

Evaluation of an oral vaccination program to control raccoon rabies in a suburbanized landscape

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Abstract: We evaluated the efficacy of an oral rabies vaccination (ORV) program conducted in Erie County, New York, from July through September, 2002–2005. Ingress of the raccoon (*Procyon lotor*) rabies variant first occurred along the southern border of Erie County, New York, during 1992 and began to spread northward at a velocity of 31 km/year. Fixed-wing aircraft dropped ORV baits in rural landscapes; helicopters, hand baiting, and bait stations distributed baits in suburban landscapes (\bar{x} bait densities ranged 59–118 baits/km²). Our study objectives were to quantify rabies case densities, evaluate efficacy of intervention efforts, and determine biological, census, geographical, and weather variables potentially affecting oral-rabies vaccination of raccoons in Erie County. Overall, 16% and 9% of the raccoons in Erie County tested positive for virus-neutralizing antibodies (VNA) at the 0.125 and 0.5 international units (IU)/ml levels, respectively. We found no differences between baiting strategies and frequencies of antibody-positive raccoons. However, adult males generally consumed baits most often, and the probability of seropositivity increased with raccoon age. Seroprevalence of VNA differed among raccoon sex and age classes, and vaccination year. A post-hoc kernel density estimation of rabies-positive raccoons and skunks (*Mephitis mephitis*) from 1992–2006 ($n = 364$) revealed clustering in northeastern Erie County. Our results should help ORV managers maximize limited resources.

Key words: human–wildlife conflicts, kernel density estimation, New York, oral rabies vaccination, *Procyon lotor*, rabies, raccoon, suburban landscapes

RACCOON (*PROCYON LOTOR*) RABIES was first recorded in Florida during the late 1940s (Winkler and Jenkins 1991) and has since spread northward along the eastern United States. In the last decade, experimental oral rabies vaccination (ORV) programs have shown progress in controlling raccoon rabies through the distribution of vaccinia-rabies glycoprotein (V-RG) baits across the landscape (Rupprecht et al. 2004). Baits consist of plastic sachets filled with vaccine that is either coated with fish meal or sealed inside a fish-meal-polymer bait matrix. V-RG is effective for immunizing raccoons (Rupprecht et al. 1986) and safe for many vertebrate species (Hanlon et al. 1998).

By 2003, 15 states had put ORV programs into effect (Rupprecht et al. 2004), and 50 million V-RG baits had been distributed (Slate et al. 2005). Bait is distributed via fixed-wing aircraft in rural landscapes, and bait stations, hand baiting, or helicopters in suburban areas (Boulanger et al. 2006).

The challenges of ORV distribution across suburban landscapes differ from those in rural areas because in suburban areas the potential for human exposure to vaccine baits is high, there is more competition by domestic pets for vaccine baits, and there are legal issues pertaining to property rights and land use that may limit access to areas of raccoon activity

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(Elvinger et al. 2001). With greater raccoon densities in suburban landscapes, there may also be more competition among raccoons for a limited number of baits (Olson et al. 2000), thus confounding ORV control.

The purpose of our study was to evaluate various ORV strategies used to control the raccoon variant of rabies virus. Our objectives were to quantify rabies case densities; record human and pet contact with baits; evaluate efficacy of intervention efforts; and determine biological, census, geographical, and weather variables associated with vaccination of raccoons in Erie County.

Materials and methods

Study area

Ingress of the raccoon variant of rabies first occurred along the southern border of Erie County, New York, during 1992, and the disease began to spread northward through the county (Trimarchi 1992). By the end of 2006, 404 terrestrial (non-bat) cases of rabies were found throughout the county. Of those cases, 321 (79%) were in raccoons and 51 (13%) were in striped skunks (*Mephitis mephitis*); other free-ranging and domestic species comprised the remainder of cases. Erie County comprises expansive suburban and rural landscapes surrounding the city of Buffalo, New York, USA. In 2002, we initiated an ORV program in Erie County to control enzootic raccoon rabies.

We used 15 years (1992–2006) of rabies surveillance data and 5 years (2002–2006) of data from an ongoing ORV intervention program in Erie County; the program was managed by Cornell University Animal Health Diagnostic Center. The vaccination area encompassed all of Erie County (42°53'N: 78°47'W; 2,484 km²) except for 229 km² of urban habitat within the city of Buffalo. We divided Erie County into 2 general study areas, suburban (591 km²) and rural (1,664 km²). Human population density within the suburban study area ranged from approximately 596 to 1,375 persons/km² versus 32 to 189 persons/km² within the rural study area (U.S. Census Bureau 2001). Elm (*Ulmus* spp.), red maple (*Acer rubrum*), and northern hardwoods comprised the dominant overstory vegetation in Erie County (Dickenson 1983, Alerich and Drake 1995).

