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A FIELD EVALUATION OF BAITS FOR DELIVERING ORAL RABIES VACCINES TO RACCOONS (*PROCYON LOTOR*)

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ABSTRACT: Eight field trials were conducted in 1989 and 1990 in Georgia (USA) and Maryland (USA) to evaluate baits and baiting strategies for delivering oral rabies vaccines to raccoons (*Procyon lotor*). Bait packets consisting of corn meal and egg batter-based baits enclosed in plastic bags were placed at 1.0-m diameter, raked tracking stations and checked daily. Packets were well accepted by raccoons; they visited 31 to 44% of the tracking stations where they removed 69 to 90% of the packets within 4 to 5 days. All or nearly all baits were removed from plastic bags and less than 1% of the baits were found only partially eaten. No rejection of water-filled paraffin ampules in baits was observed. The use of an odor attractant on bait packets did not appear to enhance bait discovery when packets were placed on raccoon travel routes. An attractant did enhance discovery when baits were placed off-road in a simulated aerial baiting test. Nontarget species comprised 31 to 53% of all visits to the stations; they took 28 to 55% of the baits but did not appear to adversely affect bait availability for raccoons. A total of 2,300 baits, each containing a wax ampule holding 10 mg of a physiological marker (iophenoxic acid), were distributed at a rate of 82 baits/km^2 on 2,800 ha of Sapelo Island, Georgia. Thirty-five (65%) of 54 raccoons collected following bait placement had eaten one or more baits as indicated by elevated levels of iodine in the blood serum.

Key words: Attractants, baits, bait consumption, field trials, oral vaccine, physiological marker, *Procyon lotor*, rabies, raccoon.

INTRODUCTION

Rabies is a persistent and widespread disease in wildlife. The Centers for Disease Control, United States Public Health Service, reported 4,881 cases of rabies in 1990 (Uhaa et al., 1992). Of these, 4,327 (89%) were in wild animals of which 1,821 (42%) were reported in raccoons (*Procyon lotor*). The two primary foci of infected raccoons in the USA were in the mid-Atlantic (Delaware, Maryland, Pennsylvania, Virginia, West Virginia) and southeastern states (Alabama, Florida, Georgia, South Carolina). More recently, rabies has spread north and eastward into New York, New Jersey, and Connecticut of the USA (Uhaa et al., 1992). The actual prevalence of rabies in raccoons remains unknown, but reported cases most likely represented only a small percentage of total occurrence.

Rabies vaccine-laden baits have been effective for orally immunizing red foxes (*Vulpes vulpes*) (Wandeler, 1991), but the technique is at an early stage of field development for raccoons (Hanlon et al., 1992). Oral immunization of striped skunks (*Mephitis mephitis*) still is at the vaccine development phase (Charlton et al., 1992). The approach appears to hold promise for controlling rabies in wildlife as well as for dogs in less developed countries.

One important component of oral vaccination is the development of effective baits and baiting strategies for vaccine delivery. Some progress already has been made on baiting methods for raccoons (Hable et al., 1992), but the World Health Organization (1990) continues to recommend further refinement of baits and baiting strategies. Toward this end, we initiated a series of raccoon bait preference tests in 1987 using captive raccoons maintained at the Centers for Disease Control facility in Lawrenceville, Georgia. That study resulted in development of a deep-fried corn meal and egg batter-based bait that was used successfully to vaccinate captive raccoons with a raccoon pox-vectored recombinant rabies vaccine (Linhart et al., 1991). Our objective of this study was to evaluate prototype raccoon baits and baiting methods under field conditions.

MATERIALS AND METHODS

Seven of the eight field trials conducted in 1989 and 1990 were located on Sapelo Island (31°28'N, 81°16'W), a barrier island located on the Atlantic coast in McIntosh County, Georgia. The island is 4,415 ha in size and has approximately 80 km of sand roads and fire trails that permitted good access to the entire island. Sapelo Island is flat (highest point = 4.9 m) and mostly timbered; dominant tree species are live oak (*Quercus virginiana*), laurel oak (*Q. laurifolia*), water oak (*Q. nigra*), longleaf pine (*Pinus palustris*), and loblolly pine (*P. taeda*). Understory vegetation is typically saw palmetto (*Serenoa repens*) and various heath species. Extensive salt marshes surround much of the island (Johnson et al., 1974). Indices of the raccoon population on this island were obtained by the Georgia Department of Natural Resources in April and November 1990 and November 1991, using a scent station index technique (Hon, 1981). The raccoon population appeared considerably higher ($\bar{x}$ visitation rate = 49%) than the mean 1982 to 1986 indices for areas on the adjacent mainland ($\bar{x}$ visitation rate = 11%).

