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Oral Rabies Vaccine (ORV) Bait Uptake by Striped Skunks: Preliminary Results

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ABSTRACT: Aerial delivery of rabies vaccine-laden bait is effective and efficient for large-scale vaccination of wildlife. Oral rabies vaccine (ORV) contained in a sachet (or blister pack) inside baits that serve as the mode of delivery currently are used for orally immunizing foxes, raccoons, and coyotes. The technique remains in the vaccine-development stage for oral immunization of skunks. Since skunks are a major vector of the rabies virus, concurrent development of a bait that is sufficiently attractive to skunks would facilitate an immediate mode of delivery once a vaccine is developed. We ran a palatability experiment with different shapes and flavors of baits to assess uptake by captive skunks. The flavors most preferred were fish and chicken. We also evaluated the fate of the sachet (punctured or not) inside baits, which would assist in assessing the delivery of a vaccine dose. On average, cylindrical-shaped baits had a higher percentage of punctured sachets than did rectangular-shaped baits, and baits with their matrix directly coated onto the sachet had a higher percentage of punctured sachets than did those baits in which the sachet was "held." We also used sulfadimethoxine, a short-term quantifiable biomarker, as a mock vaccine inside sachets in an attempt to quantify the amount of liquid ingested by skunks after consuming baits of different shape and size. While this information could have been useful for assessing the amount of vaccine delivered via sachet puncture, it could not be determined due to an aversive tasting biomarker. For effective ORV bait uptake by skunks, modifications to current baits should include a smaller size and a meat flavor matrix that is directly coated onto the sachet.

KEY WORDS: bait consumption, *Mephitis mephitis*, oral rabies vaccine, ORV, palatability, rabies, skunk, uptake, vaccine

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INTRODUCTION

More than 90% of rabies cases reported to the Centers for Disease Control and Prevention (CDC) each year occur in wildlife (Krebs et al. 2000, CDC 2001). The majority of these cases occurs in skunks (primarily *Mephitis mephitis*), raccoons (*Procyon lotor*), and bats (Order Chiroptera). Other host wildlife species of the virus include gray fox (*Urocyon cinereoargenteus*), red fox (*Vulpes vulpes*), and coyotes (*Canis latrans*). Methods to effectively control rabies in these and other species are in continual refinement.

An effective and efficient method for large-scale rabies control of wildlife is aerial delivery of vaccine-laden baits (e.g., Fearneyhough et al. 1998, Johnston and Voigt 1982, Johnston et al. 1988, Rosatte et al. 1998, Steck et al. 1982). The baits serve as the mode of delivery for the vaccine that is contained in a sachet (or blister pack) inside the baits. Vaccination of an animal is dependent upon puncture of the sachet and contact of a sufficient dose to the tonsils. Current oral rabies vaccine (ORV) campaigns are in place for foxes, raccoons, and coyotes (Esposito et al. 1988, Fearneyhough et al. 1998, Hanlon et al. 1989, Olson et al. 2000, Rupprecht et al. 1989, Wandeler 1991), but the method remains in the vaccine-development stage for immunization of skunks (Charlton et al. 1992, Tolson et al. 1987).

Skunks are especially susceptible to rabies, which makes the on-going efforts to develop a vaccine all the more imperative. There are 3 strains of skunk rabies and 3 other strains (fox, bat, and raccoon) for which skunks serve as a host. The increasing number of skunks infected with the raccoon variant of rabies is problematic in that this trend raises concerns about an independent maintenance cycle for raccoon rabies in skunks (Guerra et al. 2003). In areas where skunk rabies has become an immediate threat, trap-vaccinate-release (TVR) programs have been effective in reducing its spread. The TVR program involves live-trapping skunks, delivering an intramuscular injection of the rabies vaccine (different from an oral vaccine), and release (Rosatte et al. 1987, Rosatte and MacInnes 1987, Rosatte et al. 1990*a*). The TVR method of rabies control is effective for managing sudden outbreaks in relatively small areas (cities or towns), but it is labor-intensive and costly. Thus, the need persists for an oral rabies vaccine for skunks and a bait to effectively deliver the vaccine, similar to those used for canines and raccoons.

