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GRAY FOX RESPONSE TO BAITS AND ATTRACTANTS FOR ORAL RABIES VACCINATION

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ABSTRACT: Rabies is a widespread zoonosis that recently reached epidemic proportions in gray foxes (*Urocyon cinereoargenteus*) in central Texas. The objectives of this study were to determine bait and attractant preferences among captive gray foxes, to determine the behavioral responses of gray foxes to selected bait-attractant combinations, and to evaluate baits as a delivery mechanism of oral rabies vaccines. Trials were conducted to determine bait preferences of captive gray foxes to selected baits and attractants. Tested baits consisted of a polymer-bound cube made of either dog food meal or fish meal, a polymer-bound cylinder made of dog food meal, and a wax-lard cake that was enhanced with marshmallow or chicken flavoring. Attractants were additives to baits that exuded sweet, sulfurous, fruity, fatty, cheesy, honey, and fishy odors and flavors. Captive gray foxes (n = 31) exhibited a preference for marshmallow wax cakes and polymer dog food baits with a lard interior and granulated sugar exterior. However, gray foxes exhibited chewing behaviors consistent with ingesting an oral vaccine only with the wax cake baits.

Key words: Attractants, bait, gray fox, oral vaccination program, preference, rabies, *Urocyon cineroargenteus*.

INTRODUCTION

An epidemic of gray fox (*Urocyon cineroargenteus*) rabies began in central Texas during 1987, and by 1994, the gray fox constituted 8% of the rabies cases reported in Texas (Texas Department of Health, 1994). To combat this epidemic, an oral vaccination program using vaccine-laden baits was suggested. Programs to evaluate suitable baits for vaccine delivery have been conducted on coyotes (*Canis latrans*), red foxes (*Vulpes vulpes*), mongooses (*Herpestes javanicus*), and raccoons (*Procyon lotor*) in southern Texas (Farry et al., 1998a, b), Ontario, Canada (Johnston and Voigt, 1982), Antigua, West Indies (Linhart et al., 1993), and the mid-Atlantic and northeastern United States (Johnston et al., 1988; Hanlon et al., 1989; Linhart et al., 1991), respectively. However, little is known about bait acceptance by gray foxes.

Evaluations of carnivore bait acceptance typically have been designed using scent station methodology (Linhart et al., 1977; Guthery et al., 1984; Andelt and Woolley, 1996). However, several problems can occur assessing bait stations as the sole determination of bait preference of a species. Misidentification of tracks can occur, especially in the present case, where populations of feral dogs (*Canis familiaris*), coyotes, and foxes are sympatric. A station could be visited by multiple species in one night making it difficult to determine which species took the bait. Bait disappearance could be assigned to the species most likely to have taken the bait (Guthery et al., 1984), or attributed to each species that visited the station (Andelt and Woolley, 1996). Also, animal ingestive behavior cannot be obtained through scent station methodology. An animal may cache a bait without consumption, or it may consume a bait in a manner in which an oral vaccine would not be exposed to and absorbed in the buccal mucosa (Rupprecht et al., 1988, 1989). Therefore, knowledge of the chewing behavior by the target species is imperative. The objectives of this study were to determine bait and attractant preferences among captive gray foxes, to determine the behavioral responses of gray foxes to selected bait-attractant combinations, and to evaluate baits as a delivery mechanism of oral rabies vaccines.