Mapping rabies-positive raccoons

By 1994, rabies had spread to northern Erie County at a rate of 31 km per year. All records for animal specimens that tested positive for rabies ($n = 440$) were submitted from the Erie County Department of Health (Buffalo, N. Y.) during the years 1992–2006. Each record contained animal location, species, and date of submission. We geo-coded addresses from rabies cases using commercial mapping software (Delorme Street Atlas 2007, Yarmouth, Me.).

Rabies in big brown (*Eptesicus fuscus*; $n = 33$), little brown (*Myotis lucifigus*; $n = 2$), and hoary (*Lasiurus cinereus*; $n = 1$) bats were removed from the analysis. In order of magnitude, red foxes (*Vulpes vulpes*; $n = 14$), domestic cats (*Felis silvestris*; $n = 12$), woodchucks (*Marmota monax*; $n = 3$), and 1 each of American beaver (*Castor canadensis*), domestic cow (*Bos taurus*), and white-tailed deer (*Odocoileus virginianus*) also were removed. Although ORV specifically targets raccoons, both raccoon ($n = 321$) and skunk cases ($n = 51$) were selected for the study because rabies in striped skunks is closely related to raccoon rabies epizootics in the northeast and mid-Atlantic states (Guerra et al. 2003). After we accounted for 8 raccoon cases that could not be geo-coded due to missing addresses, the final sample size was 364 animals.

We performed a post-hoc kernel density estimation (KDE) analysis to map the cumulative densities of rabid raccoons and skunks during the 15-year period. We used geographic information system (GIS) software (ArcGIS 9.1, ESRI, Redlands, Calif.) to determine the rate of movement of the raccoon rabies epizootic and to create KDE maps. Conceptually, kernel-density estimate for point features entails fitting a smooth density function (such as a Gaussian or a quadratic function) that distributes the weight of each point smoothly over an area and then sums across these densities at each spatial location to create a smoothed surface (Zheng et al. 2004). The result is effectively a smoothed histogram of point density over the area. Using Spatial Analyst (ArcGIS extension), we generally followed procedures by Nadin-Davis et al. (2006) to acquire final number of rabies cases/km². Kernel densities were configured with a 2,750-m search radius and 10-m resolution; we reduced density classes from 9 (default) to 5 intervals to avoid replication of

classes. We estimated the northern progression of rabies spread in Erie County by measuring the distance from the first case within the southern border to the first case that arrived at the northern border, and dividing by the time interval in days (Roscoe et al. 1998).

ORV bait and distribution

Baits distributed in the rural study area consisted of a fish-meal-coated plastic sachet containing 2 ml of RABORAL V-RG® (Merial Limited, Athens, Ga.) recombinant rabies vaccine, while baits distributed in the suburban study area consisted of fishmeal polymer. In the latter bait type, sachets were sealed inside a 3.25 × 3.25 × 2 cm fish meal polymer and also contained a tetracycline biomarker (Bait-Tek Inc., Beaumont, Tex.). Fish meal is preferred by raccoons (Linhart et al. 1991). There is currently no reported difference in efficacy between the 2 bait types. Each bait type has a label with a toll-free phone number for the monitoring of human and domestic pet bait contacts. We defined 2 levels of bait contact: physical contact of the bait with an intact sachet and direct contact with liquid vaccine.

Prior to bait distribution, media announcements informed the public about the ORV program. We distributed baits during late July to early September, 2002–2005, and late October of 2006. Approximately 1,664 km² of rural Erie County were treated via fixed-wing aircraft (Ontario Ministry of Natural Resources, Peterborough, Ontario, Canada), while 491 km² of suburban landscape were treated via helicopter (New York State Police Aviation Unit, Albany, N. Y.). Both aircraft types flew parallel 1-km transects while broadcasting baits at a target rate of 75 baits/km². The helicopter also broadcast baits at a similar rate along stream, railroad, highway, and power-line corridors. During 2003–2005, 2 suburban sites (each 25 km²) received hand and bait station distribution, respectively. Each year volunteers distributed baits at hand baiting sites in likely raccoon habitat (e.g., in woodlots, along streams, behind dumpsters). In Boulanger et al. (2006), we reported design and deployment of bait stations; raccoons represented 90% of all species visiting bait stations. We sectioned 2 additional 25-km² suburban sites from a portion of the

helicopter suburban study area for comparison among the suburban bait distribution types (treatments). The minimum target density for all treatments was 75 baits/km².

