Journal of Wildlife Diseases, 38(2), 2002, pp. 363–377

A NEW FLAVOR-COATED SACHET BAIT FOR DELIVERING ORAL RABIES VACCINE TO RACCOONS AND COYOTES

Samuel B. Linhart,[1,8] John C. Wlodkowski,[2] Darrell M. Kavanaugh,[1] Laurie Motes-Kreimeyer,[2] Andrew J. Montoney,[3] Richard B. Chipman,[4] Dennis Slate,[5] Laura L. Bigler,[6] and Malcomb G. Fearneyhough[7]

[1] Southeastern Cooperative Wildlife Disease Study, College of Veterinary Medicine, University of Georgia, Athens, Georgia 30602, USA
[2] Biological Development, Biological Division, Merial Limited, 115 Transtech Drive, Athens, Georgia 30601-1649, USA
[3] U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Wildlife Services, 200 North High Street, Columbus, Ohio 43215, USA
[4] U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Wildlife Services, 1930 Route 9, Castleton, New York 12033-9653, USA
[5] U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Wildlife Services, 59 Shenell Drive, Concord, New Hampshire 03301-8548, USA
[6] Zoonotic Disease Section, Diagnostic Laboratory, College of Veterinary Medicine, Cornell University, Upper Tower Road, Ithaca, New York 14852-5786, USA
[7] Zoonoses Control Division, Texas Department of Health, 1100 West 49th Street, Austin, Texas 78756, USA
[8] Corresponding author (email: slinhart@vet.uga.edu)

ABSTRACT: Research was conducted during 1996–2000 to develop baits for delivering an oral rabies vaccine to raccoons (*Procyon lotor*) and coyotes (*Canis latrans*). A bait was sought that: (1) was attractive to the target species, (2) could be distributed by aircraft, (3) was as effective (or more so) than the currently used fish meal polymer bait, and (4) could be produced in large numbers by automated procedures and could be purchased by user groups at substantially lower cost.

Ten field trials were conducted to document raccoons' bait flavor preferences, evaluate a new vaccine sachet bait coated with various attractants, and determine if the sachet bait would effectively deliver Raboral V-RG® oral rabies vaccine (Merial Limited, Athens, Georgia, USA) to this species. Raccoons preferred fish and crustacean-based flavors over those derived from plant materials. Raccoon visits to tracking stations, frequency of bait removals, and percent of sachets discarded by this species that were emptied of placebo vaccine indicated efficacy of the new bait was equal or superior to the currently used fish meal polymer bait. A field trial conducted in fall 1998 compared aerially distributed vaccine-laden sachet and polymer baits and showed there was no difference between the percent of raccoons from the test and reference areas subsequently found positive for rabies antibody.

Four bait trials to determine coyote response to sachet baits were conducted in 1997–98. The propensity for canids to gulp or bolt smaller food items is well known. Thus, a first trial involved offering fish-flavored sachet baits of different sizes to 30 captive coyotes to determine if smaller size baits were more frequently swallowed intact. Two field trials were also conducted in fall 1997 to determine if free-ranging coyotes discriminated among sachet baits coated with different attractants. Finally, Raboral V-RG®-laden poultry-flavored sachet baits were aerially dropped and the percent of seropositive coyotes was compared with coyotes from surrounding areas where fish meal polymer vaccine baits had been distributed.

Captive coyotes did not swallow sachet baits intact, regardless of size. Bait preference field trials indicated that coyotes preferred poultry, cheese/beef tallow, and fish-flavored sachet baits and that such baits were taken at the same rate as polymer baits. A sample of coyotes from the area baited with vaccine-laden sachet baits had a markedly higher ($P = 0.01$) seropositivity rate than coyotes from areas where vaccine was distributed in polymer baits.

Sachet bait production could be facilitated by automated technology and sachet baits used either as an alternative vaccine delivery device or in combination with the fish meal polymer bait.
Key words: Baits, *Canis latrans,* coyote, oral vaccination, *Procyon lotor,* rabies, raccoon.

INTRODUCTION

The oral vaccination of terrestrial vectors of wildlife rabies in North America has progressed rapidly during the last 10–15 yr. Fifteen years ago research was still focused on developing safe and efficacious oral vaccines and testing candidate baits to determine to what extent target species would discover and ingest them (MacInnes, 1988; Winkler and Bögel, 1992). Today, millions of vaccine-laden baits are aerially distributed annually in Canada and the United States, and the disease has been nearly eliminated in red foxes (*Vulpes vulpes*) in Ontario and in coyotes (*Canis latrans*) in south Texas (Fearneyhough et al., 1998; Krebs et al., 1999; MacInnes et al., 2001). A National Working Group on Rabies Prevention and Control, under the auspices of the federal U.S. Centers for Disease Control and Prevention, has recommended further steps to implement oral rabies vaccination (ORV) of wildlife, including formulation of a national strategy and designation of a federal agency to lead wildlife vaccination efforts (Hanlon et al., 1999). Such strategies are now greatly facilitated by current understanding of phylogenetic relationships among virus variants that cause rabies in wildlife species (Smith et al., 1995), better public awareness and support for rabies control, enhanced surveillance, availability of a licensed oral rabies vaccine in the United States (Raboral V-RG®, Merial Limited, Athens, Georgia, USA), and, in some instances, increased federal and state funding to conduct research and undertake rabies control programs.

A significant aspect of recent ORV development has been efforts aimed at evaluating different bait types, bait attractants, and delivery methods in order to maximize vaccine bait discovery and ingestion by vector species (Linhart et al., 1997b; Farry et al., 1998a, 1998b; Steelman et al., 1998, 2000). In the United States, the extruded fish meal polymer bait for raccoons (*Procyon lotor*) and coyotes (Bait-Tek, Inc., Beaumont, Texas, USA) has evolved as the current bait of choice for both species (Hanlon et al., 1989; Fearneyhough et al., 1998; Olson et al., 2000). This bait is well accepted by free-ranging animals and is very resistant to adverse environmental conditions (Hanlon et al., 1989). The currently used polymer bait is square (2.0 × 3.5 × 3.5 cm) and weighs about 26 g, including vaccine, vaccine container or sachet, and the wax used to seal the sachet within the bait. Sachet insertion and pouring of wax sealant into the bait cavity is currently a manual operation and is labor intensive, thus significantly increasing the bait cost for user groups undertaking ORV programs.

The Southeastern Cooperative Wildlife Disease Study (SCWDS; The University of Georgia, Athens, Georgia, USA), began vaccine bait development in 1988, initially for feral swine (*Sus scrofa*) (Fletcher et al., 1990), mongooses (*Herpestes auropunctatus*) (Creekmore et al., 1994), and free-ranging dogs in developing countries (Linhart et al., 1997a). These investigations sought suitable vaccine containers, natural and synthetic olfactory and gustatory attractants, bait matrices, methods of formulation, and ways to evaluate bait components using both confined and free-ranging animals. The SCWDS also conducted modest feasibility trials for domestic dogs in 1994 and raccoons in 1996 to determine if vaccine containers simply surface-coated with attractants might be a less expensive option as a means of delivering vaccine to these species (Linhart et al., 1997a; Wlodkowski and Linhart, 1998).

