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BAIT DELIVERY FOR ORAL RABIES VACCINE TO GRAY FOXES

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ABSTRACT: Rabies is a widespread zoonotic disease that has reached epizootic proportions in gray foxes (*Urocyon cinereoargenteus*) in central Texas. Because each species of carnivore has different food preferences and foraging strategies, it is essential that the efficacy of a bait delivery program be examined for gray foxes prior to an oral vaccination program being attempted. Field trials were conducted to determine bait preferences of free-ranging gray foxes to selected baits and odor attractants. Baits consisted of polymer cubes made of either dog food meal or fish meal, and a wax-lard cake that was enhanced with marshmallow flavoring. Attractants added to baits exuded sulfurous, fatty, cheesy, or sweet odors and flavors. During 3,589 operable bait station nights, gray fox visitation and bait uptake rates were 9.2% and 8.3%, respectively. Gray foxes exhibited no preference in bait uptake rates between bait and odor attractant combinations. Gray foxes exhibited no difference in cumulative bait uptake rates between onroad and offroad sites; however, the uptake rate by raccoons was significantly greater for baits placed on roads than for baits randomly placed. Raccoons were the major non-target species competing for baits, being attributed with 73% of the total uptake. Visitation and bait uptake rates by raccoons significantly increased after a 7-day lethal removal of raccoons (n = 37) from the study area. Random distribution of baits is recommended; it reduced bait uptake by non-target species without adversely affecting uptake by gray foxes.

Key words: Bait, gray fox, longevity, preference, rabies, *Urocyon cineroargenteus,* vaccination program.

INTRODUCTION

Rabies, a widespread zoonotic disease, became established in the gray fox (*Urocyon cineroargenteus*) population of central Texas in 1988 (Texas Department of Health, 1994). The Texas Department of Health reported 704 cases of rabies in gray foxes during January 1988–May 1995 (Robinson et al., 1995). Forty-five counties in central Texas reported rabies cases during this time span; 37 counties were active during the first half of 1995 with approximately 185 cases of rabies in gray foxes (Robinson et al., 1995).

Baiting strategies for oral immunization have been developed for red foxes (*Vulpes vulpes*; Johnston and Voight, 1982), raccoons (*Procyon lotor*; Linhart et al., 1991), mongooses (*Herpestes javanicus*; Linhart et al., 1993), striped skunks (*Mephitis mephitis*; Gunson et al., 1978; Rosatte et al., 1992), jackals (*Canis adustus*; Bingham et al., 1993), dingoes (*Canis dingo*; Thompson and Marsack, 1992), and coyotes (*Canis latrans*; Farry et al., 1998a). Delivery vehicles of oral chemicals to wildlife have included M-44 devices (Baer, 1988), chicken heads (Wandeler et al., 1975), blister pack baits (Bachmann et al., 1990), deep fried corn meal batter (Linhart et al., 1991), meatballs (Johnston and Voight, 1982), tallow-coated sponge cubes (Bachmann et al., 1990), polyethylene capsules with fish-flavored overcoatings (Wlodkowski and Linhart, 1998), dog food-based polymer baits (Farry et al., 1998b), and fish meal-based polymer baits (Hanlon et al., 1989). No single bait has been effective as a delivery vehicle because each species of carnivore has different food preferences, foraging behaviors, and foraging strategies (Chapman and Feldhamer, 1982). Differences in masticatory behavior of carnivores could cause a bait to be consumed without the oral vaccine being exposed to and absorbed in the buccal mucosa (Rupprecht et al., 1988, 1989). For example, a rabies vaccine container and bait that effectively immunized red foxes reportedly

performed poorly for jackals (Linhart et al., 1997). Therefore, it is imperative that a bait and baiting strategy be developed for each target species.

