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## BAIT INGESTION BY FREE-RANGING RACCOONS AND NONTARGET SPECIES IN AN ORAL RABIES VACCINE FIELD TRIAL IN FLORIDA

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**ABSTRACT:** Oral rabies vaccine-laden baits, with a tetracycline biomarker, were distributed in Pinellas County (Florida, USA) by helicopter drop and from cars from January to April 1997. A total of 130,320 baits was distributed throughout the county, yielding an average bait density of 185 baits per km<sup>2</sup>. Bait ingestion was estimated by microscopic detection of tetracycline in tooth and bone samples from 244 raccoons (*Procyon lotor*), 33 opossums (*Didelphis virginianus*), 31 feral cats, and two gray foxes (*Urocyon cinereoargenteus*) that were trapped during February–April 1997. Active surveillance consisted of 17 trapping sites that were further categorized by six community descriptors. Passive surveillance consisted of animals that were collected as nuisance animals by Pinellas County Animal Services. The proportion of tetracycline positive raccoons was compared between collection techniques, among trapping sites, vegetation communities, and age and sex categories. Since there was no statistically significant difference in the frequency of tetracycline positive raccoons trapped during active surveillance (59%, 110/187) and passive surveillance (53%, 30/57), the data were pooled, resulting in a tetracycline positive frequency of 57% (140/244). The range in the positive tetracycline frequency established for raccoons from the 17 active surveillance sites was 9% (1/11) to 100% (3/3). The tetracycline positive frequency for raccoons ranged from 25% (3/12) at the dumpster sites to 78% (14/18) at the landfills. Juvenile male raccoons (71%, 34/48) were the most commonly marked age and sex class and adult females (42%, 21/50) were the least commonly marked age and sex class. Eighty-five percent (28/33) of the opossums, 3% (1/31) of the feral cats, and 50% (1/2) of the gray foxes were tetracycline positive.

**Key words:** Oral vaccine, *Procyon lotor*, rabies, raccoon, tetracycline, vaccinia recombinant virus.

### INTRODUCTION

Rabies is enzootic in raccoons (*Procyon lotor*) in the southeastern United States. The oral vaccination of raccoons against rabies has the potential to decrease the incidence of rabies and to reduce the risk of rabies to domestic animals and humans. It is generally accepted that between 60 to 70% of a population must be immune to rabies for the disease to disappear within a local population (Perry et al., 1989), although this has not specifically been tested for raccoons (Coyne et al., 1989). This immunity can be natural or vaccine induced.

A rabies vaccine, consisting of a vaccinia virus recombinant containing the rabies virus glycoprotein gene (V-RG), has been

shown to be an effective oral immunogen in raccoons (Rupprecht and Kieny, 1988) and has not caused rabies in any of the species tested (Rupprecht et al., 1992). The Raboral V-RG vaccine is currently being tested as a rabies control measure in several parts of the United States (Krebs et al., 1998; Fearneyhough et al., 1998; Roscoe et al., 1998; Robbins et al., 1998).

The vaccine is delivered to wild animal populations via vaccine-laden baits, distributed across the landscape. The proportion of free-ranging animals that consume the vaccine-laden baits can be estimated by examining tooth and bone samples for a tetracycline biomarker that is incorporated in the bait matrix. Fluorescent yellow bands of tetracycline appear in the

tooth and bone sections under ultraviolet illumination (Milch et al., 1957). Animals that have consumed the bait are marked with bands of tetracycline and animals that have not consumed the bait lack these bands, although discrepancies are known to occur (Hanlon et al., 1989; Hable et al., 1992).

Tetracycline has been used as a biomarker and incorporated in baits intended for free-ranging animals for a variety of purposes, including baits designed to carry antifertility agents to coyotes (*Canis latrans*) (Linhart and Kennelly, 1967), those designed to orally vaccinate feral pigs (*Sus scrofa*) against diseases like brucellosis and pseudorabies (Fletcher et al., 1990); and placebo baits designed to vaccinate mongooses (*Herpestes javanicus*) and coyotes against rabies (Creekmore et al., 1994; Farry et al., 1998). Tetracycline has been incorporated into rabies vaccine-laden baits since 1978 for red fox (*Vulpes vulpes*) vaccination trials in Europe (Wandeler, 1988), since 1984 in Canadian red fox oral vaccination trials (Bachmann et al., 1990), since 1990 in the United States raccoon oral vaccination trials (Hanlon et al., 1993), and since 1995 in coyote oral vaccination trials in Texas (Fearneyhough et al., 1998).

