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# EFFICACY OF BAIT DISTRIBUTIONAL STRATEGIES TO DELIVER CANINE RABIES VACCINES TO COYOTES IN SOUTHERN TEXAS

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ABSTRACT: This study sought to develop a baiting strategy to deliver an oral rabies vaccine to free-ranging coyotes (*Canis latrans*) in southern Texas. To determine bait longevity, dog food-lard baits were placed ($n$ = 50) on- and off-roads during July 1994 and January 1995. Coyote visitation and uptake rates did not differ between on-road and off-road placement of baits. To evaluate bait stations as possible visual cues, baits were placed out both with ($n$ = 50) and without ($n$ = 50) bait stations. A visual cue of a bait station did not affect coyote response to baits. Bait longevity was shorter during July (≤4 days) than January because of consumption of baits by imported fire ants (*Solenopsis invicta*). The effect of two different bait densities on coyote acceptance rates was determined on six 93.5 km$^2$ study areas in southern Texas. Three study areas received a bait density of 19 baits/km$^2$ (50 baits/mi$^2$) and the remaining three study areas received 58 baits/km$^2$ (150 baits/mi$^2$). Coyote bait uptake rates, based upon the proportion of coyotes marked with either tetracycline hydrochloride or rhodamine B or both, were 83% ($n$ = 99 coyotes) and 87% ($n$ = 101 coyotes) for the 19 and 58 baits/km$^2$ densities, respectively. Bait uptake rates did not differ ($P > 0.54$) between the two bait densities. Rodents and rabbits, which were fed baits containing tetracycline hydrochloride and a simulated oral rabies vaccine sachet containing rhodamine B, did consume the bait but not the rhodamine B sachet. These animals then were killed and fed to captive coyotes ($n$ = 9). Canine teeth were extracted from coyotes and processed for tetracycline determination. Each coyote tested negative for tetracycline. Therefore, it was unlikely that coyote bait consumption rates were overestimated because of coyotes secondarily marking themselves by ingesting prey items that consumed baits.

*Key words:* Bait, *Canis latrans*, coyote, density, longevity, rabies, vaccination program.

## INTRODUCTION

An epidemic of canine rabies transmitted by coyotes (*Canis latrans*) began in 1988 along the United States-Mexico border (Clark et al., 1994). The disease rapidly spread throughout southern Texas and resulted in two human deaths and more than 1,400 rabies exposures in which prophylactic treatment was required (Meehan, 1995).

Historically, problems involving coyotes (i.e., predation on livestock and game species) have been approached by use of lethal methods (Connolly, 1982). However, large scale depopulation programs to combat rabies in other species such as red foxes (*Vulpes vulpes*) have been controversial and questioned as to its efficacy for long-term rabies control (Rosatte, 1987). Simulation experiments suggest that depopulation efforts of coyotes by removal will stimulate density-dependent rates in natality and natural mortality, and potentially offset an overall population reduction (Connolly and Longhurst, 1975). Also, coyote removal may even intensify rabies by increasing coyote dispersal into endemic areas. Reduction of reproductive success also has been suggested as a method for reducing host population density; however, this method has not been effectively tested for disease control (Wobeser, 1994: 160).

Development of effective human post-exposure prophylaxis and mass immunizations of dogs have been effective in controlling rabies in humans and domestic animals in developed countries (Kaplan, 1985). However, immunization of free-ranging animals has been limited due to the lack of safe and efficacious vaccines for multiple species (Rosatte, 1987) and to difficulty of vaccine delivery (Baer, 1988). Trap-and-vaccinate programs have been used to control rabies in striped skunks

(*Mephitis mephitis*) in Canada (Rosatte et al., 1992). However, similar methods have not been considered feasible in southern Texas because of presumed low success rates of capturing coyotes in live traps; live-trapping has not been given as a capture option for coyotes (see Bekoff, 1977; Voigt and Berg, 1987). Propelling rabies vaccine into the mouth of captive red foxes using M-44 devices, and intramuscular inoculation of vaccine through the use of a syringe propelled by a steel trap mechanism also have been explored as delivery mechanisms; however, these methods were considered only partially successful (Baer, 1988).

