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A STUDY OF TECHNIQUES FOR THE DISTRIBUTION OF ORAL RABIES VACCINE TO WILD RACCOON POPULATIONS

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ABSTRACT: This study evaluates a technique for delivering an oral rabies vaccine to wild raccoon (*Procyon lotor*) populations. Various baits and attractants were first tested on caged raccoons and baiting trials were then conducted in two distinct physiographic regions of Virginia (USA), the coastal plain and the Piedmont plateau. Raccoon population density studies preceded the field trials. Each polyurethane sponge bait distributed contained approximately 200 mg tetracycline as a tissue biomarker, and was presented in an outer bag with a fish-based attractant. Baits were frozen until used and distributed from an aeroplane throughout two 4-km^2 sites in each region. One site received 450 baits/km^2 and the other 120 baits/km^2. Postbaiting evaluation included the direct observation of baits in the field and the examination of teeth and bone from trapped and hunted animals for evidence of the biomarker. Between 30% and 73% of the captured animals showed evidence of bait consumption. The proportion of animals with evidence of bait uptake changed when areas adjacent to the actual baiting site were included. The percentage of animals taking baits was not related to the density of baits that were distributed.

Key words: Oral rabies vaccine, raccoons, rabies, baiting, *Procyon lotor*, field study.

INTRODUCTION

The distribution of animal rabies in North America has undergone extensive changes during the past 30 yr. Prior to the 1950's, the majority of cases in the United States occurred in dogs (Tierkel, 1975). With the advent of effective canine rabies vaccines and urban dog control measures, canine rabies, and consequently human rabies, declined dramatically (Tierkel, 1975). Concomitantly, an increase in the number of rabid wild animals was reported. This increase was probably both a relative phenomenon due to improved surveillance procedures and a real increase (Winkler, 1986). Although the majority of human exposures to rabies are currently from domestic animals (primarily dogs and cats), it is believed that wild animals constitute the major reservoir for rabies from which the virus "spills over" into the domestic animal population (Helmick, 1983; Winkler, 1986).

The most common wildlife species reported with rabies in the United States are skunks (mainly *Mephitis mephitis* and *Spilogale putorius*), raccoons (*Procyon lotor*), bats (many species), red foxes (*Vulpes vulpes*) and gray foxes (*Urocyon cinereoargenteus*) (Centers for Disease Control, 1986). Two areas of the United States are presently experiencing raccoon rabies outbreaks, the southeast and the mid-Atlantic regions. An epizootic of raccoon rabies was first recognized in the mid 1950's in central coastal Florida (Bigler et al., 1973; McLean, 1975). This outbreak gradually spread north and now involves all of Florida and large areas of Georgia, South Carolina and eastern Alabama. The mid-Atlantic outbreak was first recognized on the Virginia/West Virginia border in the late 1970's (Jenkins and Winkler, 1987). It spread rapidly in all directions and is presently affecting Delaware, Maryland, Pennsylvania and the District of Columbia in addition to the two original states (Centers for Disease Control, 1986). Unlike most other wildlife rabies outbreaks, raccoon rabies occurs in dense human population centers as well as in rural areas.

Population reduction of the principal wildlife species frequently has been used in an attempt to control wildlife rabies (Lewis, 1975; Debbie, 1983; Baer, 1985), but in general this has neither been successful nor ecologically acceptable. The use of chemical reproductive inhibitors for fox rabies control has not met with much success either (Debbie, 1983). Given the disadvantages of population reduction as a rabies control tool, a number of researchers began exploring the possibility of orally immunizing wild animals against rabies (Baer et al., 1971; Debbie et al., 1972; Black and Lawson, 1973, 1980; Winkler and Baer, 1976; Steck et al., 1982; Blancou et al., 1986; Rupprecht et al., 1986), but inconsistent results and questions about safety led to a decline in such research in the United States (Baer et al., 1975; Winkler et al., 1976).

In Switzerland and West Germany successful fox rabies control campaigns have been reported using modified live rabies vaccine administered orally (Schneider et al., 1983; Wandeler, 1988). Field trials have been conducted in Ontario, also using modified live virus vaccine (Johnston et al., 1988). Researchers in the United States have reported seroconversion to rabies and resistance to challenge in raccoons administered a vaccinia-rabies glyoprotein recombinant virus vaccine by the oral route (Rupprecht et al., 1986). Safety and efficacy testing of this and other potential oral vaccines are presently under way in laboratories in the United States, Europe, Africa and China (World Health Organization, 1988). The apparent success in controlling fox rabies in Europe and the rapid advances being made on oral vaccine preparations for raccoons make it likely that an oral vaccine for raccoons will be available for field testing in the very near future.

