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Flavor Preference of Oral Rabies Vaccine Baits by Small Indian Mongooses (*Herpestes auropunctatus*) in Southwestern Puerto Rico

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ABSTRACT: The small Indian mongoose is an invasive pest species and rabies reservoir in Puerto Rico and other islands in the Caribbean. In the United States and Europe, rabies in wild carnivores is largely controlled through oral rabies vaccination (ORV), but no ORV program for mongooses exists. The oral rabies vaccine currently licensed for use in wild carnivores in the United States has not been reported as immunogenic for mongooses. A mongoose-specific bait has been developed but field-based bait flavor preference trials have not been performed in Puerto Rico. We evaluated removal of egg-flavored (treatment) vs. unflavored (control), water-filled placebo ORV baits in a subtropical dry forest in southwestern Puerto Rico from 2014-2015. During six trials at four plots we distributed 350 baits (175 treatment and 175 control) and monitored baits for five days or until at least 50% of baits had been removed or were rendered unavailable to mongooses due to inundation by fire ants. The estimated overall probability of bait removal within five days was 85% (95% CI 75-91%) and 45% (95% CI 35-55%) for treatment and control baits, respectively. Removal rate estimates in the spring were 95% (95% CI 86-98%) and 63% (95% CI 49-76%) for treatment and control baits, respectively. Removal rate estimates in autumn were 68% (95% CI 58-77%) and 30% (95% CI 22-39%) for treatment and controls, respectively. Model estimates suggest that treatment and season were more influential on bait removal rates than diel period or experimental day, although bait removal rates were higher at night than during the day, suggesting non-target bait removal by nocturnal rodents. Our results suggest that egg-flavored baits were preferred by mongooses over unflavored baits. During operational ORV bait application, non-target bait removal should be taken into consideration when calculating bait application rates.

KEY WORDS: bait flavor, disease, *Herpestes auropunctatus*, oral rabies vaccination, Puerto Rico, rabies, small Indian mongoose

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INTRODUCTION

The small Indian mongoose (*Herpestes auropunctatus*) is an opportunistic omnivore introduced in sugar cane-producing tropical islands worldwide, primarily to control rat populations (Nellis and Everard 1983, Hoagland et al. 1989). In 1872 nine mongooses were introduced to Jamaica from India to control rats (*Rattus* spp.; Espeut 1882). From 1877-1879 the progeny of mongooses from Jamaica were introduced throughout the Caribbean (Palmer 1898). While rat control by mongooses was initially successful, rat populations recovered (Seaman and Randall 1962). As a result of the inefficiency of the mongoose at suppressing rat populations and the propensity for damage to native species, the mongoose is currently considered a pest species throughout most of its introduced range (Seaman and Randall 1962, Nellis and Everard 1983).

In addition to damage to agricultural crops and native wildlife, mongooses pose a human health risk as a rabies reservoir on several islands in the Caribbean (Seetahal et al. 2018). In Puerto Rico, mongooses are the primary reservoir for rabies, comprise over 50% of reported rabid animals annually and have been associated with two fatal cases in humans (Ma et al. 2018). In the United States and elsewhere, vaccination of wild carnivores against rabies is accomplished by the distribution of oral rabies vaccine (ORV) baits (Cliquet and Aubert 2004, Slate et al. 2005). However, the vaccine currently used (RABORAL V-RG[®],

Boehringer Ingelheim Animal Health, Athens, GA) is reportedly not immunogenic for mongooses (Blanton et al. 2006). Previous studies on mongoose bait acceptance in the Caribbean suggest oral vaccination of mongooses is feasible (Linhart et al. 1993, Creekmore et al. 1994, Berentsen et al. 2014), but these studies did not involve the use of baits specifically designed to target mongooses.

A novel bait designed for mongooses (Ceva Santé Animale, Dessau Rosslau, Germany) has been developed and successfully used to deliver oral rabies vaccines to captive mongooses (Vos et al. 2013). However, bait flavor preference trials with this new bait have not been performed in free-ranging mongooses in Puerto Rico. Our objectives were to 1) evaluate bait uptake of egg-flavored (treatment) and non-flavored (control) water-filled baits; and 2) use remote cameras to determine bait removal by target and non-target species.