The eighth field trial was conducted along the Chesapeake and Delaware Canal (C&D Canal) (39°34'N, 75°42'W) which is located about 60 km east of Baltimore, Maryland, at the northern end of the Delmarva Peninsula in the states of Maryland and Delaware. In the C&D Canal trial, we sought to compare mid-Atlantic region raccoon responses to baits and attractants with those observed approximately 1,000 km to the south on Sapelo Island.

Olfactory attractants

We conducted three tests during April and May 1989, to ascertain whether odor attractants enhanced free-ranging raccoon contact with placebo oral vaccine baits. The bait consisted of a polyurethane sleeve (1.5 × 5.5 cm) dipped in a mixture of a commercial food batter (Pillsbury Product No. 6947, Pillsbury, Minneapolis, Minnesota, USA), corn meal, milk and whole eggs; coated bait was then deep fried in corn oil (Linhart et al., 1991). Unless otherwise stated, this bait served as the standard or reference bait for all trials. Single baits were placed inside a 0.7-mm thick plastic sandwich bag (13.5 × 16.5 cm) that was stapled shut. Plastic bags protected the baits from moisture and also served as a visual attractant (Johnston and Voight, 1982).

We used 30 × 30 × 6 mm squares of open-cell polyurethane foam to hold and slowly release the candidate olfactory attractants. One impregnated square was stapled to the outside of each plastic bait bag. Foam squares were prepared by dipping them into a heated liquid slow release matrix that consisted of 60% $CaSO_2$, 20% paraffin wax, and 20% beeswax (Turkowski et al., 1983). Twenty percent by weight of a given attractant was then added to the matrix. When solidified, the matrix held about 1.5 g of attractant per foam square. Foam squares without an odor attractant served as the reference. Four of five candidate attractants were prepared with the controlled release matrix. Attractants tested were synthetic fermented egg (SFE), synthetic fermented egg-fishy (SFE-F), fatty acid scent (FAS) (Bullard et al., 1983). A fourth fruit-based (FRU) attractant consisting of esters (predominantly acetates) and some volatile compounds found commonly in fruits was formulated by R. Teranishi (pers. comm.), Agricultural Research Service, U.S. Department of Agriculture. The fifth attractant was a commercial lure known as "Trappers Delight" (a proprietary blend of various processed fish oils; Norjax Industries, Jacksonville, Florida). It was directly applied by syringe (0.2 ml) to untreated foam squares.

A series of 1.0-m diameter tracking stations raked into the soil at 310-m intervals along sand roads were used for the first Sapelo Island test. Stations were located on the shoulder of the road to avoid disturbance by passing vehicles. They were alternated from left to right sides of the road to minimize the possible influence of prevailing winds. A bait packet was placed in the center of each cleared circle. The test line was divided into six segments; the five test attractants and reference were randomly assigned within each segment. Placement of segments was repeated along the route until a total of 132 stations, or 22 stations per attractant type, were set out. The total number of stations was limited by the time required to check all stations daily. Stations were checked for 5 days and bait disturbance or bait disappearance was noted, by species, based upon animal tracks left in the raked soil. All removed baits were replaced daily; however, only the first bait removal from any given station was used in the statistical analysis. There was some overlap of routes used for different trials; thus, no doubt some of the same raccoons visited stations used for several sequential field trials. The frequency of animal visitation and bait disappearance rates for each type attractant and the reference was used as an index of animal response (Linhart et al., 1977).