In this paper, we describe a collaborative effort to develop a new bait for raccoons and coyotes, designated as the "flavor-coated sachet bait," that is 6–7 times smaller (~0.5 × 2.0 × 6.0 cm) than the polymer bait, is lighter (~3.8 g) (Fig. 1), and should be less expensive to manufacture in quantity. Our ultimate objective was to develop an efficacious bait that could be commercially manufactured at low cost resulting in more widespread use of ORV to control disease in terrestrial wildlife.

MATERIALS AND METHODS

Bait formulation

Fish meal and dog food meal polymer baits were obtained either from the bait manufacturer (Bait-Tek, Inc.) or vaccine producer (Merial Limited). Merial Limited filled the

FIGURE 1. Fish meal polymer bait on left (2.0 × 3.5 × 3.5 cm, 26 g); sachet bait in center (0.5 × 2.0 × 6.0 cm, ~3.8 g); and right, typical sachet masticated, emptied of vaccine, and discarded by a raccoon.

polyethylene heat-sealed vaccine sachets with approximately 2 ml of either vaccine media (placebo) or Raboral V-RG® vaccine, depending upon objectives of the trials. Use of vaccine media-filled baits greatly facilitated ability to conduct field trials because meeting regulatory requirements could be avoided. When objectives called for the use of vaccine, Merial inserted vaccine-laden sachets into polymer baits and secured them into the bait cavity with a melted wax sealant. When the protocol called for vaccine media to be used, media-laden sachets were inserted and sealed into polymer baits and candidate attractants then surface-coated onto the polymer baits at SCWDS. The same type sachet was used for both polymer and flavor-coated sachet baits.

Unlike fish meal polymer baits, polymer baits made of dog food meal had little apparent odor and as originally envisioned (Kavanaugh and Linhart, 2000), they were surface-coated with various candidate and proprietary attractants aimed at enhancing discovery and ingestion by target species. Attractants were applied by mixing, dissolving, or suspending them, usually at a 5:95% attractant/wax concentration, in melted wax and then dipping the dog food polymer baits into the melted mixture. Other types of attractants were applied directly onto the bait by placing a given number of baits into a plastic bag, adding a liquid (~1–5%) or dry powder attractant, and shaking the bag to obtain a uniform coating on the baits' exterior.

The procedure used for surface-coating attractants directly onto sachet baits consisted of four steps: (1) coating sachets with 100% melted wax having a high tack and melting point to insure good adhesion of initial and subsequent coatings; (2) dipping sachets a second time in a 5:95% mixture of attractant/melted wax; (3) placing a ~1–5% undiluted liquid coating of attractant (i.e., 3rd coat) onto sachets by weighing both attractant and sachets, and shaking both in a plastic bag to insure a uniform coating; and (4) when commercially available, application of a fourth coat of a dried or freeze-dried powder or granulated particles of the same type attractant by shaking both in a bag and using before and after weights to estimate the amount of powder that adhered to the bait. Because of different physical properties, the amount of dried attractant that adhered varied widely (i.e., 2–14%). We used the above four steps, easily automated for possible commercial production, to insure that coatings would resist sloughing off the plastic sachets, to maximize weather resistance, and to enhance detection and ingestion by target species. The final powdered coating also was applied to minimize leakage of liquid attractant onto nearby equipment and to facilitate handling and aerial distribution of baits. Several different animal and plant-derived attractants, formulated by SCWDS or available commercially, were evaluated and compared (Tables 1, 2). Each bait type tested was individually bagged, sealed in plastic, glass, or cardboard containers, and kept refrigerated until taken to the field for evaluation.

Test sites

We used five field test sites to evaluate raccoon baits; three on the Georgia coastal plain, one on the Lake Erie coastal plain in northern Ohio (USA), and one in the St. Lawrence River valley in northern New York (USA). Two Georgia sites were on state-administered wildlife management areas located on barrier islands; the physiographic features of both Ossabaw Island (31°47′N, 81°07′W) in Chatham County, and Sapelo Island (31°28′N, 81°16′W) in McIntosh County were described (Fletcher et al., 1990; Linhart et al., 1994). The third Georgia site was located in the coastal plain of southern Georgia on the Little Satilla Wildlife Management Area (WMA) (31°27′N, 82°01′W) in Wayne and Pierce counties and consisted of cutover pine tree plantations (70%) and cypress-pine-hardwoods, ponds, swamp, and bottomlands (30%). The Ohio site (41°27′N, 82°42′W) was on a National Aeronautics and Space Administration (NASA) facility near Sandusky in Erie County and consisted of open grassland (40%), shrubs (30%), and hardwood forest (30%). The physiographic features of the fifth site (44°35′N, 75°10′W), in northern St. Lawrence and Franklin counties in northern New York were described by Will et al. (1982).

We conducted bait trials to determine the

TABLE 1. Summary of raccoon oral rabies vaccine bait preference field trials, 1997–2000.

Trial number	Location, (BEN)[a], dates of trial	Bait	Bait type and flavor[b]	Raccoon response to baits: Percent baits visited[c]	Raccoon response to baits: Percent baits taken[d]
1	Ossabaw Is., GA (375) 21–24/2/97	B	sachet, fish oil	31 (23/75)	83 (19/23)
		C	sachet, shellfish oil	24 (18/75)	61 (11/18)
		D	sachet, crayfish oil	41 (31/75)	84 (26/31)
		E	sachet, menhaden oil	29 (22/75)	64 (14/22)
		F	sachet, shrimp oil	36 (27/75)	63 (17/27)
2	Ossabaw Is., GA (375) 7–10/3/97	G	sachet, grape essence	29 (22/75)	64 (14/22)
		H	sachet, cherry essence	59 (44/75)	55 (24/44)
		I	sachet, persimmon oil	43 (32/75)	38 (12/32)
		J	sachet, corn oil	37 (28/75)	46 (13/28)
		K	sachet, anise oil	27 (20/75)	35 (7/20)
3	Sapelo Is., GA (247) 24–26/4/97	A	polymer, fish meal	56 (28/50)	61 (17/28)
		J	sachet, unrefined corn oil, sweet corn oil (99:1)	29 (14/49)	14 (2/14)
		B	sachet, fish oil	56 (28/50)	82 (23/28)
		D	sachet crayfish oil	61 (30/49)	80 (24/30)
		H	sachet cherry essence	22 (11/49)	27 (3/11)
4	Sapelo Is., GA (359) 16–19/5/97	A	polymer, fish meal	52 (93/180)	88 (82/93)
		B	sachet, fish oil	53 (94/179)	89 (84/94)
5	Erie Co., OH (400) 6–10/5/98	A	polymer, fish meal	29 (58/200)	69 (40/58)
		B	sachet, fish oil	23 (45/200)	80 (36/45)
6	Erie Co., OH (500) 11-17/5/99	A	polymer, fish meal	29 (29/100)	93 (27/29)
		L	polymer, dog food, sugar, vanillin	14 (14/100)	79 (11/14)
		M	polymer, dog food, cherry	29 (29/100)	86 (25/29)
		N	polymer, dog food, crayfish	35 (35/100)	91 (32/35)
		O	polyurethane cylinder, Mazuri® aquatic gel	35 (35/100)	83 (29/35)
7	Erie Co., OH (347) 17–20/8/99	A	polymer, fish meal	52 (36/69)	92 (33/36)
		P	polymer, dog food, Askins	47 (33/70)	91 (30/33)
		Q	polymer, dog food, Fox Hollow	61 (42/69)	93 (39/42)
		R	polymer, dog food, J. R. & Sons	53 (37/70)	84 (31/37)
		S	polymer, dog food, Wildlife control tech.	55 (38/69)	90 (34/38)
8	Ossabaw Is., GA (480) 12–15/5/00	A	polymer, fish meal. sealant wax No. 1	48 (58/120)	86 (50/58)
		T	polymer, fish meal, sealant wax No. 2	54 (65/120)	85 (55/65)
		B	sachet, fish oil	57 (68/120)	88 (60/68)
		U	sachet, fish oil, earth-colored (dyed)	45 (54/120)	89 (48/54)

[a] For each field trial, the total number of bait exposure nights (BEN) for all test bait types combined (one BEN is one bait exposed for one night).

