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# Oral Rabies Vaccination of Raccoons (*Procyon lotor*) across a Development Intensity Gradient in Burlington, Vermont, USA, 2015–2017

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ABSTRACT: Management of the raccoon rabies virus variant in North America is conducted primarily using oral rabies vaccination (ORV). When a sufficient proportion of the population is vaccinated (~60%), rabies transmission can be eliminated. To date, ORV programs have successfully controlled and eliminated raccoon rabies in rural areas, but there has been less success in urban areas. We studied the proportions of rabies virus neutralizing antibodies (RVNA) in a raccoon (*Procyon lotor*) population during a 3-yr ORV trial in developed areas of Burlington, Vermont, US. We used a modified *N*-mixture model to estimate raccoon abundance, RVNA seroprevalence, and capture rates jointly to examine factors that relate to ORV success to better inform management. We found that raccoon abundance was lower in less-developed areas compared to urban centers. Raccoon RVNA seroprevalence decreased as population abundance increased; it increased as the average age of the population increased. Nontarget opossum (*Didelphis virginiana*) captures correlated with a decrease in raccoon RVNA seroprevalence in low-development areas, suggesting that they may be competing for baits. The target bait density across the entire study area was 150 baits/km$^2$, but a hand baiting strategy was heavily concentrated on roads, resulting in uneven bait densities within sampling sites (0–484 baits/km$^2$). Uneven bait distribution across the study area may explain low RVNA seroprevalence in some locations. Our results suggest that increases in bait density across the study area may improve RVNA seroprevalence and support annual ORV to account for raccoon population turnover.

*Key words:* Field trial, ONRAB, *Procyon lotor*, rabies virus, seroprevalence, urban, wildlife disease management.

## INTRODUCTION

Rabies remains a significant wildlife management and public health challenge in the US (Pieracci et al. 2020). Among meso-carnivores, a stable focus persists in populations of raccoons (*Procyon lotor*) in the eastern US (Gilbert 2018). The US Department of Agriculture (USDA), Wildlife Services, National Rabies Management Program (NRMP) coordinates oral rabies vaccination (ORV) targeting wild meso-carnivores. More than 9 million vaccine-laden baits are distributed annually across diverse landscapes, particularly in the eastern US along the border of the area where raccoon rabies virus (RRV) is enzootic (Elmore et al. 2017).

Experimental ORV field trials to test an Ontario Rabies Vaccine Bait (ONRAB; Artemis Technologies, Inc., an indirect, wholly owned subsidiary of Ceva Sante Animale, S.A., Guelph, Ontario, Canada) targeting raccoons began during 2011 in West Virginia, US (Slate et al. 2014). Use of ONRAB in rural areas of the US has since shown promise at achieving raccoon seroprevalence close to target levels (60–80%) needed for RRV elimination (Rees et al. 2013; Reynolds et al. 2015; McClure et al. 2020). Mean post-ORV seroprevalence using 75 baits/km$^2$ during three

3-yr ONRAB trials was 52% in West Virginia (Slate et al. 2014; Johnson et al. 2021), 69% in the northeastern US (Gilbert et al. 2018b), and 58% in the St. Lawrence River region of New York state, US (Pedersen et al. 2019a).

These and other studies in eastern North America investigated how landscape composition impacts raccoon vaccine bait encounters, uptake, and rabies virus neutralizing antibody (RVNA) response in rural areas following ORV (e.g., Boyer et al. 2011; Berentsen et al. 2013; Pedersen et al. 2019b). Fewer studies have reported on the effectiveness of ORV in urban or suburban raccoon populations. Recent studies in Long Island, New York, reported lower raccoon RVNA seroconversion in medium- and high-intensity development areas, and greater success in ORV uptake with increasing distances from roads (Bigler et al. 2021a, b). The likelihood of RVNA seroconversion in raccoons following ORV with ONRAB was negatively impacted by the proportion of residential areas near the capture site (Mainguy et al. 2012). From 2012 to 2014, the NRMP conducted an ONRAB trial in urban and suburban areas near Cleveland, Ohio using 150 baits/km$^2$ in a ground bait area. The 3-yr post-ORV mean RVNA (using a 0.0625 IU cutoff) in raccoons was only 34% ($n$=1,464), suggesting challenges vaccinating populations in developed compared to rural areas (USDA 2017).

Urban challenges for ORV, such as higher raccoon densities, smaller home ranges, and fragmented habitats, are well documented and influenced by anthropogenic resources (Prange et al. 2003, 2004; Randa and Yunger 2006; Bozek et al. 2007; Rosatte et al. 2010; Berentsen et al. 2013; Slate et al. 2020). There also may be a greater abundance of nontarget bait competitors in urban areas (e.g., cats [*Felis catus*], dogs [*Canis lupus familiaris*], opossums [*Didelphis virginiana*]), which may impact ORV success targeting meso-carnivore populations. One NRMP goal is to eliminate RRV locally and nationally by moving ORV zones eastward over the next 30 yr (Elmore et al. 2017). As ORV zones move east, extensive urban and suburban habitats will be encountered, requiring a better understanding of effective strategies targeting raccoon populations in these environments.