When injected intraperitoneally, tetracycline is incorporated into growing calciphilic tissues and can be seen in bones and teeth as soon as twelve hours. (Milch et al., 1957). In the field, tetracycline has been observed in the bones and teeth of raccoons as soon as two days after bait consumption (Hanlon et al., 1989). The tetracycline deposits may last for the life of the animal when incorporated into the cementum and dentin of permanent teeth (Johnston et al., 1987). In older animals, tetracycline is most often found in the mandible, rather than the tooth itself, because of the decreased bone deposition that is associated with an increase in age (Linhart and Kennelly, 1967). Alternatively, in young animals, tetracycline deposi-

tion in the bones may be lost due to bone remodeling (Johnston et al., 1987).

The results reported here are from the third year of a raccoon oral rabies vaccination intervention program in Pinellas County; managed by the Pinellas County Animal Services (PCAS). This study was conducted in order to determine the percentage of raccoons and select nontarget species that ingested the vaccine-laden baits both at individual trapping sites and throughout this highly urbanized county. Olson and Werner (1999) reported on bait contact by target and nontarget species in several land use zones and vegetation communities in Pinellas County.

## MATERIALS AND METHODS

### Vaccine-laden bait

The Raboral V-RG rabies vaccine is contained in a plastic sachet embedded within a rectangular 2" × 1" fishmeal polymer bait. Each sachet contains (2 ml) of live Vaccinia vector rabies vaccine, manufactured by Merial Limited (formerly Rhone Merieux, Inc., Athens, Georgia, USA). The tetracycline biomarker is incorporated within the fishmeal polymer bait, which is manufactured by Bait-tek Inc. (Beaumont, Texas, USA).

### Bait distribution

Baits were distributed from January to April 1997. Baits that were distributed over undeveloped areas were broadcast from helicopters flying 200 meter transects. In the more urbanized area, baits were either distributed from helicopters flying over vegetated corridors between housing developments or from trucks. A total of 130,320 baits was distributed throughout the county yielding an average of 185 baits per km<sup>2</sup>.

### Trapping sites and vegetation communities

Pinellas County is a highly urbanized 54.4 km long peninsula located on the west-central coast of Florida (USA: 27°50'N: 82°45'W). It has the highest population density (1158.3 people per km<sup>2</sup>) in the state and includes the city of St. Petersburg. By 1990, 81% of the county's 800 km<sup>2</sup> had been developed. An additional 65.3 km<sup>2</sup> were slated for development, leaving only 86.7 km<sup>2</sup> of undeveloped land, mainly consisting of environmentally sensitive areas or nature preserves (Pinellas County Planning Department, 1995).

TABLE 1. Active surveillance trapping locations, vegetation communities, and animals in Pinellas County (Florida, USA).

Locations	Raccoons	Opossums	Cats	Gray Foxes
<b>Parks</b>				
Fort Desoto Park <sup>a</sup>	20	—	—	—
Walsingham Park <sup>a</sup>	17	2	—	—
Weedon Island Preserve <sup>b</sup>	16	2	2	—
Sawgrass Lake Park	13	6	1	—
Lake Maggiore <sup>c,d</sup>	13	2	—	—
Honeymoon Island State Recreation Area <sup>e</sup>	12	—	1	—
War Veteran's Memorial Park <sup>b</sup>	11	—	—	—
Tiki Gardens <sup>b</sup>	9	—	—	—
Lake Seminole Park <sup>a</sup>	8 <sup>f</sup>	2	—	—
John Chestnut Sr. Park	8	1	—	—
Caladesi Island State Park <sup>e</sup>	4	—	—	—
<b>Non-Parks</b>				
Higgins Power Plant <sup>c</sup>	16	2	—	—
Bay Pines VAH <sup>a</sup>	11	—	—	—
Pinellas County Solid Waste <sup>g</sup>	10	4	—	—
Toytown Landfill <sup>g</sup>	8	8	—	—
Seafood Restaurant <sup>d</sup>	8	2	—	—
Highway Site <sup>c</sup>	3	2	—	—
Nuisance	57	—	27	2
<b>Total</b>	<b>244</b>	<b>33</b>	<b>31</b>	<b>2</b>

<sup>a</sup> Pine Forest.

<sup>b</sup> Mangrove Swamp.

<sup>c</sup> Mixed Deciduous Forest.

<sup>d</sup> Dumpsters.

<sup>e</sup> Barrier Island Community.

<sup>f</sup> Includes the one raccoon obtained by passive surveillance.

