islands in marsh and maritime shrub habitat. Although longtail weasel (*Mustela frenata*), mink (*Mustela vison*) and gray fox (*Urocyon cinereoargenteus*) were historically reported from Murphy and Cedar Islands, no sign of these species was observed in more than 18 mo of field work on either Murphy or Cedar Islands. Rodent species inventoried by live-trapping or signs included eastern gray squirrel (*Sciurus carolinensis*), beaver (*Castor canadensis*) (skeletal remains only), short-tailed shrew (*Blarina brevicauda*), eastern wood rat (*Neotoma floridana*), hispid cotton rat (*Sigmodon hispidus*), rice rat (*Oryzomys palustris*) and cotton mouse (*Peromyscus gossypinus*).

Descriptive raccoon population structure

Two-hundred twenty-eight individual raccoons from study areas on Murphy, Cedar and South Islands were studied to date. On Murphy Island Study Area I (2 km^2), 43 individual raccoons (12 females and 31 males) were live-trapped during a 20-day trapping period in November and December 1987. Of these, three (7%) were sub-adults and 40 (93%) were adults. The calculated male/female sex ratio was 2.6. On Murphy Island Study Area II (0.5 km^2), 20 individual raccoons (13 males, seven females) were live-trapped during a 20-day trapping period in June and July 1988. The age class distribution was one (5%) juvenile, two (10%) sub-adults and 17 (85%) adults. The calculated male/female sex ratio was 1.9.

On South Island Study Area I (2 km^2), 117 individual raccoons, 96 males and 21 females, were live-trapped during a 20-day trapping period in January and February 1988. Of these, three (3%) were juveniles, 21 (18%) were sub-adults and 93 (80%) were adults. The calculated male/female sex ratio was 4.6. On South Island Study Area II (0.5 km^2), 34 raccoons were collected by spotlighting and shooting with a .22 caliber rifle. Fourteen of these raccoons were males, 20 were females. The calculated male/female sex ratio was 0.7 and the calculated age class distribution was two (6%) juveniles, 13 (38%) sub-adults and 19 (56%) adults. This particular South Island population was different from the other areas in that (1) it was periodically hunted/trapped for control purposes by state officials, (2) raccoons were fed routinely on corn supplements and (3) the method of collection was different, shooting versus live-trapping. Raccoons ear-tagged in one study area were not live-trapped on another, for each of the four study sites on both Murphy and South Islands.

On Cedar Island, 14 individual raccoons, seven males and seven females, were trapped, marked and released during a 20-

day trapping period. The age class distribution was one (7%) juvenile, two (14%) sub-adults and 11 (79%) adults. Placebo baits were not used on this island and a specific population estimate was not directly determined, because this island was targeted as a potential vaccine release site.

Using the census assessment technique, estimated raccoon density on Murphy Island was one raccoon per 2.7 (±9.1) ha of upland forest. The South Island study area density estimate was one raccoon per 1.8 (±12.2) ha of upland forest. Extrapolation of habitable area to derive the entire Murphy Island raccoon population based on these figures gives an overall island estimated crude abundance range of 102 to 152 raccoons. If these same two estimates are generally applied to similar upland habitat on adjacent Cedar Island, the raccoon population estimate is 59 to 65 raccoons.

Movements, home range, and habitat use

Home range calculations, using the Minimum Convex Polygon method for 10 raccoons, provided a mean home range estimate of 84 (±15.6) ha. The Harmonic Mean Transformation method generated a mean home range estimate of 138 (±22.8) ha. The mean maximum extent of range was calculated at 1.5 km. Calculations of habitat use based on 698 habitat-typed radiolocations indicated that 43% of radiolocations occurred in maritime forest and 32% occurred in marsh edge habitat with the remaining 25% in beach/dune, shrub and impoundment areas. A Chi-square analysis applied to these data revealed significantly greater values than expected based on percent habitat distribution alone ($\chi^2 = 7.2$ and 9.8, $P < 0.01$, respectively), thereby suggesting preferential use of upland and marsh habitat by raccoons.

