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EXPOSURE TIME OF ORAL RABIES VACCINE BAITS RELATIVE TO BAITING DENSITY AND RACCOON POPULATION DENSITY

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ABSTRACT: Oral rabies vaccination (ORV) baiting programs for control of raccoon (*Procyon lotor*) rabies in the USA have been conducted or are in progress in eight states east of the Mississippi River. However, data specific to the relationship between raccoon population density and the minimum density of baits necessary to significantly elevate rabies immunity are few. We used the 22-km^2 US National Aeronautics and Space Administration Plum Brook Station (PBS) in Erie County, Ohio, USA, to evaluate the period of exposure for placebo vaccine baits placed at a density of 75 baits/km^2 relative to raccoon population density. Our objectives were to 1) estimate raccoon population density within the fragmented forest, old-field, and industrial landscape at PBS; and 2) quantify the time that placebo, Merial RABORAL V-RG® vaccine baits were available to raccoons. From August through November 2002 we surveyed raccoon use of PBS along 19.3 km of paved-road transects by using a forward-looking infrared camera mounted inside a vehicle. We used Distance 3.5 software to calculate a probability of detection function by which we estimated raccoon population density from transect data. Estimated population density on PBS decreased from August (33.4 raccoons/km^2) through November (13.6 raccoons/km^2), yielding a monthly mean of 24.5 raccoons/km^2. We also quantified exposure time for ORV baits placed by hand on five 1-km^2 grids on PBS from September through October. An average 82.7% (SD=4.6) of baits were removed within 1 wk of placement. Given raccoon population density, estimates of bait removal and sachet condition, and assuming 22.9% nontarget take, the baiting density of 75/km^2 yielded an average of 3.3 baits consumed per raccoon and the sachet perforated.

Key words: Bait density, forward-looking infrared camera, oral vaccination, population density, rabies, raccoon.

INTRODUCTION

The US Centers for Disease Control (CDC) first conceived a rabies intervention strategy based on oral vaccination technology in the 1960s (Baer, 1988). By 1983, a vaccinia-rabies glycoprotein (V-RG) recombinant virus vaccine was developed (Wiktor et al., 1984; Rupprecht et al., 1995). Subsequent field studies to evaluate V-RG efficacy concentrated primarily on assessing safety concerns and oral rabies vaccine (ORV) bait acceptance. Further, these early efficacy studies also estimated seroconversion rates based on the prevalence of elevated biomarker (i.e., blood iodine) levels (Hadidian et al., 1989) or prevalence of antibody-positive raccoons (Hanlon et al., 1998; Robbins et al., 1998), but without adequate estimation of target population densities (Otis et al., 1978; Pollock et al., 1990; Rosatte et al., 2001). Because differences in patterns of rabies infections can be related to population density and life history traits of host populations (Carey and McLean, 1983), the success of individual ORV efforts likely varies with the ecology and population density of the target species (Perry et al., 1989), as well as epizootic and enzootic transmission (Hanlon et al., 1999; Slate et al., 2002) and bait distribution (Johnston and Tinline, 2002).

Using the US National Aeronautics and Space Administration (NASA) Plum Brook Station (PBS) in Erie County, Ohio, USA (41°27′N, 82°42′W), we evaluated the period of exposure of placebo vaccine baits placed at a density of 75 baits/km^2 (a target ORV bait density currently used in Ohio; Ohio Department of Health, 2002, 2003). There are no data relative to seasonal raccoon population density on the PBS. Our objectives were to 1) estimate raccoon population density within the fragmented forest, old-field, and industrial landscape at PBS; and 2) quantify the time that placebo Merial (Athens, Georgia, USA) RABORAL V-RG® vaccine baits were available to raccoons.

MATERIALS AND METHODS

Study area

The 2,200-ha PBS is enclosed by a 2.4-m high chain-link fence with barbed-wire outriggers. Habitat within PBS differs from the surrounding mix of agricultural and suburban area, comprising canopy-dogwood (*Cornus* spp., 39%); old field and grasslands (31%); open woodlands (15%); and mixed hardwood forests (11%) interspersed with abandoned and actively-used structures relating to NASA and prior operations (Rose and Harder, 1985). Also, PBS comprises a network of paved roads, and raccoons on the facility are exposed to vehicle traffic at all hours. There are no consistent human-related food resources on PBS that would concentrate raccoons in a particular area.