Sample collection

This sample collection period ranged from September through October, 2003–2005, which began 4 to 5 weeks after bait distribution. Raccoons were trapped in Tomahawk box traps (Tomahawk Live Trap Co., Tomahawk, Wis.) baited with Fur King (Blackie's Blend, Glenmont, Oh.), a commercial raccoon sweet paste. All traps in the suburban and rural study areas were placed in sets of 2 (spaced 2 m apart) and maintained for 9–10 nights; trapping sessions continued until ≥100 unique raccoons were captured per treatment type each year. We selected trap locations to maximize capture rate by targeting preferred raccoon habitat (e.g., streams, woodlots, dumpsters, agricultural lands). To avoid disturbance from domestic pets, traps were placed away from houses. To increase capture rate, we moved traps that were unsuccessful at a trap site within 3 nights ≥100 m from the original site.

Within the suburban study area, we selected 6 study sites (each 25-km²) so that they were similar to each other based on human population (\bar{x} = 914, SE = 109.9) and housing densities (\bar{x} = 343, SE = 46.2; U.S. Census Bureau 2001). We determined land use (Vogelmann et al. 2001) by using aerial digital orthophotos (U.S. Department of Agriculture Farm Services Agency Aerial Photography Field Office, Salt Lake City, Ut.), and ground observation. We assigned baiting treatments randomly (helicopter, hand baiting, bait stations) to the study sites so that each treatment was replicated twice. Each study site was comprised of 25 cells (each 1 km²). Because reported home ranges for urbanized raccoons are generally <1 km² (Prange et al. 2004), we provided for a 1-km buffer zone around each study site to help prevent cross contamination of raccoons traveling between adjacent sites. Therefore, 36 traps were restricted to the 9 1-km² grid cells located in the center of each study site; we placed 4 traps in each 1-km² grid cell such that final trap density was 4 traps/km². Trap locations were spaced ≥100 m apart within their respective cells. For each recaptured

raccoon, we recorded the distance between the 2 farthest points of capture to estimate mean linear raccoon movement.

We trapped raccoons at 47 sites within the rural study area (1,664 km²) targeting likely raccoon habitat (e.g., forests, streams, agricultural lands, culverts) where landowners gave permission. Because we considered the rural study area a single contiguous unit, trap densities ranged from 0.03 to 0.09 traps/km². Rural trap sites were irregularly distributed, but we made efforts to disperse trap sites across the entire study area. We placed 4 to 20 traps in linear arrays of 30-m intervals, and each trap location comprised 2 traps spaced 2 m apart. The nearest rural trap location was 4.7 km from the suburban study area to prevent cross contamination between areas.

We immobilized raccoons with a 10:1 dilution of ketamine:xylazine (10 mg/kg; Phoenix Scientific Inc., St. Joseph, Mo.) and applied an ophthalmic ointment (Puralube®, E. Fougere & Co., Melville, N.Y.) to prevent corneal desiccation. For serological assays of rabies virus neutralizing antibodies (VNA), we used vacutainer tubes (BD, Franklin Lakes, N. J.) to collect 10-ml blood samples from femoral veins. We extracted the first upper premolar tooth for biomarker and cementum age analyses. Raccoons were marked on each ear with numbered Monel #3 ear tags (National Band and Tag Co., Newport, Ky.). Sex, relative age (adult or juvenile), and weight of anesthetized raccoons were recorded, followed by a recovery period before release at the point of capture. We immediately released all nontarget species and within-year raccoon recaptures each morning. Low year-to-year recapture rates during this study precluded mark-recapture estimates of raccoon densities. This research conformed to the requirements of Cornell University's Institutional Animal Care and Use Committee (Protocol No. 95-79-01).

We refrigerated (4° C) blood samples for 24 hours to separate serum from clotted blood. Sera were aliquoted into 2-ml skirted screw-top tubes (Laboratory Products Sales, Rochester, N. Y.) and frozen (-20° C) for subsequent testing. The New York State Department of Health Rabies Laboratory Wadsworth Center (Slingerlands, N. Y.) conducted an *in vitro* virus neutralization test (Trimarchi et al. 1996) to detect VNA; the

minimum level of detectable VNA was 0.125 international units (IU)/ml, which served as the foundation for our comparisons.

We estimated the proportion of raccoons that had consumed V-RG baits by examining blood serum for VNA and tooth samples for tetracycline biomarker. Detection of VNA may result from ingestion of V-RG or natural immunity. In a previous study, free-ranging raccoons with naturally acquired VNA remained seropositive for 2 years (Bigler et al. 1983). The fish meal polymer bait matrix included tetracycline, which, upon consumption, forms deposits in the teeth and bones of raccoons (Olson et al. 2000). While tetracycline deposits may persist for the life of the animal (Johnston et al. 1987), detectable antibody levels peak several weeks after ingestion. Raccoons may absorb tetracycline from sources other than vaccine-laden baits, but the incidence of this occurrence is low (Nunan et al. 1994). Detection of biomarker generally runs higher than VNA, but exceptions have been reported (Johnston et al. 2005, Sidwa et al. 2005).