In theory, a long-term management of a large, contiguous endemic rabies area can include an oral vaccination program that incorporates a strategic distribution of vaccine-laden baits (Baer, 1988; Johnston et al., 1988). Successful bait delivery has been demonstrated in raccoons (*Procyon lotor*; Hadidian et al., 1989; Hanlon et al., 1989; Rupprecht et al., 1989; Linhart et al., 1991), domestic dogs (*Canis familiaris*; Kharmachi et al., 1992), red foxes, and striped skunks (Johnston et al., 1988; Bachman et al., 1990). However, each species of carnivore has different food preferences and foraging strategies (Chapman and Fieldhamer, 1982). Coyotes are a numerous carnivore in southern Texas that can transmit rabies. Therefore, it is essential to develop a comprehensive vaccine and bait-delivery program for this species.

Our objectives were to determine the longevity of a dog food-based bait placed on- and off-road, the percentage of bait consumption by free-ranging coyotes, and to determine the bait density needed to achieve ≥70% bait consumption by the coyote population. The bait consumption of ≥70% was set by the Texas Department of Health (Austin, Texas, USA) based on assumptions as to the intrinsic reproductive rate of rabies in coyotes.

## MATERIALS AND METHODS

### Study areas

Longevity of a dog food-based bait was assessed on the Santa Gertrudis Division of the King Ranch in Kleberg County, Texas (27°25′N, 97°56′W). Bait acceptance and density experiments were conducted on six ranches in southern Texas located within the current rabies epidemic. They included the Callaghan (Webb Co.), La Mesa (Webb Co.), Heard (Webb Co.), Duval (Duval Co.), Cameron (La Salle Co.), and La Campana (McMullen Co.) ranches (Fig. 1). Study sites within each ranch covered 93.5 $km^2$ and were located >32 km from each other. All areas consisted of privately owned rangeland that was used primarily for cattle grazing and oil production. Detailed topographical and climatological characteristics of the study areas are described by Farry (1995). The study areas originally supported a grassland-savannah climax community. However, grazing, suppression of fire, and other factors have resulted in plant communities dominated by dense stands of honey mesquite (*Prosopis glandulosa*), blackbrush (*Acacia rigidula*), Texas prickly pear (*Opuntia lindheimeri*), whitebrush (*Aloysia lycioides*), and spiny hackberry (*Celtis pallida*). Mammals include coyote, white-tailed deer (*Odocoileus virginianus*), javelina (*Dicotyles tajacu*), feral hog (*Sus scrofa*), raccoon, bobcat (*Felis rufus*), armadillo (*Dasypus novemcintus*), striped skunk, eastern cottontail rabbit (*Sylvilagus floridanus*), black-tailed jackrabbit (*Lepus californicus*), eastern woodrat (*Neotoma floridanus*), hispid cotton rat (*Sigmodon hispidus*), and domestic cattle (*Bos* sp.).

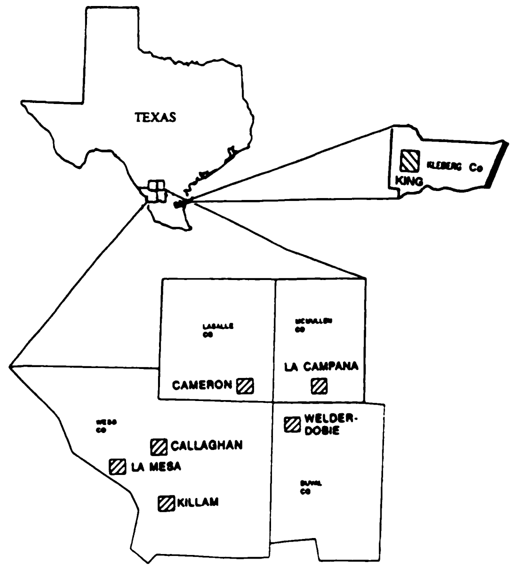


FIGURE 1. Location of bait longevity and bait density experiments for delivery of canine rabies vaccines to coyotes conducted in southern Texas during 1994–1995.