<sup>g</sup> Landfill.

Seventeen trapping sites were used as part of active surveillance: eleven parks or reserves, one sealed and one open landfill, a power plant, a county transportation storage yard, dumpsters behind a seafood restaurant, and a Veteran's hospital (Table 1). The frequency of tetracycline positive raccoons from these trapping sites was compared statistically to determine if there were differences in tetracycline uptake among raccoons from different trapping sites.

The seventeen trapping sites were pooled together by vegetation community descriptors: mangrove swamp, pine forest, mixed deciduous forest, and barrier island community. Although not actually vegetation communities, the two landfills and the two areas with dumpsters were pooled with the vegetation communities, giving a total of six community descriptors. The dominant species in mangrove swamps were red mangrove (*Rhizophora mangle*) and black mangrove (*Avicennia germinans*). Pine forests were dominated by slash pine (*Pinus elliotii*) with an understory primarily of saw palmetto (*Serenoa repens*). Mixed deciduous forests were dominated by red maples (*Acer rubrum*), live oaks

(*Quercus virginiana*), water oaks (*Q. nigra*), laurel oaks (*Q. hemisphaerica*), and cabbage palms (*Sabal palmetto*). Dominant plants in the barrier island community included beach elder (*Iva imbicata*), salt grass (*Distichlis spicata*), sea oats (*Uniola paniculata*), and an unidentified member of the goosefoot family, Chenopodiaceae. Brazilian pepper (*Schinus terebinthifolius*) and grasses (Poaceae) dominated the landfills.

#### Animal collection

Mesomammal trapping was conducted from 17 February 1997 to 30 April 1997. Tomahawk #108 traps (Tomahawk Live Trap Company, Tomahawk, Wisconsin, USA), baited with canned sardines, were set two to four weeks after the site had been baited with vaccine-laden baits. Traps were set in areas where raccoon use was evident so that captures were maximized at each site. Traps were usually set out singly, but multiple traps were set along the most heavily used animal trails. Traps were laid out and set between four and six P.M. and were

checked and collected between seven and nine the following morning. Raccoons, opossums (*Didelphis virginianus*), and cats were kept for the study. Raccoon population estimates were not quantified, however crude population abundance was described based on raccoon sign (tracks and scat).

#### Nuisance animals and land use zones

Nuisance raccoons, feral cats, and gray foxes (*Urocyon cinereoargenteus*) were included in the passive surveillance portion of the study. These animals were obtained opportunistically from the PCAS. They were trapped by members of the public and were either brought to the animal shelter or picked up as part of PCAS daily operations.

The nuisance raccoons were traced to the address where they were trapped to determine if there were differences in bait ingestion according to land use zones. These locations were then divided into four land use zones: single residential (SR), multiple residential (MR), industrial-commercial (IC), and undeveloped (UN). The SR zone consisted of areas that were primarily single family homes, excluding mobile home parks. The MR zone consisted of apartment complexes and mobile home parks. The IC zone consisted of commercial areas where houses were uncommon. The UN zone consisted of parks.

#### Animal processing

Animals were euthanized with 3–5 ml of sodium pentobarbital (Fatal Plus, Vortech Pharmaceuticals, Dearborn, Michigan USA), injected intraperitoneally. Raccoons, opossums, and gray foxes were euthanized upon arriving at PCAS and feral cats were held for 5 days before being euthanized. Animals were weighed and classified by sex and age. Raccoons were classified as adult, juvenile, or young of the year based on teat size (females) and penis extrusion (males) according to the methods of Sanderson (1961). Opossums were classified as adult or juvenile based on pelage and weight characteristics according to the methods of Seidensticker et al. (1987). Cats were classified as adults if they weighed 2 kg or more, and as juveniles if they weighed <2 kg (Latimer, 1936).

All euthanized animals were stored at –4 C. Frozen animals were decapitated and the head was cut in half longitudinally. A section containing the upper canine and maxilla was collected from each animal. These maxilla-canine samples were shipped frozen to the Rabies Section of the Viral and Rickettsial Zoonoses Branch, of the Centers for Disease Control and Prevention (CDC) (Atlanta, Georgia, USA).