MATERIALS AND METHODS

Study area

Captive trials were conducted at Texas A&M University-Kingsville (TAMUK) South Pasture Facility (Kleberg County, Texas, USA; 27°27′N, 97°53′W). Gray foxes were housed individually in either 1.8 × 1.2 × 2.4 m kennels, in 1.4 × 0.9 × 0.9 m modular kennels, or in a 1.8 × 1.2 × 1.5 m modified travel trailer, allowing accommodations for 14 foxes during each trial. Kennels were constructed with concrete floors, chain link walls and doors, and a tin roof. Doors, door frame, and the lower 0.5 m of each kennel and the travel trailer were reinforced with 1.5 × 4.5 cm rectangular mesh fencing to reduce the probability of escape and contact with neighboring foxes. Tarpaulins were used as weather breaks and attached to the top half of exterior walls. Open-ended plywood boxes measuring 30 × 30 × 60 cm were placed 45 cm above the kennel floor and attached to the back wall of the kennels to give the foxes a place to hide during cleaning and removal of bait fragments. Durizban (1%) and sevin (10%) dust (Gro Tec Inc., Madison, Georgia, USA) periodically were spread around the kennel perimeter to reduce ant populations.

Baits and odor attractants

Two bait types, four bait substrates, three bait shapes, and 11 attractants were assessed during January through December, 1995. Bait types were hollow polymer-bound cubes (Bait Tech, Orange, Texas, USA) or wax-lard cakes (Ontario Ministries Natural Resource, Ontario, Canada). Bait substrate materials were made of dog food or fish meal with a synthetic polymer binder (Bait Tech, Orange, Texas, USA), or a wax-lard combination with either chicken or marshmallow icing added as flavoring (Ontario Ministries Natural Resource, Ontario, Canada). Differences in the manufacturing process of bait types and substrates may have represented a confounding variable between tested baits. Bait shapes consisted of 5.0 × 2.5 × 3.3 cm rectangular cubes, 2.5 × 2.5 × 3.3 cm square cubes, 5.0 cm tall × 2.0 cm diameter cylinders, and 3.5 × 3.5 × 1.2 cm wax cakes. The 11 attractants included vanillin (marshmallow), valeric acid (sulfurous), raspberry/persimmon extract (fruity), beef lard (fatty), butyric acid (cheesy), phenylacetic acid (honey), butylamine (fishy), grape flavoring, molasses, powdered sugar, and granulated sugar. Selection of bait substrate material and attractants were based on food habit data of gray foxes and recommendations from trappers and personnel from Texas Department of Health (Austin, Texas, USA). Each attractant, except grape, molasses, powdered sugar, and granulated sugar, was incorporated into a heated mixture consisting of approximately 60% beef lard, 30% paraffin wax, and 10% attractant. Molasses and powdered sugar were incorporated into the polymer dog food bait substrates and comprised approximately 10% of the weight of those baits. The heated lard-wax-attractant mixture was either poured into ice cube trays, allowed to harden, and used in the attractant trials, or was poured into the hollow center of the polymer-bound cubes, allowed to harden, and used in the bait-attractant combination trials. Vanillin, valeric acid, butyric acid, phenylacetic acid, and butylamine were obtained from Aldrich Chemical Company (Milwaukee, Wisconsin, USA); beef lard and raspberry/persimmon extract were obtained from H.E.B. stores (San Antonio, Texas, USA) and Medallion International, Inc. (North Haledon, New Jersey, USA), respectively. Grape Jell-O (Kraft General Foods, White Plains, New York, USA) was mixed into a liquid solution (170g of grape Jell-O powder was dissolved in 480 ml of warm water) in which dog food baits were soaked for 24 hr. About 7–10g of granulated sugar (H.E.B., San Antonio, Texas, USA) was glued to the exterior of dog food baits with white glue (Conros Corporation, Detroit, Michigan, USA). Baits were sealed in freezer bags, stored at −23 C and removed from freezers and allowed to thaw for 24 hr prior to use in trials.

Bait and attractant trials

Fifty-three foxes were obtained from Texas Animal Damage Control (San Antonio, Texas, USA), from T.M.'s Predator Control Service (Fort Stockton, Texas, USA), and from project personnel. Foxes were brought to the TAMUK South Pasture Facility and allowed a 2 wk acclimation period before beginning trials. During acclimation, foxes were fed canned dog food (Ole Roy chicken, beef, and liver flavors, Wal-mart Inc., Bentonville, Arkansas, USA) daily and given water *ad libitum*. Kennels were cleaned twice a week and each fox was given fresh straw for bedding. Daily notes were recorded concerning percentage of food consumed, water spillage, injuries (i.e., from capture, and those incurred during captivity), and comments on behavior of each fox.