### Longevity of baits

The longevity of a dog food-based bait (Bait Tech, Orange, Texas, USA) placed on-road and baits randomly placed off-road was assessed during July 1994 and January 1995. Five transect roads, >5.0 km apart, were chosen and 20 bait stations per road were constructed. Bait stations were built at 0.81-km intervals; each station consisted of a 1-m circular plot of sifted soil that was cleared of vegetation. Ten bait stations per transect were located on the shoulder of non-paved, limited-use roads and 10 stations were located at random distances perpendicular to the roads. Random distances for off-road bait stations ranged from 3 to 99 m. A random number table was used to assign distances and direction (Steel and Torrie, 1980). If the number was even, then the perpendicular distance was measured to the right of the transect road; if the number was odd, then the perpendicular distance was measured to the left of the transect road. All bait stations were baited with dog food-lard baits (see Farry et al., 1998) and were checked daily for seven days. Animals visiting the stations were identified by their sign. Visitation and uptake rates, expressed herein as a proportion, were determined as the number of stations visited and baits removed, respectively, per the number of operative stations (Roughton and Sweeny, 1982). If >1 set of tracks were present on a station and the bait was missing, then each species that visited the station was attributed a consumption proportional to the number of species' tracks (Andelt and Woolley, 1996). Baits were not replaced once they had been removed by an animal. Z-tests were used to make comparisons of cumulative bait longevity between bait placement (Ott, 1993).

### Bait stations as visual cues

To assess the possible bias that a cleared bait station acted as a visual cue, baits were placed at stations with and without sifted soil and cleared of vegetation during January, 1995. A total of 100 dog food-lard baits were placed on the off-road bait stations, as previously described, and at an additional 50 bait stations placed at random distances perpendicular to the transect roads, as previously described, however without cleared stations and sifted soil. The same survey and statistical procedures used in the longevity tests were followed.

### Bait acceptance and density evaluation

Bait density required to mark ≥70% of a coyote population was assessed on the six ranches during March–April, 1994. Dog food-lard baits were mass produced by Texas A&M University-Kingsville (Kingsville, Texas, USA) personnel for aerial distribution (see Farry et al., 1998). Three of the six ranches received a bait density of 19 baits/km$^2$. The remaining three ranches received 58 baits/km$^2$. Bait density was assigned randomly to each ranch.

Baits were evenly distributed within a 93.5-km$^2$ study area of each ranch in early March 1994 from a Cessna 170 aircraft flying about 160 km/hr at an altitude of approximately 30 m. The aircraft navigated using Global Positioning Systems equipment to ensure precision of the flight paths and the beginning and ending points of each transect. Flight paths were 0.23 and 0.13-km apart for dropping bait densities of 19 and 58 baits/km$^2$, respectively. The baiter used a standard battery-operated clock with a second hand to standardize drop rates. Drop rates were 1 bait/5 (sec) for placing 19 baits/km$^2$ and 1 bait/3 (sec) for placing 58 baits/km$^2$. Actual drop rates varied to compensate for changes in ground speed due to wind (Bachman et al., 1990). Baits were dropped singly out of a 10 × 10 × 30.5-cm chute in the floor of the plane. One study area per day was baited. Beginning 7 days after each bait drop, coyotes were collected by aerial and ground collection. Aerial collection consisted of shooting from a helicopter. Ground collection of coyotes included shooting and M-44 devices. Coyotes were collected from within a 41.5 km$^2$ core area of each study area to minimize the effect of animals immigrating from adjacent unbaited areas. Linear distance between the peripheries of the coyote collection and baited areas was >1.6 km.