Matson's Laboratory LLC (Milltown, Mont.) conducted raccoon aging and tetracycline biomarker testing. A Buehler low-speed saw (Buehler Ltd., Lake Bluff, Ill.) cut 100- μ m sections from extracted tooth samples, which were subsequently mounted on glass slides. An epi-fluorescence microscope was used to examine the presence of biomarker (Matson and Kerr 1998); at least 1 fluorescent yellow band in a tooth section indicated a tetracycline-positive sample.

Independent variables and statistical analyses

We developed models using the information-theoretic approach (Burnham and Anderson 2002), and logistic regression models were ranked using Akaike's (1973) Information Criterion (AIC). We selected variables for regression analyses based on hypothesized biological, census, weather, and geographical variables important to raccoon vaccination. We constructed a set of 12 candidate models that included combinations of treatment year; raccoon sex and age (in years); number of days post treatment; distance from raccoon capture to nearest stream and road; \bar{x} daily temperature (°C) and total precipitation (cm) during

treatments plus 7 days; human population density per km²; land use; and a global model that included all variables. We combined the suburban treatments to include an additional variable study area (suburban vs. rural) for this analysis. We considered valid models to fall within 2 units of the minimum AIC and used them to make inferences.

We derived human population densities per km² using census data (U.S. Census Bureau 2001). We obtained data on weather variables from the National Weather Service (U.S. Department of Commerce National Oceanic and Atmospheric Administration, Silver Spring, Md.). We obtained the remaining geographic data from Cornell University Geospatial Information Repository (CUGIR; Ithaca, N. Y.).

Land use in Erie County was determined using the National Land Cover Dataset (Vogelmann et al. 2001). We reduced the individual land classes for this dataset to 7 simplified habitats: water, low-intensity residential, high-intensity residential, barren, forest, agriculture, and wetlands. Without a formal radiotelemetry study of home ranges, we assumed that buffer zones around each captured raccoon represented approximate individual raccoon home ranges. Because reported home ranges for urbanized raccoons are generally <1 km² (Hoffman and Gottschang 1977, Prange et al. 2004), we provided a 1-km² zone around each point of raccoon capture and determined the proportions of land use therein. In rural areas, raccoon home ranges vary widely (Zeweloff 2002), but are typically ≤1 km² (Prange et al. 2004). Because reported raccoon home ranges in a relatively nearby state (Massachusetts) were ≤4.4 km² (Olsen 1983), we increased the zone to 4 km² in the rural study area to account for potentially larger raccoon home ranges.

We used a quadratic kernel function described by Silverman (1986) and ArcGIS 9.1 GIS software (Spatial Analyst) to discern relative densities of rabies cases, and, thus, areas of potential virus spread. We conducted Pearson's χ^2 test to discern differences between

biomarker and VNA-positive raccoons for year, study site, sex, and age categories. We used SAS 9.1 (SAS Institute Inc. 2004) statistical software to analyze data.

Results

Descriptive analysis

The fixed-wing aircraft broadcast baits such that final bait density was approximately 75 baits/km². Helicopters broadcasted baits at approximately 118 baits/km². Each year volunteers distributed 75 baits/km² across hand baiting sites. Bait stations dispensed approximately 93, 59, and 85 baits/km² in 2003, 2004, and 2005, respectively.

Overall, rabies densities in raccoons and skunks ranged from 0–1.0 cases/km² in Erie County (Figure 1). Rabies densities were highest in northeastern Erie County; the townships of Clarence ($n = 40$), Newstead ($n = 31$), and Amherst ($n = 27$) ranked highest in terms of number of cases and comprised 26% of the total.

We observed a reduction in the number of rabies-positive raccoons and skunks in the years following the start of the ORV program in 2002 (Figure 2). After the initial peak and recovery from the rabies epizootic, the mean number of cases per year from 1997 to 2002 was 26 (SE =

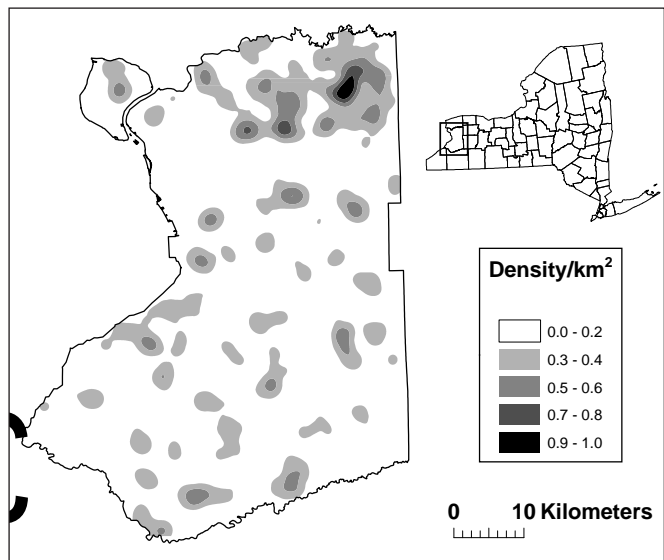


Figure 1. Rabid raccoon (*Procyon lotor*) and striped skunk (*Mephitis mephitis*) densities per km² in Erie County, New York, 1992–2006.