[b] Sources of candidate baits and flavors: for baits A and polymer dog food baits (L–N, P–S) without flavor additives (Bait-Tec, Inc., Beaumont, Texas, USA); for sachet bait without flavor additives (Merial Limited, Athens, Georgia, USA); for flavors for baits C, D, F, G, H, I, K, M, and N (Bell Flavors and Fragrances, Northbrook, Illinois, USA); for grape and cherry Kool-Aid®, (Kraft Foods, Inc., PSK-C, White Plains, New York, USA); for unrefined corn oil (bait J) (Spectrum Naturals, Inc., Petaluma, California, USA); for sweet corn oil (1%), (Bell Flavors and Fragrances); for flavors for baits B, E, and R (J.R. & Sons, Monroeville, Ohio, USA); vanillin for bait L (Merial Limited); for flavor of bait O (PMI Feeds, St. Louis, Missouri, USA); for flavors for baits P–S (P. Askins, Lititz, Pennsylvania, USA; Fox Hollow Trappers Supply, HC-1 Marble Hill, Georgia, USA; J.R. & Sons, Monroeville, Ohio, USA; Wildlife Control Technology, Cortland, USA); for sealant waxes and dye for baits A, T, and U (proprietary sources, Merial Limited).

[c] Number baits visited/BEN.

[d] Number baits taken/number baits visited.

responses of both captive and free-ranging coyotes. A captive animal trial was conducted at a field station of the National Wildlife Research Center, U.S. Department of Agriculture (USDA), Animal Plant Health Inspection Service (APHIS) located near Logan, Utah (USA). This facility maintained a colony of coyotes for conducting various ecological and behavioral studies. Field trials were conducted on a private ranch (27°17′N, 98°40′W), south of Heb-

TABLE 2. Summary of coyote oral rabies vaccine bait preference field trials, 1997.

Trial number	Location, (BEN)[a], dates of trial	Bait	Bait type and flavor[b]	Coyote response to baits: Percent baits visited[c]	Coyote response to baits: Percent baits taken[d]
1	Jim Hogg Co., Texas (692) 14–19 November 97	AA	polymer, fish meal, Hill's liquid enhancer	38 (37/98)	78 (29/37)
		BB	sachet, Cheese Plus®, beef tallow	29 (29/101)	66 (19/29)
		E	sachet, menhaden oil, fish meal	26 (25/95)	64 (16/25)
		V	sachet, menhaden oil, Hill's liquid enhancer	25 (24/98)	54 (13/24)
		X	sachet, beef tallow, Hill's dry enhancer	22 (22/102)	64 (14/22)
		Y	sachet, beef tallow, sugar	14 (14/98)	36 (5/14)
		Z	sachet, poultry oil, poultry Biodigest®	27 (27/100)	78 (21/27)
2	Jim Hogg Co., Texas (599) 10–14 December 97	AA	polymer, fish meal, Hill's liquid enhancer	34 (68/199)	82 (56/68)
		CC	sachet, poultry oil, poultry Biodigest (1 side of sachet only)	22 (44/200)	71 (31/44)
		DD	sachet, poultry oil (only)	20 (40/200)	53 (21/40)

[a] For each field trial, the total number of bait exposure nights (BEN) for all test bait types combined (one BEN is one bait exposed for one night).
[b] Sources of candidate baits and flavors: for baits AA and BB without flavor additives (AA, Bait-Tec, Inc., Beaumont, Texas, USA; BB, Merial Limited, Athens, Georgia); for baits with liquid (AA and V) and dry (X) Hill's enhancer (Hill's Pet Products, Inc., Kansas); for Cheese Plus® flavor for bait BB, (International Ingredient Corp., St. Louis, Missouri); for stabilized beef tallow for BB, X, and Y baits (Birmingham Hide and Tallow, Birmingham, Alabama); menhaden oil for baits E and V (J. R., & Sons, Monroeville, Ohio); poultry oil for baits Z, CC, and DD (The University of Georgia, Athens, Georgia); for poultry Biodigest® for baits Z and CC, (Bioproducts, Inc., Louisville, Kentucky).
[c] Number baits visited/BEN
[d] Number baits taken/number baits visited.

TABLE 3. Field trials of rabies vaccine (Raboral V-RG®, Merial Limited, Athens, Georgia) distributed in baits to immunize free-ranging raccoons and coyotes in 1998.

	Raccoon trials (New York)			Coyote trials (Texas)	
		Reference areas[a]			
	Test area	A	B	Test area	Reference area
Size (km^2)	607	937	667	2,349	39,393 km^2
Bait type	Fish flavored sachet	Square fish meal polymer	Rectangle fish meal polymer	Poultry-flavored sachet	Fish meal polymer
Estimated number baits placed (dates)	42,962	57,240 (Sept. 1998)	41,910 (Sept. 1998)	63,423 (Feb. 1998)	1,064,700 (Jan. 1998)
Number baits/km^2	75	75	75	27	27
Number sera assayed	65	170	73	35[b]	34[b]
Percent rabies seropositive[c]	45% (29/65)	46% (78/170)	31% (24/77)	94% (33/35)[b]	44% (15/34)[b]

[a] Two reference areas received two different size fish meal polymer baits; a smaller square bait (2.0 × 3.5 × 3.5 cm) on area A, and a larger rectangular bait (2.0 × 3.3 × 5.3 cm) on area B.

[b] Subadult tetracycline-negative animals only.

[c] Serum samples with rabies virus neutralizing activity at a 1:4 (raccoon) or 1:5 (coyote) dilution or greater by fluorescent focus inhibition test were considered antibody positive.

bronville, Jim Hogg County, southern Texas. This area consisted of flat pasture lands used for cattle grazing and fee hunting and was dominated by open grass-covered areas interspersed by mesquite (*Prosopi* sp.) and various shrub and herbaceous plant species.

Raccoon bait trials

We periodically conducted field trials during the period 1996–2000 to assess various bait components and potential of the sachet bait for orally vaccinating raccoons. Trials were conducted in coastal Georgia during spring, summer, and fall but not during the November to early February period because of unexplained raccoon behavior that resulted in previously documented low visitation and bait removal rates (unpubl. data, SCWDS). Each trial usually took 3–5 days to complete. Vaccine media-filled sachet baits were used for all but one of our raccoon field trials. Most trials consisted of comparing different attractant coatings on sachet baits to determine if one or more would elicit higher bait discovery and uptake. We also compared raccoon responses between sachet and fish meal polymer bait types, followed by evaluation of dog food polymer baits surface-coated with different attractants (Table 1). Last, we vaccinated free-ranging raccoons by aerially distributing sachet baits containing Raboral V-RG® in the St. Lawrence River valley area (Table 3).

