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FORMULATION AND EVALUATION OF BAITS FOR ORAL RABIES VACCINATION OF RACCOONS (*PROCYON LOTOR*)

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ABSTRACT: Captive raccoons were offered a variety of vaccine containers and bait components in a series of three-choice tests. Paraffin wax ampules were the most readily accepted vaccine container. Preferred bait components included corn and shellfish oils, deep fried corn meal batter, and egg, apple and buttermilk flavorings. These results, together with factors including ease of bait formulation, cost, and suitability for field use, were used to develop an experimental delivery system for an oral rabies vaccine. The developed system was composed of a polyurethane sleeve (1.5 × 5.5 cm) dipped in a commercial food batter mix together with corn meal, milk and egg. The sleeve was deep fried in corn oil and a 2.0 ml ampule containing a recombinant rabies vaccine was then inserted into the sleeve bait. These baits were presented to 10 captive raccoons. Nine of the 10 animals developed high levels of rabies virus neutralizing antibodies. Field tests are needed to determine if the delivery system developed also is effective for wild raccoons.

Key words: Bait preference, captivity trials, oral delivery, *Procyon lotor*, rabies, raccoons, recombinant, taste, vaccine.

INTRODUCTION

Rabies is a persistent and widespread disease in wildlife. In 1988, the U.S. Centers for Disease Control recorded 4,724 cases of rabies. Of these, 88% (4,174) were in wild animals with 35% (1,463) reported in raccoons. The two primary foci of infected raccoons are in the mid-Atlantic (Delaware, Maryland, Pennsylvania, Virginia, West Virginia) and southeastern states (Alabama, Florida, Georgia, South Carolina) (Centers for Disease Control, 1989), with cases most recently being reported from New Jersey and New York (G. Baer, pers. obs.). Public health and wildlife officials are particularly concerned about the mid-Atlantic region because it has a dense human population and the rapid spread of rabies has resulted in high levels of human and pet exposure (Jenkins et al., 1988).

Oral immunization of wildlife species (or free-roaming dogs) against rabies may be a feasible alternative to population reduction programs or trap-vaccinate-release methods (Baer, 1988; Blancou et al., 1988; Perry, 1988). Five primary requirements must be met to apply such a concept: (1) an effective vaccine, (2) baits readily accepted by target species, (3) baiting methods that reach a high proportion of the susceptible population, (4) methods and materials that can be used safely, and (5) acceptable costs for development and use. Effective baiting systems and oral rabies vaccines for red fox (*Vulpes vulpes*) have been developed, primarily for use in Switzerland (Steck et al., 1982; Wandeler et al., 1988), West Germany (Schneider et al., 1988), and Canada (Johnston, 1975; Johnston et al., 1988). The attenuated vaccine used for foxes is either not effective or only partially so (Rupprecht et al., 1989) in other wildlife species; however, recently developed genetically-engineered rabies vaccines have immunized several different species in the laboratory by the oral route (Rupprecht et al., 1986; Esposito et al., 1988; Blancou et al., 1989). Since each species of carnivore has different food preferences and foraging strategies (Chapman and Fieldhamer, 1982), it is essential that the details of effective, selective field delivery of vaccines be examined for each target species. Particular concerns unique for developing oral vaccine baits are methods for incorporating liquid formulations in baits and for obtaining rapid bait acceptance by target species before vaccines can deteriorate.

Several studies have described the use of baits for the field delivery of chemicals or biologic materials to raccoons, including beef tallow for toxicants (Schnurrenberger et al., 1964, Lewis, 1975); tallow or eggs for antifertility agents (Nelson and Linder, 1972); eggs, sausages, dog biscuits and tallow-coated sponge cubes and fishmeal polymer or sachet fox baits used in Canada and Europe for oral rabies vaccines (Debbie, 1974; Baer, 1975; Winkler et al., 1975; Johnston et al., 1988; Hadidian et al., 1989; Perry et al., 1989, Hanlon et al., 1989). However, none have extensively and systematically evaluated raccoon bait preferences.

In the investigations described below, we sought to develop a delivery system for the recombinant vaccines that would be most effective for raccoons, the major wildlife vector of rabies in the eastern U.S. First, we evaluated several capsules and ampules in baits as containers for vaccine. Second, we sequentially tested a variety of bait materials and oil, cereal and meal additives for raccoon palatability. Finally, we added recombinant raccoon poxvirus rabies vaccine (Esposito et al., 1988, 1989) to baits and assessed the efficiency of oral vaccination in captive raccoons.

METHODS

Test animals and testing procedures

Raccoons were live-trapped in early December, 1987, near Athens, Clarke County, Georgia (33°55′N, 83°24′W) and transported to the CDC rabies research containment facility in Lawrenceville, Georgia (Centers for Disease Control, Lawrenceville, Georgia 30245, USA). Each of 10 animals (seven males, three females) was held and tested while contained within separate kennels (approximately 1.0 × 2.0 × 5.0 m) having cement floors and concrete block and chain link walls. Each kennel had access by sliding metal door to a heated shelter (approximately 0.9 × 1.2 × 1.4 m). Dog food (Purina Laboratory Diet for Dogs Number 5006, Purina Mills, Richmond, Indiana 47375, USA) and water were available ad libitum, except during testing, as described below. Seven tests were conducted during 1988 at 3.5 to 10.5 wk intervals. The first six evaluations assessed the effectiveness of vaccine containers and the palatability of different baits and bait materials. The seventh test involved oral vaccination with a live recombinant virus vaccine contained in a bait considered best accepted by raccoons based upon the previous six tests.

