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ABSTRACT: The majority of rabies cases reported to the Centers for Disease Control each year occur in wildlife including skunks, raccoons, bats, foxes, and coyotes. Currently, oral rabies vaccination campaigns are employed to immunize coyotes, foxes, and raccoons. Though skunks are vectors of 6 rabies strains, there is currently no effective oral vaccine or delivery system for skunks. More information is needed to determine if baits currently used are sufficiently attractive to skunks, or if the baits are difficult for skunks to handle and consume. We observed bait manipulation by skunks in penned feeding trials to determine the bait type most conducive to ingestion and delivery of a mock vaccine to skunks. Smaller baits were easier for skunks to manipulate and consume, and vaccine containers coated with bait facilitated sachet puncture and increased the potential for vaccine delivery. Our information will be useful in the development of baits and vaccine containers for large-scale rabies vaccination campaigns that target skunks.

KEY WORDS: bait, coyote, foxes, *Mephitis mephitis*, ORV, rabies vaccine, raccoon, skunk, wildlife damage management

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INTRODUCTION

Oral rabies vaccination first became a disease management option after Correa-Giron et al. (1970) found that, when ingested, attenuated rabies virus invaded primarily through oropharyngeal mucosa and resulted in vaccination. Subsequently, the large-scale application of Oral Rabies Vaccine (ORV)-laden baits has become the favored method for combating wildlife rabies in North America and Europe. Intensive ORV baiting campaigns drop millions of baits annually in the United States and Canada and have been effective in containing and locally eliminating certain rabies strains in North America (Linhart et al. 2002).

To eliminate rabies in a local area, an estimated 60% to 80% of the vector population must be vaccinated to sufficiently reduce rabies transmission from infected to susceptible animals within and among species (Tierkal 1975, Voigt et al. 1985, Linhart et al. 2002). To control rabies in wildlife populations, researchers continually work to make ORV baiting and vaccine delivery more effective and efficient. Many factors combine to make a baiting system an effective tool. Primarily, the bait must facilitate vaccine delivery to the oropharyngeal mucosa of the target animal. To achieve this an ideal bait must 1) attract and be consumed by the target species, 2) elicit a chewing response to adequately rupture the vaccine container, 3) withstand the impact of aerial distribution, 4) protect the vaccine from solar radiation and other environmental elements, and 5) be cost effective for the purchasing/dispersing agency (Wandeler 1988, Linhart et al. 2002, Farry et al. 1998b).

The role of bait manipulation in effective vaccine delivery has received little attention (Farry et al. 1998a, Hable et al. 1992, Linhart et al. 1991). Tracking stations and biomarkers have been used to provide information about bait uptake by target and non-target species (Farry et al. 1998b, Linhart et al. 1993, Linhart et al. 1994, Linhart et al. 2002). While this information is useful in determining bait preference and proportion of baits accepted, it does not describe the fate of a bait or the vaccine container– whether it was cached, discarded, or partially or fully ingested. Knowledge of the target species' ability to manipulate baits and ingest vaccine is needed (Steelman et al. 1998). A bait that is difficult to handle, or allows an animal to separate the vaccine container from the bait matrix, may not be effective regardless of how attractive the bait. Information on the bait manipulation and ingestion behavior of species targeted in ORV campaigns (coyotes, *Canis latrans*; foxes, *Vulpes vulpes* and *Urocyon cinereoargenteus*; and raccoons, *Procyon lotor*) has been used to make ORV baits more effective and to optimize bait distribution strategies for the target species. Though striped skunks (*Mephitis mephitis*) are also vectors of rabies, there is currently no effective oral vaccine or proven mode of delivery of ORV to skunks. To target skunks, information on their preference for baits and bait manipulation abilities and behaviors is needed.

Knowledge of effective baiting systems for other species may help streamline the development of ORV baiting strategies for skunks. We review the importance of bait manipulation in the successful conveyance of vaccine to coyotes, foxes, and raccoons in current ORV campaigns. We present observations of the handling of ORV baits targeted to other species by striped skunks.

MANIPULATION OF BAITS BY SPECIES TARGETED BY ORV CAMPAIGNS

Oral rabies vaccine baits deliver a liquid vaccine dose in a container (sachet or blister pack) concealed within an attractive, edible matrix. To orally immunize an animal, the vaccine must contact and be absorbed through the oropharyngeal mucosa. Digestion of the bait or vaccine without mucosa contact is not sufficient for immunization. Bait manipulation may be a key factor in ensuring the vaccine is ingested and absorbed. Factors affecting bait manipulation include flavor, texture, size, shape, and vaccine container type and position within the bait.