Steelman et al. (1998) found that gray foxes from central Texas exhibited preferences for marshmallow wax cakes and dog food-based polymer baits with a lard interior and granulated sugar exterior; however, these preferences were determined using captive animals. All baits must be field tested to verify that captivity did not alter typical behavior of gray foxes. Also, to our knowledge, a baiting strategy has not been developed for gray foxes. Therefore, the objectives of this study were to determine bait and odor attractant preferences among free-ranging gray foxes, to determine bait uptake rate by gray foxes and non-target competitor species, and to determine the efficacy of bait placement and short duration removal of raccoons on bait uptake rates by gray foxes.

MATERIALS AND METHODS

Study areas

Field trials were conducted on the Texas A&M University Agricultural Experiment Station located 45 km southeast of Sonora, in Edwards and Sutton counties, Texas, and on the adjacent Hill Ranch (30°20′N, 100°30′W). The terrain is rolling hills with rock outcroppings. Soils are classified as stony clays and clay loams (Bryant et al., 1981). The vegetation structure was predominately an oak-grassland community with intermixed stands of juniper (*Juniperus* spp.). Woody plant species included Vasey shin oak (*Quercus pungens*), plateau oak (*Q. virginiana*), common curly mesquite (*Hilaria belangeri*), and juniper spp. (Bryant et al., 1981). Predominant herbaceous vegetation included sideoats grama (*Bouteloua curtipendula*) and Texas wintergrass (*Stipa leucotricha*) (Bryant et al., 1981). Chief industrial use of the study area was for cattle, sheep, and goat production. Wild mammalian species of the area included gray fox, red fox, coyote, ringtail (*Bassariscus astutus*), bobcat (*Lynx rufus*), white-tailed deer (*Odocoileus virginianus*), raccoon, eastern cottontail rabbit (*Sylvilagus floridanus*), javelina (*Tayassu taiacu*), black-tailed jackrabbit (*Lepus californicus*), porcupine (*Erethizon dorsatum*), eastern fox squirrel (*Sciurus niger*), striped skunk, hognose skunk (*Conepatus mesoleucus*), and feral domestic cat.

Baits and odor attractants

Twelve bait and odor attractant combinations were chosen for field trial evaluation. Bait and odor attractant combinations were selected based upon positive responses of captive gray foxes to a variety of baits and odor attractants (Steelman et al., 1998). Baits included wax-lard cakes with marshmallow icing added as flavoring (Ontario Ministries Natural Resource, Ontario, Canada), and dog food-based and fish meal-based polymer cubes (Bait Tech, Orange, Texas, USA). Polymer-bound baits were either $5.0 \times 2.5 \times 3.3$ cm rectangular cubes or $2.5 \times 2.5 \times 3.3$ cm square cubes with hollow centers. A heated wax-lard-odor attractant mixture was added to the hollow center of the polymer-bound baits in a 6:3:1 ratio. Odor attractants included beef lard, valeric acid, butyric acid, and granulated sugar, which emitted fatty, sulfurous, cheesy, and sweet odors, respectively. Beef lard and granulated sugar were obtained from H.E.B. grocery stores (San Antonio, Texas, USA) and valeric and butyric acids were obtained from Aldrich Chemical Company (Milwaukee, Wisconsin, USA). Each bait and odor attractant combination was made, except the fish meal-cheese combination, and completed baits were stored according to Steelman et al. (1998).

Field trials

Field trials were conducted twice during 1995. Preferences of the bait and odor attractant combinations were conducted during 16 March–4 May. During this time, we evaluated bait acceptance by target and non-target species before (18-day period), during (7-day period), and immediately after (12-day period) a short duration raccoon removal program. Then, we determined the longevity of baits during 18 to 30 May 1995. The trapping periods and the two field trials were separated by 6 day and 14 day periods, respectively, to reduce the probability of target and nontarget species becoming habituated to bait stations.

Bait visitation and uptake by free-ranging gray foxes and non-target species were assessed using bait station transects as described by Linhart and Knowlton (1975). Eight transect roads, >3.0 km apart, were established along paved and unimproved, limited-use ranch roads. Each transect line consisted of 13 bait stations spaced at 0.8-km intervals on alternate sides of the road. One of the bait and odor attractant combinations or a control plot (no bait) was randomly assigned daily to each of the

13 stations and placed in the center of the bait station. Each 1-m diameter bait station was cleared of vegetation, and contained 1-cm deep sifted caliche soil to aid in track identification. Bait stations were checked for animal visitations once every 24 hr and animals were identified by tracks. Camera systems were constructed according to Jones and Raphael (1993) and placed at selected stations to verify species identification at bait stations.