The same general protocol was used for all tests. Raccoons were fed one half the standard diet (approximately 130.0 g) on alternate days throughout the entire testing period. Each day at about 1700 hr, three different bait types were placed in the middle of each kennel. They were left until the following morning when each kennel was carefully searched; uneaten or partially consumed baits (or contents thereof) were removed. Daily hosing of the epoxy-coated kennel floors made it possible to recover even small pieces of uneaten baits and candidate vaccine containers. The same three bait types were provided to all 10 animals for two consecutive nights. Thus, a maximum of 20 baits of each type were available for consumption by the test group. This procedure was used for all tests excepting the first when vaccine containers were evaluated and the seventh when vaccine baits were assessed.

In Test 1, each of two bait types, with or without candidate vaccine containers, were randomly placed in individual disposable plastic dishes (13.5 × 13.5 × 6.0 cm deep) that had been equally spaced and stapled to a board (4.0 × 5.0 × 50.0 cm). Each two bait types were offered for two consecutive nights; each morning bait boards, dishes, and bait remains were removed between 900 and 1000 hr. The number of baits that were completely or partially eaten and the fate of capsules or ampules contained therein were recorded. Boards were washed with soapy water and dishes replaced daily to minimize odor contamination. Ten different vaccine containers and bait configurations were compared (some more than once) over a six day period.

Tests 2–6 (Table 1) were conducted to judge the relative attractiveness of different compounded oil additives (Test 2), bait matrices (principal material or bait substrate, Test 3), exterior bait coatings (Test 4), or combinations thereof (Tests 5–6). In each, baits were presented using a painted wooden box (10.0 × 15.0 × 50.0 cm) in each kennel, on top of which were stapled three disposable plastic cup lids

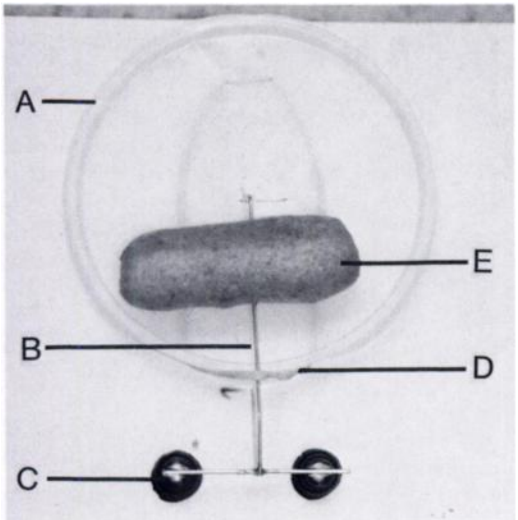

FIGURE 1. Bait platform used to determine captive raccoon bait preferences for development of oral rabies vaccination baits. Disposable plastic lid (A), bait wire (B), brass bolt head electrical contact (C), rubber band holding bait wire to bolt head (D) and test bait (E).

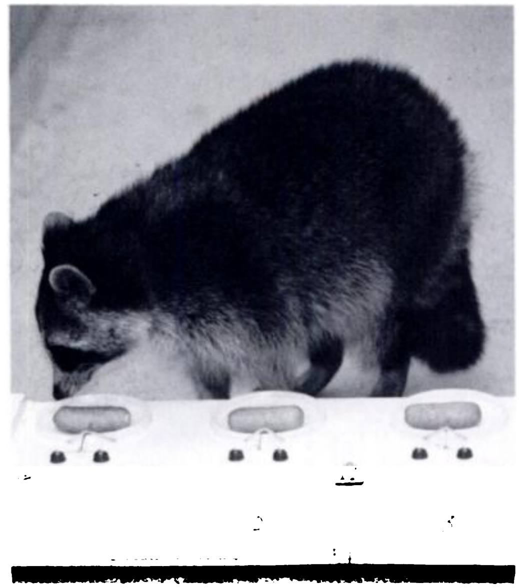

FIGURE 2. Bait platform with three bait types used to evaluate raccoon bait preferences for oral rabies vaccination.

(10.0 cm diameter), one for each of the three test baits (Fig. 1).

Each box contained three clocks to record the times and order of baits taken by test animals. The long axis (7.5 cm) of a "T"-shaped brass bait holding wire (1.1 mm diameter) was inserted into the bait and the short axis (5.0 cm) was laid across and supported by two protruding, V-slotted brass bolt heads (Figs 1, 2). These bolts served as electrical contacts; their threaded shafts extended down into the interior of the box and were wired to inexpensive battery-operated quartz alarm clocks, one for each bait type. The clocks were connected in series to a 1.5 v "D" cell, and an exterior "off-on" toggle switch that activated all three clocks. All clocks were set (hr, min and sec) at 12 and bait holding wires containing baits were placed on the plastic lids and bolt heads at the start of each test. Clocks were started by turning on the toggle switch at the time each bait box was placed in the kennel. Whenever a bait was taken by a raccoon, the associated bait wire was pulled off the bolt heads, the electrical circuit was broken, and the clock wired to the bolt heads stopped. Inspection of elapsed clock times the following morning indicated the order in which each of the three baits were taken by each test animal.

With the exception of Tests 1 and 7, both the number of baits consumed and the order in which each animal selected individual baits were recorded. The order of bait selection was not recorded in Test 1 because vaccine containers were being evaluated. Likewise, the order of bait selection was not recorded in Test 7, because only one bait type was presented.

In these screening tests, our criteria for sequential selection of specific bait components involved both raccoon response to test baits and practical considerations leading to ultimate use in the field. In advance, we decided that for further evaluation of a bait its frequency and order of consumption should be higher than its competitors. Additionally, we also considered the practicality of manufacturing such baits in quantity based on ease of preparation and cost of ingredients. The combination of quantitative and subjective selection criteria, plus our limited test population of the same 10 captive raccoons, implies that a statistical comparison of quantitative results would have little utility for choosing among bait components nor would the inferences provided be valid. For example, although bait A may have had a slightly lower consumption rate than bait B, bait B did not appear practical for mass production. Thus, bait A was selected for further sequential evaluation. The objectives and methods for each of the seven tests and the specific bait materials evaluated are listed in the following headings.