Our aim was to determine the relative impacts of baiting strategies, raccoon population characteristics, and landscape (development intensity and competitor abundance) on raccoon RVNA seroprevalence, to inform rabies management.

## MATERIALS AND METHODS

### Study area and habitat

Chittenden County, Vermont, US (44°28′55″N, 73°09′47″W) is within the urban and suburban ORV ground bait zone in the greater Burlington area. The hand-baiting zone (219 km$^2$) was overlaid with 1-km$^2$ cells and the percent of habitat types was determined for each cell using the 2011 National Land Cover Database (NLCD; Multi-Resolution Land Characteristics Consortium 2011). With an emphasis on NLCD values 21 (developed, open space), 22 (developed, low intensity), 23 (developed, medium intensity), and 24 (developed, high intensity), cells were classified into low-, medium-, or high-intensity human development (see Supplementary Materials S1.1 for details). Four nonadjacent sampling cells, separated by at least 1 km, were randomly selected for each of the three development intensities (Fig. 1A). Sampling cells had minimum spatial buffers of 1.2 km to the edge of the ground bait zone, to limit the influence of raccoon movement in and out of the ORV zone. Mean percent development across cells in each of the three intensity classes was 45% for low (range 28–58%), 67% for medium (range 54–86%), and 92% for high (range 87–96%).

### Oral rabies vaccine bait and distribution strategies

During August 2015–2017, approximately 24,000 ONRAB vaccine baits (Rosatte et al. 2009b) were distributed by hand throughout the study area at a target density of 150 baits/km$^2$ based on NLCD habitat classifications (McClure et al. 2022). Habitats with higher development intensity received fewer baits than areas with lower development intensity because of baiting off-time associated with unbaitable habitat (parking lots, large building footprints, etc.). In urban and suburban areas, baits are