In addition to a safe and effective oral vaccine, an inexpensive, efficient method of rapidly deploying oral vaccine to wild raccoon populations will be required. Several bait delivery systems have been developed and tested (Baer et al., 1971; Winkler and Baer, 1976; Johnston and Voigt, 1982; Steck et al., 1982; Schneider et al., 1983; Perry et al., 1988; Perry, 1989) but most have been directed at wild red fox and, latterly, to dog populations.

This paper describes a study of a bait delivery system suitable for the administration of oral vaccine to raccoon populations. The objective of this study was to determine the efficacy of widespread distribution of baits and attractants in two distinct raccoon habitats in Virginia.

MATERIALS AND METHODS

Bait and attractant acceptance studies in caged raccoons

Eighteen raccoons were trapped in southwest Virginia, and individually confined in stainless steel cages 70 × 95 × 80 cm. Animals were allowed to acclimatize to the caged environment for 1 wk, during which time they were fed a commercial dog food (Science Diet Canine Maintenance, Hills Pet Products, Inc., Topeka, Kansas 66601, USA). The raccoons were then divided into three groups of six animals each, with equal distribution of age, sex and place of origin in each group.

Each group was exposed to 11 different bait or attractant combinations. Eight exposures consisted of presenting three alternative foodstuff attractants simultaneously. The remaining three consisted of presenting simultaneously two alternative sponge baits combined with attractants. Test baits or attractants were placed in cages in the afternoon and removed with following morning. The commercial dog food diet was offered for 2 days between each test.

Attractants tested were hot dogs, marshmallows, glazed doughnuts, jello, molasses and apple butter, chosen for their widespread commercial availability in a standard form. All were prepared in a similar cuboidal shape of approximately 10 to 20 g for presentation. The bait tested was an open cell polyurethane sponge cube, coated with a mixture of beef tallow and histological wax (Lawson et al., 1987). Some tests utilized this bait with 100 mg of tetracycline HCl uniformly mixed with the wax before coating the sponge cube. Attractants and baits were presented either uncontained, in a polyethylene bag, or in aluminium foil.

Results were recorded as the number of raccoons that consumed or chewed on a bait, at-

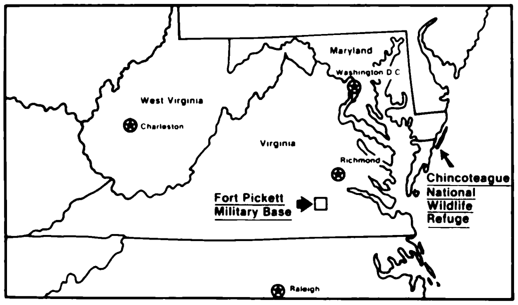

FIGURE 1. The locations of the two study areas, Fort Pickett Military Base and Chincoteague National Wildlife Refuge.

tractant, or combination. Differences between acceptance of alternative baits and/or attractants were assessed using the Student's t-test.

Field-study areas

Two areas were selected for field studies on bait acceptance by raccoon populations. Each area supported raccoon populations and contained habitats considered to be representative of other rural regions of the state in which raccoons are abundant. The areas selected were Chincoteague National Wildlife Refuge (CNWR) (37°05'N, 77°57'W) located on the coastal plain of Virginia, and Fort Pickett Military Base (FPMP) (37°58'N, 75°20'W) situated on Virginia's interior piedmont plateau (Fig. 1).

The study area at CNWR comprised 19.1 km^2 on Assateague Island, an Atlantic coast barrier island located adjacent to the mainland of Virginia. The habitat on the island consisted of a mixture of beaches, sand dunes, shrub thickets (principally containing *Myrtle* spp.), fresh and saltwater marshes, loblolly pine (*Pinus taeda*) forest and tidal mud flats.

The study area at FPMB consisted of 27.7 km^2 located in the northern section of the military base at 77–130 m above sea level. The habitat on the base consisted of an even mixture of mixed-age deciduous hardwood trees (mainly *Quercus* spp., *Carya* spp. and *Liriodendron tulipifera*) and pine forests (*Pinus* spp.) interspersed with marshes, open military operation areas, shrub fields and grasslands. Numerous streams and ponds were found in the area, which was traversed by a network of paved, gravel and dirt roads. There were no permanent human habitations in either of the study areas.

