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Research

Preference among 7 bait flavors delivered to domestic dogs in Arizona: Implications for oral rabies vaccination on the Navajo Nation

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ABSTRACT

Less than 20% of the domestic dogs on tribal lands in the United States are vaccinated against rabies. One method to increase vaccination rates may be the distribution of oral rabies vaccines (ORVs). ONRAB® (Artemis Technologies, Inc., Ontario, Canada) is the primary ORV used in Canada to vaccinate striped skunks and raccoons. To investigate the potential use of ONRAB® ORV baits to vaccinate feral domestic dogs against rabies on tribal lands and beyond, we performed a flavor preference study. A total of 7 bait flavors (bacon, cheese, dog food, hazelnut, sugar-vanilla, peanut butter, and sardine) were offered in pairs to 13 domestic dogs. Each dog was offered all possible combinations of bait pairs over a period of 10 days, with each bait offered 6 times. The proportion of times each bait was consumed first by individual dogs was calculated and comparisons among dogs were conducted using the MIXED procedure in SAS (SAS Institute, Cary, NC). Pairwise comparisons between baits were performed using "contrast" statements with sugar-vanilla flavor as the default for comparison. Type 3 tests of fixed effects showed a significant treatment effect ($F_{6,72} = 9.74$, $P < 0.0001$). Sugar-vanilla was selected first during 14% of the offerings and exhibited the least preference among all bait types ($F_{1,72} = 22.46$, $P < 0.0001$). Dog food was selected first 56% of the time, and more frequently than all other bait types ($F_{1,72} = 13.09$, $P = 0.0005$).
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Introduction

Domestic dogs are a primary vector of rabies in many countries, and the disease claims approximately 55,000 human lives annually worldwide, primarily in Africa and Asia (Knobel et al., 2005). Vaccination is an effective way to prevent rabies in domestic dogs, but there are numerous factors that contribute to a lack of vaccination in some areas. Failure to vaccinate can often be attributed to cost, lack of interest, availability of vaccine, and cultural differences in ownership practices (Bergman et al., 2008). It is estimated that on tribal lands in the United States, less than 20% of the domestic dogs are vaccinated against rabies (Bergman et al., 2008). This has implications for human health as approximately 1000 dog bites are reported annually on the Navajo Nation (Daniels, 1986).

One method to increase vaccination may be through the distribution of oral rabies vaccine (ORV) baits, which has proved successful in controlling rabies in wildlife in other areas (Blancou, 2008; Wandeler, 2009). In 1994, an outbreak of rabies in domestic dogs with subsequent spillover to coyotes (*Canis latrans*) forced a statewide health emergency and quarantine in Texas (Clark and Wilson, 1995). Widespread ORV programs have since led to eradication of the canine strain of rabies in the United States (Slate et al., 2005, 2009), but unvaccinated domestic animals are still susceptible to infection by rabies strains found in wildlife. Furthermore, affordable domestic dog vaccination programs in developing nations are lacking (Kaare et al., 2009; Tang et al., 2005).

The only ORV bait licensed for use in the United States is RABORAL V-RG® (Merial, Inc., Athens, GA), which uses a fish-flavored attractant. Research suggests that the Canadian vaccine ONRAB® (Artemis Technologies, Inc., Ontario, Canada) is more

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effective at vaccinating free-ranging raccoons (*Procyon lotor*; Fehlner-Gardiner et al., 2012; Mainguy et al., 2013). Preliminary evaluation of the efficacy of ONRAB® in vaccinating domestic dogs has been performed (Bender et al., Navajo Nation Veterinary Program, Navajo Nation Department of Agriculture, unpublished data), but it is unknown whether the sugar-vanilla flavor used to vaccinate wildlife (Rosatte et al., 1998) will be attractive to dogs. Our objective was to evaluate preference among 7 potential ORV bait matrix flavors in domestic dogs.