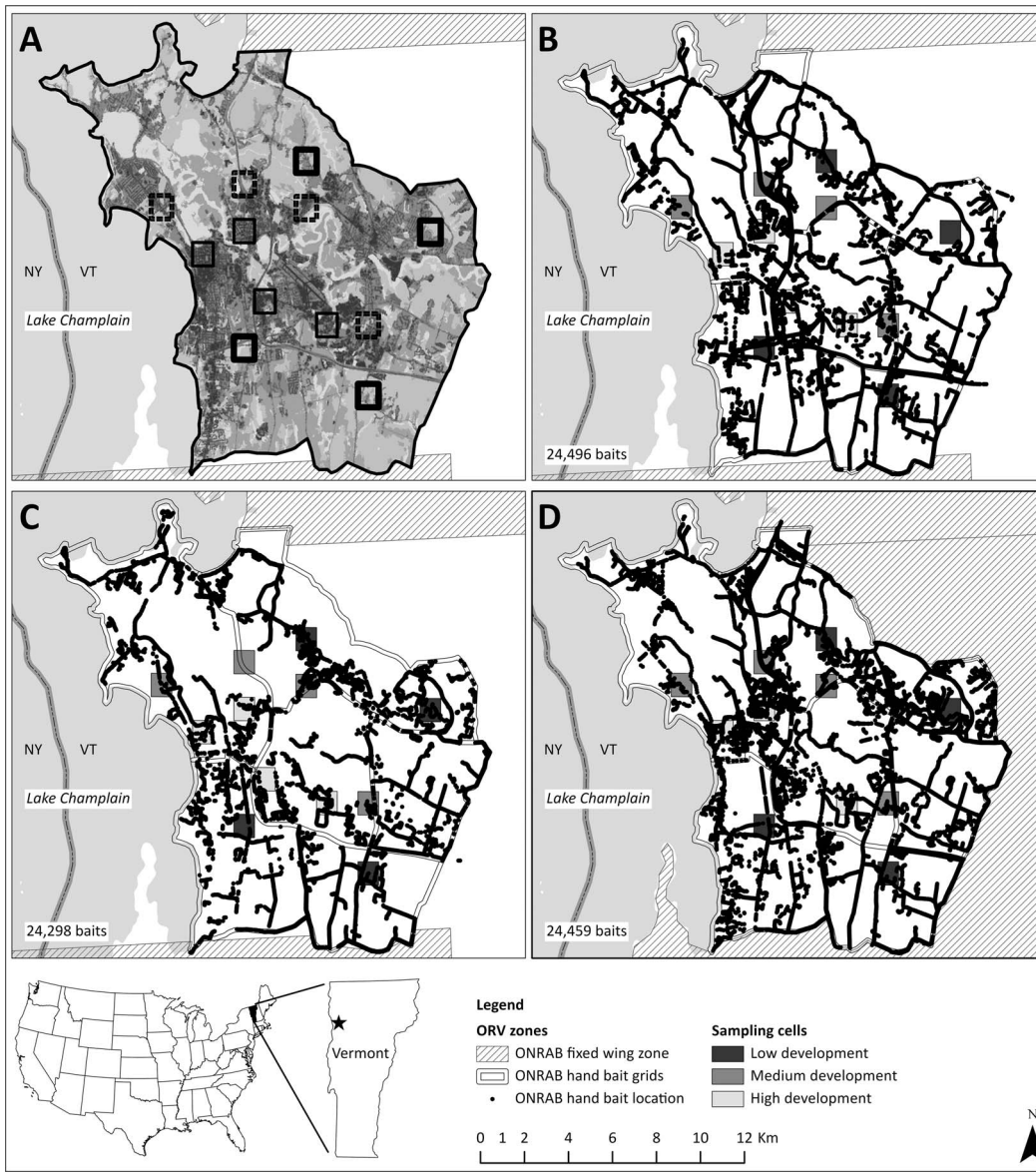


FIGURE 1. Study area where oral rabies vaccination using ONRAB (Artemis Technologies Inc., an indirect, wholly owned subsidiary of Ceva Sante Animale, S.A., Guelph, Ontario, Canada) at 150 baits/km$^2$ was evaluated in the greater Burlington, Vermont, USA area (black star on state map). (A) ONRAB hand bait zone grids (double black lines) with National Land Cover Database habitat (Multi-Resolution Land Characteristics Consortium 2011); darker shades of gray indicate higher development intensities, while lighter shades include water, wetlands, forest, and agriculture. Sampling cells were 1 km$^2$: low (thicker black squares), medium (dashed squares), and high development intensity (thinner black squares) in panel A. Panels B–D show the same hand bait grids and sampling cells (see legend) with ONRAB bait locations (black dots) for (B) 2015, (C) 2016, and (D) 2017. Areas of white indicate no baiting occurred. Baits were distributed in the same 219-km$^2$ hand bait zone annually across six grids mean area 37 km$^2$.

hand distributed using either slow moving vehicles (targeting hedgerows between properties, culverts under streets, dumpsters behind businesses), or by walking sidewalks, railroad tracks, bike paths and placing baits in areas probably used by raccoons that are less likely to be encountered by people or pets (Gilbert and Chipman 2020). Baits were distributed in the same 219-km$^2$ hand bait zone

annually across six grids of mean 37 $km^2$ (Fig. 1A). Field staff were assigned a number of baits per grid and recorded the location of baits distributed using push-button, screenless point-of-interest (POI) units (G-Log 760, Transystem Inc., Miaoli, Taiwan). We mapped POI coordinates (dots) within sampling cells and used ArcMap 10.8 (ESRI, Redlands, California, USA) to count the number of POI dots as a proxy for number of ONRAB baits distributed per cell. The study area had been previously annually baited by hand (vehicles and walking as described above) from 2002 to 2011 with RABORAL V-RG® (Boehringer Ingelheim Animal Health USA, Inc. [formerly Merial, Inc. during the years of use], Duluth, Georgia, USA) at 70–75 baits/$km^2$, then ONRAB at 75 baits/$km^2$ from 2012 to 2014.

#### Trapping, animal handling, and sampling

Sampling cells were trapped for 10 d consecutively in July (pre-ORV) and again in October (post-ORV) during 2015-2017. Each cell contained 25 live traps (model 608, Tomahawk Live Trap, LLC, Hazelhurst, Wisconsin, USA) baited with marshmallows and anise oil (Minnesota Trapline Products, Pennock, Minnesota, USA). Efforts were made to distribute traps evenly across cells given development and property access constraints. Traps were checked once daily and moved within a cell every 2–3 d if no unique target animals had been captured. Raccoons were the primary target; striped skunks (*Mephitis mephitis*), gray and red foxes (*Urocyon cinereoargenteus* and *Vulpes vulpes*), and fishers (*Pekania* (*Martes*) *pennanti*) were secondary targets because of their potential as spillover species and/or bait competitors.

Target animals were anesthetized using a 5:1 mixture of ketamine (10 mg/kg; KetaVed™, Vedco Inc., Saint Joseph, Missouri, USA) to xylazine (2 mg/kg; AnaSed®, Akorn Operating Company LLC, Gurnee, Illinois, USA) via intramuscular injection (Kreeger 1999). Under anesthesia, animals received a unique ear tag identification (Monel No. 3 tags, National Band and Tag Co., Newport, Kentucky, USA) and the sex, reproductive status, relative age (juvenile or adult), weight, and general condition were recorded. A 5–7-mL blood sample was collected from the jugular vein directly into a Vacutainer setup: 21 gauge by a 2.54-cm needle; plastic holder; and 8.5-mL tubeto containing serum separator gel (Becton Dickinson, Franklin Lakes, New Jersey, USA). A first premolar tooth (when available) was extracted using a dental elevator and extracting forceps. Target animals were released at the capture site after full recovery from anesthesia. All nontarget animals, as well as target animals recaptured within the same 10-d trapping session, were released immediately at the points of capture without sampling. Animals exhibiting abnormal behavior or severe lesions were euthanized under heavy anesthesia using potassium chloride injected intracardially (American Veterinary Medical Association 2020). A brainstem sample was collected immediately postmortem (Patrick et al. 2019), stored in a cooler with ice packs, and submitted for laboratory testing that day.