Data collected included bait presence or absence, animal sign, and animal activity on bait stations. Soil on each bait station was resifted daily. Indices as to the relative abundance of visitation and bait uptake rates for each species were calculated from equations developed by Roughton and Bowden (1979). Visitation and uptake rates were defined as the ratio of visited stations to the number of operable station nights and the ratio of stations with baits missing to the number of operable station nights, respectively. If multiple species visited a station, bait disappearance was attributed to the species most likely to have taken the bait (Guthery et al., 1984), as determined by track positioning and overlaying of multiple tracks. However, multiple visitations were infrequent and when they did occur, often involved species (i.e., rabbits and birds) that were unlikely to eat or carry off the baits. Although rabbits could conceivably eat baits, rabbit pellets were not observed on or near bait stations. Rabbits typically defecate during or immediately after eating (Rezendes, 1992, pp. 107–108).

During the trapping period of the bait preference study, raccoons were trapped with Tomahawk single-door wire-cage traps (Tomahawk Live Trap Co., Tomahawk, Wisconsin, USA) measuring 114 × 38 × 51 cm. Twenty-five traps baited with canned sardines were set each night during a 1-week period. Captured raccoons were killed with a .22 caliber rifle and the carcasses were used in a companion study.

Longevity of baits placed onroad and baits randomly placed offroad was assessed for cumulative visitation and bait uptake rates by target and non-target species. Two additional transect roads with eight onroad and eight offroad bait stations each were added to previously used transects for a total of 240 on- and offroad bait stations. Offroad bait stations were constructed as previously described and located perpendicular to the road. A random number table was used to assign distances (from 3–99 m) and direction for offroad bait stations (Steel and Torrie, 1980). If the number was even, the perpendicular distance was measured to the right of the transect road; if odd, it was measured to the left of the transect road. All stations were baited with wax-lard cakes with marshmallow icing added as flavoring and were checked daily for seven days. Identification of animal sign, presence of baits, and visitation and bait uptake rates were recorded and calculated as previously described. Baits were not replaced once they had been removed by an animal.

A completely randomized design was used for bait preference on free-ranging gray foxes. Distributions of residual errors were tested for normality using Shapiro- Wilk test (Neter et al., 1990). Non-normal datasets were log-transformed ($\log_{10}$) and retested to ensure that criteria for parametric statistical tests were met. Homogeneity of variances was verified with the Bartlett's test (Steel and Torrie, 1980). General linear analysis of variance was used to test the effect of the bait and odor attractant on the visitation and bait uptake rates of target and non-target species (PROC GLM; SAS Institute Inc., 1989). Multiple comparisons were made using the Duncan's Multiple Range procedure when a significant *F*-test occurred (Ott, 1993). Comparisons of cumulative bait longevity between bait placements were conducted using *Z*-tests (Ott, 1993). All tests were considered significant at $P < 0.05$. Descriptive statistics are reported herein as the mean ± 1 standard error ($\bar{x} \pm$ SE).

RESULTS

During 3,589 operable bait station-nights, there were 4,077 animal visits and 1,750 baits missing and presumed eaten (i.e., bait uptake). Species identified by tracks as visitors of bait stations included gray foxes, raccoons, domestic livestock (i.e., sheep, goats, cattle, horses), ringtails, red foxes, javelinas, bobcats, white-tailed deer, eastern cottontail rabbits, black-tailed jackrabbits, coyotes, skunks, and miscellaneous rodents. Raccoons were the most frequent visitors to bait stations, accounting for 40.9% of the visits (n = 1,667 of the 4,077 animal visits), followed by domestic livestock (n = 730; 17.9% of animal visits), gray foxes (n = 373; 9.1% of animal visits), ringtails (n = 134; 3.3% of animal visits), and miscellaneous species (n = 1,173; 28.8% of animal visits). Bait uptake was most frequently attributed to raccoons (n = 1,273), followed by domestic livestock (n = 206), gray foxes (n = 146), ringtails (n = 34), and miscellaneous species (n = 91).