#### Tetracycline

Samples were thawed to room temperature and then scraped of excess fur and flesh. A diamond, double-bladed Isomet low speed saw (Buehler Isomet, Techmet Scarborough, Ontario, Canada) was used for sectioning tooth and bone samples. Cleaned samples were placed in a chuck of the Isomet saw, with the cusp of the canine pointing down. A diagonal section beginning in the canine tooth and ending in the maxilla, was cut from each sample. The section of tooth and bone, approximately 400 micrometers thick, was mounted in Elvanol, on a glass slide, and covered with a cover slip. Slides were viewed with a Leitz ultraviolet illumination microscope (Johnston et al., 1987). Tetracycline deposits appeared as fluorescent yellow bands under the ultraviolet illumination. Each slide was read by two microscopists and contradictory results were reconciled by a third microscopist.

#### Statistical analyses

Tetracycline positive raccoons collected by the active and passive surveillance techniques were compared statistically with a Chi-square test in order to compare the sampling techniques. All Chi square tests were run with Minitab software (Minitab, Inc., 1996) with 0.05 as the level of significance.

Bait ingestion, measured by tetracycline, for raccoons from the 17 trapping sites were compared using the Chi-square test to test for a site effect.

Among the pooled vegetation communities of the trapping sites, bait ingestion by raccoons was analyzed with a Chi-square test. Raccoons that were trapped at Sawgrass Lake Park, John Chestnut Sr. Park, and thirteen raccoons from Higgins Power Plant were omitted from this analysis, because the actual vegetation community where individual raccoons were trapped was not recorded.

Bait ingestion among raccoon age and sex categories were compared using a Chi-square test. Young-of-year males were omitted from the analysis because of the small sample size (only three individuals). Similarly, eight raccoons that had not been assigned an age or sex category were omitted from the analyses.

## RESULTS

Tooth and maxilla sections from 244 raccoons, 33 opossums, 31 cats, and two gray foxes were examined for tetracycline deposits (Table 1). A total of 187 raccoons, 33 opossums, and four cats were trapped

TABLE 2. Tetracycline positive raccoons and trapping locations in Pinellas County (Florida, USA).

Location	Number of raccoons examined	Number of tetracycline positive raccoons	Percentage of tetracycline positive raccoons
Highway Site	3	3	100%
Toytown Landfill	8	7	88%
Sawgrass Lake Park	13	11	85%
Weedon Island Preserve	16	13	81%
Honeymoon Island State Recreation Area	12	9	75%
Pinellas County Solid Waste	10	7	70%
Walsingham Park	17	11	65%
Fort De Soto Park	20	13	65%
John Chestnut Sr. Park	8	5	63%
Nuisance	57	30	53%
Caladesi Island State Park	4	2	50%
Higgins Power Plant	16	8	50%
Lake Maggiore	13	6	46%
Bay Pines V.A.H.	11	5	45%
Tiki Gardens	9	4	44%
Lake Seminole Park	8	3	38%
Seafood Restaurant	8	2	25%
War Veterans' Memorial Park	11	1	9%
Total	244	140 (57%)	57%

as part of active surveillance and 57 raccoons, 27 cats, and two gray foxes were obtained as part of the passive surveillance. Raccoons from Lake Seminole Park included seven that were trapped as part of the active surveillance and a single raccoon obtained as a nuisance animal.

Thirty-seven of the 57 nuisance raccoons were trapped in the SR land use zone. The majority of these raccoons (25) were trapped from different addresses. In MR zones, eight nuisance raccoons were trapped from different addresses. In IC zones, six nuisance raccoons were trapped. Only one nuisance raccoon was trapped in the UN zone. Five raccoons were not placed into land use zones because of incomplete or incorrect addresses.

Overall, 57% (140/244) of the raccoons from Pinellas County were positive for tetracycline. There was no statistically significant difference between tetracycline-positive raccoons that were trapped during active surveillance (59% 110/187), and the raccoons obtained by passive surveillance (53% 30/57) ( $\chi^2 = 0.69$ ,  $P = 0.41$ ,  $df = 1$ ).

Among the raccoons trapped for active

surveillance, bait ingestion varied across a range of 9–100% among the trapping sites. There were statistically significant differences in tetracycline frequencies among trapping sites ( $\chi^2 = 32.58$ ,  $P = 0.01$ ,  $df = 16$ ) (Table 2).

Among the vegetation communities, tetracycline positive raccoons ranged from 25% (3/12) from the dumpsters to 78% (14/18) at the two landfills, but these differences in frequency were not statistically significant ( $\chi^2 = 9.42$ ,  $P = 0.09$ ,  $df = 5$ ) (Table 3). The percentage of tetracycline positive raccoons from the other four vegetation communities was between 53% and 69%.