#### RVNA determination and antigen testing

Serum samples were separated from whole blood by low-speed centrifugation (up to 1,327 × G) on the day of capture and stored in labeled cryovials at −25 to −70 C before shipment to the New York State Department of Health (NYSDOH), Albany, New York, US, for RVNA serologic analysis using a modified virus neutralization test (Trimarchi et al. 1996). Rabies titers ≥0.125 IU/mL were considered RVNA positive, as reported previously (Gilbert et al. 2018a; Pedersen et al. 2019a; Johnson et al. 2021). Additionally, RVNA titers approximately equivalent to or slightly lower than our threshold (0.05–0.11 IU/mL) had been associated with protection against a RABV challenge in an experimental study with raccoons from a population managed with ORV (Blanton et al. 2018). A meta-analysis of several experimental studies reported cutoff thresholds of 0.25–0.5 IU/mL as potential surrogates of protection against RABV challenge in orally vaccinated raccoons (Moore et al. 2017; Moore 2021). We have used the 0.125 IU/mL cutoff for purpose of ORV monitoring as an index to population immunity and for consistency and comparability with prior related studies. We have previously reported on the comparative testing of the modified virus neutralization test at this threshold with other serologic methods (e.g., rapid fluorescent focus inhibition test [RFFIT], ELISA) and laboratories (Gilbert et al. 2018a; Johnson et al. 2021). Brainstems were tested for RABV antigens by the Vermont Department of Health (VDH) Laboratory in Burlington, Vermont, US, using the direct fluorescent antibody test (Centers for Disease Control and Prevention 2017). Variant typing was not conducted for this study.

### Age determination

Teeth were shipped to Matson's Laboratory (Manhattan, Montana, USA) to determine a numeric age from cementum as described (Johnston et al. 1987). Results were returned to the nearest year: 0 for young-of-the-year juveniles and ≥1 for adults.

### Population-level analysis

We estimated raccoon abundance ($N$) and RVNA seroprevalence ($S$) post-ORV in 2015–2017 by modifying a multinomial $N$-mixture model with removal sampling (Kéry and Royle 2015). This model type uses daily counts of unmarked (unique) individuals to estimate the probability of capturing an unmarked individual during a daily count; capture probability is then used to generate abundance estimates. The model accounts for the decreasing probability of capturing a unique animal as individuals in the population are captured and marked (Kéry and Royle 2015). We modified the base model to include estimates of RVNA seroprevalence in each cell, that is, to compare daily counts of unmarked seropositive individuals to daily counts of all unmarked individuals to estimate cell-level seroprevalence (see Supplementary Materials S1.3 for more details).

We allowed abundance to vary with development intensity and capture rate to vary with trap availability (if traps were triggered by other species, they were not available to capture raccoons). We examined effects of habitat (human development level), population composition (raccoon abundance and average numeric age), competition (numbers of other species caught), and management (ORV bait density and coverage). Bait coverage was calculated by creating a 30-m buffer (McClure et al. 2022) around each POI dot to represent the area of effect for each bait. We merged all buffers into a single polygon, then calculated coverage as the proportion of the study cell that intersected the buffer polygon. Model parameters were estimated using a Bayesian hierarchical model with uninformed priors in the programs JAGS (Plummer 2017) and R (R Core Team 2021). We evaluated covariate effects using the 75% credible interval (CI) as an exploratory metric and followed up with the appropriate frequentist analysis (e.g., linear regression, ANOVA). We used the Watanabe-Akaike Information Criterion (WAIC) to perform model selection (Hooten and Hobbs 2015). We used posterior predictive checks to ensure the model was internally consistent (i.e., that model results made sense; Gelman et al. 1996, 2013) and post hoc frequentist tests to complement the Bayesian analyses. We used a randomization test (Supplementary Materials S1.3) to determine whether the model is able to distinguish significant model coefficients from random noise.

To ensure that our covariate effects were not sensitive to the RVNA cutoff we used, we conducted the population level analysis on the same data set but applying a 0.5-IU/mL RVNA cutoff.

## RESULTS

### Data summary

The number of ONRAB baits distributed within the greater Burlington area and the number of POI coordinates recorded as baits had minimal annual variation: 24,496 baits/24,111 POI dots in 2015 (−385 POI error), 24,298 baits/24,495 POI dots in 2016 (+197 POI error), and 24,459 baits/24,289 POI dots in 2017 (−170 POI error). The POI dots (proxy for number of baits distributed) in each sampling cell varied considerably by cell, development intensity, and year (Table 1).

During the 3-yr field trial, 2,274 animals were trapped during 18,000 trap nights: 1,082 (48%) were target animals sampled for RVNA (902 raccoons, 164 skunks, 11 fishers, four gray foxes, one red fox); 818 (36%) were nontarget species released without sampling (including 29 feral cat captures); and 374 (16%) were target animals recaptured during the same trapping session. Opossums represented 34% (275/818) of nontarget captures (19% in low-, 31% in medium-, and 50% in high-development areas).