Raccoon population density studies

Steel wire boxtraps (Tomahawk Live Trap Co., Tomahawk, Wisconsin 54487, USA) of dimensions 82 × 26 × 31 cm were set nightly in both study areas during the months of September and October 1986. Captured raccoons were immobilized by an intramuscular injection of ketamine hydrochloride (Vetelar, Parke Davis, Morris Plains, New Jersey 07950, USA; approximately 20 mg/kg body weight). Each raccoon was ear-tagged in both ears (Monel type, National Band and Tag Company, Newport, Kentucky 41072, USA). Physical signalment data and blood (from the anterior vena cava) were collected from each animal. Serum was separated on the day of capture and stored at −20 C. Serum samples were subsequently tested for rabies antibodies at the Centers for Disease Control (Atlanta, Georgia 30333, USA) using the Rapid Fluorescent Focus Inhibition test (Smith et al., 1973).

Raccoon density in the two study areas was calculated by the Lincoln-Peterson estimate method (Seber, 1973) using a single recapture sample of ear-tagged animals provided by hunters and trappers between November 1986 and January 1987. This allowed the calculation of a maximum and minimum raccoon density for each study area, based on a 95% confidence interval of a Poisson distribution.

Bait preparation for field distribution

Each bait consisted of a 5 × 3.5 × 2-cm white polyurethane sponge heat-sealed into an envelope of 0.8-mil polyethylene film. Baits were injected with 10 ml of a liquid (placebo for a rabies vaccine) using a syringe fitted with an 18-ga needle. The placebo comprised 10% egg yolk and 10% molasses in tap water, and contained tetracycline HCl (200 mg/bait) as a biomarker. The mixture was well agitated in an electric blender prior to injection into the baits.

Each prepared bait was placed in an outer bag (20 × 15 cm). Clear polyethylene outer bags were used at FPMB and white wax-paper bags at CNWR. An attractant consisting of a mixture of two parts hydrogenated soybean oil (previously used for the frying of fish by a restaurant chain) and one part commercial canned sardines was mixed by hand; aliquots of approximately 10 g were dispensed into each bag, allowing at least one surface of the bait to be coated.

Pilot bait acceptance tests in study areas

Thirteen "smorgasbord" bait acceptance tests were conducted in each study area to examine the efficacy of the bait with different attractants. The attractants evaluated were canned fish (sardines and mackerel), meat (suet, chicken livers, beef and turkey gravy), blue cheese, fresh fruit (banana, grapes and cherries), and sweet substances (apple sauce, grape jelly, doughnuts, and a mixture of marshmallows and honey).

Baits contained in outer polyethylene bags with 10 ml of the test attractants were left overnight in areas identified as being regularly frequented by raccoons. Thirteen different attractant bait combinations were hand-placed 2 m apart in places which allowed for identification of tracks. Six replicates of the test were conducted at a site on FPMB and seven replicates at a site on CNWR. Observations were made on the morning following the placement of the baits and attractants.

Large-scale bait distribution study

Large-scale bait distribution trials were carried out at FPMB and CNWR in October and November 1986, respectively. In each of the study areas, two test sites of approximately 4 km^2 were selected as target zones for bait distribution. At FPMB, both zones were approximately rectangular in shape, bounded on at least one side by a road, and were separated from each other by approximately 2 km. At CNWR, the northern zone (Zone 1) was approximately square in shape, broken on its western boundary by a series of bays. Zone 2 was rectangular in shape, with an irregular western boundary on the Assateague channel. Zones 1 and 2 were separated from each other by approximately 4 km. Delivery of baits in each study area was planned at predetermined densities. Delivery of approximately 500 baits/km^2 was planned for Zone 1 of each area and 125 baits/km^2 for Zone 2 of each area.

Bait delivery was accomplished within 6 hr during a single day at each study area. Baits were dropped from a Cessna 172 aircraft flying predetermined transect lines over the baiting area at an altitude of 150 to 200 meters above ground level and at a recorded air speed of 115 to 160 km/hr. The pilot was accompanied by a navigator to assist in following transect lines, and a bait distributor who released baits into a delivery tube which protruded 1.3 m through the floor of the aircraft. Baits were released at a constant predetermined rate over the target zones in order to achieve the desired density. Baits were not released over large areas of water.

Determination of bait acceptance

Bait acceptance by the raccoon populations of the study areas was assessed by two methods. Specimens of teeth and bone from animals obtained by hunters and trappers were examined for the presence of the biomarker as described below. Baits were directly examined in the field for several days following their distribution.