TABLE 1. Proportion of visitation and uptake rates of baits being tested for oral rabies vaccine by selected mammals calculated from 3,589 operable bait-station nights before, during, and immediately after lethal removal of raccoons in central Texas, March–May, 1995.

	Pre-trap[a]		Trap[b]		Post-trap[c]	
Species	Visitation	Uptake	Visitation	Uptake	Visitation	Uptake
All mammals[d]	1,777	650	735	346	1,565	754
Gray fox	8.3	9.2	9.7	6.6	9.9	8.4
Raccoon	27.5	61.7	37.3	74.6	57.8	81.4
Ringtail	4.2	3.2	2.0	1.4	2.9	1.0
Livestock	15.5	19.1	14.3	13.3	22.3	4.8
Other[e]	44.5	6.8	36.7	4.0	7.1	4.4

[a] Rates are based on 1,744 operable bait-station nights before initialization of raccoon removal.
[b] Rates are based on 648 operable bait-station nights during raccoon removal efforts. (n = 37 raccoons lethally removed from study area during the 7-day trapping period).
[c] Rates are based on 1,197 bait-station nights immediately after raccoon removal efforts.
[d] Total number of mammalian animal visits and baits missing from bait stations during each raccoon removal period.
[e] Other species included red fox, javelina, bobcat, white-tailed deer, eastern cottontail rabbit, black-tailed jackrabbit, coyote, skunk, and rodents.

Visitation rates by gray foxes to bait stations did not differ (P = 0.36) between the 12 bait and odor attractant combinations. Mean visitation rates by gray foxes ranged from 0.072 ± 0.02 for square, dog food-based polymer baits with a fatty interior odor to 0.129 ± 0.02 for rectangular, dog food-based polymer baits with a sulfurous interior odor. The mean uptake rate by gray foxes was greater (P = 0.005) for square, dog food-based polymer baits with a sulfurous interior odor (0.066 ± 0.01) than for rectangular, fish meal-based polymer baits with a sweet-flavored exterior (0.023 ± 0.01). No other differences were noted in the mean uptake rate of baits by gray foxes. Probability of bait uptake if a gray fox encountered the bait ranged from 27% for rectangular, fish meal-based polymer baits with a sweet-flavored exterior to 58% for marshmallow-flavored wax cakes.

Raccoons were the dominant competitor for baits on our study area. However, they did not exhibit a difference in mean visitation (P = 0.80) and uptake rates (P = 0.85) between the 12 bait and odor attractant combinations. Mean visitation and uptake rates by raccoons ranged from 0.412 ± 0.04 for square, dog food-based polymer baits with a sweet-flavored exterior to 0.517 ± 0.04 for rectangular, dog food-based polymer baits with a cheesy interior odor and 0.352 ± 0.03 for rectangular, dog food-based polymer baits with a fatty interior odor to 0.439 ± 0.04 for rectangular, dog food-based polymer baits with a cheesy interior odor, respectively. Probability of bait uptake if a raccoon encountered the bait ranged from 79% for rectangular, dog food-based polymer baits with a sweet-flavored exterior to 87% for marshmallow-flavored wax cakes.

Short-term raccoon removal (n = 37) did not increase the proportion of visitation and uptake rates of baits by gray foxes (Table 1). Mean visitation rates by gray foxes were similar (P = 0.96) between raccoon pre-trapping (0.077 ± 0.02) and post-trapping (0.126 ± 0.02) periods. Mean bait uptake rates by gray foxes also were similar (P = 0.34) between raccoon pre-trapping (0.032 ± 0.01) and post-trapping (0.051 ± 0.01) periods. However, mean visitation and bait uptake rates by raccoons increased ($P < 0.001$) after the lethal removal of 37 raccoons. Mean visitation and uptake rates of baits by raccoons were 0.327 ± 0.03 and 0.252 ± 0.02, respectively, during the pre-trapping period and 0.625 ± 0.04 and 0.552 ± 0.04, respectively, during the post-trapping period.