Among 902 raccoons, 482 (53%) were sampled once, 174 individuals were sampled at least twice. Three raccoons were found dead in traps, and six raccoons and three skunks were euthanized because of observed human or pet exposures, abnormal behavior, or severe lesions. All were tested for rabies; one lactating female raccoon found dead in a trap with a large open abdominal wound during the 2015 pre-ORV session tested positive. Most (42%, $n$=375) of

TABLE 1. Mean ONRAB (Artemis Technologies Inc., an indirect, wholly owned subsidiary of Ceva Sante Animale, S.A., Guelph, Ontario, Canada) bait density (per $km^2$) in cells of varying development intensities (low, medium, high) in the Burlington, Vermont, USA, area, 2015–2107. Minimum and maximum bait densities are in parentheses. Each development intensity had four cells and target bait density was 150 baits/$km^2$.

| | Development density | | |
|---|---|---|---|
| Year | Low | Medium | High |
| 2015 | 163 (18–394) | 109 (45–260) | 188 (73–386) |
| 2016 | 268 (147–431) | 142 (0–317) | 175 (86–318) |
| 2017 | 129 (85–205) | 115 (49–184) | 287 (164–484) |

the 902 raccoons were captured in the medium-development area, 33% ($n$=301) in the high-development area, and 25% ($n$=226) in the low-development area. Of the 902 raccoons, numerical ages were reported for 84% ($n$=758, range 0–11 yr) and sex was recorded for 99.8% ($n$=900). The proportion of males was highest (60%) in the low-development habitat, slightly lower (58%) in the medium-development habitat, lowest (52%) in the high-development habitat.

The RVNA seroprevalence rates and sample sizes for raccoons and skunks varied by year, sampling period, and development (Table 2). Regardless of development type, the 3-yr mean RVNA among raccoons pre-ORV was 28.5% ($n$=523, range: 24.8–32.5%) and 36.1% ($n$=379, range: 31.5–41.1%) post-ORV. Among the fishers, 5/11 (45%) were RVNA positive. All sampled foxes (four gray foxes, one red fox) were RVNA negative.

#### Population-level results

There was model uncertainty in the population level results (Supplementary Table S2.1), suggesting that no single model was strongly supported over the set examined. There were no significant interaction terms based on the 75% confidence interval (CI) and post hoc analyses; therefore, we selected and described the results from the model with all additive covariates.

The model corrected for the probability of capturing a unique raccoon decreasing when fewer traps were available to capture raccoons. Estimated raccoon abundance per cell by year ranged from 2 to 31 (median=10). Medium- and high-development cells had higher estimated raccoon abundance than low-development cells (Fig. S2.1). However, an ANOVA did not reveal differences between development categories ($P$=0.257).

Estimated post-ORV raccoon RVNA seroprevalence per cell ranged from 11.6 to 96.8% (median=39.7%) and varied by year and development class (Fig. 2). Medium-development cells tended to have lower RVNA seroprevalence compared to low development ($P$=0.077, $\eta^2$=0.144).

Raccoon abundance did not affect seroprevalence, based on the 75% CI. A linear model with estimated abundance as the predictor variable and estimated seroprevalence as the response demonstrated a negative relationship ($P$=0.020, $R^2$=0.124; Fig. 3A). Estimated raccoon seroprevalence increased as skunk captures increased ($P$<0.001, $R^2$=0.439, Fig. 3B). Opossum captures were not associated with raccoon seroprevalence based on the 75% CI or the results of a linear model ($P$=0.385, $R^2$=−0.007, Fig. 3C). Opossum captures explained 31% of the variation in raccoon seroprevalence in low-development cells ($P$=0.036, $R^2$=0.305).

The mean numerical age of captured raccoons did not impact raccoon RVNA seroprevalence based on the 75% CI (Supplementary Material Table S2.2). After removing an outlier, a linear regression suggested that raccoon populations with a lower average numeric age tend to have lower estimated RVNA seroprevalence ($P$=0.003, $R^2$=0.213, Fig. 4).

Both the actual bait density and bait coverage had 75% CI that overlapped with zero and therefore are not strong impacts on seroprevalence ($P$=0.054, $R^2$=0.078; $P$=0.709, $R^2$=−0.025, Supplementary Material Fig. S2.3). However, a weak positive relationship between increasing bait density and seroprevalence was observed.

Posterior predictive checks yielded no systemic discrepancies between the observed data

TABLE 2. Total percentage of rabies virus neutralizing antibodies for raccoons (*Procyon lotor*) and striped skunks (*Mephitis mephitis*) sampled in cells with varying levels of human development before (pre) and after (post) oral rabies vaccination using ONRAB (Artemis Technologies, Inc., an indirect, wholly owned subsidiary of Ceva Sante Animale, S.A., Guelph, Ontario, Canada) in the Burlington, Vermont, USA, area, 2015–2017. Sera with antibody titers equal to or greater than 0.125 IU/mL are considered positive. Total sample sizes across four sampling cells for each development class and cumulatively (in total columns and rows) are in parentheses.

| Species | Development density | 2015 Pre | 2015 Post | 2016 Pre | 2016 Post | 2017 Pre | 2017 Post | Total Pre | Total Post |
|---|---|---|---|---|---|---|---|---|---|
| Raccoons | Low | 32 (31) | 46 (28) | 45 (40) | 56 (27) | 14 (56) | 39 (44) | 28.3 (127) | 45 (99) |
| | Medium | 45 (64) | 26 (50) | 37 (51) | 46 (41) | 17.4 (109) | 18 (60) | 29.9 (224) | 28.5 (151) |
| | High | 35 (55) | 50 (46) | 45 (38) | 32 (41) | 13 (79) | 31 (42) | 26.7 (172) | 38.0 (129) |
| | Total | 38.7 (150) | 39.5 (124) | 41.9 (129) | 43.1 (109) | 15.2 (244) | 25.1 (146) | 28.5 (523) | 36.1 (379) |
| Skunks | Low | 0 (1) | 39 (18) | 20 (10) | 42 (12) | 8 (12) | 18 (11) | 13 (23) | 34 (41) |
| | Medium | 10 (10) | 29 (7) | 0 (4) | 0 (2) | 0 (3) | 0 (9) | 6 (17) | 11 (18) |
| | High | 4 (27) | 31 (26) | 0 (5) | n/a (0) | 0 (3) | 0 (4) | 3 (35) | 27 (30) |
| | Total | 5 (38) | 33 (51) | 11 (19) | 36 (14) | 6 (18) | 8 (24) | 7 (75) | 27 (89) |