Baits were distributed 3 wk before the start of the raccoon hunting season at FPMB, and the same interval before a planned harvest of the raccoon population at CNWR. At FPMB, the collaboration of local trappers and hunters was enlisted to provide the skull or maxilla of animals killed during the 1986/1987 hunting season (November 1986 to January 1987). Specimens were submitted to a central location on the base, stored at −20 C, and subsequently transported to Virginia Polytechnic Institute and State University (Blacksburg, Virginia 24061, USA) for examination. At CNWR, skulls or maxillae of raccoons, foxes and opossums trapped by a single individual were similarly stored and transported. At both study areas a map showing collection sites was compiled for each raccoon taken by hunters and trappers.

Canine teeth and the adjacent portion of surrounding bone were removed from each submitted animal, and were sagitally sectioned using a diamond saw (Buehler Isomet; Techmet Canada Ltd., Scarborough, Canada M1S 3P8) (Johnston and Watt, 1981). Sections of 60 to 150 μm were mounted in glycerine on glass microscope slides. Slides were then viewed under ultraviolet light (exciter filter of wavelength 390 nm and barrier filter of wavelength 420 nm) on a Nikon DIAPHOT-TMD microscope (Nikon USA, Garden City, New York 11530, USA) for the presence of fluorescing bands in the dentine and cementum layers of the teeth and in the adjacent bone (Johnston and Watt, 1981). Specimens were examined independently by two of the authors (BDP and NG) and recorded as being positive if any fluorescence was observed in dentine, cementum or bone. If the observers recorded different results, those sections were re-examined by both observers and the results reconciled.

Specimens also were aged by the examination of the pulp cavity size in the canine teeth by the method of Johnston et al. (1987), and placed into classes of juvenile (<1.5 yr) or adult (≥ 1.5 yr).

For 4 days following bait distribution at FPMB and 3 days at CNWR researchers walked through the zones to locate as many baits as possible. Located baits were examined and the surrounding habitat, nature of damage to the bait, and other features were recorded.

Data analysis

The Chi-square and the Wilcoxon signed-rank tests were used for all statistical comparisons conducted in the field studies. Significance was determined at $P \leq 0.05$.

RESULTS

Bait and attractant acceptance studies in caged raccoons

The consumption of attractants ranged from 61% to 100% ($\bar{x} = 83\%$). There were no statistically significant differences in the preferences for different attractants. Attractants placed in a plastic bag or wrapped in aluminium foil were eaten by more raccoons than uncontained attractants, but the difference between contained and uncontained attractants was not statistically significant.

In the tests of baits, uncontained sponge baits were consumed by 67% of the raccoons tested. The addition of attractants did not increase the consumption of baits; the attractant was usually licked off. Consumption rates were significantly lower (28%; $P = 0.03$) when tetracycline was incorporated into the sponge coating. When sponges containing tetracycline were presented in plastic bags, the consumption rate was increased to 56%, but this difference was not statistically significant.

Raccoon population density studies

During 238 trap-nights prior to bait distribution in the FPMB study area, 42 raccoons (20 male and 22 female) were trapped, ear-tagged, examined, blood-sampled and released at their original trapping location. During the hunting/trapping season following bait distribution, 58 raccoons were obtained from the FPMB study area. Nine of the 58 raccoons were re-captures of ear-tagged raccoons. Population size calculations by the Lincoln-Peterson estimate based on these capture-recapture data and a Poisson distribution gave a population size range of 121 to 529 raccoons (median = 325). This provides a population density estimate of 4.36 to 19.07 (median = 11.7) raccoons/km^2.

During 313 trap-nights prior to the bait distribution in the CNWR study area, 27 raccoons (13 male and 14 female) were trapped, ear-tagged, examined, blood-sampled and released at their original trapping location. Eight of the 53 raccoons were recaptures of the raccoons previously ear-tagged. Population size calculations gave a range of 76 to 367 raccoons (median = 222). This provides a population density estimate of 3.98 to 19.20 (median = 11.6) raccoons per km^2. All serum samples were negative for antibodies to rabies by the RFIT.

Small-scale bait acceptance tests in study areas

At FPMB, 64% of all baits with attractants were removed or not located, compared to 33% of those at CNWR. At FPMB, 11 of the 13 attractants were selected in three or more of the seven replicates. Sardines, black grapes, apple sauce and doughnuts were selected more often than other items. Examination of tracks and scats indicated that bait consumption was predominantly by raccoons. However, crows, ants, beetles and foxes were responsible for interfering with a small proportion of baits.