Two biomarkers, tetracycline hydrochloride and rhodamine B (Sigma Chemical Co., Kalamazoo, Michigan, USA), were used to assess bait acceptance (Ellenton and Johnston, 1975; Johns and Pan, 1981). Rhodamine B powder was dissolved in water and about 2 mL were placed in a sachet. Sachets were made of 5.3 cm diameter polyethylene tubing and sealed with an Impulse Heat Sealer (Ankon Technology Corp., Fairport, New York, USA). Rhodamine sachets were placed in the hollow cavity within baits to mimic the vaccine and sealed with a lard-wax mixture (see Farry et al., 1998 for bait development). Minimum dosage of rhodamine B was 342 mg/bait (30 mg/kg of body mass), based on the mean mass of coyotes from southern Texas (11.3 kg coyote; Knowlton, 1972). The pelage, feet, mouth, tongue, and urogenital openings (Ellenton and Johnston, 1975; Lindsey, 1983) of each collected coyote were examined for the presence of external rhodamine B staining in the field using ultraviolet light. The digestive tract from the esophagus to the anus, liver, kidneys, and bladder were collected and examined using ultraviolet light to assess internal rhodamine B staining. Claws and growing hairs on the feet also were collected and examined for the presence of fluorescent bands due to the ingestion of rhodamine B (Johns and Pan, 1981; Lindsey, 1983). Claws and hairs were examined under ultraviolet light with the aid of a variable power dissecting microscope.

The second biomarker, tetracycline hydrochloride, was incorporated directly into the polymer-based bait by the manufacturer. The tetracycline dosage was about 226 mg/bait (20 mg/kg of mean coyote body mass). Both lower canine teeth were collected from each coyote and sent to Matson's Laboratory (Milltown, Montana, USA) for determination of tetracycline hydrochloride (Ellenton and Johnston, 1975) and age by the enumeration of cementum annuli (Linhart and Knowlton, 1967). Thin undecalcified sections of the roots were examined under an ultraviolet microscope for the presence of yellow fluorescing bands within the cementum and dentine, which is characteristic of tetracycline ingestion (Johnston and Voight, 1982). Bait ingestion was defined as the proportion of all animals of a species examined that revealed internal rhodamine B staining or tetracycline deposits. Either marker was accepted as indicative of bait ingestion because staining of the gastrointestinal tract by rhodamine B was short-term (i.e., <1 wk) and incorporation of tetracycline hydrochloride into the cementum was not immediate. Bait contact included animals that exhibited either external or internal signs of the biomarkers. Additional data collected from each coyote included sex and body mass. Coyotes were classified as juveniles if they were ≤1-yr-old, and as an adult if they were >1-yr-old. Z-tests (Ott, 1993) were used to compare bait acceptance by coyotes between the two bait density strategies.

### Secondary marker evaluation

It was hypothesized that coyotes may secondarily mark themselves by consuming prey that ingested the biomarkers. To assess this possibility, 25 hispid cotton rats and 15 eastern cottontail rabbits were captured in Sherman traps and Havahart traps, respectively (Forestry Suppliers, Inc., Jackson, Mississippi, USA). Rodents were housed individually in polypropylene cages measuring 27 × 21 × 14 cm with wire lids. Rabbits were kept in wire mesh cages measuring 92 × 60 × 32 cm and were given water *ad libitum*. One dog food-lard bait containing a 2-ml rhodamine B-filled sachet was fed to each animal. Time required for complete bait consumption was noted. Immediately after bait consumption, each animal was euthanized by thoracic compression (American Society of Mammalogists, 1987: 15) and assessed for presence of external and internal rhodamine B staining as outlined for coyotes. The entire carcass of rats and rabbits were processed in a meat grinder and fed to 9 wild-caught coyotes held in captivity at the Texas A&M University-Kingsville South Pasture Facility (Kleberg County, Texas, USA; 27°27′N, 97°53′W). Coyotes were maintained for 1 wk after the ingestion of the ground meat and then euthanized by a .22-caliber gunshot to the head. A lower canine tooth was extracted from each coyote and each tooth processed for tetracycline hydrochloride determination as previously discussed.