Transect surveys

We established a 19.3-km survey route along five east-west paved-road transects across PBS, covering all habitat types. Traditional off-road transect surveys for moderate-size mammals like raccoons likely suffer from observer disturbance of target species causing flight or even attraction to the observer; either effect will potentially bias accurate measurement from the transect to the point of initial observation (Buckland et al., 1993). We conducted our surveys from a passenger van via infrared camera.

Infrared technology allows target animals to be discerned against background vegetation, an improvement over traditional sighting methods (e.g., spotlights and night-vision equipment; Belant and Seamans, 2000). We used a Raytheon (Dallas, Texas, USA) forward-looking infrared Nightsight Palm IR 250 Digital Camera (FLIR) mounted on the passenger side window to scan habitats on the right side of the vehicle. The camera was connected to a Sony (Park Ridge, New Jersey, USA) Video Walkman Digital-8 monitor.

When a raccoon or raccoon group was detected, we scanned to ensure a total count, and then recorded the number of individuals and perpendicular distance (meters) from the road to the point of the initial observation. For distances $\geq$18 m, we obtained the perpendicular distance to the point of initial detection for a single animal (or initial center of a group of raccoons; Buckland et al., 1993) by illuminating the point with a spotlight and then quantifying the distance using a Bushnell (Overland Park, Kansas, USA) Yardage Pro 1000 Laser Ranging System. For animals observed within 18 m, the observer paced the distance to the initial observation point. We did not count animals moving from the driver side of the vehicle or those that responded to the vehicle approach (e.g., escape, avoidance, or attraction behavior; Buckland et al., 1993). We also recorded the habitat category at the point of initial detection (i.e., road, grassland, shrub, or wooded; Belant and Seamans, 2000).

We began our FLIR surveys on 5 August 2002 and conducted two per week (n=8) through 31 August. Raccoons generally mate from February through June (most frequent in March) and have a gestation period of 63–65 days (Wilson and Ruff, 1999); thus, the raccoon population on PBS was likely near its peak in August. We conducted one survey per week from 1 September through 6 November. Our protocol for each survey night comprised a random selection of the starting transect (i.e., either the extreme north or south transect) and the direction of travel; observations were made from one side of the vehicle and the same side of each transect. We began each survey between 1 and 2 hr after sunset and maintained a speed between 8 and 16 km/hr. A driver and at least one observer were present for each survey. Depending upon raccoon activity, each survey required from 2 to 4 hr to complete.

Data analysis

We quantified raccoon population density from the transect data by month (combining data for 1 October through 6 November) using Distance 3.5 software (Buckland et al., 1993). Distance software calculates a detection function, $g(y)$=probability (detection | distance y), which is the probability of detecting an object given that it is at distance y from a random line. The detection function is based on the fitting of a series of a priori models to the observed data (Buckland et al., 1993; Burnham and Anderson, 1998). Final model selection is based on best fit to the observed distributions (as per the Akaike Information Criterion; Buckland et al., 1993; Burnham and Anderson, 1998, 2002).

The underlying statistical theory of distance sampling (reviewed by Buckland et al., 1993) relies on three basic assumptions: 1) $g(0)=1$, or all animals on the transect were detected with certainty; 2) animals were detected at their initial location, prior to any movement in response to the vehicle; and 3) distances were measured accurately. Given these basic assumptions, the method theoretically provides accurate esti-