The initial trial on the Little Satilla WMA, Georgia, in late May 1996 was aimed at evaluating our field trial protocol and determining the potential of the sachet bait compared with the fish meal polymer bait. Menhaden fish oil and fish meal attractants were used as the sachet coating for the above exploratory field trial as these ingredients were used in the fish meal polymer bait. The next four trials (Trials 1–4, Table 1) were conducted from February through May 1997 to compare raccoon responses to 10 attractants, five of fish or crustacean oil origin and five that were plant derived, and to eliminate from further consideration those formulations that elicited lower response rates. Two candidate coatings from each of the above categories that received the highest responses were then compared with the fish meal polymer bait (Table 1).

From May 1998 through May 2000, we conducted four more trials (Trials 5–8, Table 1), three of which were bait preference trials. Trials 5–7 were carried out near Sandusky, Ohio, and the results compared with the Georgia preference data. Trials 6–8 compared raccoon visitation to and bait removal for 10 candidate bait formulations and how they compared with the fish meal polymer bait. Five of these candidate attractants were selected based upon our earlier 1997 tests in Georgia, as well as bait types that were in use at the time by ongoing ORV programs. The other five attractants were proprietary compounded attractants (i.e., multiple ingredients) recommended and provided by commercial trapper supply companies. Attractants were surface-coated onto dog meal polymer baits using melted wax as the carrier.

Tracking station methodology was used to sequentially field test candidate attractants and bait types. Each test consisted of selecting a route along suitable rural or farm roads where animal sign, prior experience, and/or recommendations by local wildlife authorities indicated moderate to high raccoon densities. Depending upon local conditions, 100–140 tracking stations spaced 0.32 km apart and alternated on each side of the road were flagged, numbered, and maintained during the test. Each station consisted of a 1 m diameter circle of smoothly raked and sifted earth. A single candidate bait type or attractant-coated sachet was placed at each station in the center of the circle. The type of bait or attractant placed at each station was determined randomly such that equal numbers of each candidate formulation were located along the entire route. Stations were checked daily for 3–5 days, depending upon visitation rates, to determine how many of each type were visited or removed. Animal species visiting stations were identified by tracks left in the sifted soil. When the tracks of two or more different species were present at a station and the bait was removed, this activity was recorded but excluded from data analyses. A 5–10 min search was made in the immediate vicinity of stations where baits had been disturbed to locate, save, and later analyze partially eaten baits and discarded sachets left by visiting animals. Baits that were taken or partially eaten were replaced daily as needed.

Raccoon bait preferences were determined by comparing, for example, in Trial 1, Bait B with Bait C, also with D, E, and F; comparing Bait C with D, E, and F, and so on. Bait visitation and removals were compared by chi-square analysis ($P \leq 0.1$). Other criteria used to determine if a given bait or attractant should be retained for additional testing or excluded from further consideration included: ease of formulation, cost and suitability for large scale manufacture and application, frequency of sachet puncture, and vaccine media remaining within recovered sachets.

The field trial in northern New York to experimentally vaccinate raccoons with sachet baits containing Raboral V-RG® was begun in September 1998 by dropping fish-flavored sachet baits from twin engine Otter aircraft operated by the Canadian Ontario Ministry of Natural Resources (OMNR). Aircraft flew at a speed of ~290 km/hr and an altitude of ~152 m above ground level. Forty-three thousand sachet baits were dropped along transects spaced 0.75 km apart using a bait dispensing machine developed by Ontario workers (MacInnes et al., 1992). The above procedure resulted in a bait density of 75 baits/km^2 throughout the 607 km^2 test area. The raccoon population in the area was sampled in October–November 1998 by live trapping and night hunting with cooperating state-licensed hunters and their dogs. The percent of animals positive for rabies virus neutralizing antibodies by a fluorescent focus inhibition test (Smith et al., 1973) was then compared with that of raccoons collected from two other nearby comparable but discrete reference areas. Raboral V-RG® vaccine-laden fish meal polymer baits of two different sizes had been dropped on the latter two areas using the same procedures, spacing, and bait density as described for the sachet bait (Table 3).

Coyote bait trials

We conducted four trials in 1997–98 to evaluate coyote baits, one with captive coyotes and three with free-ranging animals. The captive coyote trial sought to determine if vaccine sachet configuration and size might determine whether sachets were picked up and swallowed intact (i.e., not punctured and therefore no release of vaccine into the buccal cavity). Field trials 2 and 3 were aimed at determining if free-ranging coyotes showed significant preferences for different sachet attractant coatings. Trial 4 was conducted to ascertain whether flavor-coated sachet baits containing Raboral V-RG® vaccine would immunize a local coyote population.

Adult captive coyotes ($n = 30$) were individually maintained in concrete-floored chain link runs (1.2 × 1.8 × 3.7 m) with shelter boxes and fed daily with water available ad libitum. Each coyote was offered one vaccine media-filled sachet bait coated with a mixture of the wax carrier, menhaden fish oil, and fish meal for each of 3 consecutive days. On day one, 10 coyotes were offered a single chamber sachet (0.5 × 2.0 × 6.0 cm), 10 given a two-chamber sachet (0.5 × 4.0 × 6.0 cm), and 10 a three-chamber sachet (0.5 × 6.0 × 6.0 cm). On days 2 and 3, each group received a different size sachet such that all 30 animals were exposed to three different size sachets over the 3 day period ($n = 90$). Each animal was observed for 5–10 min and its response to the sachet bait recorded. In several instances, animals refused to respond or retreated to their shelter box when we were present. In these cases, the observer moved on to the next test animal and returned 1–2 hr later to determine if the sachet had been eaten or remained undisturbed. On days 2–4, we collected feces from the floor of each cage, carefully examined them for ingested sachets, and those recovered were thoroughly washed. We recorded if the sachets were intact, were chewed, had tooth punctures, and whether the vaccine media was expelled.

The two bait preference field trials in south Texas were conducted in November and December 1997 exactly as those described earlier for raccoons, except that the interval between tracking stations was greater (i.e., 0.48 km). The November trial involved assessment of six different candidate attractants coated onto sachet baits. The fish meal polymer bait served as a control. The trial in December consisted of comparing the fish meal polymer bait with two formulations of a poultry flavor-enhanced sachet bait; the poultry formulation having received the highest ranking of all the sachet bait types tested the previous month. Bait attractants tested and formulations for each are shown in Table 2. A chi-square statistic ($P \leq 0.1$) was used to determine differences among candidate baits and attractants.