## RESULTS

### Longevity of baits

Transect surveys conducted in January 1995, resulted in cumulative on-road bait uptake by all species, which ranged from 48 to 94% after 1 to 7 days, respectively. After three days, baits at on-road stations exhibited a higher cumulative uptake rate ($P = 0.01$) than those at off-road stations (Table 1). On-road stations continued to exhibit higher bait uptake rates than off-road stations throughout the 7-day period. Coyote bait uptake for on-road and off-road stations was 36 and 27%, respectively. Coyote visitation was 47% for on-road stations and 43% for off-road stations. Bait uptake rates for other mammalian species were 64 and 73% for on-road and off-road stations, respectively. However, the likelihood of a coyote removing a bait if the bait was encountered was 77% for on-road stations and 63% for off-road stations. No difference was observed ($P > 0.16$) between on-road and off-road stations during January in coyote bait uptake, coyote visitation, non-target species bait uptake, or likelihood of a coyote removing a bait if encountered.

Transects conducted in July 1994 resulted in a cumulative on-road bait uptake by all species, which ranged from 12% after one day to 36% after four days. Cumulative on-road bait uptake was greater ($P < 0.01$) than off-road bait uptake each day (Table 1). The cumulative coyote bait

TABLE 1. Comparison of cumulative bait uptake rates of dog food-lard baits removed from on-road ($n$ = 50) and off-road ($n$ = 50) stations during July 1994 and January 1995 in southern Texas.

| | Proportion of cumulative bait uptake[a] | | | | | |
|---|---|---|---|---|---|---|
| | January | | | July | | |
| Day | On-road | Off-road | $P$-value[b] | On-road | Off-road | $P$-value[b] |
| 1 | 0.480 | 0.420 | 0.7280 | 0.320 | 0.120 | 0.0080 |
| 2 | 0.700 | 0.500 | 0.1294 | 0.460 | 0.200 | 0.0029 |
| 3 | 0.840 | 0.540 | 0.0107 | 0.520 | 0.280 | 0.0071 |
| 4 | 0.900 | 0.680 | 0.0338 | 0.620 | 0.360 | 0.0047 |
| 5 | 0.920 | 0.700 | 0.0106 | — | — | — |
| 6 | 0.940 | 0.700 | 0.0076 | — | — | — |
| 7 | 0.940 | 0.740 | 0.0027 | — | — | — |

[a] Cumulative proportion of baits removed by all species.
[b] Calculated using Z-test.

uptake rates for on-road and off-road stations were 16 and 33%, respectively. Coyote visitation rates were 19% for on-road stations and 39% for off-road stations. Bait uptake by other mammalian species was 84 and 67% for on-road and off-road stations, respectively. The likelihood of bait removal if a coyote encountered the bait was 84% for on-road stations and 86% for off-road stations. No difference was observed ($P > 0.07$) between on-road and off-road stations during July in coyote bait uptake, coyote visitation, other mammalian bait uptake, or likelihood of bait removal if encountered by coyotes.

Coyote visitation and bait uptake rates did not differ ($P < 0.31$) for off-road stations between July and January. However, coyote visitation, total bait uptake, and coyote bait uptake rates for on-road stations were greater ($P < 0.03$) in January than July (Table 2).

The presence of fire ants (*Solenopsis invicta*) negatively affected the longevity of baits during the bait assessment trials in July. The useful life of baits during July was limited to ≤4 days. Within 24 hr after bait placement on a station, fire ants were present on 50% of the baits remaining on-road and 43% of the baits placed off-road. After 48 hr, 89 and 68% of remaining baits on-road and baits placed off-road, respectively, had fire ants present. During January, fire ants were absent from all bait stations for the duration of the 7 day trial.

