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RACCOON (*PROCYON LOTOR*) RESPONSE TO ONTARIO RABIES VACCINE BAITS (ONRAB) IN ST. LAWRENCE COUNTY, NEW YORK, USA

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ABSTRACT: Oral rabies vaccination (ORV) campaigns have been conducted annually in the US over the past two decades to prevent raccoon (*Procyon lotor*) rabies, which is enzootic along the eastern region of the country from southeastern Canada to Alabama. Because raccoon rabies has been eliminated from neighboring Canadian provinces, continued detection of the variant in the US is of concern due to the potential for infected raccoons to cross the border via the St. Lawrence River. Ontario Rabies Vaccine Baits (ONRAB) containing a live, recombinant human adenovirus expressing the rabies virus glycoprotein have been under experimental use in the US since 2011. We distributed ONRAB in St. Lawrence County, New York, from 2013 to 2015 as part of field trials to evaluate serologic responses in raccoons. Prior to ONRAB distribution, rabies virus neutralizing antibody (RVNA) seroprevalence in raccoons was 45.2% (183 of 405) and increased to 57.7% (165 of 286) after 3 yr of ONRAB baiting. Postbait RVNA seroprevalence increased each year, with a lower response observed in juvenile compared with adult raccoons. The pre-ONRAB seroprevalence detected in 2013 was relatively high and was likely impacted both by elevated rabies activity in the county and the use of ORV with a different vaccine bait for 14 consecutive years prior to our study. Tetracycline biomarker prevalence increased from 1.4% prior to ONRAB baiting to 51.3% from 2013 to 2015, demonstrating bait palatability to raccoons. These data complemented related field trials conducted in West Virginia and the northeastern US.

Key words: Oral rabies vaccination, ONRAB, *Procyon lotor*, rabies, rabies virus neutralizing antibodies, raccoon, St. Lawrence County.

INTRODUCTION

The St. Lawrence River not only serves as an important waterway connecting the US and Canada, but it also provides important riparian habitat for various wildlife species. The corridor is heavily used by raccoons (*Procyon lotor*), a ubiquitous mammal native to North America, and the primary reservoir for raccoon rabies virus in the eastern US (Monroe et al. 2016). Rabid raccoons from the enzootic area in St. Lawrence County, New York (as supported by phylogenetic data), have been identified in Ontario, Canada (Rosatte et al. 2001; Nadin-Davis et al. 2006). Despite coordinated oral rabies vaccination (ORV) campaigns in the US and Canada to prevent and control spread of the disease (Slate et al. 2005; Rosatte et al. 2009a), cross-border viral transmission along this corridor remains a concern (Cullingham et al. 2009). Within St. Lawrence County, New York, specifically, ORV had occurred annually for 14 yr prior to our study, yet raccoon rabies cases have continued to occur, suggesting the need to evaluate different strategies, including raccoon serologic responses to a different vaccine bait.

Because raccoon rabies is considered enzootic in St. Lawrence County and other parts of New York, Vermont, and New Hampshire, ORV had been conducted previously with RABORAL V-RG® (V-RG; a registered trademark in the US and elsewhere of Merial, Inc., now Boehringer Ingelheim, Athens, Georgia),

in an attempt to halt spread of the virus. Ontario Rabies Vaccine Bait (ONRAB) is an oral rabies vaccine bait that has been linked to seroconversion rates of 66–84% in raccoons in southwestern Ontario with bait densities ranging from 75 to 400 baits/km^2 (Rosatte et al. 2009b). In a field trial conducted in northern New York, Vermont, and New Hampshire to evaluate the effectiveness of targeting raccoons, a similar seroprevalence (69%) to that reported in Ontario was observed although different serologic tests were used for analyzing samples (Gilbert et al. 2018).