Vaccine containers

Oral rabies vaccine can lose potency for many reasons, but sealed, sterile containers are critical for preventing rapid deterioration. Thus, it was

TABLE 1. Raccoon bait materials tested, number of baits consumed, and order of bait selection for Tests 2 to 6, March to November 1988, CDC, Lawrenceville, Georgia (USA).

Test number	Test days	Bait matrix	Bait additive	Number of baits consumed (of 20 available)	Order of bait selection[a] 1st	2nd	3rd
2	1–2		1% within matrix				
		Tallow (90%) beeswax (10%)	Grape	8	4	2	2
		Tallow (90%) beeswax (10%)	Persimmon	8	2	4	1
		Tallow (90%) beeswax (10%)	Cherry	10	3	2	4
	3–4	Tallow (90%) beeswax (10%)	Clam	12	5	4	2
		Tallow (90%) beeswax (10%)	Shellfish	12	5	4	1
		Tallow (90%) beeswax (10%)	Shrimp	7	2	1	3
	5–6	Tallow (90%) beeswax (10%)	Sweet corn	13	7	3	3
		Tallow (90%) beeswax (10%)	Anise	11	1	4	6
		Tallow (90%) beeswax (10%)	Smoke	13	5	5	2
	7–8	Tallow (90%) beeswax (10%)	Grape	12	1	2	9
		Tallow (90%) beeswax (10%)	Shellfish	13	6	7	0
		Tallow (90%) beeswax (10%)	Sweet corn	13	7	3	3
3	1–2	Fruit bar	NONE	12	5	3	2
		99% Tallow (80%) beeswax (20%) 1% methyl anthranilate in 35% starch	NONE	8	2	3	1
		Milk chocolate (80%) beeswax (20%)	NONE	12	3	4	3
	3–4	Sardines (80%) beeswax (20%)	NONE	14	4	6	2
		Corn meal batter deep fried in corn oil	NONE	18	11	5	1
		Fishy cat food (50%) tallow/beeswax (50%)	NONE	10	2	2	5
	5–6	Peanut butter (80%) beeswax (20%)	NONE	13	0	2	3
		Fruit bar	NONE	15	3	3	1
		Corn meal batter deep fried in corn oil	NONE	17	9	1	1
4	1–2		Exterior meal/powder				
		Tallow (80%) beeswax (20%)	Cheese whey	4	1	2	0
		Tallow (80%) beeswax (20%)	Cheddar cheese	9	4	2	3
		Tallow (80%) beeswax (20%)	Buttermilk	10	6	4	0
	3–4	Tallow (80%) beeswax (20%)	Apple	8	2	3	1
		Tallow (80%) beeswax (20%)	Banana	8	3	2	1
		Tallow (80%) beeswax (20%)	Methyl anthranilate	4	1	0	2

TABLE 1. Continued.

Test number	Test days	Bait matrix	Bait additive	Number of baits consumed (of 20 available)	Order of bait selection[a] 1st	2nd	3rd
	5–6	Tallow (80%) beeswax (20%)	Corn	8	1	1	6
		Tallow (80%) beeswax (20%)	Fish	10	1	7	2
		Tallow (80%) beeswax (20%)	Egg	14	12	2	0
	7–8	Tallow (80%) beeswax (20%)	Buttermilk	11	4	4	3
		Tallow (80%) beeswax (20%)	Apple	10	5	2	3
		Tallow (80%) beeswax (20%)	Egg	11	5	3	3
5	1–2[b]	Tallow (80%) beeswax (20%)	1% sweet corn oil	2	—	—	—
		Tallow (80%) beeswax (20%)	1% sweet corn oil & apple powder	3	—	—	—
		Tallow (80%) beeswax (20%)	1% sweet corn oil & egg powder	3	—	—	—
	3–4[b]	Tallow (80%) beeswax (20%)	1% shellfish oil	1	—	—	—
		Tallow (80%) beeswax (20%)	1% shellfish oil & apple powder	2	—	—	—
		Tallow (80%) beeswax (20%)	1% shellfish oil & egg powder	2	—	—	—
	5–6[b]	Tallow (80%) beeswax (20%)	NONE	0	—	—	—
		Tallow (80%) beeswax (20%)	1% shellfish oil & egg powder	1	—	—	—
		Tallow (80%) beeswax (20%)	1% sweet corn oil & egg powder	1	—	—	—
	7–8	Tallow (80%) beeswax (20%)	1% sweet corn oil & egg powder	1	0	0	1
		Foam sleeve dipped in tallow (80%) beeswax (20%)	1% sweet corn oil & egg powder	7	2	5	0
		Foam sleeve dipped in tallow (80%) beeswax (20%)	Exterior coating of used soybean oil (66%) & sardines (33%)	10	8	2	0
	9–10	Foam sleeve dipped in tallow (80%) beeswax (20%)	Exterior coating of used soybean oil (66%) & sardines (33%)	9	2	2	4
		Corn meal batter deep fried in corn oil	NONE	18	13	3	1
		WISTAR polymer fishmeal bait	NONE	11	4	5	1
6	1–2[b]	Foam sleeve	Dipped in corn oil w/1% sweet corn oil	3	—	—	—
		Foam sleeve	Dipped in corn oil w/10% sweet corn oil	1	—	—	—
		Foam sleeve	Dipped in corn oil w/50% sweet corn oil	1	—	—	—
	3–4[b]	Foam sleeve	Dipped in corn oil w/1% sweet corn oil	3	—	—	—
		Foam sleeve	Dipped in corn oil	2	—	—	—
		Foam sleeve	Dipped in used corn oil	3	—	—	—

TABLE 1. Continued.