#### Bait stations as visual cues

Comparison of baits placed on off-road stations, and baits placed off-road without stations during January 1995, resulted in bait uptake rates of 74 and 70%, respectively. After seven days, bait uptake rates did not differ ($P > 0.30$) between baits placed on and without stations (Table 3).

#### Bait acceptance and density evaluation

Two hundred coyotes were collected by aerial and ground hunting; 99 coyotes were collected from ranches that received 19 baits/km$^2$, and 101 coyotes were collected from ranches that received 58 baits/km$^2$. Baiting densities of 19 and 58 baits/km$^2$ resulted in external rhodamine B staining rates of 30 and 38%, respectively; internal rhodamine B staining rates of 55 and 58%, respectively; tetracycline biomarker rates of 51 and 66%, respectively; and bait ingestion rates of 84 and 87%, respectively. Evaluation of rhodamine B staining of claws and growing hairs was inconclusive due to difficulty in detecting fluorescence within these materials and therefore not used in bait acceptance estimates.

No difference was observed ($P > 0.15$) in occurrence of external or internal rho-

TABLE 2. Comparison of on-road and off-road coyote visitation, coyote bait uptake, and total bait uptake rates by all species of dog food-lard baits in southern Texas during July, 1994 and January, 1995.

| | July | | January | | |
|---|---|---|---|---|---|
| | $n$[a] | Proportion | $n$[a] | Proportion | $P$-value[b] |
| Coyote uptake | | | | | |
| On-road | 31 | 0.161 | 47 | 0.362 | 0.0268 |
| Off-road | 18 | 0.333 | 37 | 0.270 | 0.3156 |
| Coyote visitation | | | | | |
| On-road | 31 | 0.193 | 47 | 0.468 | 0.0066 |
| Off-road | 18 | 0.389 | 37 | 0.432 | 0.3821 |
| Total mammalian uptake | | | | | |
| On-road | 31 | 0.620 | 47 | 0.900 | <0.0010 |
| Off-road | 18 | 0.360 | 37 | 0.680 | <0.0010 |

[a] Number of operable bait stations.
[b] Calculated using Z-tests.

damine B markings, tetracycline marking, bait ingestion, or bait contact by coyotes between the two bait density strategies (Table 4). A possible trend was observed with a greater rate of ingestion ($P > 0.05$) in adult coyotes than in juveniles (Table 4). No difference was observed ($P > 0.18$) in occurrence of any biomarker between male and female coyotes (Table 4). There also was no difference observed between sex ratios ($P > 0.56$) and age structure ($P > 0.79$) of coyotes collected between the sites of differing bait densities.

### Secondary marker evaluation

Cottontail rabbits and cotton rats readily consumed the dog food-lard baits containing a 2-ml rhodamine B-filled sachet. Rabbits and rodents consumed the entire bait within 3 days ($\bar{x} \pm SE = 2.5 \pm 0.5$ days) and within 8 days ($5.9 \pm 0.8$ days), respectively. Neither rabbits nor rats consumed the rhodamine B-filled sachet. Upon necropsy, 3 of the 25 cotton rats (12%) had a trace of rhodamine B staining on their front feet. No rhodamine B staining was found on the rodent pelage or in internal organs. No trace of rhodamine B was found on rabbits or in their internal organs. Of the 9 coyotes fed the carcasses of the rats and rabbits, 6 coyotes were considered to be tetracycline negative, while the remaining 3 coyotes were classified as questionable negative (i.e., the tetracycline biomarker was probably absent, but there appeared to be very weak fluorescence).