Raccoon natality, morbidity, and mortality survey

Potentially significant factors affecting natality, morbidity and mortality were identified in the study site population. A variety of techniques were used including: field necropsy, histopathology and serological surveys. Fifty-eight percent of 24 adult female raccoons necropsied during the spring of 1988 were in reproductive condition, of which 25% were pregnant. Major signs of overt illness observed in South Carolina barrier island raccoons to date have been diagnosed as due to canine distemper, as described elsewhere (Johnson, 1970; Cunningham, 1962), and to related secondary bacterial invaders. Thirty-eight percent of 30 raccoons and one of three gray foxes collected on South Island during a late winter/early spring 1988 suspected distemper epizootic had intracytoplasmic eosinophilic inclusion bodies diagnostic of canine distemper upon histopathological examination; canine distemper antibodies were not determined. No gross or microscopic evidence suggestive of any other viral etiology was found.

Results of a parasite survey are summarized in Table 2; samples have been deposited in the U.S. National Parasite Collection (Beltsville, Maryland 20705). Incidental lesions noted on post-mortem examination included: stomach nodules caused by *Gnathostoma procyonis* and coccidial enteritis, possibly occurring secondary to canine distemper. Periodontal disease and gingivitus also were frequently observed, perhaps occurring secondarily to mechanical trauma sustained while foraging upon crustaceans and shellfish. In this preliminary survey, South Carolina coastal raccoon populations had no apparent mortality directly attributable to starvation, parasitism, predation or severe climatic conditions (e.g., hypothermia). Observed bobcat or alligator predation upon raccoons was minimal; no authorized public raccoon sport hunting was allowed.

All raccoon serum samples tested to date from Murphy and Cedar Islands were negative for rabies virus neutralizing antibodies (≤0.2 IU/ml, $n = 67$). Serological surveys for raccoon poxvirus also were negative, with no inhibition of cytopathic effect observed in 49 samples tested. A

TABLE 2. Parasites of raccoons from South Carolina barrier islands.

Parasite	Site of infection	Number and percent of hosts infected	Range in number of parasites	Mean parasite intensity (±SE)
Nematoda				
Gnathostoma procyonis (80973)[a]	S[b]	4/15 (26.7)	1–3	1.8 (±0.4)
Physaloptera rara (80974)	S	8/15 (53.3)	1–40	8.2 (±4.3)
Arthrocephalus lotoris (80975)	SI	2/15 (13.3)	1–12	2.2 (±3.9)
Molineus barbatus (80976)	SI	1/15 (6.7)	1	1
Capillaria sp.[c]	E, T	5/15 (33.3)	ND[d]	ND
Unidentified Microfilariae[c]	B, LU	12/15 (80.0)	ND	ND
Trematoda				
Carneophallus turgidus (80977)	SI	7/15 (46.7)	25–500	223.3 (±61.2)
Phagicola angrense (80978)	SI	1/15 (6.7)	6	6
Phagicola longa (80979)	SI	2/15 (13.3)	10–123	62 (±43.1)
Eurytrema sp.[c]	PD	2/15 (13.3)	ND	ND
Cestoda				
Atriotaenia procyonis (80980)	SI	2/15 (13.3)	1–11	6 (±3.5)
Acanthocephala				
Macracanthorhynchus ingens (80981)	SI	9/15 (60.0)	2–48	13.1 (±4.8)
Sarcocystis sp.[c]	SM	6/15 (40.0)	ND	ND
Hepatozoon sp.[c]	H, SM	7/15 (46.7)	ND	ND
Eimeria spp.[c]	SI	1/15 (6.7)	ND	ND

[a] Parasite accession number.
[b] E = esophagus, S = stomach, SI = small intestine, LU = lungs, PD = pancreatic duct, SM = skeletal muscle, H = heart, T = Tongue, B = blood.
[c] Histologic diagnosis.
[d] ND = Not determined.

serologic survey for canine adenovirus 1 and 2 found significant inhibition of cytopathic effect in four of 49 (8%) and seven of 49 (14%) samples, respectively.