Large-scale bait distribution study

In Zone 1 of FPMB, 2,078 baits were distributed, providing a bait density of 428 baits/km^2. In Zone 2, 500 baits were distributed, giving a bait density of 133 baits/km^2. In Zone 1 of CNWR, 1,934 baits were distributed, giving a bait density of 493 baits/km^2. In Zone 2, 481 baits were distributed, giving a bait density of 107 baits/km^2.

Determination of bait acceptance

At FPMB 116 raccoons and an unknown number of red and gray foxes and opossums (*Didelphis marsupialis*) were obtained by hunting and/or trapping during the 1986/1987 season. Canine teeth and accompanying bone samples taken from 94 raccoons, one red fox and one gray fox were examined for the presence of tetracycline. Of these, 58 from raccoons and two from foxes were from the study area (Table 1).

In Zone 1 (high density baiting zone), 11 of 21 raccoon specimens (52%) were marked; in Zone 2 (low density baiting zone), five of eight specimens (63%) had been marked (Fig. 2). The proportion of positive specimens increased for both zones when specimens submitted from animals collected in a 0.5-km-wide strip surrounding the perimeter of the baiting zones were

TABLE 1. Summary of the results of examinations of canine tooth and accompanying bone from raccoons for the presence of tetracycline following aerial distribution of baits in two localities.

	FPMB[a]			CNWR[b]		
	Number examined	Number positive	%	Number examined	Number positive	%
Total specimens retrieved	94	34	36		29	35
Specimens from study area	58	32	55	83	27	51
Specimens from baiting Zone 1				53		
(high bait density)	21	11	52		7	64
				11		
Zone 1 + 0.25 km	23	13	56		—	—
Zone 1 + 0.5 km	30	18	60	—	9	70
Zone 1 + 1 km	—	—	—	13	12	75
Zone 1 + 1.5 km	—	—	—	16	15	65
Zone 1 + 2 km	—	—	—	23	18	60
				30		
Specimens from baiting Zone 2						
(low bait density)	8	5	63	12	7	58
Zone 2 + 0.25 km	9	6	67	—	—	—
Zone 2 + 0.5 km	11	8	73	16	7	44
Zone 2 + 1.0	—	—	—	18	7	39
Zone 2 + 1.5	—	—	—	21	7	33
Zone 2 + 2	—	—	—	23	7	30

[a] Fort Pickett Military Base, Virginia.
[b] Chincoteague National Wildlife Refuge, Virginia.

included in the calculations (Fig. 3). There was no statistically significant difference between the positivity rates of specimens from the two zones. Of the eight ear-tagged raccoons from which specimens were obtained, six (75%) were positive for tetracycline. The two fox specimens from Zone 1 were positive for tetracycline. Two of 35 control specimens from raccoons hunted or trapped at a distance of ≥16 km from either baiting zone were weakly positive for tetracycline.

At CNWR, 85 raccoons, 10 red foxes and two opossums were trapped during the 1986/1987 season. Canine teeth and accompanying bone samples retrieved from 83 raccoons and all of the foxes and opossums were examined for the presence of tetracycline. Of the submitted specimens, 53 raccoon and seven red fox specimens were taken in the study area.

In Zone 1 (high density baiting zone), seven of 11 raccoon specimens (64%) had evidence of tetracycline deposits and in Zone 2 (low density baiting zone) seven of 12 specimens (58%) had evidence of tetracycline (Fig. 4a, b, c). The proportion of positive specimens for Zone 1 increased when specimens retrieved from a 1-km-wide strip surrounding the perimeter of the baiting zone were included in the calculations (Fig. 3). The proportion of pos-

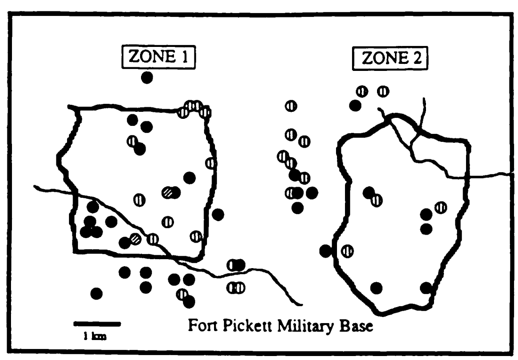

FIGURE 2. Location of raccoons and the tetracycline deposit status of specimens submitted from Fort Pickett Military Base. ●, tetracycline-positive raccoon; O, tetracycline-negative raccoon; O, tetracycline-positive fox; — baiting zone boundary; ⌒, river.