The second trial involved testing the identical bait and odor attractants along the C&D Canal in June 1989. In this trial we sought to determine if raccoons located on the coastal plain of the mid-Atlantic region would respond in the same manner as that observed on Sapelo Island. Differences between the two test areas included time of year (April for Sapelo versus June for the C&D Canal) and the presence of more nontarget species and more varied raccoon habitat along the C&D Canal. The latter factor required that some bait stations be placed off-road near water sources and small waterways in order to encourage raccoon visitation and to reduce bait disturbance by the red foxes that frequented the upland areas. Animal visitation to stations and bait disappearance rates were used to compare results between the two areas.

The third trial of olfactory attractants was conducted on Sapelo Island in May 1989 to simulate aerial application of baits to determine whether an odor attractant attached to bait packets would enhance discovery and bait take by raccoons. This test was conducted using 60 bait stations comprised of 20 stations for each of three different treatments (A, B, C). All stations were located 20 m off and at a right angle to the roadway. Treatment A consisted of a bait packet with an unscented foam square that served as a control; treatment B was identical but each foam square contained a mixture of 0.2 ml of shellfish and menhaden fish oils (1:8; M&M Fur Company, Bridgewater, South Dakota, USA). Treatment C was a bait packet with an unscented foam square placed at a station where approximately 0.5 l of sand saturated with 20.0 ml of menhaden fish oil had been sprinkled on top of the circle of raked soil. Treatment C simulated a technique used decades ago for toxic bait treatments in the western United States whereby small lard or tallow baits were placed in a paper bag containing sand saturated with seal oil. Such bags when dropped from aircraft would break upon impact and would scatter oil-soaked sand that served as a draw station to attract coyotes to the baits. A modification of this technique was thought to have possible application for distributing oral vaccine baits to raccoons. All stations were checked daily for tracks, bait packet penetration, and bait disappearance, and rates between treatments were compared. Baits taken were replaced daily.

Modified baits and bait packets

Four field tests were conducted on Sapelo Island in May (tests 1, 2, 3) and December (test 4) 1989. Our objectives were to determine if raccoon discovery and bait take differed between heat-sealed versus staple-closed plastic bait bags, baits with and without 0.5% sodium benzoate (to prevent formation of mold growth on baits), baits with and without 2.0-ml water-filled paraffin wax ampules (W&F Mfg. Co., Inc., Buffalo, New York) to simulate vaccine, and if aerially dropped bait packets would penetrate the loblolly pine and live oak forest canopy on the island. Tests 1, 2, and 3 were conducted by alternating bait or bait packet types at tracking stations placed along sand roads at 320-m intervals. Forty stations per test were prepared and checked daily for 3 days resulting in 120 bait nights per test or 60 bait nights for each of the paired bait types evaluated.

Test 4 was conducted at two bait drop sites, both approximately 0.4 ha in size and having relatively sparse ground cover. The first site (Moses Hammock) consisted of an even-age stand of 25% loblolly pine and 75% live oak approximately 25 m in height; tree canopy closure was visually estimated at about 70%. The second site contained 100% loblolly pine of uneven age (20 to 40 cm diameter breast height) with the largest trees about 26 to 30 m high; canopy closure was estimated at about 90%. At each site, a biologist first hand-placed 10 marked bait packets and did not participate in the bait searches following the aerial drops. Unmarked bait packets (25 per site) were dropped from a Rockwell Jet Ranger helicopter hovering at an altitude of about 60 m above ground level. A four-person search crew then systematically searched the sites for marked and unmarked packets. The percentage of marked bait packets found by the crew provided an estimate of the percentage of aerially-dropped packets that reached the ground and were found by searchers. The resultant value was used to estimate the percentage of dropped packets that failed to reach the ground.

Placebo vaccine baiting trial

This trial was conducted on Sapelo Island during the period 24 April to 16 May, 1990. We used an oral physiological marker, iophenoxic acid (IA; Aldrich Chemical Company, Inc., Milwaukee, Wisconsin, USA), in baits to determine the percentage of the raccoon population that could be reached by baits. Iophenoxic acid binds to protein in the blood serum and can be detected for weeks or months following ingestion of a single oral dose (Larson et al., 1981). We live-trapped (26 × 32 × 82-cm traps; Tomahawk Live Trap Company, Tomahawk, Wisconsin) 23 adult raccoons prior to bait distribution, sedated them with 5 mg/kg ketamine (Veterinary Products, Bristol Laboratories, Syracuse, New York) given intramuscularly, bled them, and submitted the blood sera for analysis (SmithKline Beecham, Clinical Laboratories, Van Nuys, California, USA). Serum iodine was determined using baseline Technicon Autoanalyzer methodology (Technicon Instrument Corporation, Irvine, California) (Henrey, 1964). Results were expressed as the amount of iodine in microliters (μl) per 100 ml of serum.