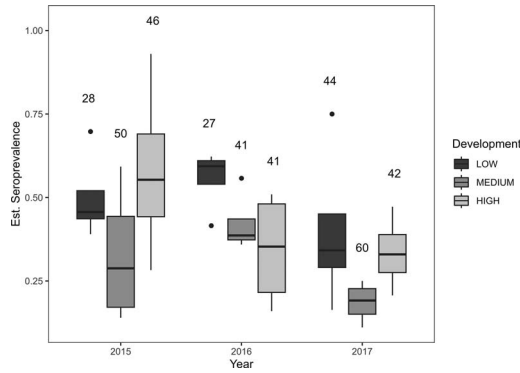


FIGURE 2. Estimated raccoon rabies virus neutralizing antibody seroprevalence across human development classifications, based on National Land Cover Database habitats (Multi-Resolution Land Characteristics Consortium 2011), associated with a 3-yr oral rabies vaccination trial with ONRAB (Artemis Technologies Inc., an indirect, wholly owned subsidiary of Ceva Sante Animale, S.A., Guelph, Ontario, Canada) at 150 baits/km$^2$ in the greater Burlington, Vermont, USA, area. Boxes represent quartiles, whiskers represent the 95% confidence interval, and dots represent outliers.

and data generated by the model (Fig. S2.4). The distributions for the simulated data had slightly longer tails. A randomization test indicated that covariate estimates are precise enough to identify significant covariates should any be present (Fig. S2.5). Covariate impacts were similar for both the 0.125 and 0.5 IU/mL RVNA cutoffs (S3), suggesting that the resulting relationships were not sensitive to the choice of RVNA cutoff.

## DISCUSSION

The post-ORV raccoon RVNA seroprevalence was well below the 60–80% target levels recommended for RRV elimination in a developed area (Reynolds et al. 2015; McClure et al. 2020) except for four cells that reached 60% estimated seroprevalence at least once during the study. Multiple environmental factors may affect raccoon RVNA seroprevalence in the greater Burlington area, where medium-development sites had lower seroprevalence compared to low-development sites, with no clear differences

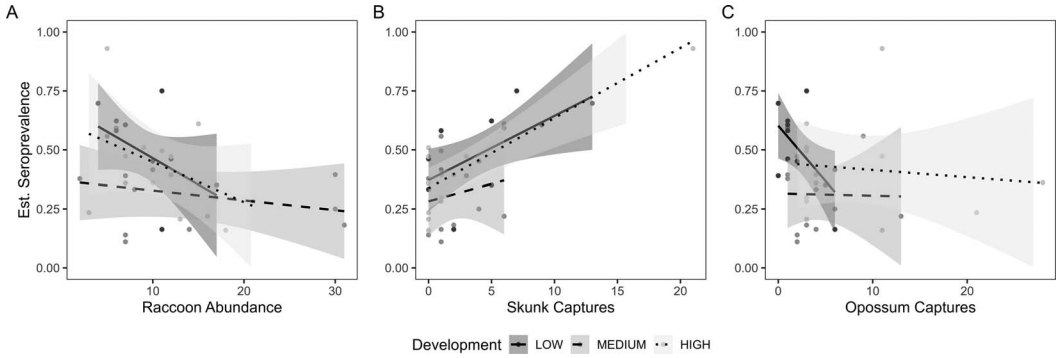


FIGURE 3. Estimated raccoon rabies virus neutralizing antibody (RVNA) seroprevalence tends to decrease with estimated increased raccoon (*Procyon lotor*) abundance in the greater Burlington, Vermont, USA, area (2015–2017) based on the results of a linear model (A; $F_{1,34} = 5.941$, $P = 0.020$, $R^2 = 0.124$), yet to increase with increased skunk (*Mephitis mephitis*) captures, based on the results of a linear model (B; $F_{1,34} = 28.4$, $P < 0.001$, $R^2 = 0.439$). Estimated raccoon RVNA seroprevalence in cells classified as low development tended to decrease with increasing opossum (*Didelphis virginiana*) captures (C; $F_{1,10} = 5.828$, $P = 0.036$, $R^2 = 0.305$). This association was not present in cells classified as medium and high development.

between sites comparing high-development to low- or medium-development areas (Fig. 2).