Bait contact and acceptance

Bait contact (defined as either consumption of at least half of the bait matrix, vaccine capsule penetration, or missing baits) on day two post-deployment ranged from 59% for the Canadian sachet baits used on Murphy Island Study Site I to 99% for the fishmeal polymer baits used on South Island Study Site II. By day seven post-bait deployment, 93 to 100% of the baits had been contacted on all study sites regardless of bait type, baiting density or season. Bait acceptance was defined by analysis of biomarker incorporation into selected tissues of collected animals. Seventy-one percent (12 of 17) of the raccoon mandibular bone samples examined from Murphy Study Area I had been marked by ingestion of one or more baits. With regards to non-target species, 80% of opossums (four of five), none of five small mammals, nor a single female bobcat showed evidence of tetracycline-induced fluorescence. Eighty-five percent (17 of 20) of the raccoons sampled from Murphy Island Study Area II had eaten at least one of the fishmeal polymer baits deployed at 10 baits/ha. One of two opossums sampled was tetracycline positive. None of 12 small mammals had evidence of tetracycline fluorescence. Forty-nine percent (19 of 39) of the raccoons sampled from South Island Site I had been marked by eating at least one of the tallow/sponge baits or German sachet baits. Of non-target species, two river otters and 28 small mammals were not tetracycline positive. Despite a high day two post-deployment bait disturbance (99%), the observed acceptance rate for

South Island Study Area II was only 46% (11 of 24 raccoons), where rhodamine B was used instead of tetracycline as the biomarker.

Tracking pit data collected during the fishmeal-polymer baiting trial indicated that 83% of the pits where baits were consumed or missing contained raccoon tracks. Only 1% (1/80) of tracking pits with non-target species tracks (i.e., small mammal, passerine birds, etc.) but no raccoon tracks had bait partially consumed or missing. Six additional tracking pits, where only non-target tracks were present, still had intact baits. There was no evidence of unauthorized human contact with placebo baits on any island.

DISCUSSION

Reliable, inexpensive bait delivery systems are needed to consistently deliver an immunizing dose of a safe efficacious oral rabies vaccine to ≥70% of targeted free-ranging wildlife populations, a percentage generally cited as necessary to achieve sufficient anti-rabies herd immunity (Wandeler, 1991), but which has not been sufficiently tested in any mammalian species except the red fox (Bacon, 1985). Additionally, a biosecure field site with well-studied target species population parameters, non-target species inventory and preliminary ecological assessments are prerequisite to the successful initiation of any novel wildlife vaccine field trial, especially when anticipating the use of genetically-modified agents.

Historically, the finding that ingested attenuated rabies virus (ERA strain) invaded primarily through the lingual and buccal mucosa (Correa-Giron et al., 1970) established the potential for rabies vaccination by the oral route. Baer et al. (1971) were the earliest workers to test and champion the concept of oral immunization as a unique method of wildlife rabies control. Initially, laboratory trials concentrated on the red fox due to its prominence as a rabies vector in Europe and North America; after determination of the efficacy of the ERA virus by the oral route, baits were developed to efficiently deliver vaccine. Baer (1975), Winkler and Baer (1976) and Black and Lawson (1973) successfully vaccinated foxes using a variety of vaccine-filled baits.