Due to the importance of the St. Lawrence River Valley as a potential corridor for movement of rabid raccoons into Canada, we evaluated the impact of ONRAB by assessing rabies virus neutralizing antibody (RVNA) seroprevalence and tetracycline biomarker response rates in raccoons prior to and after ONRAB baiting in St. Lawrence County from 2013 to 2015. Because ONRAB contains a tetracycline biomarker that was not present in the V-RG baits that had been distributed prior to our study, the tetracycline response data allowed for assessment of bait uptake in a biomarker naïve population.

MATERIALS AND METHODS

Study area

The study area was located near the St. Lawrence River in St. Lawrence County, New York, at 44°41′N, 75°17′W (Fig. 1). The design consisted of two 127-km^2 study cells (PBG09 and PBG10) within a larger ORV zone separated by ≥5-km buffer zones (Fig. 1). Both study cells were within an area that had been baited annually at 75 baits/km^2 with V-RG since 1999 and consisted of similar habitat, including primarily forest, agriculture, and wetlands or water based on the 2011 National Land Cover Database (Homer et al. 2015).

Bait and bait distribution

The Ontario Rabies Vaccine Bait (ONRAB Ultralite baits, Artemis Technologies, Inc., Guelph, Ontario, Canada) consist of a polyvinyl chloride blister pack containing 1.8 mL of the human adenovirus recombinant rabies vaccine coated with a dark green wax containing 100 mg of tetracycline hydrochloride. The baits were distributed in August each year (2013–15) by fixed-wing aircraft along parallel flight lines at 750-m intervals at a target bait density of 75 baits/km^2 throughout the ORV zone (Fig. 1).

Trapping

Random points (34–36) were generated along roadways each year for the four quadrants of each study cell to guide trap placement by using Hawth's tools (Beyer 2012), and 25 of the points were selected based on property access, permission to trap, and spatial distribution within the cell. Four to six traps (depending on surrounding habitat at the point) were placed within 800 m of six of the selected points in three of the quadrants and seven in the fourth quadrant, with a minimum of 30.5 m between traps, for a total of 150 traps per cell.

Trapping occurred annually for 10 consecutive days approximately 2–4 wk prior to ORV in July and August and again 4–5 wk after ORV in September and October. Tomahawk live traps (model 108; Tomahawk Live Trap, Hazelhurst, Wisconsin, USA), baited with marshmallows and anise oil (Minnesota Trapline Products, Inc., Pennock, Minnesota, USA), were used to trap animals. Traps were checked daily and moved every 2–3 d if no unique raccoons had been captured.

Animal handling and sampling

All unique raccoons, striped skunks (*Mephitis mephitis*), foxes (*Vulpes vulpes* and *Urocyon cinereoargenteus*), coyotes (*Canis latrans*), and fishers (*Martes pennanti*) captured during the study were considered target animals due to their importance as rabies vector species. All target animals were anesthetized by intramuscular injection of a 5:1 mixture of ketamine (10 mg/kg) and xylazine (2 mg/kg) on the basis of estimated body weight. While anesthetized, weight, sex, and relative age were recorded, each animal was uniquely numbered with an ear tag (National Band and Tag Company, Newport, Kentucky, USA), and approximately 5 mL of blood was collected via a peripheral vein. A premolar tooth was collected, whenever possible, for age determination and biomarker (tetracycline) analysis.

Animals were released at the point of capture after sampling and upon recovery from anesthesia, unless they were sick, acting strangely, or seriously injured. These animals were euthanized with carbon dioxide in accordance with the American Veterinary Medical Association's guidelines on euthanasia (Leary et al. 2013). All