Characteristics of raccoon populations explain some of the variation in RVNA seroprevalence, as areas of greater raccoon abundance exhibited lower seroprevalence (Fig. 3A). Furthermore, estimated raccoon abundance was lower in low-development compared to medium- or high-development sites. Raccoons may thrive in moderate levels of human development, with residential areas and nearby forested areas (e.g., cemeteries and parks) close to the urban core where they can forage for anthropogenic food sources such as garbage, bird feeders, pet food, and vegetable gardens (McKinney 2002; Randa and Yunger 2006). Our classifications of low, medium, or high human development are all within the Burlington metropolitan area; even the low cells averaged 45% developed and should not be considered rural. Many studies have documented greater raccoon densities in urban and suburban compared to rural habitats (Schinner and Cauley 1974; Riley et al. 1998; Prange et al. 2003; Slate et al. 2020). Greater raccoon densities in urban areas may contribute to the lower RVNA seroprevalence observed in the Burlington study area when compared to similar studies (and serology methods) from rural areas.

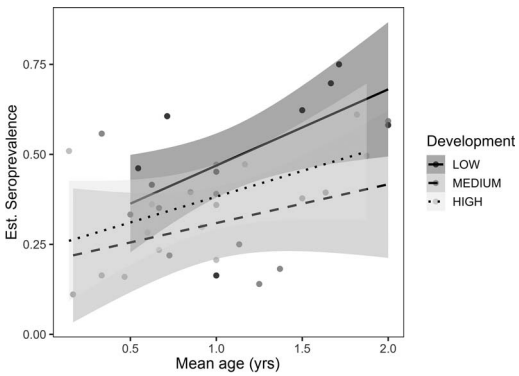


FIGURE 4. Study cells with a higher proportion of juvenile raccoons tended to have lower estimated rabies virus neutralizing antibody seroprevalence than cells with a higher proportion of adults for an oral rabies vaccination trial with ONRAB (Artemis Technologies Inc., an indirect, wholly owned subsidiary of Ceva Sante Animale, S.A., Guelph, Ontario, Canada) at 150 baits/km$^2$ in the Burlington, Vermont, USA, area, 2015–2017. Results are based on a linear regression after an influential outlier was removed ($F_{1,33}$= 10.22, $P$=0.003, $R^2$=0.213).

Older raccoons had a higher probability of being RVNA seropositive than younger raccoons. Juvenile raccoons typically travel in family groups and may be inexperienced in foraging and encountering baits. During our post-ORV sessions, juveniles had been exposed to only one baiting event, while adults had potentially encountered multiple baiting events across previous years. Several studies have reported

greater RVNA seroprevalence among adult compared to juvenile raccoons (Boulanger et al. 2008; Rosatte et al. 2009a; Horman et al. 2012; Mainguy et al. 2012; Slate et al. 2014; Pedersen et al. 2019a), where RVNA seroprevalence increases with animal age (Fig. 4 and Supplementary Material Fig. S2.5A; Gilbert et al. 2018a; Johnson et al. 2021) and cumulative exposure to annual ORV baiting programs. Overall, 45% of raccoons in our study were juveniles, similar to Mainguy et al. (2012) and Bigler et al. (2021a), but this varied by development type (37% in low, 46% in medium, and 49% in high), which may relate to seroprevalence differences by development class. We concur with prior work suggesting that a pulse of susceptible juveniles entering the population each year underscores the need for at least annual ORV to maintain levels of population RVNA seroprevalence and is also consistent with modeling predictions (Mainguy et al. 2012; McClure et al. 2020).

Skunk and opossum relative abundance were important factors affecting raccoon RVNA seroprevalence. In low-development areas when there were more opossums captured, raccoon RVNA seroprevalence was lower during the ORV trial. Although this relationship between the number of opossums captured and raccoon seroprevalence only occurred in low development, it suggests potential bait competition between opossums and raccoons. Opossums made up one third of all nontargets captured during our study and have been recognized as bait competitors with raccoons previously (Olson and Werner 1999; Olson et al. 2000; Smyser et al. 2010; Dixon et al. 2023).

Sites with greater skunk captures demonstrated higher raccoon RVNA seroprevalence. Except for one high-development cell in 2015 with an unusually high number of unique skunks (25/km$^2$ during prebait and 19/km$^2$ during postbait), skunk captures tended to be greatest in the low-development cells, followed by medium-development cells and least frequent in high-development cells. Although skunks consume ORV baits, their dependence on urban green spaces (Greenspan et al. 2018) may reduce their likelihood of encountering baits distributed along roads. Additional research is needed to explain how interspecific encounters of target meso-carnivores may affect bait uptake and RVNA seroconversion in raccoons.

The target bait density for the Burlington ground zone was 150 baits/km$^2$, which is commonly used within urban areas with higher raccoon densities (Slate et al. 2020). Our baiting grids averaged 37 km$^2$ and 4,070 baits distributed per grid. Within sampling cells, the actual bait densities varied from 0 to 484 baits/km$^2$. There is a known number of baits per grid but, depending on habitats encountered while driving, distribution may be uneven or patchy within grids (Fig. 1B–D). Additionally, concentrating delivery along roads may lead to bait distribution in suboptimal raccoon habitat (e.g., roadside ditches, under shrubs on front lawns), potentially reducing raccoon bait encounters (Bigler et al. 2021b). There was a slight positive association between actual bait density and raccoon RVNA seroprevalence (Supplementary Material Figure S2.3); however, this was not a strong influence compared to other factors measured during the study. In contrast, rural studies in Ohio (Sattler et al. 2009) and West Virginia (Johnson et al. 2021) demonstrated increases (about twofold) in raccoon seroprevalence in areas using 300 baits/km$^2$ when compared to 75 baits/km$^2$. Further evaluation of increased bait densities should be studied in urban and suburban areas.