A third field trial, conducted in early February 1998, sought to determine efficacy of the sachet bait for delivering Raboral V-RG® to coyotes. It was conducted in the same manner as described earlier for raccoons in northern New York. However, 63,423 sachet baits enhanced with poultry attractants were aerially dropped by OMNR planes at a density of 27 baits/km^2 along transects spaced at 0.81 km intervals throughout a 2,349 km^2 test area. A core area ($\sim$777 km^2) was later sampled and the percent of coyotes seropositive for rabies virus antibodies (by a fluorescent focus inhibition test) was compared with the surrounding areas where Raboral V-RG® contained in fish meal polymer baits was dropped under identical conditions (Table 3). One factor that complicated the above trial was that the test site and surrounding areas were treated annually (January–February) with Raboral V-RG® and tetracycline laden fish meal baits in previous years. Thus, an unknown percent of surviving coyotes older than 1 yr of age may have been either marked and/or had rabies virus neutralizing antibodies resulting from prior exposure to baits. Field trials with polymer baits containing the biomarker tetracycline (but no vaccine) also were conducted the previous year (November–December, 1997). Therefore comparison of rabies seroprevalence was limited to unmarked (i.e., tetracycline negative) subadult coyotes. This cohort of the population was never exposed to vaccine laden baits as they were born in spring 1997. Presence or absence of tetracycline in the teeth of coyotes was determined by longitudinally sectioning canine teeth with a diamond saw blade and looking for fluorescence under a compound microscope equipped with a UV light and appropriate filter (Fletcher et al., 1990).

One of us (LLB) expressed concern at the onset that the field protocol did not allow for ascertaining whether rabies antibodies present in adult females previously exposed to vaccine baits might be transferred in utero to their offspring and thus subsequently augment the percent of seropositive subadult coyotes. However, we felt that research to determine this possibility was beyond the scope of the field trial.

RESULTS

The tracking station technique was generally satisfactory for determining which species, target and nontarget, visited stations and took baits. Both raccoons and coyotes often discriminated among certain bait and attractant formulations but not others. Thus, as field trials proceeded, we observed which formulations elicited higher rates of station visitation and bait removal. Along with other criteria, we then decided whether to advance a given candidate formulation to the next test or to drop it from further consideration.

The tracking station technique also was useful to determine if candidate formulations elicited the same level of bait uptake as a control or reference polymer bait. For example, the initial May 1996 raccoon field trial at Little Satilla WMA to validate the tracking station technique and to compare an early prototype fish-flavored sachet bait with the fish meal polymer bait, showed no significant difference in bait uptake. The sachet bait received 44 raccoon visits of which 33 baits were taken (75%), whereas the polymer bait had 60 visits and 42 were taken (70%).

Both raccoon and coyote field trials revealed that time of year or season was important in obtaining adequate visitation and bait removal. Raccoon trials generally ran for 3–5 days and coyote trials for 4–7 days. For coyotes, the spring months were not used because seasonally windy conditions in Texas often obliterated animal tracks. Because of the above seasonal factors, as well as geographic variations in habitat, food availability, and population densities, bait removal in relation to the number of bait exposure nights was not considered a meaningful measure of target species bait preference.

Raccoon bait trials

A total of 3,083 bait exposure nights, consisting of 58 comparisons among 21 different formulations both for visitation and bait removal, were completed during the eight raccoon bait preference field trials (Table 1). We were unable to discern the relative importance of raccoon visitation to different bait formulations, versus actual bait removal from stations (Table 1). Obviously, raccoons must approach and discover baits before ingesting them. Therefore, both data sets were used to evaluate candidate formulations. At the $P = \leq 0.10$ level, 34% (20/58) of the bait visitation comparisons and 21% (12/58) of the bait uptake comparisons differed. These differences occurred in five of eight field trials but were heavily skewed toward the first three trials when we compared animal versus plant-derived attractants.

Four of 58 bait comparisons sought to determine if sachet bait coated with fish-flavored attractants differed from the fish meal polymer bait. All four trials (Trials 3, 4, 5, and 8) showed there was no difference between either bait visitation or bait removal.

Raccoon visitation to and removal of five oil-based fish and crustacean sachet attractants (Trial 1) ranged from 24–41% and 61–84%, respectively. The crayfish attractant was visited more often than shellfish bait and was removed more often than shellfish bait, menhaden oil bait, or shrimp oil bait (Table 1).

Trial 2 compared five plant-derived attractants used by raccoon trappers. Six of 10 visitation comparisons, and two of the bait take comparisons, differed. The cherry-coated sachet bait was visited more often than grape bait, persimmon bait, corn bait, or anise bait but was not taken more often than any of the other candidate plant-derived attractants. Persimmon bait was visited more than grape bait or anise bait, but was removed less often than grape bait, while grape bait was removed more than anise bait. Based on the results of this trial, ease of formulation, and other factors, the cherry and a modified corn formulation baits (Table 1) were retained for further evaluation (Trial 3).

Trial 3 consisted of comparing the four "best" formulations from trials one and two; that is, fish bait, crayfish bait, a mixture of corn oils and cherry "essence" bait, with the fish meal polymer bait. Visitation and removal rates were both much higher for the fish bait, crayfish bait, and fish meal polymer bait formulations (56%, 61%, and 56% for visits; 82%, 80%, and 61% for bait removal) than for either the cherry bait or corn oil bait coatings (27% and 14%) (Table 1).

The fourth trial sought to confirm, by larger sample size, trial 3 results, that is, a comparison of the fish oil/fish meal sachet coating with the fish meal polymer bait (Table 1). We found no differences in either visitation or bait removal between the two bait types.

The fifth trial, undertaken in north-central Ohio, sought to determine if raccoons in that region of the country responded to identical baits in the same manner as those tested in Georgia. Raccoons in Ohio visited a lower percent of both fish meal polymer baits (29%) and fish oil-coated sachets (23%). These percentages were considerably lower than those reported earlier from the Georgia coastal areas, possibly because of fewer raccoons and greater densities and more diverse carnivore community of vertebrate scavengers present at the mainland Ohio site. However, the percent of polymer and sachet baits removed from stations visited by raccoons in Ohio was similar to that recorded for Georgia (Table 1).

Trial 6, also in Ohio, sought to determine how raccoons responded to dog food polymer baits coated with attractants compared to the fish meal polymer bait (Table 1). These formulations were tested, in part, because dog food polymer baits do not have much odor. We also elected to test a bait, similar to that described earlier by Linhart et al. (1994) that consisted of a polyurethane sleeve saturated with a proprietary fish-based commercially available fish food product (Mazuri® aquatic gel; Bait O; Table 1). Six of 10 paired bait comparisons differed and in general substantiated findings of earlier trials. The fish meal polymer bait, cherry and crayfish flavored dog food polymer baits, and Mazuri® sleeve baits were visited more often than the sugar-vanillin bait used by the Texas ORV program for gray foxes (*Urocyon cinereoargenteus*). Crayfish and Mazuri®-flavored baits were visited more than cherry-flavored baits. Frequency of bait removal, regardless of attractant type, did not differ (Table 1).

Trial 7 (Table 1) compared a few of the numerous commercially available raccoon attractants marketed to raccoon trappers. Three of the four attractants contained crayfish oil. The four candidate attractants were compared with each other and also with the fish meal polymer bait. There was no difference among the five bait types either with respect to visitation or removal.

Trial 8 (Table 1) determined how raccoons responded to two different waxes used to seal sachets within the fish meal polymer bait. A new high tack sachet wax sealant was selected to more securely seal the vaccine sachet within the polymer bait to reduce the likelihood of the sachet being removed from the bait by raccoons (Olson and Werner, 1999). There was no difference in raccoon removal of baits from stations containing either the old or new sealants. However, we were unable to recover enough discarded sachets or baits to determine if fewer sachets were lost from the high tack sachet wax sealant bait or to compare frequency of sachet puncture between the two sealant types. A companion test determined if raccoons would discriminate between the "standard" fish-flavored sachet bait and an identical bait having the wax coating dyed earth colored to reduce human detection of baits following aerial distribution. There was no difference between the two in visitation or bait removal (Table 1).