TABLE 3. Comparison of dog food-lard baits removed from off-road locations with ($n$ = 50) and without ($n$ = 50) cleared bait stations during January in southern Texas, 1995.

| Day | Proportion of bait uptake without stations[a] | Proportion of bait uptake with stations[a] | $P$-value[b] |
|---|---|---|---|
| 1 | 0.330 | 0.420 | 0.1401 |
| 2 | 0.410 | 0.500 | 0.1469 |
| 3 | 0.470 | 0.540 | 0.2090 |
| 4 | 0.650 | 0.680 | 0.3557 |
| 5 | 0.670 | 0.700 | 0.3557 |
| 6 | 0.690 | 0.700 | 0.4483 |
| 7 | 0.700 | 0.740 | 0.3050 |

[a] Cumulative proportion of baits removed by all species.
[b] Calculated using Z-test.

## DISCUSSION

A bait placement strategy away from secondary roads appears consistent with the goal of >70% of the population of coyotes ingesting baits. Even though cumulative bait uptake was greater when baits were placed on secondary roads, non-target species removed a significant number of baits. Coyote visitation and uptake rates appeared unaffected by bait placement. Therefore, a bait placement strategy that reduces the likelihood of bait encounters by non-target species is appropriate.

TABLE 4. Proportion of bait acceptance by coyotes for bait densities of 19 baits/km$^2$ and 58 baits/km$^2$ in southern Texas during March, 1994.

| | Bait density | | | | | Age class | | | | | Sex | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 19 baits/km$^2$ | | 58 baits/km$^2$ | | | Juvenile | | Adult | | | Male | | Female | | |
| Markings | $\bar{x}$ | SE | $\bar{x}$ | SE | P-value[a] | $n$[b] | Proportion | $n$[b] | Proportion | P-value[a] | $n$[b] | Proportion | $n$[b] | Proportion | P-value[a] |
| External[c] | 0.2973 | 0.07 | 0.3792 | 0.09 | 0.5229 | 45 | 37.8 | 116 | 33.6 | 0.3085 | 88 | 35.2 | 102 | 31.4 | 0.2912 |
| Internal[d] | 0.5455 | 0.06 | 0.5803 | 0.10 | 0.7831 | 33 | 45.4 | 71 | 56.3 | 0.1492 | 53 | 52.8 | 64 | 56.3 | 0.3520 |
| Tooth[e] | 0.5100 | 0.09 | 0.6606 | 0.10 | 0.3113 | 55 | 52.7 | 118 | 55.9 | 0.3438 | 82 | 50.0 | 104 | 56.7 | 0.1841 |
| Ingestion[f] | 0.8326 | 0.48 | 0.8678 | 0.02 | 0.5432 | 45 | 77.8 | 94 | 88.3 | 0.0526 | 68 | 83.8 | 81 | 86.4 | 0.3264 |
| Contact[g] | 0.8627 | 0.35 | 0.9505 | 0.04 | 0.1577 | 45 | 86.7 | 97 | 91.8 | 0.1711 | 71 | 90.1 | 83 | 90.4 | 0.4761 |

[a] Calculated using Z-test.
[b] Number of coyotes exhibiting a particular mark.
[c] Rhodamine B staining of pelage, feet, or external surfaces of mouth.
[d] Rhodamine B staining of digestive tract.
[e] Tetracycline fluorescence in teeth.
[f] Rhodamine B staining of the digestive tract and/or tetracycline fluorescing in teeth.
[g] Rhodamine B staining of pelage, feet, external surfaces of mouth and/or tetracycline fluorescence in teeth and/or rhodamine staining of the digestive tract.

In this study the stimulus of a bait station as a visual cue did not influence the removal of baits by coyotes. Coyotes appear to rely on their olfactory sense more than vision to locate food items (Fagre et al., 1984).

Our data indicated that the presence of fire ants during summer limited the life of baits and deterred potential coyote removal. Fire ants would build a mound around a bait and encapsulate the bait within three days. Even though the bait stations with fire ants would be visited by coyotes, the bait would not be removed. Therefore, a bait distribution conducted during winter, which coincides with fire ant inactivity, would be most effective in achieving maximum coyote uptake rates.