Test number	Test days	Bait matrix	Bait additive	Number of baits consumed (of 20 available)[a]	Order of bait selection[b] 1st	2nd	3rd
	5–6	Foam sleeve	Dipped in used corn oil	4	1	1	1
		Foam sleeve	Coated w/corn meal batter deep fried in corn oil	17	11	4	0
		Corn meal batter deep fried in corn oil	NONE	18	4	10	2
	7–8	Foam sleeve dipped in tallow (80%) beeswax (20%)	Exterior coating of used soybean oil (66%) & sardines (33%)	7	1	3	3
		Foam sleeve	Coated w/corn meal batter deep fried in corn oil	17	16	1	0
		WISTAR polymer fishmeal bait	NONE	12	3	6	3

[a] Totals differ from numbers of baits consumed in some instances because individual clocks failed to start due to faulty electrical contacts with bait wires.
[b] Order of bait selection not determined due to very poor bait consumption.

important to identify inexpensive containers that would both protect the vaccine and be compatible with bait ingredients. On the basis of these criteria, two water-filled container types were selected. The first were 2 or 4 ml capacity thin-walled soft polyethylene capsules (KAFKO International, LTD., Chicago, Illinois 60657, USA) and the other was a 2 ml thin-walled paraffin wax ampule (W & F MFG. Co., Inc., Buffalo, New York 14240, USA).

Both capsules and ampules were incorporated into two different bait types. The first type involved pouring a small amount of melted, rendered beef tallow (90%) obtained at a local rendering plant, and yellow beeswax (10%) obtained from a trappers supply house, into a cylindrical plastic vial with movable bottom. The capsule or ampule was placed into the vial and the remainder of the vial was filled with the melted matrix. Once the matrix was solidified, the vial bottom was pushed upward to extrude the bait. The second type bait involved insertion of a capsule or ampule into a cylindrical, polyurethane, open cell foam sleeve (1.5 × 5.5 cm with 0.6 cm wide cavity, Goody Products, Inc., New York, New York 10121, USA) used for manufacturing women's hair curlers. Sleeves were briefly dipped into the same melted matrix mentioned above and cooled at room temperature. All baits, including controls identical to the above but without capsules or ampules, were dipped in glycerol containing 1% shellfish oil, allowed to drain, and then surface-coated with fish meal. Raccoon consumption of tallow and beeswax baits and foam sleeve baits, both with and without capsules or ampules, was then compared as previously described.

Compounded oil additives

Various blended essential oils are available from the food flavoring industry and from companies that sell supplies to trappers who use them to make lures and baits for trapping furbearers. The objective of this test was to determine if the addition of various oils to tallow and beeswax baits would enhance raccoon bait consumption. We selected nine oils for evaluation; three represented common fruits eaten by raccoons (grape, persimmon, cherry), three were seafood by-products (clam, shellfish, shrimp oils) and three were unrelated substances (sweet corn oil, anise oil and a liquid "smoke" flavor used for flavoring barbecued meats). One percent by weight of each of the above oils was added to the melted tallow and beeswax matrix and baits were molded as described earlier. The three different baits in each of the above categories were first compared using the three choice bait test; the most preferred baits in each category were then compared against each other (Table 1).

Bait matrices

This test was conducted to determine raccoon response to seven different bait matrices. Tallow and/or beeswax was used in several of the test baits either as binders or to elevate the melting temperatures of the primary matrix material. The seven bait matrices tested were: (1) a mixture of Hershey's milk chocolate (80%) and beeswax (20%), (2) peanut butter (80%) and beeswax (20%), (3) sardines in olive oil (80%) and beeswax (20%), (4) canned fish cat food (50%) and a tallow and beeswax mixture (50%), (5) a 5.0 cm length of commercially sold fruit bar (Sunkist Strawberry N' Grape Two-T-Fruit), (6) a corn meal bait (approximately 2.0 × 5.0 cm) made of a corn meal and water batter deep fried in corn oil (Baer et al., 1989), and (7) a melted tallow (80%) and beeswax (20%) mixture to which had been added methyl anthranilate (MA), a food flavoring (grape) that is aversive to birds (Mason et al., 1989). One percent of a mixture containing 35% MA and 65.0% starch was added to the tallow-beeswax to make this test bait. The MA-treated bait was of particular interest to us because crows (*Corvus* sp.) are a major non-target scavenger of baits intended for raccoon and red fox. A grape-flavored bait readily accepted by raccoons but avoided by crows would be particularly desirable. Baits 1, 2 and 3 and baits 4, 5 and 6 were separately compared in three choice bait tests and those found most preferred in each category were then compared with bait No. 7 (Table 1).

Exterior bait coatings

Carnivore baits used in the past were often coated with various substances (e.g., sugar, or blood, fish or liver meals) to enhance consumption. We therefore conducted a test using beef tallow (80%) and yellow beeswax (20%) baits coated with various meals or powders to determine which, if any, were preferred over tallow baits without coatings. Nine different coatings were applied by shaking tallow and beeswax baits in a plastic bag containing a candidate material. Depending upon their physical properties, the amount of test material that adhered to baits varied from 2 to 6% of total bait weight. The nine dried or freeze-dried coatings tested were; group 1: cheese whey (3%), cheddar cheese (3%), buttermilk (3%); group 2: apple (2%), banana (6%), methyl anthranilate (3% of MA-starch mixture described in Test 3), and group 3: corn meal (4%), fish meal (2%) and powdered egg (6%). Each of the above groups were tested sequentially and the best accepted coatings in each group were then compared against each other (Table 1).