We recovered 632 sachets that had been picked up and then discarded by raccoons; 291 were flavor-coated sachet baits, and 341 were sachets contained within polymer baits that had been masticated. Raccoons ruptured 96.6% (281/291) of the coated sachet baits and 85.3% (291/341) of sachets contained within polymer baits. More coated sachets were completely emptied of vaccine media (91.5%, 258/282) following mastication than were the sachets recovered from polymer baits (78.3%, 246/314). Both of the above differences were highly significant ($P = \geq 0.01$). These results indicate that the flavor-coated sachet bait most likely would deliver oral vaccine more effectively to this species.

The field trial of fish-flavored V-RG® vaccine sachet baits in the St. Lawrence River valley provided data that showed this bait was as effective as the fish meal polymer bait for orally immunizing raccoons. Of the raccoons collected following aerial distribution of Raboral V-RG® sachet baits, the number rabies virus seropositive was the same as for raccoons sampled from the test area where the smaller square fish meal polymer bait was used. Fewer raccoons (31%) collected from the third test site where the larger rectangular fish meal polymer bait was dropped were rabies seropositive (Table 3).

Coyote bait trials

Captive coyotes were observed approaching and picking up 60 of the 90 sachet baits offered and their ingestive behavior was noted. Disposition of the remaining 30 sachets was determined by returning later in the day to check for bait consumption. One coyote refused to ingest any of the three sachet baits offered over the 3-day test period. The other 29 animals picked up and ingested baits. Of the 60 direct observations where coyotes were seen to pick up baits, all three sizes, regardless of configuration, were masticated and none were bolted or gulped without being chewed. Combining direct and in-direct observations, 93% (84/90) of the sachet baits were swallowed, with no observed differences among bait sizes. A few (3/90) of the baits were chewed, ruptured, and subsequently discarded.

Remains of 47 sachets (56% of total ingested) were collected from feces from individual runs on days 2, 3, and 4 of the trial and examined each morning. All had been thoroughly chewed or shredded regardless of size, and all were completely emptied of vaccine media. These results dispelled concerns that sachet size might influence frequency of sachet rupture and probable exposure of Raboral V-RG® vaccine to the buccal cavity in this species.

A total of 1,291 bait exposure nights were recorded during the two coyote bait preference trials. Coyotes in the November 1997 field trial discriminated among sachet baits coated with six different attractants, as well as among sachet baits and the polymer control bait. Of the 21 comparisons made between paired attractants ($P = \leq 0.10$), 38% (8/21) showed differences in visitation to tracking stations, and 33% (7/21) differed in frequency of bait removal. The fish meal polymer control bait with Hill's Liquid Enhancer® bait elicited the highest visitation and bait removal (38 and 78%, respectively). However, these did not differ statistically from the best performing sachet bait containing both poultry oil and poultry Biodigest® (27 and 78%, respectively). Conversely, a sachet bait containing beef tallow and sugar had the lowest visitation and bait removal (14 and 36, respectively). Visits and bait removal for all other bait types fell between the above values (Table 2).

The second bait preference trial in December 1997 revealed that when the poultry Biodigest® coating was reduced or eliminated, poultry-flavored sachets were visited less often than fish meal polymer baits. Fewer sachets were removed from stations with less or no Biodigest® in the formulation. However, when sachets coated with poultry oil and Biodigest® on both sides (Trial 1) were compared with the visitation and removal of fish meal polymer bait tested in Trial 2, there was no difference (Table 2).

We were unable to find discarded sachets that had been removed by free-ranging coyotes. Presumably sachets were swallowed as was observed in captive coyotes.

Fifty-nine of 175 (34%) coyotes collected in March 1998 from the test area in south Texas baited with poultry-flavored sachet baits containing Raboral V-RG® vaccine were subadults based on age determination by tooth pulp cavity size and cementum annuli counts. This cohort of the population was born in spring, 1997. Thirty-five of the 59 subadults were tetracycline-negative. Therefore, they were not previously exposed to baits containing tetracycline, nor were they born when Raboral V-RG® baits were dropped in the area by the Texas ORV program in the winter of 1997. Ninety-four percent (33/35) of this subsample of the population had rabies virus antibodies. This exceptionally high seroconversion rate was achieved despite concern that a faulty sachet flavor coating procedure, which caused a partial sloughing of the sachet coating, might have jeopardized coyote bait ingestion. In comparison, the seropositivity rate (44%, Table 3) of subadults on the area baited with vaccine-laden fish meal polymer baits was significantly lower ($P = >0.01$).

Bait removal by nontarget species

Removal of raccoon baits at tracking stations by nontarget species varied markedly between the Georgia and Ohio test sites. This difference was primarily because the Georgia barrier islands supported a limited mammalian fauna as well as a large feral swine population on Ossabaw Island. Combining data from five field trials conducted on Sapelo and Ossabaw Islands, a total of 116 baits (6.3%) were taken by nontarget species whose tracks could be identified at tracking stations the day after bait removal. The only species that removed significant numbers of baits was feral swine. Swine took 87.9% (102/116) of all baits removed by nontargets. A few baits were taken by donkeys (5), unidentified bird species (2), rodents (5), an armadillo (*Dasypus novemcinctus*) (1), and a domestic dog (1). Some baits were removed by species whose tracks could not be identified (n = 34), or where two or more species visited a station on a given night and it was impossible to tell which had removed the bait (n = 81). Some of the unidentified tracks and situations where multiple species visited stations and took baits were undoubtedly raccoons. However, the percent of baits removed, both by identified nontarget species and unknown species combined, was only 12.6% of all baits exposed during the five trials (231/1,836).

Three field trials in Ohio produced different results. Of identified nontarget species, opossums (*Didelphis virginiana*) took the most (4.6%, 57/1,247). Unidentified birds, red foxes, coyotes, and domestic cats combined took another 4.7%. Combining all bait removals not attributable to raccoons, a maximum of 22.9% of all baits exposed (286/1,247) could have been taken by nontarget species. The rather high number of tracks that could not be identified (n = 68) was attributed to initially using poor soil tracking medium (loam) that did not provide good tracks and a trial when a combination of wind and dried out tracking medium (fine sand) resulted in poor track definition. We believe that neither the loss of 12.6% of the bait in Georgia nor the loss of 22.9% of the baits in Ohio would have had a great effect on the immunization rate of raccoons had vaccine been used in sachet and polymer baits. Although white-tailed deer (*Odocoileus virginianus*) and cottontail rabbits (*Sylvilagus floridanus*) were common in most of our test sites, neither species disturbed baits.

In two coyote bait preference trials in south Texas, nontarget species bait removal was minimal. Nontarget animals, all species combined, accounted for removal of only 8.7% (113/1,291) of all baits exposed. Feral swine accounted for 84.9% (96/113) of these removals. A few baits were removed by other species: unidentified lagomorphs (5), raccoons (6), badgers (*Taxidea taxus*) (1), gray fox (4), and domestic dog (1). No instance was recorded where rodents removed baits. Combining totals for baits taken by identified nontarget and unidentified species, and those instances where multiple species tracks were present and bait removed, a maximum of 11.8% (152/1,291) of all baits exposed could have been removed by species other than coyotes.