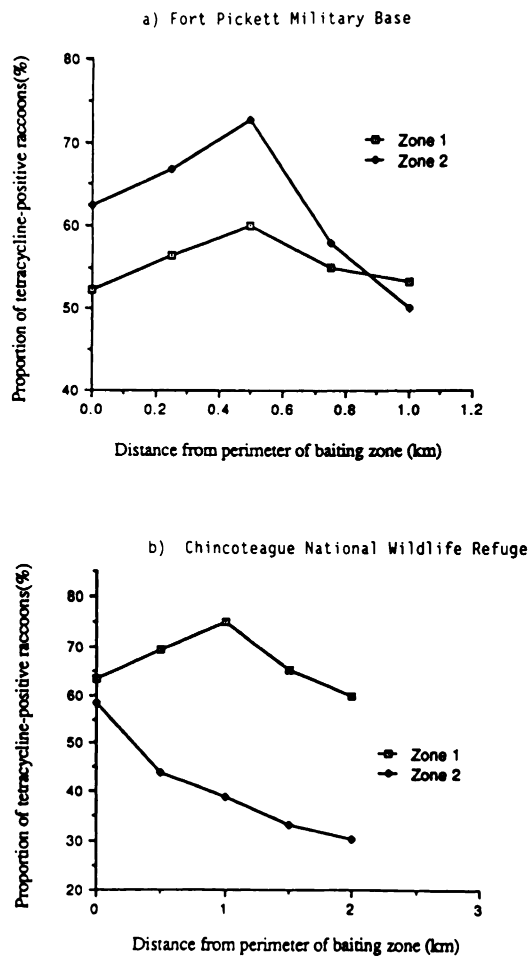

FIGURE 3. Proportion of tetracycline-positive raccoons within the baiting zones and including strips of designated width surrounding the baiting zone perimeters: (a) Fort Pickett Military Base, (b) Chincoteague National Wildlife Refuge.

itive specimens for Zone 2 decreased when any areas outside the perimeter of the zone were included. There was no significant difference between the positivity rates of specimens from the two zones when a 0.5-km-wide strip surrounding the perimeter of the zones was included. However, positivity rates were significantly higher in Zone 1 than in Zone 2 ($P = 0.03$) when a 1-km-wide strip surrounding the perimeter of each zone was included.

Four of the seven specimens from red foxes (57%) submitted from the baiting area

TABLE 2. Location of tetracycline deposits in canine teeth and bone of raccoons and foxes from Fort Pickett Military Base and Chincoteague National Wildlife Refuge.

Location of deposits	Raccoon Adult (n = 36)	Raccoon Juvenile (n = 27)	Fox Adult (n = 6)
Bone	30 (83)[a]	25 (93)	5 (83)
Cementum	22 (61)	24 (89)	1 (17)
Dentine	14 (39)	27 (100)	3 (50)
Bone only	9 (25)	0	2 (33)
Cementum only	1 (3)	0	0
Dentine only	3 (8)	2 (7)	1 (17)
Bone and cementum	12 (33)	0	1 (17)
Bone and dentine	2 (6)	1 (4)	2 (33)
Cementum and dentine	2 (6)	0	0
Bone, cementum and dentine	7 (19)	24 (89)	0

[a] Number positive (%).

were positive for the presence of tetracycline. Of 27 control specimens from raccoons trapped at a distance of over 2.5 km from either baiting area, two were positive for tetracycline presence.

A summary of the anatomic location of tetracycline deposits detected in submitted specimens from FPMB and CNWR is given in Table 2. Over 95% of the independent observations by the two observers were in agreement. The remainder were re-examined and the results were reconciled.

Direct examination of 135 baits at FPMB revealed that 21% of the observed baits in the high density zone and 57% in the low density zone had been chewed or eaten (Table 3). The proportions reported from the evaluation of 343 baits at CNWR were 33% in the high density zone and 21% in the low density zone (Table 4).

DISCUSSION

The initial cage trial studies demonstrated that a wide variety of food substances were potential candidates as attractants for the omnivorous raccoon. They also showed that presentation of baits in a plastic bag or an aluminium wrapper probably took advantage of the inquisitive nature and manual dexterity of the raccoon and en-