Mammals differ greatly in their relative susceptibility both to rabies street virus infection and to oral vaccination. For example, raccoons, a major rabies reservoir in the southeastern and the mid-Atlantic regions of the USA, are somewhat refractory to conventional oral immunization (Rupprecht et al., 1989) when compared to foxes, even with two apathogenic variant clones of CVS-11 and ERA fixed rabies virus strains (Rupprecht et al., 1986). Therefore, other approaches were necessary to focus upon vaccines displaying greater efficacy for raccoons, while relatively safe for routine field use. Wiktor et al. (1984, 1985) developed a V-RG recombinant virus that induced high levels of circulating rabies virus-neutralizing antibodies in a variety of animals after direct parenteral administration, while Rupprecht et al. (1986) demonstrated the safety and efficacy of this vaccine in raccoons when the V-RG recombinant virus was administered orally in baits. The V-RG recombinant virus vaccine has proven safe and effective for major domestic and wildlife rabies vectors; to date, more than six avian and 35 mammalian species have been tested in confinement (Rupprecht and Kieny 1988). No untoward effects have been associated with the use of the V-RG vaccine for fox rabies control in Europe (Pastoret et al., 1988), prompting interest in field use for North America.

Basic mammalian species inventories conducted on South, Cedar and Murphy Islands confirmed the presence of at least 13 mammalian species representing seven families (Swiderek, 1982). Regarding target species, relatively high density raccoon populations (estimated at one raccoon per 1.8 to 2.7 ha) exist on the three islands. Coastal maritime forest and marsh habitats support a high density of raccoons in South

Carolina, perhaps due to the diversity of water-associated foods, fruit (e.g., yaupon), abundance of acorns and mast, and refuge from hunting. Although differences in methodology and specific habitats make direct comparisons difficult, raccoon population estimates reported elsewhere usually range from about one raccoon per 8 to 10 ha (Kaufmann, 1982), but have been cited as dense as one raccoon per 0.4 ha (Twichell and Dill, 1949) to less than one raccoon per 100 ha (Fritzell, 1978). Sex ratios reported in most raccoon studies are skewed towards male by as much as two to one (Kaufmann, 1982), similar to the mean of 2.2 males to females on South, Cedar and Murphy Islands. Age class distributions have been normally used as an index of wildlife population growth phases. A low, immature distribution has been associated with declining populations; conversely, a high, immature distribution has been associated with growing populations (Kaufmann, 1982). Southeastern United States raccoon populations typically exhibit low proportions of immature animals, on the order of 20–32% (Cunningham, 1962; Johnson, 1970). Data reported here support these findings, with an average of 23% immature raccoons in the five study populations examined. While live traps may obviously contribute to bias in estimating particular population characteristics, it is obvious that the island raccoon populations are older aged animals because of the normal potential for otherwise trapping an abundance of juveniles.

There is tremendous variation in home range size reported for raccoons, but most home ranges average 100 to 300 ha (Kaufmann, 1982). Radio-collared raccoons on South Island maintained small, seasonally stable home ranges approximately 83 to 138 ha. Although seasonal variations in range utilization (presumably reflecting food item availability) do occur, major seasonal shifts in raccoon home ranges were not observed. Ellis (1964) concluded that raccoons move less and use smaller home ranges when their population density is high. Similar results were reported for red foxes in Europe (Harris and Trewhella, 1988).

Comparative raccoon morbidity and mortality were of interest for several reasons. Raccoons have been used extensively as indicator species for monitoring environmental pollutants and zoonoses such as rabies (Bigler et al., 1973, 1975). With the exception of canine distemper, overt disease may play only a minor role in actual raccoon mortality, especially when population densities are low (Johnson, 1970). As previously reported for raccoons in Alabama and South Carolina, canine distemper appears to be the principal agent of disease-induced mortality on South, Cedar and Murphy Islands. Thirty-eight percent of 30 raccoons sampled had intracytoplasmic eosinophilic inclusion bodies diagnostic of canine distemper upon histopathological examination. Perhaps moderate amounts of exposure to various adenoviruses also contribute to canine distemper virus-induced morbidity and mortality in affected raccoons, but these have not received systematic study. Importantly, prior knowledge of incidental lesions in any targeted wildlife population assists in the differentiation of acute health risks that could be mistakenly associated with V-RG recombinant or other genetically-modified agent vaccine use in the field.