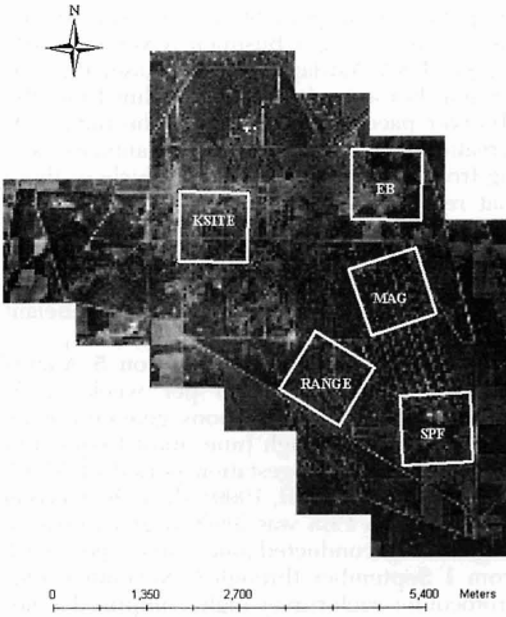

FIGURE 1. Location of five 1-km² bait grids on the US National Aeronautics and Space Administration (NASA) Plum Brook Station (PBS) for evaluation of the exposure time relative to raccoon population density of oral rabies vaccination baits placed at 75 baits/km² from 20 September through 18 October 2002.

mates of density, despite the fact that animals go undetected during transect surveys (Buckland et al., 1993).

Simulated ORV baiting

We delineated five 1-km² bait grids on PBS using a Garmin (Olathe, Kansas, USA) GPSMap 76S with Garmin MapSource software and located the grids to maximize coverage of all habitat types, including or in proximity to bodies of water (Fig. 1). Random location of grid corners was, however, not possible because of restrictions around certain NASA operations. The mean (SE) distance between centers of adjacent grids was 2.1 km (0.5 km). Each grid comprised nine 1-km transects with nine bait points at 125-m intervals (i.e., 81 points). Prior to distributing baits and because of inherent variability in repeatedly locating GPS waypoints, we marked each bait point with plastic flagging. We randomly selected six points per grid for omission of baits to yield 75 baits/km². Also, where grid points fell on roads or in bodies of water, we placed baits on the periphery of the road or water at intervals less than 125 m (fewer than five of the 375 points).

We used Merial fish-meal polymer placebo baits. These baits were physically the same as the Merial RABORAL V-RG® vaccine baits, including the sachet within the bait, but contained no vaccine (J. L. Maki, Merial Limited). Our protocol involved baiting and monitoring of one to two grids over a 7-day period. We randomly selected two grids (EB and RANGE; Fig. 1) on north and south sides of PBS (2.8 km between grid centers) and, beginning at 8:00 AM on 30 September and 1 October 2002, hand-placed 75 baits on each. The Ohio Department of Health's ORV program has conducted baiting during October (Ohio Department of Health, 2002, 2003). We placed wire flags approximately 1 m from the bait point to aid in locating baits on subsequent checks.

On 10 and 11 October, we baited KSITE grid on the north side of PBS and SPF grid on the south (4.9 km between grid centers; Fig. 1) at 75 baits/km². We baited our last grid, MAG, on 24 October. We returned to MAG and RANGE after 3 days of bait exposure, then again after 1 wk of exposure. We checked all other grids after 5 and 7 days of exposure. Each grid check began by 9:00 AM. Upon returning to each grid point, we noted presence or absence of the bait; condition of the bait (if present); presence or absence of the sachet; condition of the sachet (if present); and any animal sign (e.g., scat or tracks) near the site. When a bait was missing and a sachet was not immediately noticed, each observer searched the immediate area around the point of bait placement (i.e., within a minimum 3-m radius) for evidence of the bait and sachet, as well as examining obvious trails to the bait point.

During field trials on PBS to evaluate bait flavor preferences for a new coated-vaccine sachet, Linhart et al. (2002) estimated that 22.9% of all baits exposed were taken by nontarget species, with opossums (*Didelphis virginiana*) the most common nontarget species. To provide an index of potential nontarget take in our ORV baiting simulation, we placed TrailMaster (Lenexa, Kansas, USA) TM35-1 camera kits with TM1550 and TM700v active infrared trail monitors at randomly selected bait points each week. During our first week of baiting, only six camera kits were available; we used 11 camera kits in subsequent weeks.