METHODS

Study Site

We conducted this study on the east and west sides of the Cabo Rojo National Wildlife Refuge (CRNWR), Cabo Rojo Municipality, SW Puerto Rico (Figure 1). The habitat is classified as a sub-tropical dry forest. Dominant habitat types are mangrove, littoral woodland, mesquite, semi-evergreen woodland, coastal shrub, and deciduous woodland. Annual temperatures range from 25 to 32°C with an annual rainfall of 114 cm, approximately 50% of

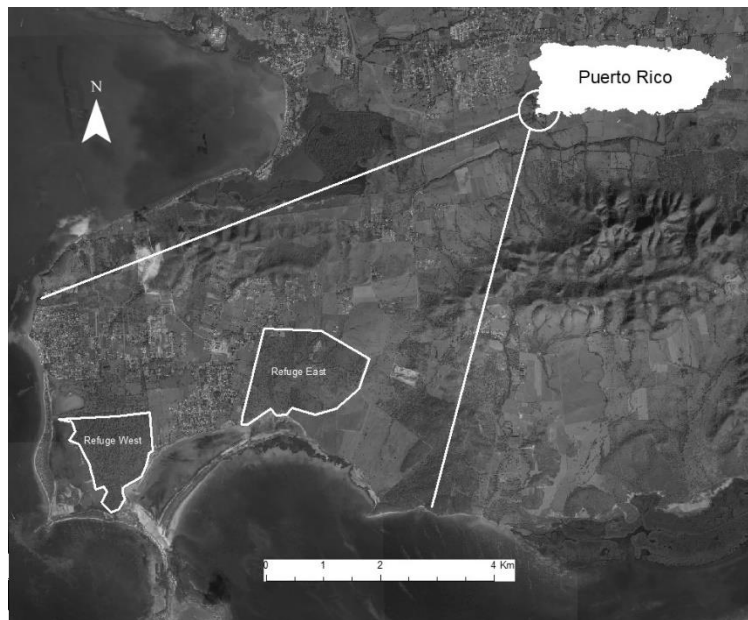


Figure 1. Location of study sites, SW Puerto Rico.

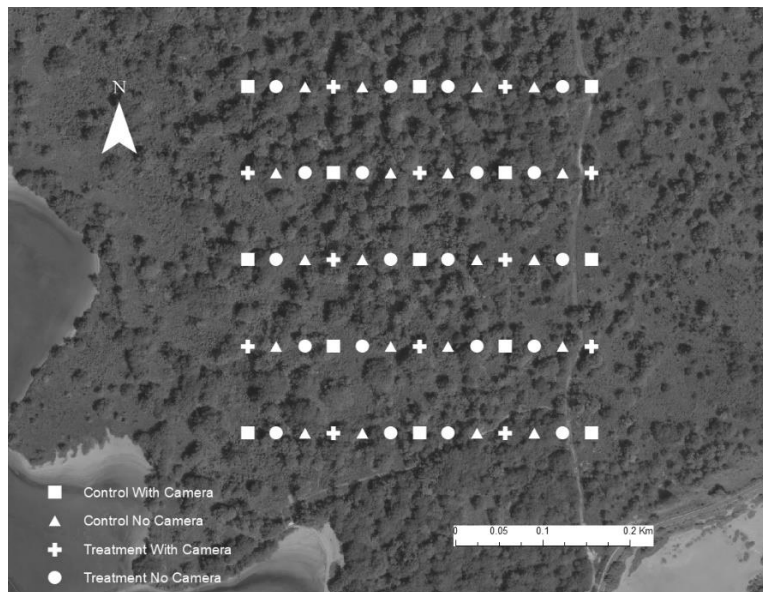


Figure 2. Example of bait and camera transect, 100 m between transects, 33 m between stations, Cabo Rojo National Wildlife Refuge, SW Puerto Rico.

which falls from August to November (USFWS 2011).

Baits

Each bait consists of a $28 \times 20 \times 9$ mm (0.7 ml) foil blister pack with an external bait matrix composed of gelatin and powdered whole chicken eggs. Baits were water-filled placebos containing no vaccine. Throughout the study, ‘treatment’ refers to egg-flavored baits, and ‘control’ refers to unflavored baits.

Bait Transects and Data Collection

We established four 5×13 grids (~ 0.25 km², 65 baits per grid; Figure 2) at CRNWR (two grids on the east and west sides) with 100 m between transects and 33 m between baits. We alternated treatment and control baits. We placed a remote camera programmed to record a series

of three photographs at every third or fourth bait (for an even distribution of cameras among treatment and control baits) with a one-second interval between photo events. We placed baits in the morning ~ 1 hr. after sunrise and checked them daily at approximately sunrise and sunset until at least 50% of the baits had disappeared and/or were rendered “unavailable,” or for up to five days. We considered baits unavailable to mongooses if baits were inundated or buried by fire ants (*Solenopsis invicta*). We reviewed the photos in an effort to determine which species removed the bait.