Coyotes

For coyotes, the chewing response is key to vaccine delivery. Coyotes take an entire bait into their mouths for mastication and may gulp or swallow a bait that is too small or does not stimulate adequate chewing (Linhart et al. 1994). Farry et al. (1998a) found that the number of times a coyote chewed a bait affected the degree of sachet puncturing and vaccine release. It is suggested that both hard bait matrixes (Farry et al. 1998a) and sugar coating (Steelman et al. 1998) can increase the chewing response in canids. Farry et al. (1998a) used Rhodamine B, a short-term topical biomarker, to assess vaccine delivery by polymer baits to coyotes and found all specimens stained on the tongue, upper palate, oropharyngeal region, and esophagus illustrating adequate vaccine-membrane contact for vaccination. Oral rabies vaccination campaigns in the United States successfully employ large, hard-shelled polymer baits; for example, the canine strain of rabies was eliminated from coyotes in southern Texas (Fearneyhough et al. 1998).

Foxes

Foxes are less likely to enclose a whole bait in their mouths during consumption. For foxes and other smaller species, the shape and ease of handling of baits becomes more important. Steelman et al. (1998) observed that even though gray foxes showed equal preference for fishmeal polymer baits and marshmallow wax cake baits, only the wax cakes elicited a manipulation response conducive to vaccine delivery. Wax cake baits were chewy and stuck to the teeth and gums, stimulating grey foxes to tilt their heads back to chew and dislodge waxy pieces, thus directing the liquid over the oropharyngeal region (Steelman et al. 1998). Hard polymer baits, which crumbled as eaten, stimulated foxes to incline their heads downward, allowing pieces of the bait and much of the liquid to fall from their mouths. Steelman et al. (1998) used rhodamine B to assess vaccine delivery of these polymer baits to gray foxes. They determined that most sachet puncturing occurred as the bait was broken apart, so that the liquid was absorbed by the bait matrix and then ingested without being absorbed by the oropharyngeal mucosa. Thus, while 45% of the animals had discolored feces from digesting the mock vaccine, none showed any staining of the oral region. In this case, manipulation rendered even attractive baits less effective. Winkler and Baer (1976) found that red foxes also broke apart hard-shelled baits and dropped pieces during consumption, sometimes separating the vaccine container. Through the use of wax cake style baits, ORV campaigns in Canada have eliminated fox rabies in some metropolitan areas (Rosatte et al. 1992)

Raccoons

Raccoons are more dexterous than canids; and thus, their ability to handle baits is an important factor in effective oral vaccine delivery. Raccoons use their paws to hold and manipulate baits and vaccine containers. Raccoons may use their manual dexterity and tactile sensitivity to select food portions of a bait and reject synthetic vaccine containers. Hable et al. (1992) reported that unpunctured sachets were discarded commonly. Linhart et al. (2002) found 30% of sachets separated from fishmeal polymer baits and still intact after raccoons ate the bait. They demonstrated that sachets coated directly with bait were equally attractive as polymer baits to raccoons and prevented the separation of the sachet from the food portion of the bait. A greater percentage of flavor-coated sachets was ruptured and emptied of vaccine than sachets from polymer baits, suggesting that direct flavoring of the sachet may increase the efficiency of vaccine delivery. Despite some sachet rejection, fishmeal polymer baits have been used successfully to maintain a barrier to the spread of raccoon rabies in the eastern United States (Slate et al. 2003).

MANIPULATION OF BAITS BY STRIPED SKUNKS

Bait preference and manipulation by skunks has not been addressed previously. Without an effective vaccine and method of delivery for skunks, the containment and elimination of many rabies strains remains challenging (Slate et al. 2003). Skunk rabies has the broadest geographical distribution of all terrestrial rabies strains in the United States (Krebs et al. 1995). Skunks are also susceptible to raccoon, fox, and bat strains of rabies, making skunks a problematic part of the maintenance cycle of numerous rabies strains across the country (Krebs et al. 2002). Raboral VR-G, the only oral rabies vaccine approved for use in the United States, does not effectively immunize skunks (Tolson et al. 1987). The ORV technique is still in the vaccine-development stage for skunks. Concurrent development of a bait that is sufficiently attractive to skunks would facilitate delivery once a vaccine is developed. Skunks are incidental non-target consumers of fox, raccoon, and coyote baits (Roscoe et al. 1998, Bachmann et al. 1990) which suggests the potential for vaccination of skunks via ORV-laden baits.