We considered each grid as an experimental unit (i.e., a sampling unit from PBS as a whole) and evaluated the period of bait exposure via descriptive statistics only. A comparison of bait exposure periods among grids would, by necessity, require replicates of habitat types (e.g., six grids per type) to gain insight into specific habitat effects. However, the addition of grids to meet sample size requirements would have placed our study area within NASA operational areas.

RESULTS

Raccoon population density

One survey (22 August) was stopped after approximately 7.2 km due to heavy rain; however, we considered the habitat covered as representative of PBS and included these data in our analyses. The majority (68.8%) of raccoon sightings (n=269) were in grass or on paved road (transects and others), with 16.0% in wooded areas and 2.2% in shrubs. The majority of observations were of individuals, although group size ranged from one to five animals (mean=1.4 animals [SD=0.8]). For our analysis, we assumed each raccoon represented an individual data point. Also, based on the frequency distributions of the monthly transect data (i.e., number of observations versus perpendicular distance), we truncated our data at 110 m (removing a mean of 7.3% of observations per period [SD=0.06]). Generally, at least 5% of data are truncated to remove outliers (Buckland et al., 1993). To adjust for possible bias due to movement of raccoons ahead of the vehicle, which can lead to "heaping" of observations on or near the transect, we grouped the data within 10-m intervals. This type of grouping has little effect on efficiency as indicated by the sampling and can improve the robustness of the estimator (Buckland et al., 1993).

Our detection function, $g(y)$=key function (y)[1+series expansion (y)], for each survey period comprised a half-normal as the key function and a cosine series expansion as:

$$G(y) = e^{(-y^2/2\sigma^2)}\left[1 + \sum_{j=2}^{m} a_j \cos\left(\frac{j\pi y}{w}\right)\right]; \quad (1)$$

cosine adjustments were of orders of two to five (i.e., the order of polynomial necessary for model fit), where y is the detection distance, w is the truncation point, and a is the area of interest (Fig. 2A,B,C). Estimated population density (raccoons

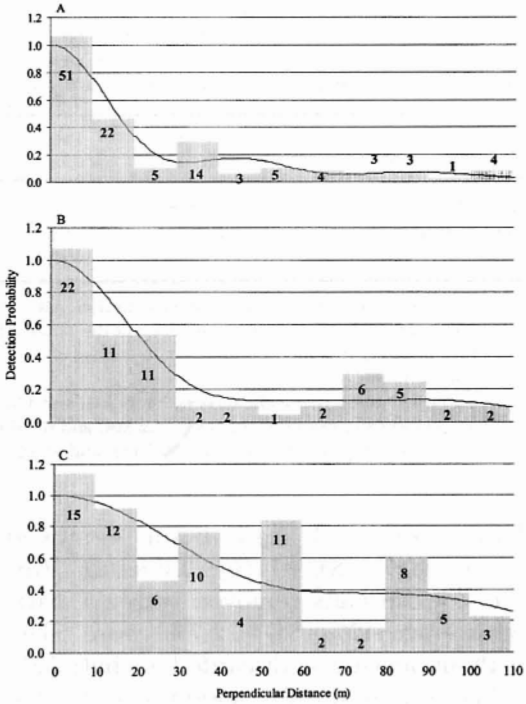

FIGURE 2. Detection probability (curve) of raccoons based on half-normal detection function with cosine series expansion fit for (A) August (cosine adjustment order=5), (B) September (cosine adjustment order=4), and (C) October through 6 November 2002 (cosine adjustment order=2) relative to the frequency distribution (bars) of raccoons observed during nighttime surveys of the US National Aeronautics and Space Administration's (NASA) Plum Brook Station (PBS). Surveys were conducted using a forward-looking infrared camera along 19.3 km of paved roads (extending out 110 m perpendicular to the route of travel) during 2002. Observed numbers of raccoons at 10-m intervals are noted for each bar on survey-specific graphs.

per square kilometer) on PBS (Table 1) decreased from 1 September through 6 November, yielding a monthly mean (SD) of 24.5 (10.1) raccoons/km^2.