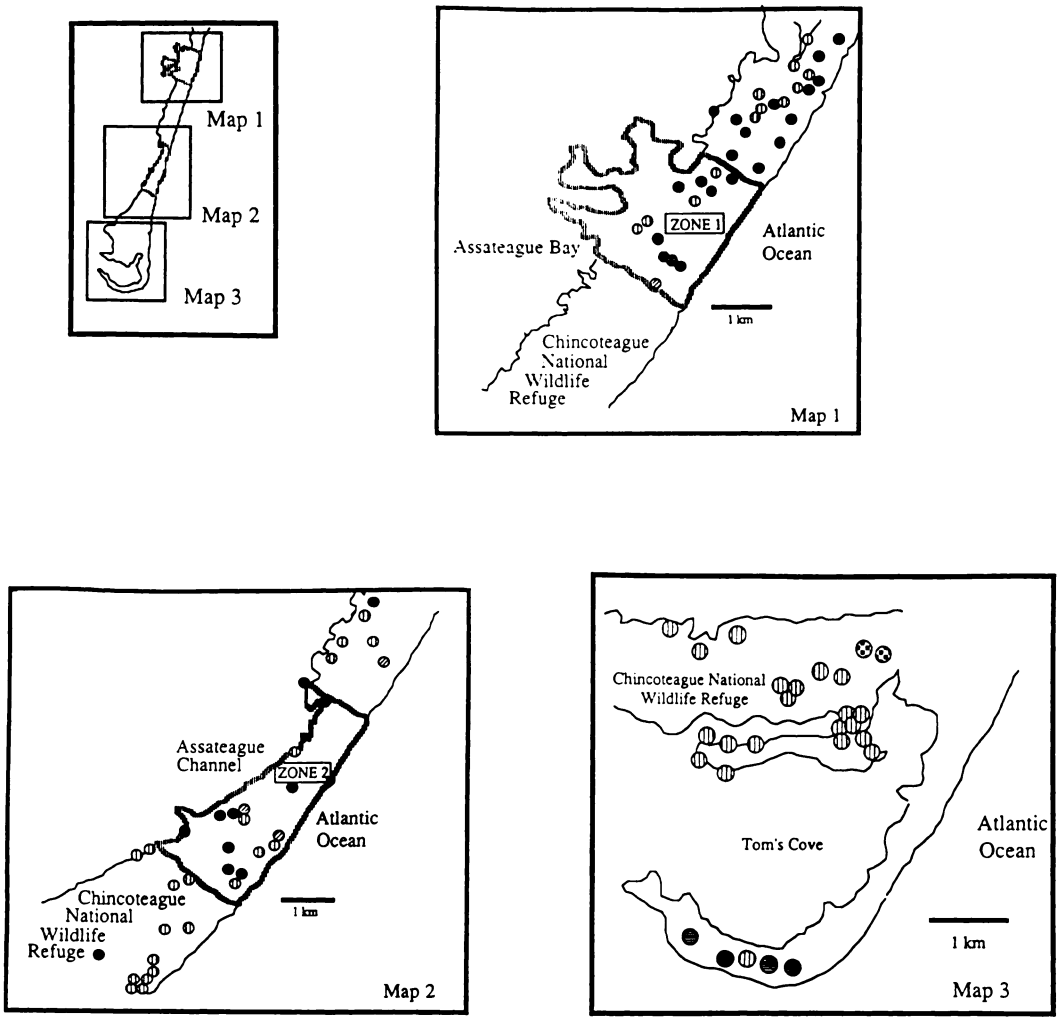

FIGURE 4. Location of raccoons and the tetracycline deposit status of specimens submitted from Chincoteague National Wildlife Refuge. Map 1, northern area; Map 2, central area; Map 3, southern area. ●, Tetracycline-positive raccoon; O, tetracycline-negative raccoon; O, tetracycline-positive fox; O, tetracycline-negative fox; ⊗ tetracycline-negative opossum; —, baiting zone boundary.

hanced the uptake of the less attractive baits. The polyurethane sponge which was eventually used in the field study was not available at the time of the cage trials.

In the "smorgasbord" studies of attractants, canned sardines in oil, which were not evaluated in the cage trial, were the most frequently selected attractant when results from FPMB and CNWR were combined. For this reason they were subsequently selected for incorporation in the attractant used at the time of large-scale bait distribution.

In the field studies, bait uptake rates in raccoons were measured using tetracycline as a biomarker, a technique now well established for such studies (Linhart and Kennelly, 1967; Steck et al., 1982; Schneider et al., 1983; Johnston et al., 1987). Although the bait uptake rates reported in this study were lower than that achieved with foxes in studies in Switzerland, Ger-

TABLE 3. Bait examination and retrieval following distribution at Fort Pickett Military Base in three habitat classifications in the two baiting zones.