Methods

In trials with penned skunks, we evaluated a variety of bait flavors and shapes to assess the preference and bait manipulation abilities of striped skunks. We used currently available, mass-produced ORV baits; rectangular and cylindrical polymer baits (Bait Tek, Orange, TX), Ontario slims (Artemis Technologies, Inc., Guelph, ON, Canada), and flavor-coated sachets (Merial, Athens, GA). Feeding behaviors and bait manipulation of penned skunks were observed using video recordings to collect data without disturbing or influencing behavior. We determined bait preference by recording the order in which baits were examined (sniffed or touched) and the order in which baits were selected (chewed or consumed). We characterized bait manipulation by recording the time spent on each bait and manipulation activity (handling, chewing, and maneuvering). We also recorded the fate of vaccine containers (punctured, discarded, consumed, and liquid spillage).

Results and Discussion

Skunks examined all baits offered, indicating an initial attraction to each of the candidate baits. A greater preference was shown for meat flavors; Jojola et al. (2004) provide more information on bait preference. Manipulation of baits was not determined by flavor, but depended primarily on size, shape, and texture. Ontario slims were held on end and bitten from the corners with the teeth penetrating the blister pack from the top and bottom of the thin bait. The blister packs were not under pressure, and most of the liquid dripped out of puncture holes onto the pen floor. The waxy texture appeared to make chewing difficult for skunks. The bait would often stick to skunks' teeth or the roof of the mouth, stimulating them to gag and spit out pieces of the bait, sometimes leading to rejection of bait.

Skunks held polymer baits (rectangular and cylindrical) open end up and ate them from the outer edges. The bait matrix was chewed and often separated from the inner sachet. Due to the thickness of the bait wall and breakage of rectangular polymers, sachet contact was minimal and seldom at an angle to facilitate the delivery of liquid to the throat. The smaller circumference and smooth shape of cylindrical polymer baits encouraged skunks to insert the entire end into their mouths and bite through the cylinder. The degree of insertion and the thinner bait wall allowed more contact with the sachet. Sachets were punctured as the skunks bit into the outer matrix. As sachets were packed tightly into the hollow baits, they were pressurized, which aided vaccine delivery.

Skunks picked up flavor-coated sachets in the forepaws and, holding them vertically, bit into the end of the bait and punctured the sachet, often biting off the end. Sachets were either chewed thoroughly before discarding or the entire sachet was chewed and swallowed. The insertion of the sachet into the mouth directed liquid into the oral cavity.

Skunks have the ability and inclination to consume flavorful bait materials while selecting against synthetic vaccine containers. The direct flavoring of the vaccine container made it more difficult for them to separate the container from the bait matrix. The small size of flavor-coated sachets encouraged the animals to take the entire sachet into their mouths for mastication, which maximized sachet puncturing and achieved angles more conducive to vaccine delivery than baits with thicker matrix walls.

CONCLUSIONS

The manipulation of ORV baits by skunks sheds light on their low uptake of baits designed for other species. A primary reason is the size of baits. Skunks have significantly smaller body mass, jaw size, and oral cavity size than other species targeted by currently available ORV baits. Large baits, such as the polymer baits (the type most common in current U.S. baiting campaigns), were difficult for skunks to manipulate for ingestion. Except for the flavor-coated sachet, all trial baits were too large to be fully inserted into a skunk's mouth and chewed. Instead, baits were held on the ground and chewed, and often broken apart. Even with several tooth punctures in the sachet, this angle allowed much liquid to be spilled rather than being directed toward the oropharyngeal mucosa.

Comparing the bait handling and uptake of different species illustrates the importance of variety and adaptability in ORV delivery systems to accommodate species-specific behaviors and preferences. Many factors affect the attractiveness and delivery potential of a bait. Key factors to consider are bait flavor, texture, size, shape, and vaccine container type and position within the bait. A bait that allows the vaccine container to be swallowed unpunctured or spilled cannot deliver an effective vaccine dose. For coyotes, large size and hard texture seem to ensure the most thorough chewing response (Farry et al. 1998a). Texture responses can vary by species, as seen with foxes where hard textures minimized vaccine contact and waxy baits elicited a more effective chewing response (Steelman et al. 1998). With more dexterous animals such as raccoons and skunks, it is important that the sachet be difficult to separate from the bait matrix. Size and shape may also be imperative to the bait being held and eaten in a manner to deliver the vaccine to the target membranes. Future ORV baits can be made more effective by tailoring baits to species-specific preferences and handling behaviors to increase target uptake and improve vaccine delivery. Development of ORV baits for skunks should consider small size and direct sachet coating to optimize vaccination potential.

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