Bait exposure and condition

After 3 days of exposure, 67% (n=50) and 73% (n=55) of baits had been removed from MAG and RANGE, respectively. Bait removals ranged from 48% to 81% per grid (EB, KSITE, and SPF) by day 5. A minimum of 68% of all baits (i.e., across five grids) had been removed after 5 days of exposure. By day 7, 64–91% of

TABLE 1. Raccoon population density estimates on the US National Aeronautics and Space Administration's Plum Brook Station in Erie County, Ohio, based on data from nighttime forward-looking-infrared camera surveys along 19.3 km (extending out 110 m perpendicular to the route of travel) during 2002, and estimated using a half-normal detection function and a particular order of cosine series expansion (CSE).

Survey period	CSE (AIC wt.)[a]	Density (raccoons/km^{-2})	% CV[b]	Deg. of freedom	Lower 95% CL[c]	Upper 95% CL
August[d]	5 (0.66)	33.4	20.1	13	21.7	51.3
September[e]	4 (0.35)	26.6	20.8	12	17.0	41.7
October–6 November[f]	2 (0.50)	13.6	27.8	8	7.3	25.5

[a] Proportional weight (Akaike Information Criterion, AIC, weight) of evidence in favor of a particular model that minimizes the "information" lost between the model and the observed data (see Burnham and Anderson 1998; 2002).

[b] Coefficient of variation.

[c] Confidence limit.

[d] Survey period comprised eight surveys, 143.8 km, and 115 observations.

[e] Survey period comprised four surveys, 77.2 km, and 66 observations.

[f] Survey period comprised five surveys, 96.5 km, and 78 observations.

baits per grid had been removed (mean=83% [SD=11%]; Table 2). Further, of the baits removed within 7 days, on average 42% (SD=12.7%) were taken without leaving a discarded sachet; 52% (11.8%) of removals yielded a perforated sachet (Table 2). Evidence from sign at bait sites and our photographs (from 11 of 28 camera placements) indicated that, in addition to raccoons (16 individuals photographed, one to two individuals per camera placement, sign at one bait point); opossum (five individuals photographed, one to two individuals per camera placement); groundhog (*Marmota monax*; one individual photographed); fox squirrel (*Sciurus niger*; one individual photographed); and white-tailed deer (*Odocoileus virginianus*; sign at bait point) investigated baits. Equipment failure and varying TrailMaster sensitivity (e.g., adjusting for leaf litter vs. animal movement) contributed to fewer camera placements recording animal visits. In addition, during FLIR surveys, we commonly observed white-tailed deer and occasionally opossums, striped skunks (*Mephitis mephitis*), and coyotes (*Canis latrans*). Red (*Vulpes vulpes*) and gray (*Urocyon cinereoargenteus*) fox have also been observed on PBS (T.W.S. and J.D.C., unpubl. data).

DISCUSSION

Rupprecht et al. (1995) noted that understanding the relationship between animal population density and the minimum density of ORV baits necessary to confer herd immunity is a critical component of an effective immunization program. In addition, a national working group on pre-

TABLE 2. Fate of placebo raccoon oral rabies vaccination (ORV) baits placed at 75 baits/km^2 on each of five 1-km^2 grids located on the US National Aeronautics and Space Administration's (NASA) Plum Brook Station (PBS) in Erie County, Ohio, and exposed for up to 7 days from 30 September through 18 October 2002.

Grid	Intact baits recovered	Intact vaccine sachets recovered	Perforated vaccine sachets recovered	Baits removed and sachet not recovered	Total baits removed
EB	13	3	25	34	62
KSITE	27	7	27	14	48
RANGE	9	3	26	37	66
MAG	7	3	41	24	68
SPF	9	1	43	22	66
Total	65	17	162	131	310

vention and control of rabies in the USA reiterated the need for a better understanding of the dynamics of host populations in relation to proposed disease control through, for example, oral vaccination (Hanlon et al., 1999). A lack of understanding of target species' population demographics will affect not only program design and implementation, but also the cost effectiveness of the ORV program (Rupprecht et al., 1995; Meltzer, 1996).