DISCUSSION

Placing baits at tracking stations spaced at regular intervals along rural roads through habitats supporting moderate to high densities of target species generally provided data adequate to compare visitation and bait uptake rates by the various species present. However, factors that sometimes limited or prevented data collection included: (1) tracks obliterated by rain or wind, (2) soil conditions not conducive to good track impressions and identification, (3) greatly reduced raccoon visitation to stations during the early winter months in the southeast, (4) low target species densities that resulted in inadequate sample sizes for chi-square analyses, (5) questionable usefulness of the technique in areas having moderate to high human densities and landscape alteration, and (6) a limited network of rural roads.

It was sometimes helpful to conduct a 2–3 day pretest survey in potential new test areas to determine if visitation rates by target species justified longer and more costly bait preference trials. These pretest surveys were conducted by placing a limited number of tracking stations (n = 30–50) at intervals throughout the proposed test area and checking them for 1–2 days to determine the extent of visitation to a standard chemical attractant (fatty acid scent) absorbed into a plaster of paris disk (Roughten and Sweeney, 1982) available from Wildlife Services (U.S. Department of Agriculture, Pocatello Supply Depot, Pocatello, Idaho, USA). Test sites where nightly raccoon and coyote visitation rates were about 30 and 20%, respectively, were generally used.

Soil types and soil moisture were extremely important factors affecting track identification. For example, in Ohio in May, 1998 (Trial 5), loam top soil was purchased and used to make tracking stations. Raked and sifted stations made of this type soil initially appeared satisfactory. However, dry soil particles later collapsed into individual tracks resulting in a series of unidentifiable depressions in the soil. Forty-seven percent of all tracks at stations made with loam soil could not be identified the following morning. The loam soil was then covered with a layer of moist, fine-grained sand, and the percent of unidentified tracks was subsequently reduced to 25%. When the surface of this sand was then saturated with water sprayed from a garden pump-type sprayer, the percent of unidentified tracks the next day was further reduced to only 2%. Finely powered caliche soil, containing large amounts of deposited salts, used for tracking stations in south Texas proved to be ideal as a tracking medium.

Problems associated with delivery of oral rabies vaccine were reported as early as 1976 by Winkler and Baer (1976) who found that red foxes often bit vaccine baits into pieces that then fell to the ground, resulting in the loss of liquid vaccine from vaccine containers. Our data show that bait manipulation by raccoons sometimes results in consumption of polymer baits without rupturing the enclosed vaccine-laden sachet. For example, in Ohio in May 1998 (Trial 5), 39 sachets from fish meal polymer baits taken by raccoons were recovered. Of these, 12 of 13 sachets remained intact within uneaten or partially eaten baits (30%). These results were similar to those reported in Florida by Olson and Werner (1999). We addressed this problem by testing (Trial 8) a different wax sealant having greater tack or adhesive property for holding sachets more securely within polymer baits. Raccoons did not discriminate between polymer baits containing the standard wax sealant and the new wax with the higher adhesive characteristic.

Merial Limited has conducted both laboratory and field tests to address environmental and physical factors that might adversely affect bait integrity or vaccine potency over time. They found that aerial bait drops did not physically damage either polymer or coated sachet baits (unpubl. data). White, opaque polyurethane plastic film used to fabricate sachets protected Raboral V-RG® vaccine from loss of titer by ambient ultraviolet light. Heavy rainfall may wash off some of the exterior oil-based flavor overcoating on sachet baits. Extremely high temperatures may also cause some melting of the high temperature resistant wax used for both bait types. However, field observations and results of vaccine field trials indicated these factors do not seriously affect immunization of target species (unpubl. data, Merial Limited). No trials of other oral rabies vaccines (SAG2, SAD strains) currently being operationally used in the field have been evaluated in either polymer or sachet-type baits.

Our nontarget species disturbance data from the Georgia barrier islands was not indicative of what might be expected for mainland areas in the southeast. The barrier islands had far fewer nontarget species and on Ossabaw a much higher density of feral swine than found on the mainland. For example, one of our earliest field trials in south Georgia on the Little Satilla WMA in May 1996, provided a more realistic indication of nontarget activity. Ten different species, of which five took baits, visited tracking stations. Of 63 total visits recorded, 52% of baits were removed by all species. Raccoons took 49% (16/33) and identified nontarget species removed 36% (12/33). Dogs, coyotes, and gray foxes were the principal nontarget species taking baits intended for raccoons. These results are more comparable to those reported by Olson and Werner (1999) in Florida, although their nontarget species composition differed from ours.

Primary advantages of the sachet bait are the potential for automated manufacture in large quantities, less weight and volume than polymer baits such that more efficient aerial distribution can be achieved through the use of smaller and less expensive aircraft, and reduced cost of purchase and distribution by user groups (e.g., state and county agencies). Our observations (Kavanaugh and Linhart, 2000) and others who have used them suggest that both sachet and polymer-type baits should be available so that ORV program managers can decide which best fit the conditions prevailing in their areas.

Sachet baits do have several limitations. One problem is that, unlike the polymer baits, their light weight and physical configuration make it impossible to throw them any distance (i.e., into culverts and creek drainages) from vehicles when ground distribution of vaccine baits is needed. Another is that the lighter sachet bait when dropped from aircraft drifts further from the transect lines being flown (data on file, Rabies Unit, OMNR). Aerial drift is not a major problem when sachet baits are distributed in uninhabited areas, but becomes more important when aircraft approach highly populated areas or large bodies of water.

Our field trial data are geographically limited and thus do not characterize raccoon and coyote response to candidate vaccine baits throughout the range of these two species. Moreover, we were able to field test only a few of the many potential attractants for sachet baits. Nevertheless, our results provide convincing and consistent evidence that the sachet bait is attractive to raccoons and coyotes and can effectively deliver oral rabies vaccine to these two species. These findings resulted in the recent amendment of Merial Limited's federal licensure of Raboral V-RG® to include the fish-flavored sachet bait as an alternate method for orally vaccinating raccoons. Further evaluation of alternate baiting strategies, as well as ongoing field use of the vaccine-laden sachet bait along with extensive application of fish meal polymer vaccine baits in various states, will provide additional data on their efficacy.

ACKNOWLEDGMENTS

We acknowledge field assistance of C. Baumann, D. Gregory, M. Hensley, R. Long, and A. Meadows, SCWDS, University of Georgia. V. Nettles, SCWDS Director, ably administered and provided guidance to the project from inception to completion. C. McElwee and K. King, SCWDS, provided administrative support. Wildlife Services (APHIS, USDA) field and supervisory staff in New York, Ohio, and Texas provided field and logistic support. The National Wildlife Research Center (NWRC, APHIS, USDA), Logan, Utah field station, furnished captive coyotes, facilities, and support for evaluation of the sachet bait. P. Savarie and R. Engeman, NWRC, suggested bait formulations and provided statistical expertise, respectively. S. Blom, Wildlife Services, Pocatello, ID, made many helpful suggestions on potential bait attractants and their formulation. L. Paulik, staff librarian at the NWRC provided assistance with the literature. Personnel in the Departments of Health in New York, Ohio, and Texas also provided logistic and field assistance during the course of field trials, as did the Zoonotic Disease Section, College of Veterinary Medicine, Cornell University. The Georgia Wildlife Resources Division gave permission to use several of their Wildlife Management Areas for field tests and provided facilities and vehicles. The Rabies Research Unit, Ontario Ministry of Natural Resources, Canada, provided aircraft and technical expertise for aerial distribution of V-RG laden baits. Financial support for this project was provided by Merial Limited, the Georgia Research Alliance, the University of Georgia, Wildlife Services (APHIS, USDA), and the sponsorship of the Southeastern Association of State Fish and Wildlife Agencies.