Materials and methods

We conducted the study at the Navajo Nation Animal Control Facility on the Navajo Nation, AZ. All dogs were either surrendered by owners to the Animal Control facility or were captured as feral dogs by Animal Control Officers. A licensed veterinarian (SB) performed a physical examination of all dogs before selection. The first 13 healthy dogs (7 males, 5 females, and 1 unrecorded) that became available were included in the study. Any overtly aggressive dog or a dog with medical issues requiring extensive treatment (i.e., tumors and so on) was excluded. Any dog showing overt symptoms of disease (i.e., symptoms compatible with canine parvovirus infection, canine distemper, tumors, fever, injuries, and so on) were excluded from participation in the study. No advanced diagnostics (i.e., complete blood count and so on) were performed as the objective of the study was to be representative of the free-roaming population. All dogs accepted into the study were vaccinated for canine parvovirus, canine distemper virus, adenovirus type 2, coronavirus, and parainfluenza. Testing for heartworm was not necessary as it is not present in the Navajo Nation. All dogs received a broad-spectrum deworming treatment and treatment specifically for tapeworm infection. Because only approximately 50% of the dogs were accustomed to human handling, dogs were grouped (no more than 2 per group) and kenneled by behavior toward the other dogs for 2 weeks to acclimate to other dogs and the presence of humans. Dogs ranged in age from 6 months to 4 years, based strictly on dental wear and condition and body condition score (BCS) ranged from 2 to 5 (maximum: 10). No dog was excluded owing to low BCS. Dogs were housed in kennels consisting of an indoor and outdoor section that were joined by a sliding door used to separate co-kenneled dogs during individual bait trials. Kennel dimensions (length × width × height) were $2.0 \times 1.0 \times 1.5\ m^3$ and $1.8 \times 1.0 \times 2.2\ m^3$ for the indoor and outdoor sections, respectively. Dogs were fed a daily ration of commercial dog food ("Ol' Roy, Adult;" Wal-Mart, Bentonville, AR), with water available *ad libitum*. Dogs were housed at the shelter for total of 6 weeks.

The bait matrix consisted of food-grade partially hydrogenated vegetable shortening, Microbond® wax (International Group, Inc.), stearine, vegetable oil, a food dye, and flavor ingredients (Rosatte et al., 2009). Commercially produced food-derived products were used to formulate the bait flavors as such products were readily available. All bait flavors were manufactured by Artemis Technologies, Inc., and the recipes are proprietary. A total of 7 flavors were evaluated: bacon, cheese, dog food flavor, hazelnut, sugar-vanilla, peanut butter, and sardine. Sugar-vanilla flavor was selected as the *a priori* reference bait because it is the standard flavor used for oral rabies vaccination of wildlife in Canada (Rosatte et al., 2009). We offered baits as $1.0 \times 1.0 \times 0.5\ cm^3$ cubes, each weighing 1.0 g.

Baits were paired so that each possible combination was represented resulting in 21 pairs of baits with each bait flavor introduced to each dog 6 times. Pairs were randomly assigned to individual dogs and offered at 2 pairs per day (3 pairs on the final study day) for 10 consecutive days. Baits were offered at 09:00 and 15:00 hours with an additional offering at 12:00 hours on the final trial day. Dogs were fed their daily ration 1 hour after the final bait trial, which corresponded to the normal daily feeding schedule. Dogs were exposed to each bait pair until at least 1 of the baits was consumed with a 2-minute time limit. Baits not consumed within the time limit were removed and classified as "not eaten" for that trial. Fresh baits were used for each trial. All dogs were exposed to all bait combinations in a randomized block design with baits as treatments and individual dogs as blocks. The proportion of times each bait flavor was consumed first was calculated and compared using the MIXED procedure with pairwise comparisons using "contrast" statements in SAS (SAS Institute, Cary, NC). We accepted statistical significance at P value lower than 0.05.

Table 1
Proportion of times each bait was selected first when offered

Bait type	Proportion
Dog food	0.56
Bacon	0.54
Cheese	0.49
Sardine	0.45
Peanut butter	0.29
Hazelnut	0.19
Sugar-vanilla	0.14

Results

Sugar-vanilla flavor was selected first the least frequently, and dog food flavor was selected first the most frequently overall (Table 1). Type 3 tests of fixed effects showed an overall treatment effect ($F_{6,72} = 9.74$, $P < 0.0001$). Sugar-vanilla and dog food flavors were selected least ($F_{1,72} = 22.46$, $P < 0.0001$) and most ($F_{1,72} = 13.09$, $P = 0.0005$) frequently, respectively, when compared with all other flavors combined. Pairwise comparisons using sugar-vanilla as the reference flavor indicate no difference in preference between sugar-vanilla and peanut butter ($F_{1,72} = 3.96$, $P = 0.051$) or hazelnut ($F_{1,72} = 0.44$, $P = 0.509$; Table 2). Using the most frequently selected flavor (dog food) as the reference flavor, we found no difference in preference between dog food and bacon, cheese, or sardine flavors. Sugar-vanilla flavor was least preferred (Table 3).