Within land use zones, 51% (19/37) of the raccoons in the passive surveillance from the SR zone were tetracycline positive, as were 62% (5/8) from the MR zone, 33% (2/6) from the IC zone, and 100% (1/1) from the UN zone. Three of the five raccoons that were not traced to an address were positive for tetracycline (Table 4).

Tetracycline positive raccoons included 71% (34/48) of the juvenile males, 63%

TABLE 3. Tetracycline positive raccoons from the six vegetation communities in Pinellas County (Florida, USA).

Vegetation community	Number of raccoons examined	Number of tetracycline positive raccoons	Percentage of tetracycline positive raccoons
Landfills	18	14	78%
Barrier Island	16	11	69%
Pine Forest	56	32	57%
Deciduous Forest	15	8	53%
Mangrove Swamp	36	19	53%
Dumpster Sites	12	3	25%
Total <sup>a</sup>	153	87	57%

<sup>a</sup> Raccoons from Sawgrass Lake Park, John Chestnut Sr. Park, and 13 raccoons from Higgins Power Plant were not included in this analysis because the vegetation community from which they were trapped was not recorded.

(37/59) of the juvenile females, 59% (45/76) of the adult males, 42% (21/50) adult females, and none (0/3) of the young of the year. The differences in frequency of tetracycline among the four age and sex categories were statistically significant ( $\chi^2 = 9.07$ ,  $P = 0.028$ ,  $df = 3$ ).

A total of 28/33 (85%) of the opossums, 1/31 (3%) of the cats, and 1/2 (50%) of the gray foxes was tetracycline positive. Tetracycline positive opossums included 1/1 (100%) juvenile male, 10/11 (91%) adult males, and 17/21 (81%) adult females.

#### DISCUSSION

Fifty-seven percent of the sampled raccoons were tetracycline positive, which is within the ranges that have been previously reported in placebo trials and the two published vaccine trials (Rupprecht et al., 1987; Perry et al., 1989; Hanlon et al.,

1989; Hable et al., 1992; Hanlon et al., 1993, 1998; Roscoe et al., 1998).

Potential explanations for the differences in the percentage of tetracycline positive raccoons among sites include differences in sample size, bait availability, and raccoon populations. For example, the number of raccoons sampled from the Highway Site and Toytown Landfill was low. Only three raccoons from the Highway Site were examined for tetracycline, so the 100% positive frequency could have been due to sampling chance. At Toytown Landfill, raccoon abundance, based on raccoon sign (Olson, 1998) was extremely low, so individual raccoons were more likely to come into contact with baits, resulting in a high percentage of tetracycline positive animals. Conversely, at War Veterans' Memorial Park and the seafood restaurant, raccoon abundance, based on raccoon

TABLE 4. Tetracycline-positive raccoons and passive surveillance land use zones in Pinellas County (Florida, USA).

Land use zone	Number of raccoons examined	Number of tetracycline positive raccoons	Percentage of tetracycline positive raccoons
Undeveloped	1	1	100%
Multiple Residential	8	5	62%
Zone Unknown	5	3	60%
Single Residential	37	19	51%
Industrial-Commercial	6	2	33%
Total	57	30	53%

signs, was high. Raccoons from Tiki Gardens and the Veteran's hospital, the sites with the lowest percentage of tetracycline positive raccoons, were trapped as nuisance animals, so the populations were probably fairly high. Individual raccoons from the areas with high raccoon abundance would likely have less of an opportunity to encounter a bait, based on the sheer number of animals and a limited number of baits. At the remaining locations, raccoon abundance, based on sign, was neither exceptionally low nor high.

The low proportion of tetracycline positive raccoons from the dumpster sites most likely reflects a dense population that is foraging in a limited feeding area. The raccoons that frequent the dumpsters probably spend most of their foraging time within and around the dumpsters, and have less of an opportunity to come into contact with the vaccine-laden baits. Conversely, the low population density, based on raccoon signs is reflected in the high proportion of tetracycline positive raccoons from the landfills.

The third highest proportion of tetracycline positive raccoons was from the pine forests, which are not known to support high raccoon densities (Stuewer, 1943). In contrast, the lowest percentages of tetracycline positive raccoons were from the mangrove swamp and deciduous forest, all of which bordered on lakes or ponds. Mangrove swamps and other wetlands generally support high densities of raccoons (Leberg and Kennedy, 1988). An exception to this general pattern is the barrier islands, (Caladesi Island State Park and Honeymoon Island State Recreation Area) which had the second highest proportion of tetracycline positive raccoons. The two barrier islands had relatively high raccoon abundance, based on signs, and the second highest percentage of tetracycline positive raccoons.