LITERATURE CITED

CREEKMORE, T. E., S. B. LINHART, J. L. CORN, M. D. WHITNEY, B. D. SNYDER, AND V. F. NETTLES. 1994. Field evaluation of baits and baiting strategies for delivering oral vaccine to mongooses in Antigua, West Indies. Journal of Wildlife Diseases 30: 497–505.

FARRY, S. C., S. E. HENKE, A. M. ANDERSON, AND

M. G. FEARNEYHOUGH. 1998a. Responses of captive and free-ranging coyotes to simulated oral rabies vaccine baits. Journal of Wildlife Diseases 34: 13–22.

———, ———, S. L. BEASOM, AND M. G. FEARNEYHOUGH. 1998b. Efficacy of bait distributional strategies to deliver canine rabies vaccines to coyotes in southern Texas. Journal of Wildlife Diseases 34: 23–32.

FEARNEYHOUGH, M. G., P. J. WILSON, K. A. CLARK, D. R. SMITH, D. H. JOHNSTON, B. N. HICKS, AND G. M. MOORE. 1998. Results of an oral rabies vaccination program for coyotes. Journal of the American Veterinary Medical Association 212: 498–502.

FLETCHER, W. O., T. E. CREEKMORE, M. S. SMITH, AND V. F. NETTLES. 1990. A field trial to determine the feasibility of delivering oral vaccines to wild swine. Journal of Wildlife Diseases 26: 502–510.

HANLON, C. A., D. E. HAYES, A. N. HAMIR, D. E. SNYDER, S. JENKINS, C. P. ABLE, AND C. E. RUPPRECHT. 1989. Proposed field evaluation of a rabies recombinant vaccine for raccoons (*Procyon lotor*): Site selection, target species characteristics, and placebo baiting trials. Journal of Wildlife Diseases 25: 555–567.

———, J. E. CHILDS, AND V. F. NETTLES. 1999. Article III: Rabies in wildlife. Journal of the American Veterinary Medical Association 215: 1612–1618.

KAVANAUGH, D. M., AND S. B. LINHART. 2000. A modified bait for oral delivery of biological agents to raccoons and feral swine. Journal of Wildlife Diseases 36: 86–91.

KREBS, J. W., J. S. SMITH, C. E. RUPPRECHT, AND J. E. CHILDS. 1999. Rabies surveillance in the United States during 1998. Journal of the American Veterinary Medical Association 215: 1786–1798.

LINHART, S. B., F. S. BLOM, R. M. ENGEMAN, H. L. HILL, T. HON, D. I. HALL, AND J. H. SHADDOCK. 1994. A field evaluation of baits for delivering oral rabies vaccine to raccoons (*Procyon lotor*). Journal of Wildlife Diseases 30: 185–194.

———, G. M. BAER, J. M. BALDERAS TORRES, R. M. ENGEMAN, E. FLORES COLLINS, F.-X. MESLIN, C. L. SCHUMACHER, A-H. TAWEEL (EL-), AND J. C. WLODKOWSKI. 1997a. Acceptance of candidate baits by domestic dogs for delivery of oral rabies vaccines. Onderstepoort Journal of Veterinary Research 64: 115–124.

———, A. KAPPELER, AND L. A. WINDBERG. 1997b. A review of baits and bait delivery systems for free-ranging carnivores and ungulates. *In* T. J. Kreeger (Technical Coordinator). U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Washington, D.C., Technical Bulletin No. 1853, pp. 69–132.

MACINNES, C. D. 1988. Control of wildlife rabies: The Americas. *In* Rabies, J. B. Campbell and K. M. Charlton (eds.). Kluwer Academic Publishers, Boston, Massachusetts, pp. 381–405.

———, D. H. JOHNSTON, P. BACHMANN, B. A. POND, C. A. FIELDING, C. P. NUNAN, N. R. AYERS, D. R. VOIGHT, K. F. LAWSON, AND R. L. TINLINE. 1992. Design considerations for large-scale aerial distribution of rabies vaccine-baits in Ontario. *In* Wildlife rabies control, K. Bögel, F.-X. Meslin and M. Kaplan (eds.). Wells Medical Limited, Kent, England, pp. 160–167.

———, S. M. SMITH, R. R. TINLINE, N. R. AYERS, P. BACHMAN, D. G. A. BALL, L. A. CALDER, S. J. CROSGREY, C. FIELDING, P. HAUSCHILDT, J. M. HONIG, D. H. JOHNSTON, K. F. LAWSON, C. P. NUNAN, M. A. PEDDE, B. POND, R. B. STEWART, AND D. R. VOIGT. 2001. Elimination of rabies from red foxes in eastern Ontario. Journal of Wildlife Diseases 37: 119–132.

OLSON, C. A., AND P. A. WERNER. 1999. Oral rabies vaccine contact by raccoons and nontarget species in a Florida field trial. Journal of Wildlife Diseases 35: 687–695.

———, K. D. MITCHELL, AND P. A. WERNER. 2000. Bait ingestion by free-ranging raccoons and nontarget species in an oral rabies vaccine field trial in Florida. Journal of Wildlife Diseases 36: 734–743.

ROUGHTON, R. D., AND M. W. SWEENY. 1982. Refinements in scent-station methodology for assessing trends in carnivore populations. Journal of Wildlife Management 46: 217–229.

SMITH, J. S., P. A. YAGER, AND G. M. BAER. 1973. A rapid tissue culture test for determining rabies neutralizing antibody. *In* Laboratory techniques in rabies, M. M. Kaplan and H. Koprowski (eds.). World Health Organization, Geneva, Switzerland, Monograph Series No. 23, pp. 354–357.

———, L. A. ORCIARI, AND P. A. YAGER. 1995. Molecular epidemiology of rabies in the United States. Seminars in Virology 6: 387–400.

STEELMAN, H. G., S. E. HENKE, AND G. M. MOORE. 1998. Gray fox response to baits and attractants for oral rabies vaccination. Journal of Wildlife Diseases 34: 764–770.

———, ———, AND ———. 2000. Bait delivery for oral rabies vaccine to gray foxes. Journal of Wildlife Diseases 36: 744–751.

WILL, G. B., R. D. STUMVOLL, R. F. GOTIE, AND E. S. SMITH. 1982. The ecological zones of northern New York. New York Fish and Game 29: 1–25.

WINKLER, W. G., AND G. M. BAER. 1976. Rabies immunization of red foxes (*Vulpes fulva*) with vaccine in sausage baits. American Journal of Epidemiology 103: 408–415.

———, AND K. BÖGEL. 1992. Control of rabies in wildlife. Scientific American 266: 85–92.

WLODKOWSKI, J. C., AND S. B. LINHART. 1998. Raccoon acceptance of a new coated-capsule bait. Wildlife Society Bulletin 26: 575–577.

Received for publication 3 March 2001.