The potential of virus recombination occurring during a proposed use of V-RG vaccine in raccoons has also been considered. For significant viral recombination, simultaneous animal infection should occur between related orthopoxviruses. The only documented New World orthopoxvirus thought to occur in the eastern United States is the so-called raccoon poxvirus. Documentation for raccoon poxvirus was more than 25 yr ago from a single site in Maryland (Herman, 1964); no new published virus isolations have yet occurred. Although some 20% of raccoons sampled from Maryland were reportedly seropositive for raccoon poxvirus, actual virus was

isolated from only two animals (Alexander et al., 1972). Pilot serological surveys for raccoon poxvirus on Cedar and Murphy Islands have been negative thus far, making the probability for V-RG viral recombination and subsequent regeneration of a thymidine kinase-positive phenotype an extremely remote possibility.

In addition to describing ecological components of a proposed recombinant vaccine trial, the results of this pre-vaccine deployment study demonstrated that it was feasible to consider vaccination of a significant proportion (>70%) of a barrier island raccoon population that can be selectively targeted, marked and monitored using an oral bait delivery system. Bait acceptance rates for raccoons between 49 and 85% were obtained by selecting various combinations of preferred habitat for bait placement and target species population assessment, attractant, biomarker, bait matrix, baiting density and distribution method. This acceptance rate was favorably high when compared to those documented for other furbearers to date (Johnston and Voight, 1982; Johnston et al., 1988; Schneider et al., 1988; Wandeler, 1988; Bachmann et al., 1990). Bait acceptance differences from trial to trial may be attributed to a number of variables including: specific habitat, season, bait type, method of distribution, baiting density, target and non-target species density, biomarker employed, and tissue collection techniques, among others. Considering that even under ideal laboratory conditions, oral vaccination resulting in adequate seroconversion and longterm protection against virulent rabies challenge may be demonstrable but is rarely 100% efficacious (Rupprecht and Kieny, 1988), it is critical to maximize bait acceptance in the field to promote sufficient herd immunity.

Direct observations of presentation trials, conducted in the laboratory and in the field, as well as larger scale bait trials have demonstrated the utility of the experimental fishmeal polymer bait (Hanlon et al., 1989). It is environmentally durable, resists insect fouling over longer periods than strictly organic-based baits, is attractive to raccoons and is readily consumed with no apparent adverse clinical effects. Furthermore, this bait matrix can be economically mass-produced in a variety of shapes and sizes with multiple odor/flavor attractants and biomarkers incorporated as regional food preferences of target species suggest. Unlike other bait systems tested thus far, the fishmeal polymer system did not contain a well-defined synthetic vaccine chamber, such as the foil and polystyrene blister packs used in the Canadian and German baits. The lack of synthetic vaccine chamber components (versus paraffin or gelatin capsules) should greatly reduce the chances of outright vaccine chamber rejection or inadequate vaccine chamber penetration by carnivore teeth, thus minimizing vaccine loss and environmental contamination. Raccoons are selective omnivores with great manual dexterity and tactile sensitivity, enabling them to reject synthetic components of a bait-vaccine system. It was not uncommon to recover sachet baits in which the outer matrix had been consumed but the inner sachets remained unpunctured. Although shellfish essence was incorporated in the attractant slurry in all bait trials, the fishmeal polymer bait itself possesses a fish-like odor (due to incorporated fish oils) attractive to raccoons, which may be adequate alone.