There is limited literature on skunks as nontarget consumers of ORV baits in raccoon and coyote ORV campaigns (e.g., Bachmann et al. 1990, Rosatte et al. 1990*b*). That skunks occasionally will chew on baits intended for other species indicates the potential exists for baiting skunks with ORV in the field. Low uptake by skunks may be an indication that current baits are not suitable for skunks and that species-specific modifications to the bait are necessary. Development of a bait for skunks that considers both flavor and shape will be critical. Enhancing a bait's attractiveness and palatability may increase uptake, which in turn may increase the likelihood of vaccination via puncture of the sachet. However, if a bait is sufficiently palatable to skunks, its shape may hinder vaccine delivery due to skunks' physical characteristics (e.g., small jaws) that make processing more difficult. Additionally, different shapes of bait may facilitate sachet puncture more readily, or may deliver a higher dose of vaccine than others. So, how skunks process or manipulate various shapes of bait is valuable information in development efforts to optimize the potential for vaccine delivery.

Rabies immunization is critically dependent on puncture of the sachet and delivery of a sufficient vaccine dose. Most ORV baits contain a biomarker (typically tetracycline hydrochloride) in the bait matrix that can be used as an index of an animal's exposure to the ORV. However, the disadvantage of this index is that uptake of the bait does not necessarily indicate exposure to the vaccine. It is possible that sachets within baits could be discarded without puncture, or that the sachet is punctured but due to shape or placement within the bait it does not deliver a sufficient dose of vaccine to the oral cavity. In either case, an ORV campaign that observes high bait uptake by the target species does not necessarily imply a high vaccination rate. Currently, vaccination can only be verified by collecting blood samples and verifying titer levels, which can be extremely time consuming and costly for large-scale projects. More specific information is needed to assess the dose delivered by sachet punctures inside ORV baits.

Although an ORV for skunks does not yet exist, an effective bait and sachet will be required once one is developed to expedite large-scale campaigns for the control of skunk rabies. In this study, we assessed sachet fate in relation to bait shape and flavor. Our information will assist in the development of an ORV bait that will enhance palatability to skunks, which may increase uptake by skunks, thereby increasing vaccination potential. We also assessed the potential dose delivered by using a quantifiable biomarker as a mock vaccine, but we terminated the trial soon after inception due to an aversive-tasting biomarker. The biomarker data were intended to be used as an indication of approximately how much liquid is delivered by a sachet puncture, which would be useful for determining bait dispersal densities in the field.

METHODS

Experiment 1: Sachet Fate Relative to Bait Shape and Flavor

The purpose of this experiment was to assess the puncture rate of placebo (distilled water) sachets presented in different shapes and flavors of baits. Sachet puncture (yes or no) was determined only for those baits that were qualified as consumed. A consumed bait was one in which at least half the mass of the bait was eaten (pre-test bait mass – post-test bait mass = mass consumed).

We ran 4 cafeteria-style palatability trials with baits from 3 different bait companies (Bait-Tek, Orange, TX; Artemis Technologies, Inc., Guelph, Ontario, CAN; Merial, Athens, GA). Each trial consisted of 5 test days. The same suite of baits was offered for 2 hours during each of the 5 test days. Bait arrangement was rotated daily. The sample size was 24 skunks, adult and young of the year, male and female.

Trial 1 presented meal polymer baits (Bait-Tek) to skunks. The baits included cylindrical-shaped alligator bait and chicken meal flavors, and rectangular-shaped fish meal and cat food flavors. Trial 2 presented these same flavors but alternated shape (i.e., cylindrical-shaped fish meal and cat food flavors, and rectangular-shaped alligator bait and chicken meal flavors). Trial 3 presented only fish-flavored baits from each of the bait companies: cylindrical and rectangular polymer fish meal baits, a fish crumble-coated sachet (Merial), and a vegetable shortening-based seafood Ontario slim (Artemis Technologies, Inc.). Trial 4 presented 5 flavors of the Ontario slim bait: seafood, sugar, chicken stew, apple, and cherry.