Paraffin wax ampules containing 10 mg of IA and a green food dye in 1.5 ml of propylene glycol (Aldrich Chemical Company, Inc., Milwaukee, Wisconsin) were inserted into the previously described corn baits which had been deep-fried in a 50:50 mixture of corn oil and melted paraffin. The corn and paraffin mixture was used to make baits water and mold resistant. Baits were individually packaged in sandwich bags that contained a 20 × 40 mm printed label that provided explanatory information and the phone number of the resident island manager. A ground crew of five used pickup trucks and all terrain vehicles (ATV's) to distribute 2,300 baits on the northern 2,800 ha of the island (82.0 baits/km^2) during a 1.5-day period (1 and 2 May). Bait distribution was carried out by dividing the study area into three quadrants within which transect lines were spaced at approximately 100-m intervals. Vegetation permitting, ATV's were driven along transect lines and single baits were placed about 100 m apart. Raccoon travel routes such as firebreak trails, crossroads, culverts, drainage ditches, and inland creeks were baited more heavily with two to four baits per site. One hundred baits (4.3% of the total) were placed on tracking stations (one bait per station) located at 320-m intervals along perimeter island roads. These baits were checked for 2 days following placement to record bait disturbance and animal tracks in the raked soil. The fate of these 100 baits was used to estimate the activity for all baits. The area was trapped post-baiting on 11 to 16 May and raccoons were bled and serum analyzed for iodine content. A comparison of pre- and posttreatment iodine levels was then used to determine which animals ate baits and to assess the efficacy of the baiting effort.

Where applicable, product-limit survival curves (Kaplan and Meier, 1958) were used to compare the rates of bait removal for different treatments. To avoid potential learning effects, only the first baits placed at a station were considered for these analyses. Missing baits not identified as having been removed by raccoons were excluded from the analyses. Proportions of baits removed where the time course of the removal was not followed were compared using chi-square contingency table tests. For 2 × 2 contingency tables where the assumptions for a valid chi-square test were violated, the unconditional test of McDonald et al. (1977) was applied.

RESULTS

Olfactory attractants

Raccoons readily took baits during the April 1989 Sapelo Island test regardless of associated odor attractant. Of 584 bait nights (one BN = one bait exposed for one night at one station), raccoons visited stations 205 times (35% visitation rate) and removed 162 bait packets (79%) (Table 1). Typical bait removal behavior consisted of carrying the intact bait packet some distance ($\bar{x}$ = 2.7 m, range = 0 to 15 m, SE = 0.27 m) from the station, biting a small hole in the plastic bag and removing the bait. Of 127 bags recovered (75% of total bags taken by raccoons), all had baits removed; only four partially eaten baits were found. We estimated that, had baits not been replaced daily, 29% of all bait packets would have been removed by all species combined on the first night, 48% by the second night, 67% by the third night, and 76% by the fourth night.

All five odor attractants as well as the reference packet were exposed for 97 to 98 bait nights each. Raccoon visitation rates among the attractant stations varied from 27 to 45%, with stations having commercial lure and reference packets receiving the lowest visitation (27 and 30%, respectively) (Table 1). However, there were no significant differences among the bait sur-

TABLE 1. Raccoon response to odor attractants and baits on Sapelo Island, Georgia, and the Chesapeake and Delaware (C&D) Canal, Maryland and Delaware, April and June, 1989, respectively.