Combined bait ingredients

We reviewed the results of the tests described above and, on the basis of (1) raccoon bait consumption, (2) order of bait selection, (3) ease of bait preparation and (4) cost of ingredients, we formulated and compared 10 different bait types using the three choice bait test (Table 1, Test 5). Tallow (80%) and beeswax (20%) baits containing either 1.0% sweet corn oil or 1.0% shellfish oil were compared with identical baits coated with either dried egg or apple powder to determine which combinations were best accepted. Two of the above "best" combinations were then compared with a tallow and beeswax bait containing no additives. The "best" two baits resulting from the first six days of testing were then compared against a raccoon bait similar to that reported by Johnston and Lawson (1987) and successfully used for field tests conducted in Virginia by Perry et al. (1989). We simulated the Virginia bait by using the previously described foam sleeve dipped in melted beef tallow (80%) and beeswax (20%). Once solidified, the bait was again dipped in a liquid mixture of hydrogenated soybean oil (66%) that was used to deep fry seafood (obtained from a restaurant), and sardines canned in soybean oil (33%). The last two test days (days 9–10) involved a three-choice comparison of the most promising bait from test days 1–8 (the designated "Virginia" bait) with the previously described deep-fried corn meal bait, and a fishmeal polymer bait developed by The Wistar Institute (Philadelphia, Pennsylvania 19104, USA) (Hanlon et al., 1989). For this test, we used the Wistar bait designated DuPont No. 1232 which consisted of fish oil (5%), fish meal (70%), "sludge," consisting of various fish and shellfish byproducts (15%) and 10% of a plastic polymer binder (E. I. DuPont de Nemours & Co.; Smith and Daigle, 1988), that resulted in a bait impervious to water (Table 1).

Corn oil and corn meal formulated baits

Our prior tests indicated that of all baits evaluated, the deep fried corn meal bait was most preferred by raccoons. However, because this bait could not be easily molded to hold a vaccine container, we formulated and tested seven different modifications of corn meal or corn oil-based baits, all contained within or surface-coated on polyurethane sleeves. The first 2-day comparison sought to determine if raccoons would consume sleeves that had been simply dipped in 1, 10, or 50% concentrations of sweet corn oil using commercial corn cooking oil (Mazola) as a carrier. On days 3–4 we compared 1% sweet corn oil in a corn oil carrier, 100% unused corn oil and 100% corn oil that had been used to cook corn meal baits. All three of these baits were prepared by dipping sleeves into each type oil and allowing the excess oil to drain off. On days 5–6 we compared the best accepted corn oil bait with the original deep fried corn meal bait and a commercial corn muffin batter mix (Jiffy, Chelsea Milling Co., Chelsea, Missouri 48118, USA) to which had been added fresh egg, milk and methocel, the latter to enhance adhesion of the batter to the sleeve. The batter-coated sleeve was then deep fried for 20–30 seconds in corn oil heated to 190 C. The last test on days 7 and 8 compared the best accepted bait from days 5–6 (corn muffin batter bait) with the previously described Virginia and Wistar baits (Table 1).

Oral rabies vaccination of raccoons

We selected the 2.0 ml capacity paraffin wax ampule and the polyurethane sleeve coated with corn muffin batter mix as the vaccine container and bait most likely to result in the best acceptance by raccoons. The same 10 animals used for prior tests were used for this vaccination trial. A raccoon poxvirus rabies virus-glycoprotein recombinant preparation (RCN-KB3-JE13) developed as described by Esposito et al. (1988, 1989) was used as the test vaccine. It consisted of 2×10^8 PFU/2 ml of recombinant in phosphate buffered saline (PBS) containing 20% fetal bovine serum. Because ampules were from an experimental lot made to have thicker side walls than usual, capacity varied somewhat and thus volume of vaccine dispensed in each varied from 1.4 to 1.6 ml (dosage approximated 1.5×10^7 PFU). Melted paraffin wax (74 C) was used to seal the open end of the ampule. A 0.5 cm thick circular closed cell foam plug was placed on top of the vaccine in order to prevent the hot wax from coming into direct contact with the vaccine, possibly causing a loss of potency. After the sleeve bait had been deep fried, the exterior of each ampule was lubricated with corn oil and then inserted into the cavity within the sleeve.

We simultaneously presented each test animal with two of the above baits; one contained a water-filled ampule and the other a vaccine-containing ampule. This was done to determine if differential consumption might be indicative of vaccine rejection due to an aversive taste. The epoxy floors of each kennel were carefully examined the morning after baits were placed and all bait and ampule remains were removed and identified for later comparison with results of sera titrations for rabies antibody levels. All 10 test animals were sedated with ketamine hydrochloride and bled 5 days pre- and 10, 30 and 60 days post-vaccination and sera were tested for rabies virus neutralizing antibodies by the rapid fluorescent focus inhibition test (Smith et al., 1973).

RESULTS

Vaccine containers

Raccoons discriminated between candidate containers, both with respect to their size and the material from which they were fabricated. For example, raccoons ate 15 of 20 tallow beeswax baits without containers. They ate 14 of 20 identical baits containing 2.0 ml polyethylene capsules and in only one case was a capsule not ruptured and emptied of water. However, while 16 of 20 baits containing 4.0 ml capsules were eaten, four of these larger capsules were found intact with no evidence of being chewed. Thus, use of smaller capsules should enhance vaccine delivery.

Capsules ($n = 52$) were never consumed by raccoons, but were usually emptied by chewing and loss of water through tooth punctures in the plastic. However, several were only partially emptied and as none were swallowed during the six day test period, it was evident that raccoons were able to distinguish between bait material and this type of container. Conversely, except for small fragments of wax, all wax ampules ($n = 23$) were completely consumed along with bait material; raccoons apparently did not distinguish between the bait matrix and wax ampule.

Compounded oil additives

We compared nine different oils (1.0% by wt., Test 2), each blended into tallow-beeswax baits in an 8-day test (Table 1). None of the three fruit flavored oils was greatly preferred. Of the three seafood-based oils, both clam and shellfish-flavored baits were taken more frequently than baits containing shrimp oil. Sweet corn oil and the smoke-flavored barbecue product rated equal whereas anise oil, an attractant commonly used by trappers, ranked somewhat lower. When a preferred oil from each of the above three categories was selected and the three were compared (grape, shellfish and sweet corn), nearly equal numbers of each bait type were consumed but grape was selected first only once whereas shellfish and corn oils were selected first 6 and 7 times, respectively.