Of the 53 foxes obtained, 31 foxes remained healthy for the duration of their respective trial periods and exhibited normal behaviors relative to diseased animals. Only healthy animals were used in the preference trials. Losses and mortality attributed to non-infectious disease factors included stress myopathy (1), escape (3), intussusception of the large intestine (1), and euthanasia due to severity of trap wounds (3). Mortality attributed to infectious diseases included canine distemper (11) and rabies (3). Instances of fatal canine distemper virus infections was induced by modified live-virus vaccines (Henke, 1997).

Preference trials were conducted using cafeteria style trays containing seven compartments. Trays were made of wood and each compartment measured 15 × 10 × 10 cm. Trays were wired to the kennel door to prevent spillage of tray contents by foxes.

Fifteen of each bait substrate or attractant were weighed and placed into a randomly selected compartment at the beginning of each trial. Baits were reweighed at 6, 12, 18, 24, 36, 48, 60, and 72 hr after the initiation of a trial. Chewing behavior of foxes with each bait type was noted. Bait consumption was measured as the area under the plotted consumption versus time curve when the proportion of each bait consumed was plotted against time (see Krebs, 1989:399–404 for details of cafeteria experiments). Preference trials 1–4 lasted 3 days and were conducted on a 7 day interval. Food was withheld for one day prior to the start of the trial. Trials started at 1800 hr. The morning following trials, kennels were cleaned, fresh straw added, and food given. Trials 5–8 were conducted on consecutive 2 day intervals. Food was withheld for 24 hr prior to trials. Trials started at 1800 hr and lasted for 24 hr with baits being weighed every 6 hr. Foxes were used in multiple trials; however, a fox was not given the same style of bait more than once in order to avoid the animal becoming habituated to a particular bait.

Eight preference trials were conducted. Trials 1 and 2 compared responses of gray foxes to bait substrates and shapes (previously described), trials 3 through 5 compared responses of gray foxes to attractants, trial 6 compared responses of gray foxes to 10, 20, and 30% concentration levels of the attractant, vanillin, and trials 7 and 8 compared responses of gray foxes to combinations of baits and attractants, as previously described. Baits and attractants that received low preference rankings were excluded from subsequent trials. Program RODGERS (Krebs, 1989:600–602) was used to calculate the area under the plotted consumption versus time curve (from here on referred to as area) and the bait preference index for each bait by each fox. Program RODGERS is an accepted method to determine bait preferences (Krebs, 1989); however, several qualifications require discussion. It is only practical to compare bait preferences between foxes if each animal had the same choice of baits. If one bait differed between trials, then bait preferences only were compared between foxes within a trial and not between trials. Also, gray foxes cache food items (Fritzell, 1987). However, all bait fragments must be accounted for to effectively measure consumption rates. Otherwise, bait consumption will be overestimated. To avoid overestimating food consumption, bedding such as straw was not given during trials. In addition, bait fragments of similar hues and odors were difficult to identify. Therefore, lard-wax-attractant mixtures were color coded to aid in identification of fragments. Colors were randomly assigned to attractants before each trial. Color coding each bait type aided the process of replacing baits that were removed but not consumed from the cafeteria trays by gray foxes, and thus, in identifying bait fragments not eaten.

A completely randomized design was used for preference trials on captive gray foxes. Distributions of residual errors were tested for normality using the Shapiro-Wilk test (Shapiro and Wilk, 1965). 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 analyses of variance were used to test the effects of bait and attractants on the Rodger's preference indices and the area (SAS Institute, Cary, North Carolina, USA). Multiple comparisons were made using the Duncan's Multiple Range procedure when a significant F-test was noted (Ott, 1993). All tests were considered significant at $P \leq 0.05$. Descriptive statistics are reported herein as the mean ± 1 standard error ($\bar{x} \pm$ SE).