We believe the baiting strategy of aerially-placed baits would be successful in delivering an oral vaccine to about 80% of free-ranging coyotes within 1 mo. This conclusion is based on the finding that after 3 wks of potential contact with baits, >80% of the coyotes collected on the study areas exhibited markings consistent with the biomarkers mixed with our bait.

Bait acceptance rates from our study concur with previous research (Linhart et al., 1968). Johnston et al. (1988) reported an 85% acceptance rate by coyotes to a 30-g meat-ball bait. Guthery et al. (1984) and Farry et al. (1998) reported that coyotes consume baits 83 to 98% of the time if a bait was encountered.

We were conservative in our approach toward biomarker assessment; therefore, bait acceptance rates by free-ranging coyotes were potentially higher. For example, a dye was present within the cyanide capsules of M-44 devices. Because the presence of this dye fluoresced under ultraviolet light similar to rhodamine B, the upper digestive tracts of coyotes collected by M-44 devices were re-assessed. To verify the extent of M-44 dye contamination, twenty coyotes from central Texas (i.e., away from the bait density assessment sites to avoid cross-contamination) were collected by M-44 devices. Each of the 20 animals exhibited dye in the mouth and esophagus, and 10% of the coyotes had the dye in their stomach. Therefore, to be identified as ingesting a bait, coyotes collected by M-44 must have possessed visible rhodamine B staining in the lower digestive tract, or other corroborating signs of ingestion such as bait material in the stomach. Coyotes obtained by M-44 devices that exhibited fluorescent staining solely in the mouth, esophagus, and stomach were regarded as questionable and recorded as negative with regards to bait acceptance. During the rhodamine B assessment of nails and growing hairs, it was noted that white-tipped hair or white-colored scratches on nails would appear as fluorescence under ultraviolet light. Rather than risk misidentification of the biomarker, claws and growing hairs were not used in bait acceptance estimates. In general, if rhodamine fluorescence was in question, then the animal was considered negative for bait ingestion and not included in our bait acceptance rates. Matson's Laboratory, which assessed the tetracycline biomarker, listed coyotes in one of four categories according to the degree of tetracycline fluorescence: positive, negative, questionable positive, and questionable negative. A rating of questionable positive was given if the biomarker was probably present in the tooth section but fluorescence was weak, and a rating of questionable negative was given if the tooth section had a very weak tetracycline-like fluorescence. Only positive ratings of tetracycline fluorescence were used in our bait acceptance estimates.

Prevalence of naturally occurring tetracycline-like fluorescence has not been evaluated in coyotes. However, tetracycline-like fluorescence was found to be in <1% of populations of red foxes, raccoons, and striped skunks (Nunan et al., 1994). The low occurrence of such marks supports the use of tetracycline as a marking agent in baiting trials (Nunan et al., 1994). Therefore, there was no reason to suspect the prevalence of coyotes that have naturally occurring tetracycline-like fluorescence to be greater than that documented for other omnivores. We also demonstrated that it was equally unlikely that coyotes could secondarily mark themselves with tetracycline hydrochloride or rhodamine B by consuming prey that had come in contact with baits.

The lower of the two bait density strategies was effective in attaining our *a priori* goal of >70% of a free-ranging coyote population consuming baits. For a vaccination program of wildlife to be economically feasible, one required factor is an acceptable cost for bait development and use (Linhart et al., 1991); hence, a low bait density. Our study determined the maximum bait density required to mark coyotes from southern Texas; however, a minimum bait density that achieves similar bait acceptance rates is needed. Also, we used an even distributional strategy of aerially-placed baits. Different distributional strategies could yield different bait acceptance rates.

The results of our study support the potential of delivering a vaccine-laden bait to free-ranging coyotes. However, the proportion of coyotes that require vaccination to halt the spread of this epidemic needs to be verified, and documentation of coyote seroconversion resulting from consumption of a vaccine-bait are necessary.

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