Several biomarkers were tested with South Carolina Island raccoons, but tetracycline appears to be the most reliable and practical post-mortem indicator. Lindsey (1983) successfully marked mountain beaver (*Aplodontia rufa*) in the field in Washington (USA) with the industrial dye rhodamine B. When beaver facial vibrissae and hair follicles were examined under ultraviolet light, bands of bright orange fluorescence were observed for up to 7 mo. We were unable to obtain similar consistent results with rhodamine B in raccoons; the duration of marking and the anatomic site marked were extremely

variable. In contrast, the serum-protein marker iophenoxic acid (a-ethyl-2-hydroxy-2,4,6-triiodebenzenepropanoic), initially developed by Larson et al. (1981), has been used effectively for raccoons (Hadidian et al., 1989) and represents an alternative ante-mortem choice for a biomarker. Sample collection is relatively simple (by venipuncture) and adequate marking may be obtained for 6 to 8 wk. Unfortunately, the current assay procedure is complex, relatively expensive, and uses hazardous chemicals for analysis. While efforts are under way to develop a less expensive and more efficient protocol, the estimated cost of serum iodine analysis (>$20.00 (US)/sample) may be prohibitive for use in widespread carnivore vaccination projects. Tetracycline is a proven inexpensive post-mortem calciphilic biomarker (Johnston and Voight, 1982) and its use in field baiting trials has been adequately demonstrated (Steck et al., 1982; Schneider and Cox, 1983; Wandeler, 1988). For raccoons, cancellous bone samples of mandibular origin should be utilized to obtain the greatest yield. This is especially true with slow-growing, older animals (adults comprised 70 to 80% of the raccoon populations of South Carolina barrier islands). Although tetracycline deposition in cementum/dentin layers of teeth is well documented (Johnston and Voigt, 1982), we found as much as 78% false negatives when teeth alone (premolar or canine) were used without adjacent cancellous bone samples.

Bait densities should be tailored to regional target and non-target population densities. The bait densities utilized in this study (2.0 baits/ha to 10 baits/ha) were somewhat higher than typical bait densities reported elsewhere (0.8/ha to 6.0/ha). Optimal bait densities used successfully for foxes in Europe and Canada (15–20 baits/km^2) are not necessarily adequate for raccoons, whose population densities can be greater than one raccoon per ha. Lower baiting densities may have been responsible for the lower acceptance rates in some of the bait trials reported herein and elsewhere (Hadidian et al., 1989). Although Perry et al. (1989) reported no significant difference between acceptance rates of baits by raccoons in their high and low density areas, the density difference they used (1.25/ha versus 5/ha) may have been inadequate to detect what may be an exponential relationship between acceptance rates and bait density. In areas with average raccoon densities of one raccoon per 5 ha, a minimum baiting density of five baits/ha may achieve a 70% or greater acceptance rate. Furthermore, an even higher baiting density (7 to 10 baits/ha) or multiple baiting campaigns may be necessary if a greater proportion of the target population is to be reached, further complicated if a more heterologous fauna of medium-sized mammals are present. As yet, the relationship between assumed target species density and the bait density necessary to obtain greater than 70% of marked animals has not been critically determined.

Test site biosecurity must be a primary consideration in the development of protocols for initial testing of recombinant vaccines in the field. Test sites in South Carolina were carefully selected from numerous candidate sites examined from Virginia to Florida. In addition to natural water barriers, climatic considerations, and habitat restrictiveness, test site biosecurity should be enhanced throughout any proposed vaccine field trial by direct personal surveillance, complemented by state game enforcement officials, when possible. Island sites are administered and managed by the South Carolina Wildlife and Marine Resources Department (Columbia, South Carolina 29202) and the Tom Yawkey Wildlife Center, as part of the Santee Coastal Reserve System. State biologists and law enforcement officials routinely patrol adjacent waterways. Public access to the islands is restricted; islands are posted and access is by boat or helicopter only. Legal public use of these islands is limited to the mean high tide zone on the beaches. An-

ticipated illegal trespass, state-guided tours and research projects overall are limited to <300 human hr/yr.