Odor attractant	Sapelo Island			C&D Canal		
	Bait nights	Number of visits (%)	Number of baits taken (%)	Bait nights	Number of visits (%)	Number of baits taken (%)
Control	97	29 (30)	26 (90)	56	33 (59)	31 (94)
FAS	97	36 (37)	33 (92)	56	32 (57)	29 (91)
Fruity	97	38 (39)	32 (84)	56	26 (46)	24 (92)
SFE	98	44 (45)	33 (75)	56	24 (43)	22 (92)
SFE-Fishy	97	31 (32)	17 (55)	56	16 (29)	12 (75)
Lure	98	27 (28)	21 (78)	56	18 (32)	16 (89)
Totals	584	205 (35)	162 (79)	336	149 (44)	134 (90)

FAS = fatty acid scent; SFE = synthetic fermented egg; SFE-Fishy = synthetic fermented egg with enhanced fishy odor.

vival curves for attractant types ($P \geq 0.45$). The frequencies at which bait packets were removed by raccoons were clustered (75 to 92%) and did not differ significantly among attractant types or the reference. However, only 55% of the baits associated with SFE-F were taken; thus this odor may have had a repellent effect.

Nontarget species comprised 31% of all animal visits to stations and resulted in 28% of bait take. Opossums (*Didelphis virginiana*), crows (*Corvus brachyrynchos*), dogs (*Canis familiaris*), and cats (*Felis catus*) were the four most common species visiting stations. Opossums took five of nine of the baits visited, crows took six of six, domestic cats took 16 of 28, and dogs took 16 of 26; all other species (including humans and unidentified animals) took 21 of 24 baits at the stations visited.

We observed similar results with the second odor attractant field trial on the Delmarva Peninsula. We recorded 149 (44%) visits by raccoons during the 336 nights of bait exposure; 134 (90%) of the 149 baits were removed from the stations (Table 1). In no instance did we find bait remnants at the sites where raccoons opened bait packets. Bait removal by all species combined was more rapid than on Sapelo Island, with 75% removed on night 1, 87% by night 2, and 94% by night 3.

All odor attractant type and reference packets were exposed for the same number of bait nights (56 per treatment). Raccoon visitation rates varied from 29 to 59%, with visits to bait packets having no odor attractant as high, or higher, than visits to packets having attractants. The visitation rate to the most visited bait packet type (control) was double that of the least visited, SFE-F (Table 1). However, no differences among bait survival curves were detected for the different attractants ($P \geq 0.81$). On comparing the Sapelo Island and C&D Canal attractant data, we noted no consistent patterns other than that baits in SFE-F packets were taken less often at both study areas (Table 1).

Nontarget species disturbance of baits was more common along the C&D Canal than on Sapelo Island. At the C&D Canal, they comprised 53% of all animal visits to stations and 55% of all baits taken. Red foxes were the primary species involved; this animal visited 84 stations and took 75 baits. Opossums, crows, cats and dogs only minimally disturbed baits (six of six, one of one, two of three, and two of five baits eaten, respectively). However, rain obliterated tracks and made identification difficult; this resulted in a large number of visits and bait removals (22% and 26%, respectively) being placed in the "species undetermined" category.

On Sapelo Island, raccoon visits to baits placed off-road to simulate aerial distribution (test 3) were significantly different among treatments ($P < 0.02$). Only 12% of the reference packets (A baits) were

TABLE 2. Raccoon visits and bait disturbance at off-road stations with and without odor attractant during simulated aerial application trials, Sapelo Island, May 1989.

Attractant	Bait nights	Number of visits (%)	Number of baits taken (%)
A—No attractant (control)	80	10 (13)	6 (60)
B—0.2 ml fish and shellfish oil	80	30 (37)	14 (53)
C—20 ml fish oil in sand	80	34 (42)	29 (85)
Totals	240	74 (31)	51 (69)

visited compared with three times as many visits (37%) to packets with foam squares containing 0.2 ml of shellfish and fish oil (treatment B). Bait packets placed on fish oil-soaked sand (treatment C) were visited 42% of the time (Table 2). If a bait packet was discovered, the reference baits were removed as often (60%) as the baits with the scented foam squares (53%). However, use of the fish oil-soaked sand resulted in more baits being removed (85%).