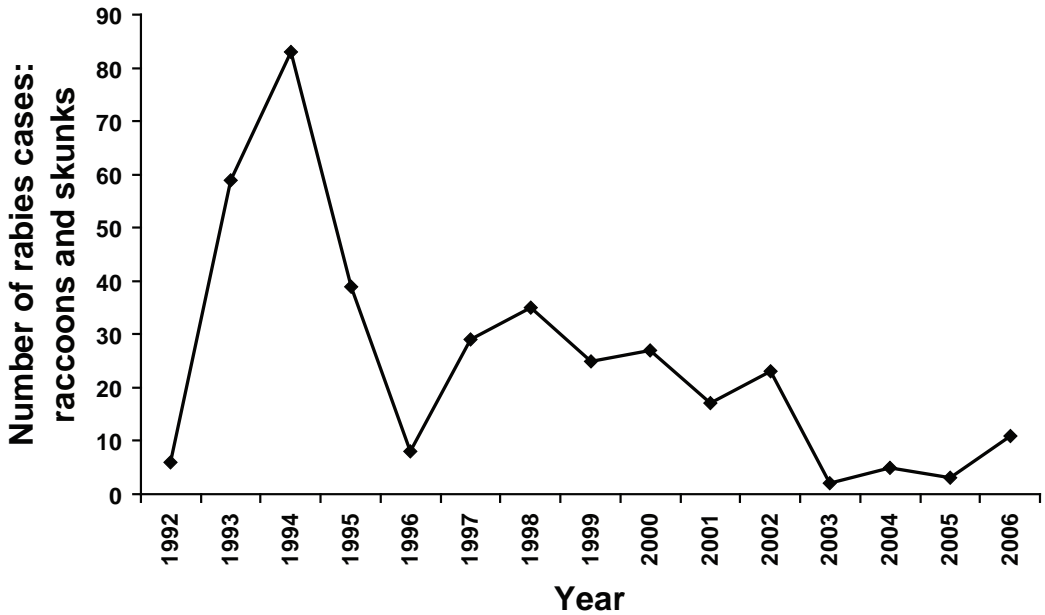


Figure 2. Frequency of positive raccoon and skunk rabies cases in Erie County, New York, 1992–2006. Oral rabies vaccination (ORV) initiated in 2002.

2.3). Thereafter, the mean number of cases from 2003 to 2006 dropped to 5 (SE = 2.0) per year.

Reported human and domestic pet contact with liquid vaccine remained relatively low during the study period. During 2002–2006, 101 phone calls pertaining to bait contacts were recorded, with 53% ($n = 54$) and 46% ($n = 46$) of the calls coming from the suburban and rural study areas, respectively. There were 117 human bait contacts, including 8 exposures to liquid vaccine, 67 contacts with baits in the suburban study area, and 50 contacts in the rural study area. Within the 6 suburban study sites, 2 bait contacts by humans originated from the hand baiting and bait station treatment sites during 2003–2005; no contacts originated from the 2 helicopter study sites. In addition, 53 dogs (*Canis familiaris*) contacted baits, including 29 exposures to liquid vaccine. Only 2 domestic cats contacted baits during the study.

Serologic evaluation

We received sera results from 1,255 raccoons and removed 14 samples due to lysis, resulting in 1,241 usable samples. Overall, 16% (202 of 1,241) and 9% (108 of 1,241) of the raccoons in Erie County tested positive for VNA at the 0.125 and 0.5 IU/ml levels, respectively.

The percentage of raccoons that tested

seropositive for rabies did not increase after each annual bait distribution. In 2003, 19% (63 of 328) of sampled raccoons had detectable levels (0.125 IU/ml) of VNA. Antibody responses were 14% (66 of 480) in 2004 and 17% (73 of 433) in 2005. These differences, however, were not statistically significant ($\chi^2_2 = 4.42$, $P = 0.11$). Treatment year differed ($\chi^2 = 6.31$, $P = 0.04$) relative to antibody response at the 0.5 IU/ml level. Antibody responses at the 0.5 IU/ml level ranged from 6% in 2004 to 11% in 2003.

There was no difference among bait distribution type (helicopter, bait station, hand baiting, fixed-wing aircraft) and antibody positive raccoons at the 0.125 ($\chi^2_3 = 1.57$, $P = 0.67$) and 0.5 IU/ml levels ($\chi^2_3 = 0.99$, $P = 0.80$; Table 1).