Discussion

It is interesting to note that sugar-vanilla flavor had the lowest preference among the baits tested. Rosatte et al. (1998) found no statistical difference in bait acceptance between cheese and sugar-vanilla flavors by raccoons (no data are presented on flavor preferences of striped skunks, *Mephitis mephitis*), but the sugar-vanilla flavor was more practical to manufacture and is now the flavor used

Table 2
Comparison of proportions with sugar-vanilla as the reference bait

Contrast	F	Degrees of freedom numerator	Degrees of freedom denominator	P value
Sugar-vanilla vs. dog food	29.93	1	72	<0.0001
Sugar-vanilla vs. bacon	26.41	1	72	<0.0001
Sugar-vanilla vs. all others	22.46	1	72	<0.0001
Sugar-vanilla vs. cheese	20.03	1	72	<0.0001
Sugar-vanilla vs. sardine	15.83	1	72	0.0002
Sugar-vanilla vs. peanut butter	3.96	1	72	0.051
Sugar-vanilla vs. hazelnut	0.44	1	72	0.509

Table 3
Comparisons with dog food flavor as the reference bait

Contrast	*F*	Degrees of freedom numerator	Degrees of freedom denominator	*P* value
Dog food vs. sugar-vanilla	29.93	1	72	<0.0001
Dog food vs. hazelnut	23.11	1	72	<0.0001
Dog food vs. all others	13.09	1	72	0.0005
Dog food vs. peanut butter	12.12	1	72	0.0009
Dog food vs. sardine	2.23	1	72	0.1401
Dog food vs. cheese	0.99	1	72	0.3232
Dog food vs. bacon	0.11	1	72	0.7412

for vaccinating raccoons and striped skunks in Canada (Rosatte et al. 1998, 2009). In our study, peanut butter flavor did not differ in preference when compared with sugar-vanilla flavor, but the borderline statistical result ($P = 0.051$) may be a function of relatively small sample sizes. Hazelnut performed nearly as poorly as sugar-vanilla flavored bait. Although many dogs tend to show a preference for sugars such as sucrose, fructose, and glucose (Ferrell, 1984; Houpt et al., 1979), our results suggest that sweet-tasting flavors such as sugar-vanilla flavor or hazelnut may not be suitable for use in the ONRAB® Ultralite bait matrix for domestic dogs.

Dog food flavor was selected first more often than all other bait types. This result may be confounded by the study subjects' familiarity with commercial dog food as their daily food source, although different brands were used as the daily ration ("Ol' Roy Adult") and in the bait matrix formulation (Iams, Mason, OH). The apparent preference of dog food flavor may also be a function of access to commercial dog food by feral dogs through intentional or unintentional (food left out for kept dogs) feeding. In our study, bacon, cheese, and sardine flavors were selected with a similar frequency as dog food. Interestingly, Linhart et al. (2002) found that other canids also preferred cheese- and fish-flavored baits. Thus, dog food, bacon, cheese, or sardine may be equally suitable flavor matrix choices for domestic dog ORV baiting on the Navajo Nation. However, caution must be exercised when making inferences to other regions. In developing nations, feral dog diets range from human waste and animal carcasses in Zimbabwe (Butler and du Toit, 2002), invertebrates and small mammals in Brazil (Campos et al., 2007), and human-derived garbage in India (Vanak and Gompper, 2009). Selection of an appropriate bait flavor for ORV applications in developing nations may be site specific and requires further investigation.

Conflict of interest

The authors declare no conflict of interest. The idea for the article was conceived by Scott Bender, Kurt C. VerCauteren, and David Bergman. The experiments were designed by Kurt C. VerCauteren, David Bergman, Scott Bender, and Are R. Berentsen. The experiments were performed by Peggy Bender, Scott Bender, Krista Hausig, David Bergman, and Kurt C. VerCauteren. The data were analyzed by Are R. Berentsen and Kurt C. VerCauteren. The article was written by Are R. Berentsen, Kurt C. VerCauteren, Scott Bender, Peggy Bender, Krista Hausig, and David Bergman. Funding was provided by the National Rabies Management Program. This research was approved by the USDA National Wildlife Research Center Institutional Animal Care and Use Committee under protocol QA-2053.

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