Modified baits and bait packets

Visitation and bait removal rates encountered during the modified bait field trials were similar to those previously described. Combining data from bag closure, sodium benzoate, and ampule and bait tests conducted on Sapelo Island in May 1989, raccoons visited 33% of all stations and took 88% of the bait packets they discovered. We observed a difference ($P = 0.01$) between the numbers of baits taken from stapled bags (100%) and from heat-sealed bags (73%). The number of baits taken with (67%) and without (100%) 0.5% sodium benzoate also differed ($P = 0.01$). There was no difference in the numbers of baits taken with and without water-filled ampules ($P = 0.82$; Table 3). We inspected 56 sites where raccoons had removed baits from plastic packets. A single partially eaten ampule was found at one site, one partially eaten bait and ampule were discovered at a second site, and the solid paraffin wax plug from another ampule was recovered from a third locale.

TABLE 3. Raccoon visits and disturbance of modified baits, Sapelo Island, May 1989.

Bait or packet modification	Bait nights	Number of visits (%)	Number of baits taken (%)
Stapled bag	60	7 (12)	7 (100)
Heat-sealed bag	60	11 (18)	8 (73)
Sodium benzoate (0.5%)	60	15 (25)	10 (67)
No sodium benzoate	60	19 (32)	19 (100)
Ampule	60	31 (52)	28 (90)
No ampule	60	37 (62)	34 (92)
Totals	360	120 (33)	106 (88)

Nontarget species accounted for 38% of all animal visits to bait stations and they removed nearly half (46%) of the total number of baits taken by all species (raccoons and nontargets combined). Opossums took 25 of 29 baits, crows 32 of 37, cats 25 of 31 and dogs 10 of 15. Unidentified and miscellaneous species accounted for an additional 62 visits (21%) and for 58 bait packets (52%) removed from stations.

Based on our aerial distribution of bait packets, nearly all packets penetrated the forest canopy and reached the ground. The search crew that recovered bait packets immediately after the two aerial drops found eight of 10 ground-placed marked baits and 18 of 25 air-dropped unmarked baits at the Moses Hammock site. Considering the difference between recovery of ground-placed and air-dropped baits, only 0.8% or about two of the air-dropped bait packets had fallen through the forest canopy to the ground but were not found by the search crew. The remaining five missing baits evidently had been snagged by the branches, leaves or needles of the live oak and loblolly pine forest overstory. We returned to this study site daily for 2 days following bait distribution and searched for additional bait packets, as we speculated that windy conditions subsequent to the aerial drop might dislodge baits that had hung up in trees. We found one additional marked bag and an additional six air-dropped baits or 24 of a total of 25. At the second aerial drop site (uneven age loblolly pine) we found all 10 of the ground-placed baits and 22 of 25 air-dropped baits on the first day. Two days later we found two more air-dropped baits (24 of 25) that had fallen from the trees.

Placebo vaccine baiting trial

The 100 baits containing the physiological marker (IA) were rapidly removed from tracking stations. By day 1, 61 of 100 baits had been taken by all species combined (n = 58) or destroyed by ants (n = 3). Raccoons visited 36 stations and took 34 baits. By day 2, only 16 baits remained intact. Of the 84 stations showing animal activity, raccoons had visited 46 stations and taken 44 baits (96%). Ants damaged (n = 12) or destroyed (n = 5) 17 baits and were the most significant nontarget problem. We assumed that these findings were indicative of the fate of all 2,300 baits distributed during this field test.

Maximum daytime air temperature when baits were placed was about 33 C with resultant higher ground temperature in direct sunlight. We checked 102 baits on day 3 of the trial and observed that partial melting of some wax ampules within baits had occurred in 30 (29%) of the 102 baits. Dye was typically visible at one end of the bait indicating collapse of the distal end of the wax ampule.

The sex ratio of adult raccoons trapped before and after baiting was skewed strongly to males (15 females, 61 males). The mean iodine serum level of 23 raccoons trapped pre-treatment was 9 μl/100 ml (range 5 μl to 19 μl/100 ml, SE = 3 μl/100 ml). Of the 54 raccoons trapped (one raccoon per 7.7 trap nights) following bait distribution, 35 of 54 (65%) exhibited high iodine blood levels ($\bar{x}$ = 3,028 μl/100 ml, range 40 μl to 9,920 μl/100 ml, SE = 520) indicative of consumption of one or more baits. Since the iodine levels of the 19 trapped raccoons considered unmarked were 35 μl/100 ml or less, we estimated that at least 65% of the raccoon population on Sapelo Island was reached by baiting.