Antibody positive raccoons at the 0.125 IU/ml level included 23% (70 of 311) of the adult males, 23% (79 of 346) of the adult females, 10% (30 of 292) of the juvenile females, and 8% (23 of 292) of the juvenile males. At the 0.5 IU/ml level, we detected VNA in 12% (37 of 311) of the adult males, 11% (39 of 346) of the adult females, 7% (19 of 292) of the juvenile females, and 4% (13 of 292) of the juvenile males. The differences in frequency of VNA among the 4 sex and age categories were significant at the 0.125 ($\chi^2_3 = 42.61$, $P < 0.01$) and 0.5 IU/ml ($\chi^2_3 =$

Table 1. Bait treatment type segregated by frequencies of rabies neutralizing antibody titer at the 0.125 and 0.5 IU/ml levels in Erie County, New York, 2003–2005.

| Treatment | Titer (≥ 0.125 IU/ml) ^a | | | | Titer (≥ 0.5 IU/ml) ^b | | | |
|--------------|--|--------------|----------|------------|--|--------------|----------|------------|
| | Negative | | Positive | | Negative | | Positive | |
| | % | <i>n</i> | % | <i>n</i> | % | <i>n</i> | % | <i>n</i> |
| Helicopter | 82.7 | 272 | 17.3 | 57 | 91.8 | 302 | 8.2 | 27 |
| Bait station | 82.8 | 259 | 17.3 | 54 | 90.4 | 283 | 9.6 | 30 |
| Hand baiting | 85.9 | 268 | 14.1 | 44 | 92.3 | 288 | 7.7 | 24 |
| Fixed-wing | 83.6 | 240 | 16.4 | 47 | 90.6 | 260 | 9.4 | 27 |
| Total | | 1,039 | | 202 | | 1,133 | | 108 |

^aOverall $\chi^2_3 = 1.57$; $P = 0.67$

^bOverall $\chi^2_3 = 0.99$; $P = 0.80$

15.28, $P < 0.01$) levels.

Suburban biomarker analysis

Of the 965 unique raccoons we captured in the suburban study area, 9% (85) were recaptured during the last 2 years of this study. The mean linear distance traveled between the 2 farthest points of capture for recaptured raccoons was 0.52 km (SE = 0.05). Most ($n = 77$) raccoons were recaptured <1 km from the first capture location. Six raccoons traveled between 1 km and 2 km, and 2 raccoons traveled >2 km.

We examined tooth sections from 958 suburban raccoons for deposits of tetracycline biomarker. The final sample size was 954 after removing 4 individuals due to questionable biomarker results. Overall, 38% (358 of 954) of the suburban raccoons in Erie County tested positive for biomarker.

The percentage of biomarker-positive raccoons did not show an increase between 2003 and 2004, but showed an increase between 2004 and 2005. Positive biomarker rates were 35% (107 of 302) in 2003, 32% (103 of 327) in 2004, and 46% (148 of 325) in 2005. These differences were statistically significant ($\chi^2_2 = 14.46$, $P < 0.01$).

No difference ($\chi^2_2 = 5.02$, $P = 0.081$) existed between treatment type (helicopter, hand baiting, bait stations) and biomarker frequencies across all years (2003–2005) combined. Biomarker-positive raccoons included 37% (114 of 310) of the adult males, 30% (102 of 343) of the adult females, 30% (88 of 293) of the juvenile females, and 22% (64 of 295) of the juvenile males. The differences in biomarker frequency among the 4 sex and age categories were significant ($\chi^2_3 = 16.51$, $P < 0.01$).

After accounting for missing data ($n = 13$), 25% (90 of 353) of biomarker-positive raccoons were also positive for VNA at the 0.125 IU/ml level in the suburban study area. Conversely, 59% (90 of 153) of antibody-positive (0.125 IU/ml) raccoons were also positive for biomarker.

Factors related to raccoon vaccination

Three of 12 candidate models describing the presence or absence of VNA were within 2 units of the minimum AIC value for both 0.125 and 0.5 IU/ml antibody level model sets, and models that included age had the lowest (best) Δ AIC (Δ AIC = 0–10.8, models 1–5 in Table 2). In the most parsimonious model, raccoon age alone best explained the presence of VNA at the 0.125 IU/ml level, and was 0.5 AIC units ahead of its closest competitor (Table 2). A yearly increase in raccoon age was associated with a 30% increase in the predicted odds of antibody response (odds ratio [OR] = 1.30, 95% confidence interval [CI] = 1.21–140). Confidence intervals for remaining variables in the 0.125 IU/ml model set included one. At the 0.5 IU/ml level, raccoon age and year of study comprised the most parsimonious model, and was 1.4 AIC units ahead of its closest competitor (Table 2); a yearly increase in raccoon age was associated with a 15% increase in the predicted odds of antibody response (OR = 1.15, 95% CI = 1.05–1.26). The odds of VNA prevalence in 2005 were about 1.6 times the odds in 2004; however, the CI included one (OR = 0.61, 95% CI = 0.37–1.00). Confidence intervals for remaining variables in the 0.5 IU/ml model set also included one.