Bait system target species selectivity should also contribute to biosecurity. With the exception of the opossum, bait uptake by non-target species was minimal on all study sites. Opossum acceptance rates using the fishmeal-polymer bait was at times near 50%. This is lower than the acceptance rate for other bait types tested where bait consumption by opossum was 70 to 80%.

Limited raccoon mobility and slow population turnover rate enhance site biosecurity. Target raccoon populations on these islands maintain relatively small home ranges (83 to 138 ha) and the extent of their range averages only 1.52 km. No radio-collared raccoons apparently left their resident island in 11 mo of extensive monitoring. No inter-island exchanges of ear-tagged raccoons were found in 228 individuals live-trapped on five different areas on three adjacent islands. These results are consistent with a non-hunted, undisturbed population where food and available cover may not be limiting factors (Cunningham, 1962; Johnson, 1970). Furthermore, review of the age structure, sex ratio and reproductive and health status of the raccoon populations on these islands suggests a population with long-lived adults, at or above carrying capacity. This relatively stable raccoon population may be limited primarily by available upland habitat, high juvenile mortality, and periodic canine distemper epizootics. Regarding off-island dispersal, although several species of mammals endemic to the study island(s) are potentially capable of dispersal from the island(s) (e.g., river otter, rice rat, raccoon, deer, etc.), the probability for widespread dispersal is considered to be generally low.

Enzootic rabies need not be a prerequisite feature of intended study areas; if present, it could provide logistical dilemmas concerning inapparent rabies exposure to field personnel. Rather, initial recombinant vaccine environmental release should be perceived as a limited trial concentrated upon biosafety features and dynamics of vaccine in the field, rather than as an efficacy experiment, per se. As such, high anticipated bait distribution (e.g., 10 baits/ha) should allow maximum contact and multiple consumption by individual raccoons to monitor health effects. While rabies has not been reported from the South Carolina study sites, on a state-wide basis, 59 positive cases of rabies were reported in South Carolina during 1987, 127 in 1988, 192 in 1989 and 130 in 1990; 70%, 64%, and 63% of these cases, respectively, were diagnosed from raccoons. In the immediate area, Charleston County reported two rabies cases in 1987 and Georgetown County reported its first case of rabies in October 1988; yet, both historical records and serological surveys to date support a rabies-free status of the islands, which would obviously minimize handling constraints of otherwise potentially rabid animals by study personnel.

Several additional factors related to V-RG recombinant virus vaccine itself significantly reduce its perceived hazards (i.e., overt mortality; gross lesions; vaccine virus spread beyond target populations; human health risks; etc.) Extensive safety and efficacy studies have been completed in captive wild target and non-target species and in laboratory animal species (Rupprecht and Kieny, 1988). These studies have been carried out in laboratory confinement, in compliance with international guidelines, and demonstrate that the V-RG recombinant vaccine virus is not actively shed from vaccinated animals to seronegative contact animals; has not been recoverable after 48 hrs from any tissue of any animal species tested in the laboratory; is lost after 3–4 induced serial passages; is non-pathogenic for target and non-target species; is further attenuated by inactivation of the thymidine kinase gene; and cannot cause vaccine-induced rabies (because the recombinant only contains the rabies virus glycoprotein gene). These previous findings and current results, which demon-

strate an effective and safe method for the delivery of an oral recombinant vaccine to as much as 85% of free-ranging coastal raccoons, have generated prerequisite data for limited field trials with a V-RG recombinant vaccine in North America. Without such limited safety trials at reasonably biosecure sites, progress towards larger, more complex, and more biologically relevant mainland field trials will be severely curtailed. Limitations to preliminary field testing of recombinant vaccines in North America would make realistic export of applied biotechnology unconscionable to developing countries, where human rabies mortality can be directly traced to uncontrolled enzootic canine rabies. Only a multi-disciplinary systems approach to oral vaccination, with broad multi-national collaboration and support, will bring the practical control of rabies in free-ranging carnivores to its fruition.

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