We classified baits as 1) removed/punctured/chewed, 2) available but unpunctured/unchewed, and 3) unavailable (baits inundated or buried by fire ants). We evaluated the west grids twice during the spring of 2014 and autumn of 2015. We evaluated the east grids once each during the

autumn of 2014 and spring of 2015 (one spring of 2015 east grid was conducted with 25, rather than 65 baits). Data for all grids were pooled for analysis.

Data Analysis

We estimated bait removal rates using a nest survival model in Program MARK (White and Burnham 1999). This model assumes perfect detection, and can evaluate the impact of multiple covariates on survival rates (Cooch and White 2016). In this study, the survival rate was equivalent to the non-removal rate of a bait on the landscape. We censored unavailable baits. Baits were considered “available” (or alive) at the last time point then omitted from further analysis. Baits were checked twice a day and therefore we estimated bait survival at 12-hour intervals. We examined the relationships between bait survival (non-removal) and the flavor treatment, the time of day (day or night), the season, and the time since bait deployment. We compared all combinations of covariates and used the second order Akaike’s Information Criterion (AICc) to evaluate model performance and models within 2 AICc of the top model were considered competitive (Burnham and Anderson 2002).

RESULTS

During six trials at four grids we distributed 350 baits (175 treatment and 175 control). The bait removal rate varied with treatment, time of day, season, and time since deployment (Table 1). The estimated overall probability of bait removal within five days was 85% (95% CI 75-91%) and 45% (95% CI 35-55%) for treatment and control baits, respectively. Removal rate estimates in the spring were 95% (95% CI 86-98%) and 63% (95% CI 49-76%) for treatment and control baits, respectively. Removal rate estimates in autumn were 68% (95% CI 58-77%) and 30% (95% CI 22-39%) for treatment and controls, respectively. Model estimates suggest treatment and season were more influential on bait removal rates than diel period or experimental day (Table 2). Based on this parameterization of the model (censoring the unavailable baits) we did not have the power to quantify the impact of fire ants on the bait uptake. Results of overall bait removal by bait type and season are presented in Table 3. Results of bait removal by bait type, season and diel period are presented in Table 4.

Remote cameras captured 209,134 photographs, 473 of which included animals. Of these, 313 (66.1%) were mongooses, 68 (14.4%) were rodents (i.e., *R. rattus* and

Table 1. Model selection results showing the impacts of covariates on bait survival (or non-removal) rates by mongoose in Cabo Rojo National Wildlife Refuge, Puerto Rico, 2014-2015. Trt = treatment, AMPM = time of day, Duration = time since bait deployment, Season = Indicator for autumn or spring.

Model	AICc	Delta AICc	AICc Weights	Model Likelihood	Num. Par	Deviance	-2log(L)
Trt+AMPM+Duration+Season	844.63	0.00	0.97	1.00	5	834.58	834.58
Trt+AMPM+Season	851.76	7.13	0.03	0.03	4	843.73	843.73
Trt+Duration+Season	862.65	18.01	0.00	0.00	4	854.61	854.61
Trt+Season	864.35	19.72	0.00	0.00	3	858.33	858.33
Trt+AMPM+Duration	873.56	28.93	0.00	0.00	4	865.53	865.53
AMPM+Duration+Season	889.43	44.80	0.00	0.00	4	881.40	881.40
Trt+Duration	890.66	46.03	0.00	0.00	3	884.64	884.64
Trt+AMPM	902.30	57.66	0.00	0.00	3	896.28	896.28
AMPM+Season	903.17	58.54	0.00	0.00	3	897.15	897.15
Duration+Season	906.69	62.06	0.00	0.00	3	900.67	900.67
Trt	908.23	63.60	0.00	0.00	2	904.22	904.22
AMPM+Duration	912.28	67.65	0.00	0.00	3	906.26	906.26
Season	913.36	68.72	0.00	0.00	2	909.35	909.35
Duration	928.91	84.28	0.00	0.00	2	924.90	924.90
AMPM	950.61	105.98	0.00	0.00	2	946.60	946.60
Intercept only	955.00	110.37	0.00	0.00	1	953.00	953.00

Table 2. Parameter estimates for top model relating bait survival (non-removal) rates and covariates.