Only 51% (19/37) of the nuisance raccoons from the SR land use zone were tetracycline positive. This result is important given that animals in residential zones are

likely to come in contact with humans and their companion animals. The small sample sizes from the MR, IC, and UN zones (8, 6, and 1 respectively), prevented any countywide extrapolation.

Male raccoons usually have larger home ranges than females (Gehrt and Fritzell, 1997). This larger home range allows greater opportunity to encounter vaccine-laden baits. For example, the average home range of suburban raccoons in Ohio was largest for adult males, and was followed by yearling (juvenile in this study) males, yearling females, and lastly, adult females (Hoffmann and Gottschang, 1977). Adult females with young generally have the smallest home range (Kaufmann, 1982) and so are the least likely to encounter vaccine-laden baits.

In this study, juvenile males consumed the bait most often, followed by juvenile females, adult males, and lastly adult females, which was statistically significant. Differences in the marking ability of tetracycline according to age and movement patterns according to age and sex may further influence these differences. The deposition of tetracycline in teeth and bones can partially explain the differences in the age classes. Animals with growing teeth and bones are most easily marked with tetracycline. Although the tetracycline can be remodeled in young animals (Johnston et al., 1987), trapping at each site was conducted two to four weeks post baiting, so remodeling is unlikely. The adults are not as likely to be marked with the biomarker (Linhart and Kennelly, 1967; Taylor and Lee, 1994). However, these results are interesting since it might be expected that at least some of the adults may have contacted vaccine-laden baits, and have been marked, during the two previous years of PCAS baiting.

Forty-two percent of the adult female raccoons were tetracycline positive. Low bait uptake by females may be partially due to the smaller home range of females during pregnancy and parturition when they decrease the size of their home range



(Gehrt and Fritzell, 1997). These results suggest that baits may have to be applied at greater densities to reach the breeding females.

Of the three nontarget species (opossum, cat, gray fox) examined for tetracycline deposits, the opossum was the species that most often consumed the vaccine-laden bait, which was also reflected in opossum contacts with baited tracking plates (Olson and Werner, 1999). In this study, 85% of the sampled opossums were tetracycline positive. Hable et al. (1992) found similar results; 80% (4/5), and 50% (1/2) of the opossums sampled from Murphy Island (South Carolina, USA) were tetracycline positive. Only one of the 31 cats was tetracycline positive, suggesting that cats do not consume a large number of the vaccine-laden baits. However, in New Jersey (USA) domestic cats were major competitors for the baits (Roscoe et al., 1998). One of two gray foxes was positive for tetracycline, but the small sample size does not permit interpretation.

Direct comparisons among studies are difficult because of differences in bait formulation, distribution and bait density, and bone sample differences. Bait density within this study was not uniform. Baits that were distributed over undeveloped land were broadcast from a helicopter, whereas in the more urbanized areas, the baits were distributed from trucks or other vehicles. This unavoidable bait distribution pattern does not allow accurate bait density to be determined within specific areas. Additionally, comparisons are limited since in most other studies, tetracycline was most often examined in the lower canine and the attached mandible, whereas in this study, the upper canine and the attached maxilla were used.

Animals may receive tetracycline from sources other than tetracycline treated baits, but it is thought to be very low. In a study in Ontario (Canada), the number of animals that received tetracycline from the environment was only (6/744, 0.8%) for raccoons, (5/3406, 0.2%) for red foxes,

and (5/1103, 0.4%) for striped skunks (*Mephitis mephitis*) (Nunan et al., 1994). The animals that were tetracycline positive were believed to have eaten afterbirths, from cows which had been treated with tetracycline. No such study exists for Pinellas County; however there are few farms in Pinellas County, so the animals most likely acquired the tetracycline from the vaccine-laden baits.

Future biomarkers should be incorporated into the sachet with the vaccine instead of into the bait matrix. The present bait and vaccine configuration allows the proportion of animals that ingest the bait to be determined, but not the proportion of animals that actually ingest the vaccine. This subtlety is especially important when the target animal is as dexterous as the raccoon, and able to consume the bait matrix, but reject the vaccine chamber (Olson and Werner, 1999).

Research on raccoon ecology, especially in urban and suburban landscapes must continue. Basic information on urban raccoon population interactions is required in order to design the most effective baiting strategies. Information on home range size, distances traveled, preferred urban habitats must be determined. Baiting strategies in oral rabies vaccination programs will be strengthened if the basic ecology of the target species is better understood.

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