Raccoon population densities vary widely (e.g., 1–100 animals/km^2; Rosatte, 2000) and are primarily a function of available resources (e.g., increased food and shelter opportunities in suburban/urban areas; Gehrt and Fritzell, 1998; Rosatte, 2000). Urban (1970) estimated an average of 17.5 raccoons/km^2 on a managed waterfowl marsh in Ohio on western Lake Erie. Results from spring live trapping in a semiurban area of northeastern Illinois indicated mean annual raccoon densities ranging between 13 and 49 animals/km^2 (Gehrt et al., 2002). We estimated that the raccoon population on the PBS exceeded 700 animals (>33 raccoons/km^2) during August 2002 and decreased to just under 300 animals (>13 raccoons/km^2) through the first week of November. This pattern of decline in population density is consistent with a peak in the population annual cycle, reflecting adults and young-of-the-year present during late summer (Whitaker and Hamilton, 1998) followed by natal dispersal (Stuewer, 1943; Gehrt and Fritzell, 1998); emigration; and mortality (e.g., Gehrt and Fritzell, 1999).

As potential food resources changed with season (e.g., ripening mast [*Quercus* spp.], corn [*Zea mays*]), the frequency in which we observed animals on or close to the survey route decreased (Fig. 2A,B,C). However, our ability to detect animals with the FLIR increased as cover thinned with leaf drop (October to 6 November). Further, our data reflected consistent variation in density between months (mean percentage coefficient of variation=22.9% [SD=4.2%]). We contend, therefore, that our data are representative of the late summer and fall components of the annual population cycle for raccoons on PBS. Although a separate and independent density estimate might have provided additional information, mark-recapture methods would have introduced the confounding influence of baited traps and temporary removal of animals during the placebo-bait phase of the study.

Relative to potential bias due to visual attractants, we considered that scent cues from the bait and human presence within the grids during delineation and when checking bait points would serve as the predominant attractants, not flagging. The potential bias of attractants (i.e., decreased exposure time per bait) was uniform across grids. However, scent cues along baited operational flight lines are likely not uniform, and the discovery of ORV baits by raccoons and nontarget species are far from random. Specifically, scent, bait appearance, and bait density bias any assumption that individual baits are statistically independent units. We consider potential scent and visual biases due to revisiting bait points as unavoidable in the effort for a timely assessment of the period of bait exposure. For example, trapping within grids to obtain teeth for biomarker analysis introduces the potential confounding influence of baited traps and temporary removal of animals, as well potential age-related effects in the timing of marker binding (Linhart and Kennelly, 1967).

Thus, given an estimate of raccoon population density on PBS, an evaluation of baiting density relative to baits available per animal is possible. Making the unrealistic assumption that no nontarget species consumed baits, we found that on average 62 (SD=8.1) baits per grid were consumed by raccoons within 7 days. However, if we adjust for the percentage of intact sachets (i.e., across the total number of removed baits; 6.0% [SD=2.2%]) and potential nontarget take (i.e., assume 22.9% nontarget take as per Linhart et al., 2002), on average 45 baits/grid were consumed by raccoons and the sachet perforated. Therefore, given our estimate for raccoon population density on PBS during October 2002 and assuming 22.9% nontarget take, 75 baits/km^2 yielded 3.3 placebo vaccines consumed per raccoon and the sachet perforated.

We recognize, however, that in ORV programs bait consumption by nontarget species, effective delivery of the bait (Johnston and Tinline, 2002) and vaccine, and effective serologic conversion are all uncertainties. Still, an estimate of seasonal raccoon population density within the program area will allow for adjustments to bait distribution plans (e.g., bait density, frequency of baiting, and program duration) to ensure a reduction in the density of rabid raccoons. A cost-efficient ORV program is one that achieves a 70% population bait consumption level and with, on average, one bait per individual (Johnston and Tinline, 2002). To further evaluate the effectiveness of ORV bait density on PBS, sero-conversion rate in the raccoon population should be quantified relative to ORV bait density estimated from aerial distribution and relative to raccoon population density; this work began in spring of 2003.

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