Covariate	Estimate	SE	LCI	UCI
Intercept	-0.40	0.24	-0.87	0.08
Treatment	1.24	0.19	0.87	1.60
Time of Day	0.85	0.19	0.47	1.22
Experimental Day	0.19	0.07	0.06	0.32
Season	1.10	0.20	0.71	1.49

Table 3. Overall number of baits offered, available and removed by season and bait type. Cabo Rojo National Wildlife Refuge, Puerto Rico, 2014-2015.

	Autumn		Spring		Total
	Treatment	Control	Treatment	Control	
Number Offered	98	97	77	78	350
Number Available	81	91	63	73	308
Available Removed	57	27	49	28	161
Proportion	70.4%	29.7%	77.8%	38.4%	52.3%
95% LCL	59.7%	21.3%	66.1%	28.1%	46.7%
95% UCL	79.2%	39.7%	86.3%	49.8%	57.8%

Table 4. Overall number of available baits removed by season, bait type and diel period. Cabo Rojo National Wildlife Refuge, Puerto Rico, 2014-2015.

	Autumn				Spring			
	Treatment		Control		Treatment		Control	
	Day	Night	Day	Night	Day	Night	Day	Night
Number Removed	23	36	13	14	28	21	12	16
Proportion Removed	40.4%	59.7%	48.2%	51.9%	57.1%	63.6%	42.9%	57.1%
95% LCL	28.6%	46.7%	30.7%	34.0%	43.3%	30.0%	26.5%	39.1%
95% UCL	53.3%	71.4%	66.0%	70.8%	70.0%	56.7%	60.9%	73.5%

Mus musculus), 60 (12.7%) were birds (primarily common ground doves; *Columbina passerina*), 48 (10.1%) were green iguanas (*Iguana iguana*), 45 (9.5%) were the Puerto Rican giant ground lizard (*Pholidoscelis exsul*), 12 (2.5%) were domestic dogs, (*Canis familiaris*), 9 (1.9%) domestic cats (*Felis domesticus*), and one (0.2%) of an unknown species of non-native, invasive primate. Of the mongoose photographs, four (1.27%) documented mongoose-bait interaction. The only non-target species documented removing baits were rodents.

DISCUSSION

Our objective was to evaluate bait preference and removal rates of egg-flavored oral rabies vaccine placebo baits in small Indian mongooses on Puerto Rico. We determined that the egg-flavored baits were preferred over unflavored baits and that after five days of exposure the probability of treatment baits being removed was 95% vs. 63% for control baits. Over 70% of egg-flavored baits were removed vs. 34% of control baits. Photographic documentation of species responsible for bait removal was scant and is likely due to camera sensitivity. There were many photographs that recorded no animal activity, yet the camera had been triggered. There were also cases where mongooses were seen on camera prior to and following a bait disappearing, but no mongoose-bait interactions were captured. We attribute this to high ambient temperatures relative to the sensitivity of the camera that resulted in false triggers as well as interference by vegetation, despite efforts to clear potentially obstructive vegetation from the cameras' sensors.

Our determination that rodents are likely responsible for a relatively high proportion of bait removal stems from evaluating bait fate in the morning (to determine nighttime removal rate) and the afternoon (to determine daytime removal). While it is possible that other non-target species were responsible for bait removal, there are few terrestrial mammals on the study sites (i.e., feral dogs, cats, horses, and non-native primates) and very few photographs were obtained of these mammals interacting with baits. Nor

were many non-target mammals seen by personnel during field activities. One photograph was obtained of what appeared to be the hind leg of a non-native primate, but the bait remained undisturbed. Reptiles (iguanas and lizards) and birds showed little to no interest in baits and the few photos of domestic cats showed them more interested in playing with baits than removing them. Given the relative lack of non-target species and an abundance of rodents at these study areas (Berentsen et al. 2018) we believe rodents are the most likely culprit in nighttime bait removal with approximately 50% of all baits removed after sunset.

Burial or inundation of baits by fire ants was relatively uncommon with approximately 12% of all baits affected. Bait loss to fire ants was largely restricted to the egg-flavored baits. Few plain flavored baits seemed to be attractive to ants. Our research suggests that fire ants are unlikely to have a significant effect on bait availability.

The delivery of pharmaceuticals or toxicants to wildlife by bait must take into consideration non-target consumption. An understanding of bait loss to non-target species can provide guidance in determining application rates and whether modifications to account for non-target bait consumption are warranted. While non-target bait loss in our study was estimated to account for roughly half of the baits distributed and was factored into target bait density calculations for a placebo bait field trial to target mongooses, the target host density and relative impact of non-target loss may vary in other habitats or regions.

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