Exploratory post-hoc stepwise logistic regression analysis of the global model at 0.125 IU/ml corroborated the results of a priori

Table 2. Model rank, model, number of estimable parameters (K), maximized log-likelihood (log [L]), Akaike's Information Criterion (AIC), Δ AIC, and Akaike weights (w_i) for top 5 logistic regression models with an antibody response of 0.125 IU/ml^a and 0.5 IU/ml^b. Models were ranked by AIC.

| Rank | Model ^c | K | log (L) | AIC | Δ AIC | w_i |
|----------------|--------------------|----|----------|--------|--------------|-------|
| 1 ^a | Age | 2 | -509.143 | 1022.3 | 0 | 0.41 |
| 2 | Age+ year | 3 | -507.390 | 1022.8 | 0.5 | 0.32 |
| 3 | Age + sex | 3 | -509.136 | 1024.3 | 2.0 | 0.15 |
| 4 | Age + area + year | 4 | -507.299 | 1024.6 | 2.3 | 0.13 |
| 5 | Global | 11 | -504.553 | 1033.1 | 10.8 | <0.01 |
| 1 ^b | Age + year | 3 | -342.824 | 693.6 | 0 | 0.50 |
| 2 | Age + area + year | 4 | -342.509 | 695.0 | 1.4 | 0.25 |
| 3 | Age | 2 | -345.781 | 695.6 | 2.0 | 0.18 |
| 4 | Age + sex | 3 | -345.761 | 697.5 | 3.9 | 0.07 |
| 5 | Global | 11 | -339.499 | 703.0 | 9.4 | <0.01 |

^c The following variables were considered: age (in years), sex, area (suburban versus rural), year, days since end of treatment, human population density/km², \bar{x} temperature during treatment plus 7 days, \bar{y} precipitation during treatment plus 7 days, distance of capture to closest road, and distance of capture to closest stream

analyses. However, only raccoon age ($P < 0.01$) was retained in the 0.5 IU/ml model; a yearly increase in raccoon age was associated with a 16% increase in the predicted odds of antibody response (OR = 1.16, 95% CI = 1.05–1.27). Although the overall χ^2 was significant ($P < 0.01$) for both post-hoc models, the area under the receiving operating characteristic, a measure of predictive accuracy, was low for 0.125 IU/ml (0.65) and 0.5 IU/ml antibody titer (0.61) levels (SAS Institute, Inc. 2004). Multicollinearity precluded the use of land use in the models.

Discussion

Epizootic movement of terrestrial rabies in Erie County (31 km/year) fell within the lower range of those previously reported. In Connecticut, estimated wave front velocities ranged from 30 km/year (Wilson et al. 1997) to 46 km/year (Lucey et al. 2002). Other reported velocities include 38 km/year in Pennsylvania (Moore 1999), 40 km/year in New York (Raczkowski and Trimarchi 2001), and 47–50 km/year in New Jersey (Roscoe et al. 1998). Differences in land use and habitat likely explain the varying velocities in the aforementioned studies, but unintentional translocation of raccoons may be another factor. For example, the spread of rabies jumped

100 km from Connecticut to Massachusetts due to suspected translocation of raccoons in refuse trucks (Wilson et al. 1997).

We identified rabies clustering in the towns of Amherst, Clarence, and Newstead, New York. Expansive suburban sprawl (83 km²) and woodland corridors (69 km²) that make for ideal raccoon habitat characterize Amherst and Clarence. Amherst ranks second in terms of human population density within Erie County (U.S. Census Bureau 2001). Land use within Newstead is characterized by agricultural land (94 km²) and forests (32 km²), but also includes a suburban center. Suburban areas are associated with higher densities of raccoons (Riley et al. 1998), which may explain the higher densities of rabies cases in northeastern Erie County. While we can use kernel density estimate to assess rabies densities in enzootic areas, this approach may offer little information in areas of new rabies outbreaks where few positive cases exist.

Without the availability of a formal radiotelemetry study of home ranges, we used the mean distance between the 2 farthest points of capture (Schinner and Cauley 1974, Hoffman and Gottschang 1977) to determine whether cross contamination of raccoons traveling between adjacent treatment areas was

problematic. In this study, only 9% ($n = 8$) of recaptured raccoons traveled distances greater than buffer zones separating treatments (i.e., 1 km).