Bait matrices

This 6-day test compared seven different bait materials or matrices (Table 1, Test 3). The first 2-day test compared a 5.0 cm length of fruit bar with a milk chocolate/beeswax bait and a tallow/beeswax/methyl anthranilate (MA) bait. Of these, the MA baits were eaten less often than the other two bait materials. On days 3–4, sardines and beeswax, fish-flavored canned cat food and a deep-fried corn meal batter were tested. The corn meal bait was highly preferred whereas both sardine and cat food baits were less so and were also of soft consistency making them difficult to handle and impractical for field use. When the fruit bar and corn meal baits were compared with a peanut butter/beeswax bait on days 5–6, raccoons continued to show a clear preference for the corn meal bait.

Exterior bait coatings

An 8-day test of nine different bait coatings provided information on which would best enhance raccoon acceptance of tallow and beeswax baits (Table 1, Test 4). Slightly more buttermilk powder baits were eaten than those coated with either cheddar cheese or cheese whey (days 1–2). Baits coated with MA were eaten less frequently than either apple powder or dried banana coated baits (days 3–4). Corn meal coated baits were eaten less often than those having fish meal or egg powder; the latter bait was eaten first 12 of 14 times (days 5–6). When one preferred coating from each category was selected and the three compared, buttermilk, apple powder and egg powder were all eaten at the same frequency. We anticipated that the fish meal-coated bait would be highly attractive (days 5–6), but subsequent to the test learned that the fish meal used had little odor and was of unknown age and origin. A higher rating might have been achieved had we used fresh material.

Combined bait ingredients

We selected and combined various previously tested materials based upon the data collected in Tests 2–4 and the selective factors outlined earlier and prepared candidate baits for a 10 day test (Table 1, Test 5). We observed that all tallow and beeswax bait types tested during the first 6 days were very poorly accepted regardless of additives. In view of the good acceptance of the above bait components earlier in our study, we speculated that the poor acceptance was due either to inadvertently using old (perhaps rancid) tallow in our baits, or to a seasonal change in bait preference among our test animals (this test was conducted during mid-August, a high temperature month in Georgia). However, baits formed over polyurethane sleeves were much better accepted. For example, on test days 7–8, using the same lot of tallow/beeswax as on days 1–6, both the sleeve bait dipped in tallow and beeswax containing 1.0% sweet corn oil and coated with egg powder, and the simulated Virginia bait also using a polyurethane sleeve, were preferred over solid tallow and beeswax baits containing sweet corn oil and egg powder. When we compared the Virginia bait with the previously described Wistar fishmeal polymer bait and a deep-fried corn meal bait on days 9–10, corn meal baits were both eaten and selected first more frequently than the other two candidates.

Corn oil and corn meal-formulated baits

Since previous tests showed that raccoons highly preferred deep-fried corn meal-based baits, we conducted an 8-day test comparing different bait types formulated from sweet corn oil, cooking corn oil and corn muffin batter mix (Table 1, Test 6). We observed that raccoons did not readily consume polyurethane sleeves sim-

TABLE 2. Postvaccination rabies virus-neutralizing antibodies of raccoons fed a bait containing a poxvirus-vectored rabies virus glycoprotein recombinant.[a]

Animal number	10 days	30 days	60 days
1	—[b]	—[b]	—[b]
2	14.4	25.6	27.2
3	22.2	32.8	27.2
4	14.4	25.6	4.3
5	15.5	46.8	5.4
6	14.4	25.6	4.3
7	15.5	46.8	5.4
8	0.6	5.5	5.4
9	12.2	18.7	6.5
10	35.1	32.8	3.4

[a] All animals were negative prior to feeding. Each animal was fed one bait (foam sleeve with deep fried muffin batter mix coating) containing a wax ampule with 2 × 10^8 PFU/2 ml of recombinant (Esposito et al., 1988; 1989) in PBS containing 20% fetal bovine serum. Recombinant was rabies glycoprotein-vectored raccoon poxvirus. Results are expressed as International Units (I.U./ml) as determined by the rapid fluorescent focus inhibition test (Smith et al. 1973).

[b] <0.1 I.U./ml.

ply dipped in three different concentrations of sweet corn oil (1, 10, 50%) dissolved in corn oil (days 1–2). They also largely refused to eat sleeves saturated with unused corn oil, corn oil previously used to deep fry a corn meal batter and corn oil containing 1% sweet corn oil (days 3–4). Acceptance of polyurethane sleeves saturated with used corn oil was also poor in comparison with the previously described deep-fried corn meal batter bait and a bait made from a polyurethane sleeve dipped in corn muffin batter mix and deep-fried (days 5–6). On days 7 and 8 we compared the simulated Virginia bait, the Wistar fishmeal polymer bait and the corn muffin batter mix deep fried on polyurethane sleeves and again found, as in Test 5, that raccoons preferred the corn meal-based baits (consumption rates of seven of 20, 12 of 20 and 17 of 20, respectively). Raccoons readily consumed sleeve baits coated with the muffin mix formula and deep fried. However, we found it difficult to make large numbers of baits with this mix because it rapidly changed consistency and required the frequent addition of water to maintain the batter density needed to dip, coat, and deep fry baits. We later found that a corn dog batter mix (Pillsbury Product No. 6947, Pillsbury, Minneapolis, Minnesota 55402, USA) used by fast food restaurants maintained its consistency for a much longer interval and was equally well accepted by raccoons. We formulated this mix by using 43% Pillsbury batter, 8% yellow corn meal, 10% whole milk, and 39% whole eggs. We mixed the above ingredients in an electric mixer for 5 min and added a small amount of milk or corn dog mix until a satisfactory dipping consistency was achieved. The polyurethane sleeves were slipped onto 2.5 cm diameter wooden dowels and dipped into the above mixture. They were then drained for a few seconds, deep fried in corn oil (190 C) for 20 to 30 seconds, removed from the dowels and frozen (−12 C) until used.