Evaluation of vaccine delivery and fox behavior

Eleven foxes were given dog food substrate, square shaped, lard interior baits with a 2 mL rhodamine B (Sigma Chemical Company, St. Louis, Missouri, USA) dye-filled sachet incorporated into the center of the bait. Rhodamine B dye was used to simulate the vaccine and aided our evaluation of vaccine delivery and gray fox behavior with baits. The rhodamine B dosage was 150 mg/bait, or 30 mg/kg of body mass, based on a mean mass of 5 kg for gray foxes (Fritzell, 1987). Foxes were euthanized and necropsied 24 hr after delivery of baits. An ultraviolet light was used to note the presence of rhodamine B dye. Chi-square analyses with the Yates Correction for Continuity (Steel and Torrie, 1980:484) were used to determine differences in chewing behaviors between male and female foxes by comparing the location of dye marks on internal organs.

RESULTS

During trial 1, a difference was exhibited in the area and preference indices ($P = 0.0025$ and $P = 0.0001$, respectively) between marshmallow wax cakes and all other baits tested (Table 1). Trial 2 was similar to trial 1 except chicken wax cakes were not tested. This time no differences in area ($P = 0.36$) and preference ($P = 0.34$) were noted between bait substrates (Table 1); however, the statistical power of the test was low ($\beta = 0.68$).

Gray foxes exhibited a difference in preference indices ($P = 0.0001$) and areas ($P = 0.0001$) between attractants during trial 3 (Table 1). A granulated sugar exterior was preferred over all other attractants, followed by fatty, fruity, and sulfurous attractants, and then cheesy, honey, and fishy attractants. In trial 4, honey and fishy attractants were replaced with powdered sugar and molasses as attractants; there were no differences in preference ($P = 0.38$) and area ($P = 0.40$) between these attractants (Table 1).

No differences were observed during trial 5 in area ($P = 0.54$) and preference indices ($P = 0.42$) between grape and marshmallow attractants incorporated into dog food substrate, square shaped baits (Table 1). During trial 6, gray foxes exhibited a preference ($P = 0.012$ and $P = 0.007$ for area and preference indices, respectively) for baits with 10% vanillin concentrations incorporated into the lard-wax mixture more so than baits of higher vanillin concentrations (Table 1). During trials that tested bait-attractant combinations (i.e., trials 7 and 8), the area and preference indices exhibited by gray fox were greater ($P < 0.001$) for the dog food substrate, fatty interior, granulated sugar exterior bait-attractant combination than the other tested combinations (Table 1).

Of the 11 foxes in the vaccine delivery trial, none showed signs of rhodamine dye on the buccal mucosa of the back throat region or on the esophagus (Table 2). Seventy-three percent showed evidence of sachet perforation within the mouth and 45% showed signs of rhodamine consumption in the feces. There were no differences ($P > 0.12$) in rhodamine markings between males and females.

DISCUSSION

Gray foxes are omnivores and their diets are known to vary seasonally (Smith, 1979; Trani, 1980). However, sweet baits and attractants ranked highest in every trial conducted throughout the year. This suggests that sweet baits and attractants are universal in time and would yield a high consumption rate by gray foxes regardless of season.

Polymer-bound baits did allow for incorporation of multiple flavors and fragrances (i.e., interior and exterior attractants), which enhanced gray fox consumption. Although not quantified, the addition of granulated sugar to the exterior of polymer-based baits increased chewing and licking behaviors in gray foxes. Teranishi et al. (1981) reported that the addition of sucrose solution to lures elicited a greater chewing response in coyotes. However, the wax cake baits displayed several advantages over the polymer-bound baits. Gray foxes exhibited increased chewing behavior with wax cakes, which in turn, may increase the probability of vaccine absorption into the buccal mucosa. Gray foxes typically bit the middle of the wax cakes, which resulted in the sachets being ruptured. Foxes consuming polymer-bound baits were inclined to first chew the ends of the baits. This behavior exposed the sachets, which were pulled out and discarded by foxes.