Our observed proportion of raccoons with VNA was less than the lowest range (30–70%) of previously reported field trials (Hanlon et al. 1996, 1998; Robbins et al. 1998, Roscoe et al. 1998). In the suburban study area, observed biomarker levels fell within the lower range of previously reported placebo and vaccine trials (Perry et al. 1989, Hable et al. 1992, Hanlon et al. 1993, Roscoe et al. 1998, Olson et al. 2000). It is difficult to make direct comparisons among studies because of differences in bait and raccoon densities, tooth and bone samples used for biomarker testing, seroconversion cut-off levels, bait type and distribution method, and land use. Olson et al. (2000) suggested that low proportions of tetracycline-positive raccoons might be a reflection of high raccoon densities. In addition, greater competition for baits from nontarget wildlife species may exist in urban and suburban landscapes. Virginia opossums (*Didelphis virginiana*) and striped skunks in urban habitats may share food resources with raccoons in urban habitats (Prange and Gehrt 2004). Rosatte et al. (1991) and Broadfoot et al. (2001) reported elevated skunk densities in urban Ontario, Canada, but it is unknown whether opossum densities are greater in urban areas (Prange and Gehrt 2004). Using a higher density of baits might improve the proportion of raccoons testing positive for VNA and biomarker.

Despite relatively low proportions of antibody- and biomarker-positive raccoons, we noted a reduction in terrestrial rabies in Erie County since the first ORV distribution in 2002. Guerra et al. (2003) reported 4-year to 5-year epizootic cycles in the mid-Atlantic states for raccoons and skunks. Childs et al. (2000) reported a 4-year period between the beginning of the first and second rabies epizootics, which was consistent with our data. Based on the Childs et al. (2000) study, however, we expected to see a second increase in rabies. Instead, we saw a decrease prior to 2002. Without more data, it is difficult to determine whether a natural decrease in rabies occurred concomitantly with ORV control during 2002, or if the reduction in rabies occurred primarily from ORV.

Results from this study indicated that the treatment year did not influence 0.125 IU/ml antibody rates. However, the treatment year differed relative to antibody response at the 0.5 IU/ml level and to biomarker response in the suburban study area. In 2004, the proportion of raccoons testing positive for rabies antibody and biomarker was lower than in other years. Heavy rains caused several inches of flooding in portions of the study area in 2004, which may have impeded raccoon foraging. Alternatively, a greater abundance of natural food may have been available during 2004.

Unexpectedly, no differences existed between treatment type (i.e., fixed-wing aircraft, hand, bait station, and helicopter deployments) and antibody positive (0.125 and 0.5 IU/ml) raccoons across all years combined. For example, we expected greater antibody response either in the hand-baiting study sites where we targeted raccoon habitat or in the helicopter sites that received the highest density of baits. Bait distribution densities in our study ranged from 59 to 118 baits/km². Therefore, it appears that variation in bait density at these levels did not significantly increase vaccination rates. Perry et al. (1989) found no significant difference between differing bait densities (450 versus 120 baits/km²) and bait uptake levels. Robbins et al. (1998), however, reported significantly increased vaccination rates of raccoons when distributing extra baits to prime raccoon habitats.

Within the suburban study area, biomarker rates were higher than seroconversion rates. Moreover, not all raccoons testing positive for biomarker had detectable levels of antibody and vice versa. Different sample populations, time of consumption of bait relative to sampling, and ingestion of the bait matrix, but not V-RG, may explain these differences (Roscoe et al. 1998). Differences in raccoon immunity, degradation of V-RG, and dilution of V-RG by concurrent consumption of other food and water may also explain the higher biomarker rates (Sidwa et al. 2005). A recent study that investigated tetracycline stability in fishmeal polymer V-RG baits suggests that tetracycline may convert to epitetracycline during the manufacturing process; approximately 40% of the target quantity of biomarker was unavailable for absorption, possibly resulting in low biomarker detection rates (Johnston et al. 2005).

Based on an Ontario, Canada, study in an area free of ORV control efforts, only 0.2% and 0.4% of raccoons and skunks, respectively, exhibited tetracycline-like fluorescence in their teeth (Nunan et al. 1994). In that study, the tetracycline-positive animals had likely eaten afterbirths from cows treated with tetracycline. In our study, tetracycline incorporated into fishmeal polymer baits was restricted to the suburban study area where there were few farms. Thus, background tetracycline from sources other than ORV efforts in Erie County was likely low.

Management implications

Future efforts to control the spread of raccoon rabies should quantify the relationship between raccoon seasonal population density and the minimum density of baits necessary to confer population immunity (Rupprecht et al. 1995, Blackwell et al. 2004). Specifically, managers should consider target bait densities in excess of 75 baits/km² in areas of higher risk. In addition, KDE should be used to prioritize bait distribution in rabies enzootic areas where ORV rabies control is considered.

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