Zones	Number of baits observed	Number of baits showing interference[a]	Calculated interference rate (%)
Total Zone 1 (high bait density)	107	22	21*
By habitat:			
Forest	40	11	28*
Roadside	33	7	21
Field	34	4	12
Total Zone 2 (low bait density)	28	16	57*
By habitat:			
Forest	14	13	93*
Roadside	9	2	22
Field	5	1	20

[a] Outer bag or sponge chewed or missing.
* Significant difference ($P = 0.001$) between interference rate in the two baiting zones.

TABLE 4. Bait examination and retrieval following distribution at Chincoteague National Wildlife Refuge in nine habitat classifications in the two baiting zones.

Zones	Number of baits observed	Number of baits showing interference*	Calculated interference rate (%)
Total Zone 1 (high bait density)	232	76	33*
By habitat:			
Salt marsh	32	12	38
Freshwater marsh	10	4	40
Forest	103	39	38
Shrubland	35	7	20
Dunes	13	0	0
Beach	0	0	0
Field/grassland	20	8	40
Wash flat	16	5	31
Roadside	3	1	33
Total Zone 2 (low bait density)	111	29	26*
By habitat:			
Salt marsh	14	3	21
Freshwater marsh	0	0	0
Forest	29	7	24
Shrubland	25	2	8
Dunes	14	5	36
Beach	2	0	0
Field/grassland	24	11	46
Wash flat	1	1	100
Roadside	2	0	0

* No significant difference between interference rate in the two baiting zones.

many and Canada (Schneider et al., 1983; Johnston et al., 1988; Wandeler, 1988), they are higher than the rates previously achieved for raccoons in Canada (Johnston et al., 1988). Raccoons were not, however, the principal target species in the latter study. For dog rabies control, it has been suggested that 70 to 80% of the dog population must be immunized to reduce sufficiently the contact rate between rabid and susceptible dogs in order to eradicate the disease (Tierkel, 1975). There is no target figure available for wildlife populations based on definitive studies; however, mathematical models have suggested that an immunization cover of 60% in the red fox population in Europe would be effective in eradicating the disease (Voigt et al., 1985). This figure will vary with the ecology and population density of the target species, and its relevance to raccoon populations has not been defined.

Baiting was conducted in October and November to conveniently precede hunting activities in the two areas. However, this may not be the most appropriate time to distribute vaccine to obtain maximum population coverage. As bait acceptance rates will probably vary at different times of the year, further studies during other seasons may be necessary.

The bait densities used in this trial were considerably higher than those used for foxes in other trials. Johnston et al. (1988) used bait densities of 18 to 48 baits/km^2 in Ontario, while Steck et al. (1982) used bait densities of 12 to 15 baits/km^2 in Switzerland. However, the fox population densities, estimated to be 0.8 to 6/km^2 in Switzerland, (Steck and Wandeler, 1982) were usually considerably lower (0.7 to 24 times) than raccoon densities in the present study area although interspecies comparisons may not be valid. The small sample size in studies of raccoon density at FPMB and CNWR resulted in a wide range in the calculated density, and this further limits valid comparisons with other studies. Nevertheless, in the present study there were was no significant difference between uptake levels of baits by raccoons in the high and low bait density zones.

Two tetracycline-positive raccoons were found in control specimens from each study area. At FPMB, these were found in close proximity to a swine farm on the perimeter of the military base, where chlortetracycline and oxytetracycline had been used as a feed additive for the pigs during the previous months. At CNWR, the tetracycline-positive controls were both juvenile male raccoons, probably dispersing from the baited areas.

The use of direct observation of baits was helpful in understanding the fate of individual baits, but the small sample sizes and bias due to the inaccessibility of some areas precluded using this data to draw any conclusions. More baits were observed in habitats that had easier human access and the proportion of baits that were disturbed was directly related to the length of time after the bait distribution that the observations were made. To eliminate bias, samples of a similar size would have to be examined in each habitat and bait density zone on the same days. Standardized evaluation of bait disturbance could help in planning strategies for baiting various habitats. In addition if correlations between proportion of baits disturbed and percentage of animals marked are established, direct examination of baits could be used as a predictor of bait uptake.

The results of this study clearly indicate that aerially distributed baits can effectively deliver liquid substances such as rabies vaccine contained within the bait to a substantial proportion of the target raccoon population. With relatively small baiting zones in both study areas (approximately 4 km^2), there was considerable movement of raccoons in and out of the baited area, which may have caused a dilution effect with respect to the proportional uptake of baits. Thus, the proportional uptake of baits increased in three of the four baiting zones when strips of up to 1 km outside the perimeter of baiting zones were included in the target zone analyses. The uptake rates achieved in this study may therefore be considered at the lower end of the range of uptake rates possible if baits were to be distributed on a larger scale.

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