Oral rabies vaccination of raccoons

Nine of 10 raccoons ate one or both paired baits (vaccine and placebo) on the first night of exposure. Food was withheld from the 10th animal and 48 hr later it ate the vaccine bait but rolled the placebo under the kennel door making it unavailable for consumption. Eight of 10 animals completely consumed the vaccine-containing baits, but two animals consumed only 75% of the polyurethane sleeve. All ampules were apparently consumed. Of the placebo baits, seven were eaten completely, two only partially and one, as mentioned above, was moved out of reach. Bait consumption was comparable to that of similar baits previously used in Tests 5–6. These results also indicated that the vaccine preparation had no adverse affect on bait consumption. No wax ampules were found intact in kennels where one or both baits were consumed. Ampule remains found in six kennels consisted of small fragments or only the wax plug used to seal them. Nine of 10 raccoons developed high rabies antibody titers while the 10th animal never seroconverted (Table 2). This individual was one of two that consumed only 75% of the polyurethane sleeve.

DISCUSSION

The procedure we used to evaluate baits worked well and enabled us to determine which test baits were preferred by raccoons. However, individual clocks within bait boxes occasionally failed to start when the electrical circuit was activated. These failures are reflected in Table 1, where the number of baits shown as consumed (Column 5) exceed the total numbers shown in the order of bait selection column (Column 6) (e.g., persimmon and cherry bait additives, Test 2, days 1–2). We found that sanding of the v-slot in the brass bolt heads prior to and midway through each test was necessary to remove corrosion that prevented good contact between bait wire and bolt heads.

Reading, recording and resetting clocks took considerable time. Our subsequent experience suggested that a device consisting of a water-tight plastic box with a clear lid, three magnets, three digital clocks and three reed switches wired in series to a 1.5 v battery, would be more efficient as well as suitable for field use. Baits attached by nylon line to magnets located on top of the lid and over reed switches, when removed by animals, would activate reed switches to complete circuits and start clocks. Elapsed clock times would then indicate the order in which baits were taken.

We were concerned that a bait position bias might influence results and that raccoons might select more baits from one position on the box than from others. We therefore tabulated all observations (n = 120) where raccoons had eaten all three bait types on a given night. These data showed that 43 and 44 baits, respectively, were taken first from either end position whereas only 33 were selected first from the middle bait position. Thus, while baits situated at either end tended to be selected first more frequently, the daily random positioning of baits on the platform minimized this bias.

We also questioned whether the order in which baits were selected or removed from bait platforms actually represented the order in which they were consumed. For example, a raccoon might remove a bait from the box, discard it, eat the second and third choice baits and later return to consume the one initially selected. Our data suggested that this was not a serious problem because first choice baits were also most often consumed. A comparison of the numbers of baits consumed and their order of selection shows this relationship (e.g., Table 1: Test 3, days 3–4 and 5–6; Test 4, days 5–6; Test 5, days 9–10). For some tests, there were few differences in either numbers of each bait type consumed or the order of selection (e.g., Test 2, days 1–2; test 4, days 7–8). However, some indiscriminate selection and consumption of baits was anticipated since we tested only those baits we believed would be palatable to raccoons. The order of bait selection sometimes appeared to be a more sensitive measure than did consumption rates in that the numerical differences between bait types for order of selection were greater than for the numbers of each bait type consumed (e.g., Test 3, days 5–6; Test 6, days 7–8). Thus, both consumption rates and order of selection should be considered when screening candidate baits or bait components.

Although field confirmation is needed, our data from captive raccoons show that a bait consisting of a paraffin wax ampule, an open cell polyurethane sleeve and deep-fried cornmeal, milk and egg-based surface coating can effectively deliver an oral vaccine to this species. Further, because of its capacity to absorb liquids (approximately 20 times sleeve weight), this bait type can be modified to contain biomarkers, food preservatives, water proofing substances and different flavored coatings, such as egg or fish meal-based materials to vary bait attractiveness and thus increase acceptance by target animals. This latter factor is important because raccoon bait preference may vary seasonally and geographically and alternate flavors might maximize the percent of local populations reached by baits. Sleeve baits also protect the vaccine ampule from direct exposure to the sun and from shock, prevent vaccine loss by absorbing it once the ampule is ruptured and bait components are inexpensive (approximately $0.02 to 0.03 (U.S.) when produced in large numbers). Many other factors, such as vaccine type and potency, bait densities and delivery systems, safety, selectivity and economics are important determinants of successful oral vaccination of wildlife species. However, an inexpensive and highly palatable bait capable of effective vaccine delivery is the first step toward developing such methodology.

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LITERATURE CITED

BAER, G. M. 1975. The oral rabies immunization of foxes and dogs with sausage baits. Developments in Biological Standards 33: 417--423.

———. 1988. Oral rabies vaccination: An overview. Reviews of infectious Diseases 10: S644–S648.

———, R. C. BROOKS, AND C. M. FOGGIN. 1989. Oral vaccination of dogs fed canine adenovirus in baits. American Journal of Veterinary Research 50: 836–837.

BLANCOU, J., P. P. PASTORET, B. BROCHIER, I. THOMAS, AND K. BÖGEL. 1988. Vaccinating wild animals against rabies. Revue Scientifique et Technique Office International des Epizooites 7: 1005–1013.

———, M. ARTOIS, B. BROCHIER, I. THOMAS, P. P. PASTORET, P. DESMETTRE, B. LANGUET, AND M. P. KIÉNY. 1989. Innocuité et efficacité d'un vaccin antirabique recombinant des virus de la vaccine et de la rage administré par voie orale au renard, au chien et au chat. Annales de Recherches Véterinaires 20: 195–204.