The lard and wax formulation of the cake baits caused these baits to stick to the teeth of foxes upon chewing. The stickiness of these baits would cause foxes to tip their heads back; and therefore, the liquid within the vaccine packet of cake baits remained in the mouth of foxes. In contrast,

TABLE 1. Responses of captive gray foxes to bait substrates, shapes, and attractants during penned trials conducted in southern Texas, 1995.

Trial	n[a]	Bait type[b]	Area $\bar{x}$[c]	Area SE[c]	Preference $\bar{x}$[d]	Preference SE
1	7	Marshmallow cake	4,830.2A[e]	826.2	0.704A	0.073
		Dog food rectangle	1,731.8B	639.9	0.247C	0.069
		Fish meal rectangle	1,595.6B	389.5	0.409B	0.077
		Dog food square	1,099.7B	492.1	0.117C	0.059
		Chicken cake	946.2B	368.2	0.130C	0.043
		Dog food cylinder	620.1B	304.6	0.136C	0.051
		Fish meal square	592.7B	172.2	0.151C	0.043
2	4	Marshmallow cake	8,280.6A	1,855.8	0.706A	0.130
		Dog food square	4,230.9A	1,566.5	0.272A	0.118
		Dog food rectangle	3,369.9A	1,563.7	0.365A	0.126
		Dog food cylinder	124.2A	57.4	0.010A	0.004
		Fish meal rectangle	64.2A	46.1	0.046A	0.032
		Fish meal square	38.7A[e]	25.8	0.003A	0.002
3	8	Granulated sugar	13,330.3A	1,140.0	0.880A	0.053
		Fatty	3,288.8B	468.8	0.320B	0.054
		Fruity	1,248.8C	295.2	0.166C	0.045
		Sulfurous	574.6D	175.0	0.074D	0.031
		Cheesy	310.2D	93.1	0.053D	0.024
		Honey	181.2D	52.7	0.059D	0.032
		Fishy	146.7D	40.6	0.035D	0.017
4	4	Granulated sugar	5,771.0A	3,449.1	0.494A	0.285
		Molasses	3,620.0A	3,490.7	0.261A	0.247
		Fatty	2,783.0A	2,088.0	0.277A	0.226
		Fruity	1,396.0A	1,153.2	0.392A	0.233
		Powdered sugar	215.0A	214.5	0.015A	0.015
		Sulfurous	181.0A	62.1	0.011A	0.007
		Cheesy	135.0A	94.8	0.011A	0.007
5	12	Grape	1,652.5A[e]	311.6	0.696A	0.073
		Marshmallow	1,429.6A	230.3	0.601A	0.086
6	11	10% Vanillin	401.2A	63.3	0.824A	0.045
		20% Vanillin	155.0B	25.9	0.486B	0.060
		30% Vanillin	111.1B	20.7	0.368B	0.057
7	10	Dog food-fatty-sugar	1,807.8A	258.2	0.732A	0.083
		Grape-fatty-sugar	1,075.5B	155.4	0.510B	0.073
		Dog food-vanillin-sugar	1,020.9B	199.3	0.438B	0.088
		Grape-vanillin-sugar	819.0BC	146.9	0.391B	0.069
		Grape-fatty-no sugar	430.1CD	72.2	0.212C	0.048
		Grape-vanillin-no sugar	250.4D	26.1	0.122C	0.017
		Dog food-vanillin-no sugar	71.5D	19.0	0.035C	0.011
8	10	Dog food-fatty-sugar	2,563.8A[e]	201.2	0.966A	0.032
		Grape-vanillin-sugar	621.1B[e]	140.6	0.293B	0.071
		Dog food-vanillin-sugar	602.2B	181.8	0.202BC	0.064
		Dog food-vanillin-no sugar	298.6BC	99.3	0.127CD	0.037
		Grape-vanillin-no sugar	59.1C	16.4	0.021D	0.006

[a] Number of gray foxes used in a trial.