Within-cell bait coverage did not strongly influence raccoon RVNA seroprevalence, perhaps because raccoon movements in developed areas may exceed the cell size (1 km$^2$): Prange et al. (2004) documented raccoon movements and home ranges in urban and suburban areas during the summer that exceeded the width and area of our cells. There was some evidence that bait coverage at a scale larger than the 1-km$^2$ sampling cells was important, as a portion of the greater Burlington area was not baited during 2016 (Fig. 1C) and raccoon RVNA seroprevalence

rates were unusually low during 2017 (Supplementary Material Fig. S2.2C). Sattler et al. (2009) and others have found a cumulative effect of baiting for maintaining raccoon RVNA seroprevalence rates, considering the pulse of naive juveniles entering the population annually. A cumulative effect might explain the lower seroprevalence rates in medium-development sites, as many of these sites were in or adjacent to the areas without baits. Future research should consider the potential benefit of reducing the size of baiting grids, which may result in fewer spatial gaps in baiting and increased raccoon RVNA seroprevalence.

There was considerable variability of raccoon RVNA seroprevalence estimates among sampling cells. However, we observed a high probability of raccoon detection (capture) in our model, suggesting that this variability was not due to observation error. Detection-based models are useful in situations where bias or error in capture rates may introduce error in the observed data (Iknayan et al. 2014; Kellner and Swihart 2014). Our detection model estimated relatively high capture rates, increasing our confidence that the seroprevalence estimates accurately represent the raccoon population in this area. The model also corrected for a decrease in raccoon captures as more traps are occupied, which is important at sites with high numbers of recaptures or nontargets. The addition of seroprevalence to the base *N*-mixture model with removal sampling was also important for accurately modeling seroprevalence, as the model was able to estimate abundance and seroprevalence jointly, the former of which often affects the latter in wild populations (Mainguy et al. 2012).

The use of multiple analytical methods to support and expand upon the results of the *N*-mixture model shed additional light on the factors that may influence raccoon population RVNA seroprevalence associated with ORV. Evaluation of the 75% CI missed many important variables because of outliers (Supplementary Material Table S2.2) or because the pattern only held in one development class (Fig. 3C). Additionally, small sample sizes and high variability between sites probably presented obstacles to interpretation of model covariates. An individual-level analysis (Supplementary Material S1.4) also supported the finding that seroprevalence was influenced by the mean numerical age of the population, as we found that the probability of being seropositive increased with the age of an individual (Supplementary Material Fig. S2.6A). Our consideration of multiple analytical approaches was useful for teasing apart complex relationships between biological and landscape factors in this urban environment.

During 2014 (before our study), there were 30 cases of RRV within our study area (43 negatives, 41% positive), declining to seven (66 negatives, 10% positive) and one case (32 negatives, 3% positive) detected during 2015 and 2016, respectively. During 2017–2021, there were no cases of RRV in this area, with 106, 87, 91, 65, and 65 negatives, respectively. Despite the relatively low RVNA response in this study when compared to rural ONRAB studies, case reduction and apparent elimination during the study was observed. This phenomenon of low RVNA response with case reduction has also been observed in urban Hamilton, Ontario, Canada, where seroprevalence was only 6–14% (Acheson et al. 2023), yet RRV cases declined from 256 in 2016 to 23 in 2022 (Ontario Ministry of Natural Resources and Forestry 2023). Despite 5 yr of no cases in our study area, 19 cases of RRV were reported in 2022 (174 negatives, 10% positive) and five cases between January and March 2023. This new outbreak further emphasizes the need for more research aimed at improving urban management of RRV using ORV. Future research should focus on a more comprehensive understanding of the interplay between RVNA seroprevalence and case reduction of RRV.

Future studies in developed areas should investigate potential factors among the ecological community of meso-carnivores and nontarget animals that may impact ORV effectiveness for raccoons (e.g., population densities, movements, home ranges, habitat use and selection, bait

consumption). A comprehensive ecological understanding may inform refinement of baiting strategies for raccoons in urban and suburban environments. Bait stations require additional study, such as incorporating them with hand baiting, locating them farther from roads to potentially bolster bait encounters as suggested by (Bigler et al. 2021b), and expanding on work by Bjorklund et al. (2017) to improve specificity of access by raccoons. Bigler et al. (2021b) found significantly greater raccoon seroconversion from helicopter bait distribution when compared to vehicle (hand) distribution. Some low-development areas that are difficult to access for ORV (few roads) result in lower raccoon seroprevalence and may warrant conversion to helicopter baiting in our study area followed by re-evaluation.

As the NRMP continues to work toward regional RRV elimination over the next 30 yr while moving the ORV zone eastward, the challenges associated with ORV in urban areas will become more prominent. Continued investigation of ORV targeting raccoons in urban and suburban habitats will be critical to successful elimination of RRV from North America.

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## SUPPLEMENTARY MATERIAL

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