Experiment 2: Assess the Potential Vaccine Dose with a Mock Vaccine

The purpose of this experiment was to assess the volume of liquid that is delivered orally by the sachet contained inside baits, and whether bait shape influenced the volume delivered. We used a short-term, quantifiable liquid biomarker, sulfadimethoxine (SDM), as a mock vaccine to quantify the dose delivered by punctured sachets. Sulfadimethoxine is detected in sera and can be analyzed using an enzyme-linked immunosorbent assay (ELISA). A concentration of 80 mg SDM/ml of distilled water was manually injected into empty sachets and resealed. The treatment sachets were offered to skunks and serum samples were collected on days 1, 3, and 6 post-consumption.

RESULTS

Experiment 1: Sachet Fate Relative to Bait Shape and Flavor

The puncture rates of sachets relative to shape (cylindrical versus rectangular) were comparable among the meal polymer baits. On average, 91% of cylindrical baits and 84% of rectangular baits that were consumed had punctured sachets. Although this difference seems slight, cylindrical baits would optimize vaccination potential in an ORV campaign.

The fish and chicken flavors of the meal polymer baits were preferred (70 - 73%, respectively) over alligator and cat food (26 - 29%, respectively). Puncture rates of sachets relative to flavor were also comparable. On average, 93% of fish baits, 83% of alligator baits, 85% of cat food baits, and 90% of chicken baits that were consumed had punctured sachets. Following these trials, it appears that a fish or chicken flavor of cylindrical shape might have ideal uptake in the field and a relatively high rate of sachet punctures.

The fish crumble-coated sachet comes in one shape, so we did not test for a difference in puncture rates based on shape. The puncture rate of consumed coated sachets was 100%. Many of these coated sachets (27%) were thoroughly chewed, then completely ingested by skunks and excreted in a day or two. The consumption rate of coated sachets (42%) was comparable to the meal polymer baits (45%) in the third bait trial (fish flavor of all baits).

The Ontario slim bait also comes in one shape. The puncture rate of blister packs for consumed baits was 100% for all flavors. The seafood flavor was most consumed (17%), followed by apple and sugar (13%). The uptake rates of these slims were low, compared to the meal polymer (45%) and coated sachet baits (42%), and may have been influenced by bait texture (wax-like).

Experiment 2: Assess the Potential Vaccine Dose with a Mock Vaccine

It appeared that SDM had an aversive taste. Very few (19%) of all biomarker-injected baits were consumed by skunks. Meal polymer baits had a consumption rate of 33% and a puncture rate of 58%. The coated sachets had a 0% consumption rate but an 89% puncture rate. During palatability trials the coated sachets were ingested at a rate of 27%, but none were ingested during the biomarker trial. The Ontario slim baits had a consumption rate and puncture rate of 11%. This experiment was discontinued soon after inception, since the taste aversion interfered with making an accurate assessment of the potential dose delivery by the sachet.

DISCUSSION

Preliminary results suggest that of the baits we tested, the most effective in terms of uptake rate and sachet puncture rate was the fish crumble-coated sachet. The fish meal and chicken meal polymer baits had a comparable consumption rate but did not have as high of a sachet puncture rate as did the coated sachets. Conversely, the Ontario slim had a comparable sachet puncture rate to the flavor coated sachet but a much lower consumption rate. Bait development for skunks should take into consideration both flavor and shape to maximize potential uptake and potential vaccine delivery.

Although SDM has an aversive taste, the search for a less aversive biomarker, or masking the aversion to SDM, would benefit ORV campaigns for skunks. Assessing the potential vaccine dose delivery by a sachet will be useful information for determining bait density dispersal in the field.

This study illustrated that bait manipulation, consumption, and the probability of sachet puncture (i.e., potential for vaccine dose delivery) are influenced by bait flavor and shape, and that the bait matrix should be directly coated onto a sachet. Flavor and shape of baits and access to the sachet within baits must be taken into consideration in ORV bait development for skunks in order to be effective. These findings contribute to the development of ORV baits for skunks and will help expedite field operations once an ORV is developed. Future studies should include examination of powerful and persistent attractants to increase the range at which skunks can detect baits in the field, of alternative bait shapes that increase the puncture rate of sachets, and of alternative biomarkers to monitor uptake by skunks in the field until a vaccine is developed.

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