DISCUSSION

On Sapelo Island, we found that the use of odor attractants on bait packets did not enhance raccoon bait take when packets were placed along raccoon travelways. We obtained similar results approximately 1,000 km to the north along the C&D Canal. However, when bait packets were placed off-road in a simulated aerial drop, odor significantly increased bait discovery by raccoons. We believe that when larger quantities of attractant were used, it resulted in more exploratory behavior at sites and thus a greater likelihood of raccoons opening packets and taking baits. Baits and packets modified to enhance mass production by heat-sealing plastic bags and incorporating a mold retardant (0.5% sodium benzoate) into baits significantly reduced bait take by raccoons. Acceptance of baits with and without paraffin wax ampules did not differ. Unless raccoons had discarded baits or ampules some distance from where packets were opened, we infer that raccoon avoidance or partial consumption of ampules was not a problem. Nearly all bait packets air-dropped into live oak and loblolly pine stands readily penetrated the 70 to 90% forest canopy closure and were found on the ground.

Raccoon bait station visits and bait removal rates were quite consistent between tests and locales. Raccoons visited 31 to 44% of stations and removed 69 to 90% of the baits they discovered. Nontarget species frequently visited bait stations (31 to 53%) where they took 28 to 55% of all baits. Nonetheless, we conclude that nontarget bait removal did not seriously impair bait availability for raccoons. It is noteworthy that red foxes on the Delmarva study area took 89% of all baits they visited suggesting a potential for this bait as a vehicle for delivering oral vaccine to this species. Bait consumption by ants concurrent with the onset of warm weather in late spring/early summer was noted; incorporation of an insect repellent into baits might be desirable. Baits rapidly disappeared in three to five days and few bait or wax ampule remains were found either at bait stations or where plastic bags had been discarded by raccoons.

Relative to biomarker analysis, we considered any level of iodine in raccoon serum that was over twice the highest pretreatment value (19 μl/100 ml) indicative of bait or IA ingestion. Use of this criterion equated with considering an animal marked if the plasma iodine exceeded the mean by 8.4 standard deviations. This is a more conservative approach than that used in an earlier field test when IA was used to mark wild swine where a measurement in excess of three standard deviations above the mean was the criterion for considering an animal as marked (Fletcher et al., 1990). Such a conservative interpretation may be desirable due to the variability often encountered in blood iodine assays.

The 65% of the marked raccoons on Sapelo Island reached by baiting was achieved on a larger test area (28 km^2) and at a lower bait density (82 baits/km^2) than has been previously reported (Rupprecht et al., 1987; Hadidian et al., 1989; Perry et al., 1989; Hanlon et al., 1989, 1992; Hable et al., 1992). It is interesting to note that more male (80%) than female (20%) raccoons were captured, most likely because our study coincided with the peak of raccoon parturition (Sanderson, 1987) when female movement (and thus bait discovery) was restricted.

One objective of oral rabies vaccination programs should be to determine the minimum number of baits needed per unit of land area to immunize sufficient individuals in the population so as to result in elimination or control of the disease. The number of vaccine baits required may vary by geographic area, season, availability of natural foods, the densities of target and nontarget species, and the extent to which baits are consumed. Such data can be acquired only through repetitive field trials. For raccoons, there now exist a number of studies (Rupprecht et al., 1987; Hadidian et al., 1989; Hanlon et al., 1989, 1992; Perry et al., 1989; Hable et al., 1992) in which biomarkers were used in baits to simulate vaccine. Bait types and densities, methods of application, biomarkers used, and other parameters varied amongst these studies. Thus far, there appears to be no obvious relationship between bait density and the percentage of test populations reached by baits. Based on these results, we believe that more definitive studies are needed to determine this relationship and thereby keep bait, vaccine, and application costs to a minimum and yet achieve the desired level of immunity in the population. We also believe that future field studies would be enhanced by experimental designs that allow examination of correlations between bait density, target and nontarget species densities, and the percentage of local populations of these species reached by baits. Such information, along with active rabies surveillance, will be needed to plan timely application of vaccine baits in the most cost-effective and efficacious manner.

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