Bait longevity surveys resulted in cumulative onroad bait visitation rates by all species that ranged from 25% after 1 day

TABLE 2. Cumulative bait visitation and uptake relative abundances of baits being tested for oral rabies vaccine for all mammals, gray foxes, and raccoons during 7-day bait longevity trials in central Texas, May, 1995.

Species	Day	Visitation			Bait uptake		
		Onroad	Offroad	*P* value[a]	Onroad	Offroad	*P* value[a]
All mammals							
	1	0.250	0.133	0.011	0.200	0.075	0.002
	2	0.433	0.350	0.093	0.375	0.225	0.006
	3	0.692	0.442	<0.001	0.567	0.333	<0.001
	4	0.808	0.533	<0.001	0.708	0.445	<0.001
	5	0.858	0.617	<0.001	0.767	0.542	<0.001
	6	0.867	0.625	<0.001	0.775	0.550	<0.001
	7	0.892	0.658	<0.001	0.825	0.650	0.001
Gray fox							
	1	0.033	0.000	—[b]	0.017	0.000	—[b]
	2	0.042	0.092	0.061	0.017	0.058	—[b]
	3	0.117	0.150	0.227	0.050	0.100	0.071
	4	0.125	0.158	0.233	0.050	0.100	0.071
	5	0.158	0.167	0.425	0.075	0.117	0.136
	6	0.167	0.167	0.500	0.075	0.117	0.136
	7	0.167	0.167	0.500	0.075	0.117	0.136
Raccoon							
	1	0.150	0.117	0.227	0.108	0.058	0.081
	2	0.292	0.258	0.278	0.242	0.108	0.003
	3	0.467	0.292	0.003	0.383	0.183	<0.001
	4	0.592	0.475	0.035	0.500	0.258	<0.001
	5	0.633	0.408	<0.001	0.500	0.325	<0.001
	6	0.650	0.408	<0.001	0.525	0.325	<0.001
	7	0.650	0.408	<0.001	0.525	0.333	<0.001

[a] Calculated using Z-test.
[b] Normal approximation to binomial was violated.

to 89% after 7 days and uptake rates from 20% after 1 day to 82% after 7 days (Table 2). Cumulative offroad bait visitation and uptake rates by all species ranged from 13% after 1 day to 66% after 7 days and 7% after 1 day to 65% after 7 days, respectively (Table 2). Cumulative bait visitation and uptake rates by all mammals was greater ($P < 0.011$) for onroad bait placement than for offroad bait placement during days 1 to 7, except for the cumulative visitation rate during day 2 in which no differences between the two bait placements were noted ($P = 0.093$).

Gray fox cumulative visitation and bait uptake rates did not differ ($P > 0.061$) between bait placements during the 7 day period (Table 2). Cumulative visitation and bait uptake rates by gray foxes ranged from 0% after 1 day to 17% after 7 days and 0% after 1 day to 12% after 7 days, respectively. The likelihood of bait uptake by a gray fox if it encountered a bait was 45% for onroad and 70% for offroad stations.

Onroad bait stations exhibited higher cumulative visitation rates ($P < 0.035$) and cumulative bait uptake rates ($P < 0.003$) by raccoons than those at offroad stations after 3 and 2 days, respectively (Table 2). Cumulative onroad visitation rates by raccoons ranged from 15% after 1 day to 65% after 7 days, whereas cumulative offroad visitation rates ranged from 12% after 1 day to 41% after 7 days. Cumulative raccoon bait uptake rates for onroad and offroad stations ranged from 11% after 1 day to 52% after 7 days and 6% after 1 day to 33% after 7 days, respectively. The likeli-

hood of bait uptake by a raccoon if it encountered a bait was 81% for onroad and 82% for offroad stations.