CENTERS FOR DISEASE CONTROL. 1989. Rabies Surveillance, United States, 1988. *In* CDC Surveillance Summaries, August 1989. MMWR 1989;38 (No. SS-1): 1–21.

CHAPMAN, J. A., AND G. A. FELDHAMER (editors). 1982. Wild mammals of North America. Biology, management, and economics. The Johns Hopkins Press, Baltimore, Maryland, 1147 pp.

DEBBIE, J. G. 1974. Use of inoculated eggs as a vehicle for the oral rabies vaccination of red fox (*Vulpes fulva*). Infection and Immunology 9: 681–683.

ESPOSITO, J. J., J. C. KNIGHT, J. H. SHADDOCK, F. J. NOVEMBRE, AND G. M. BAER. 1988. Successful oral rabies vaccination of raccoons with raccoon poxvirus recombinants expressing rabies virus glycoprotein. Virology 165: 313–316.

———, G. M. BAER, AND F. W. CHANDLER. 1989. Oral immunization of animals with raccoon poxvirus expressing rabies virus glycoprotein. *In* "Vaccines '89: Modern approaches to new vaccines including the prevention of AIDS," R. A. Lerner, H. Ginsberg, R. M. Chanock, and F. Brown (eds.). Cold Spring Harbor Laboratories, Cold Spring Harbor, New York, pp. 403–408.

HADIDIAN, J., S. R. JENKINS, D. H. JOHNSTON, P. J. SAVARIE, V. F. NETTLES, D. MANSKI, AND G. M. BAER. 1989. Acceptance of simulated oral rabies vaccine baits by urban raccoons. Journal of Wildlife Diseases 25: 1–9.

HANLON, C. L., D. E. HAYES, A. N. HAMIR, D. E. SNYDER, S. JENKINS, C. P. HABLE, 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.

JENKINS, S. R., B. D. PERRY, AND W. G. WINKLER. 1988. Ecology and epidemiology of raccoon rabies. Reviews of Infectious Diseases 10: S620–S625.

JOHNSTON, D. H. 1975. The principles of wild carnivore baiting with oral rabies vaccine. WHO consultation on oral vaccination of foxes, Frankfurt am Main, Item 5, Document 7. Geneva, Switzerland.

———, AND K. F. LAWSON. 1987. Oral immunization of mammals, U.S. patent No. 4,650,673, 1987, U.S. Government Printing Office, Washington D.C.

———, D. R. VOIGHT, C. D. MACINNES, P. BACHMANN, K. F. LAWSON, AND C. E. RUPPRECHT. 1988. An aerial baiting system for the distribution of attenuated or recombinant rabies vaccines for foxes, raccoons and skunks. Reviews of Infectious Diseases 10: S660–S664.

LEWIS, J. C. 1975. Control of rabies among terrestrial wildlife by population reduction *In* The natural history of rabies, G. M. Baer, (ed.), Academic Press, New York, New York, pp. 243–266.

MASON, J. R., M. A. ADAM, AND L. CLARK. 1989. Anthranilate repellency to starlings: Chemical correlates and sensory perception. The Journal of Wildlife Management 53: 55–64.

NELSON, R., AND R. L. LINDER. 1972. Percentage of raccoons and skunks reached by egg baits. The Journal of Wildlife Management 36: 1327–1329.

PERRY, B. D. 1988. The oral immunization of animals against rabies. *In* The veterinary annual, 29th ed., G. S. G. Grunsell, M. E. Raw, and F. W. G. Hill (eds.). Butterworth Scientific, London, England, pp. 37–47.

———, N. GARNER, S. R. JENKINS, K. MCCLOSKEY, AND D. H. JOHNSTON. 1989. A study of techniques for the distribution of oral rabies vaccine to wild raccoon populations. Journal of Wildlife Diseases 25: 206–217.

RUPPRECHT, C. E., T. J. WIKTOR, D. H. JOHNSON, A. N. HAMIR, B. DIETZSCHOLD, W. WUNNER, L. T. GLISKMAN, AND H. KOPROWSKI. 1986. Oral immunization and protection of raccoons (*Procyon lotor*) with a vaccinia-rabies glycoprotein recombinant virus vaccine. Proceedings of the National Academy of Science of the United States of America 83: 7949–7950.

———, B. DIETZSCHOLD, J. H. COX, AND L. G. SCHNEIDER. 1989. Oral vaccination of raccoons (*Procyon lotor*) with an attenuated (SAD-B_{19}) rabies virus vaccine. Journal of Wildlife Diseases 25: 548–554.

SCHNEIDER, L. G., J. H. COX, W. W. MILLER, AND K. P. HOHNSBEEN. 1988. Current oral rabies vaccination in Europe: An interim balance. Reviews of Infectious Diseases 10: S654–S659.

SCHNURRENBERGER, P. R., J. R. BECK, AND D. PEDEN. 1964. Skunk rabies in Ohio. Public Health Reports 79: 161–166.

SMITH, J. S., P. A. YAGER, AND G. M. BAER. 1973. A rapid reproducible test for determining rabies neutralizing antibody. Bulletin of World Health Organization 48: 535–541.

SMITH, M. S., AND C. J. DAIGLE. 1988. Longlife semi-artificial water borne feed. U.S. Patent No. 4741904. U.S. Government Printing Office, Washington D.C.

STECK, F., A. WANDLER, P. BICHSEL, S. CAPT, AND L. SCHNEIDER. 1982. Oral immunization of foxes against rabies. A field study. Zentralblatt fuer Veterinarmedizin [B]29: 372–396.

WANDELER, A. I., S. CAPT, A. KAPPELER, AND R. HAUSER. 1988. Oral immunization of wildlife against rabies: Concept and first field experiments. Reviews of Infectious Diseases 10: S649–S653.

WINKLER, W. G., R. G. MCLEAN, AND J. C. COWART. 1975. Vaccination of foxes against rabies using ingested baits. Journal of Wildlife Diseases 11: 382–388.

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