[b] In trials 1 and 2, bait type consisted of hollow polymer-bound baits made of either dog food or fish meal and formed to rectangle (5.0 × 2.5 × 3.3-cm), square (2.5 × 2.5 × 3.3-cm), or cylinder (5.0 cm tall × 2.0-cm-diameter) shapes, or consisted of wax-lard cakes with either chicken or marshmallow icing added as flavoring. In trials 3–6, bait type odor consisted of 30% wax-60% lard-10% attractant combination formed to ice-cube shape. Granulated sugar consisted of polymer dog food baits cut to square shape and granulated sugar glued to the exterior. Grape baits were made of dog food polymer baits soaked for 24 hr in grape Jell-O (170 g dissolved in 480 ml water) solution. Trials 7 & 8 were combinations of bait types.

[c] Mean ($\bar{x}$) ± standard error (SE) of the area under the plotted consumption versus time curve when the proportion of each bait consumed was plotted against time for each fox.

[d] Rodger's preference index as calculated by Program RODGERS (Krebs, 1989), expressed in terms of $\bar{x}$ ± SE.

[e] Means with the same letter are not different ($P > 0.05$) within a trial.

TABLE 2. Results of internal staining by rhodamine B in gray fox that consumed polymer-bound dog food baits with a fatty interior attractant during captive trials in southern Texas, 1995 (n = 11 foxes).

Tissue	Proportion marked by rhodamine dye				
	Males[a]	Females[b]	Total[c]	χ^2 value	P-value
Tongue	83	40	64	2.213	0.14
Upper palette	83	60	73	0.749	0.39
Tonsils	83	40	64	2.213	0.14
Esophagus	0	0	0	0.000	0.00
Stomach	0	20	9	1.320	0.25
Feces[d]	67	20	45	2.396	0.12

[a] Proportion marked based on 6 males.
[b] Proportion marked based on 5 females.
[c] Proportion marked based on 11 foxes.
[d] Feces represents excretions, not intestinal material.

the polymer-bound baits would crumble upon being chewed, which caused foxes to tip their heads down and allowed the dye to drip from their mouths to the ground.

Another advantage of wax cake baits was their resistance to melting even when temperatures approached 33 C. The interior attractant within the polymer-bound baits tended to soften and melt when ambient temperatures exceeded 26 C. Because the interior attractant was used to hold the sachet in place, if it melted, the sachet would typically fall out of the bait when picked up by a fox, and thus, the vaccine would not reach the oropharynx.

During the rhodamine trials, even though the foxes were puncturing the sachets and consuming the polymer-bound baits, most of the dye was spilled onto the kennel floor. There was evidence of rhodamine in the feces; therefore, the dye had passed through the digestive tract. However, foxes only tended to rupture the sachets in an attempt to free them from the baits. During the process, dye would leak from the sachets and be absorbed by the baits. Therefore, upon ingestion, liquid dye would not stain the esophagus but feces would contain rhodamine-stained baits.

Although marshmallow wax cakes and sweet flavored, polymer-bound dog food baits were preferred by gray foxes, wax cake baits caused foxes to exhibit the type of chewing behavior necessary to achieve successful immunization. Even though the addition of granulated sugar glued to the exterior of polymer-bound baits elicited a chewing and licking behavior in gray foxes, much of the simulated vaccine would spill on the ground. Further research is needed to quantify the minimum amount of vaccine absorption required to provide rabies immunization for gray foxes. However, the marshmallow wax cake baits appear to be a highly palatable and viable vaccine delivery system for gray foxes.

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