DISCUSSION

Free-ranging gray foxes did not exhibit a preference for any bait and odor attractant combination. However, marshmallow wax cakes had the highest percent uptake rate by gray foxes when they encountered the bait. It is reasonable to select a bait with the best bait uptake rate because bait uptake by ≥70% of a gray fox population speculatively is required to effectively lower the susceptible population below the density threshold needed for continuance of disease spread (Texas Department of Health, 1994). The bait uptake of ≥70% was set by the Texas Department of Health (Austin, Texas, USA) based on assumptions of the intrinsic reproductive rate of rabies in gray foxes. However, the percentage of the population of gray foxes needed to become vaccinated to halt the spread of rabies in central Texas may be less. Voigt et al. (1985) suggested that immunization of 60% of the red fox population of Ontario should be effective in eradicating rabies. Vaccination rates as low as 50% have been considered successful in suppressing fox rabies in Europe (Schneider et al., 1988; Bachmann et al., 1990).

An additional advantage of marshmallow wax cakes was their resistance to melting up to 37 C. In contrast, the wax-lard interior of the polymer-based baits melted when temperatures exceeded 27 C. Because the wax-lard interior of the polymer-based baits holds the vaccine sachet in place, its melting could facilitate loss of the vaccine sachet, making it unavailable to vaccinate an animal. Therefore, the polymer-based baits would be limited to winter use in central Texas; whereas the marshmallow wax cakes could be used throughout the year. Lastly, gray foxes exhibit chewing behaviors of the wax cake baits that would facilitate ingesting an oral vaccine (Steelman et al., 1998).

Although bait visitation and bait uptake rates by gray foxes were low (9% and 8%, respectively) during our study, rates may be underestimated due to raccoon foraging behavior. Visitation and uptake rates were based on the total number of operable bait-station nights. However we noted that raccoons were observed foraging earlier during evenings than gray foxes. Thus it is possible that fewer baits were available to gray foxes than to raccoons. Therefore, our estimates of bait visitation and uptake should be viewed as the minimum percentage of baits visited and taken by gray foxes.

A short duration removal program of raccoons actually intensified bait visitation and uptake rates by raccoons. Adult male raccoons in southern Texas are members (typically 3–4 individuals) of spatially distinct social groups that are capable of exploiting temporary and unpredictable resources (Gehrt and Fritzell, 1998). Male raccoons typically maintain consistent home range sizes throughout the year (Gehrt and Fritzell, 1997), except upon the death of an adjacent male, upon which another male will shift his territory to encompass a part of the vacant area (Fritzell, 1978). Because 59% (n = 37) of the raccoons removed during our study were males, it is possible that males from surrounding areas quickly moved into the newly-vacated territory, visited bait stations and took baits. Chamberlain et al. (1999) also noted that summer hunting of raccoons did not reduce raccoon populations. Therefore, a short-term raccoon population control program may not increase the availability of baits to gray foxes.

A bait placement strategy away from roads is consistent with the goal of having as many gray foxes as possible encounter baits. Gray fox visitation and uptake rates appeared unaffected by bait placement. However, raccoon visitation and uptake rates were much greater for baits placed on unimproved ranch roads. Because marshmallow wax cake baits exhibited the highest percent uptake rate when they were encountered by raccoons (the same

trend observed by gray foxes for this bait), it is reasonable to select a bait placement strategy that reduces the likelihood of bait encounters by non-target competitor species.

The results of our study support the potential of delivering a vaccine-laden bait to free-ranging gray foxes. However, the minimum bait density required to reduce the proportion of susceptible gray foxes to rabies may be problematic due to the confounding effects of non-target competitor species. In addition, the proportion of gray foxes that require vaccination to halt the spread of rabies and the minimum number of vaccine-laden baits that must be consumed to result in seroconversion needs to be determined for gray foxes. Also, because domestic animals had a high rate of visitation and bait uptake, more safety trials with the rabies vaccine are warranted prior to such a field program being initiated due to the possibility of exposure of people to these animals.

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