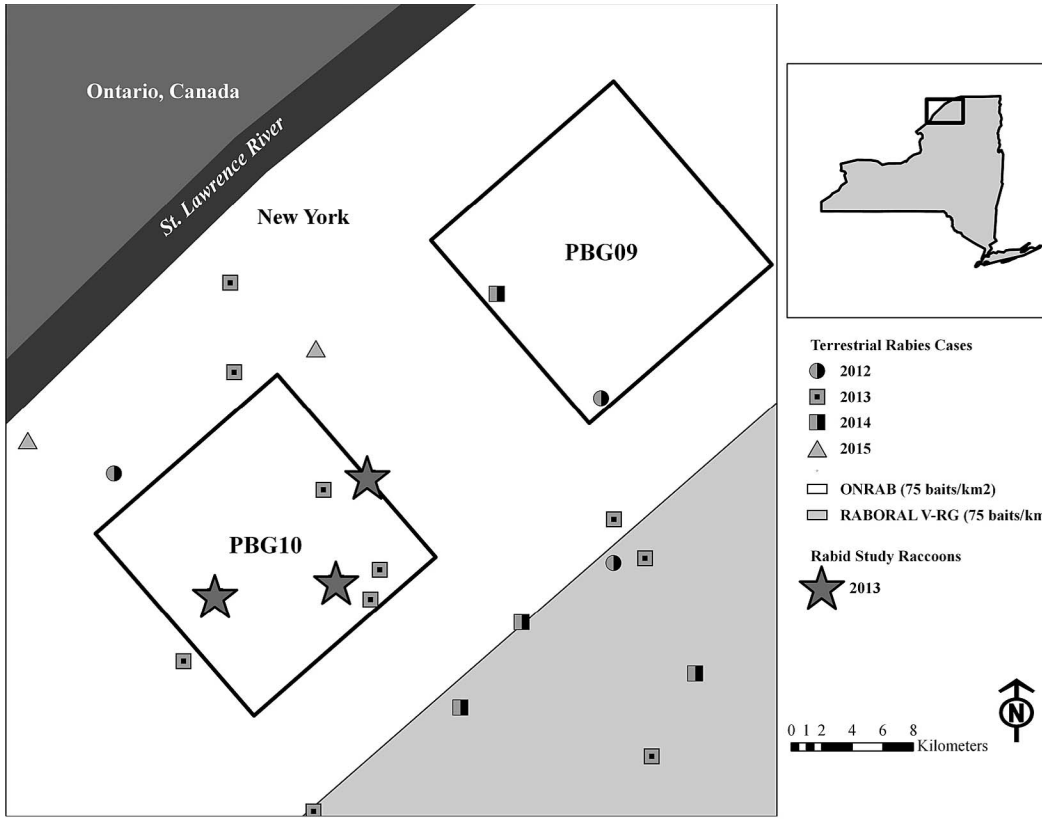

FIGURE 1. Two study cells within a larger oral rabies vaccination zone where Ontario Rabies Vaccine Baits (ONRAB) were distributed from 2013 to 2015, with the raccoon (*Procyon lotor*) rabies cases in the study area in St. Lawrence County, New York, USA, reported by the New York State Department of Health from 2011 to 2018 (no cases were reported in the study area in 2011 or after 2015).

nontarget species were released at the point of capture without anesthesia or sampling.

Laboratory testing

Serum samples were submitted to the New York State Department of Health, Wadsworth Laboratory (Slingerlands, New York, USA) to test for RVNA by using a modified neutralization test (Trimarchi et al. 1996). Sera with RVNA titers $\geq$0.125 IU/mL were considered positive, and sera with RVNA titers $<$0.125 IU/mL were considered negative. Sera from 41 raccoons (2%) that could not be evaluated at the cut-off value due to poor sample quality were excluded from further analyses. The entire heads from 11 euthanized animals were sent to New York State Department of Health for testing of brain tissue by direct fluorescent antibody test (CDC 2005), and rabies virus variant typing was performed on positive samples (Szanto et al. 2011).

Age determination and biomarker analysis

Teeth were submitted to Matson's Laboratory LLC (Manhattan, Montana, USA) for age determination and biomarker analysis. Age was determined to the nearest year by using a compound microscope and ultraviolet light with filters to detect and count cementum or dentin annuli (Johnston et al. 1999). Animals $<$1 yr old were classified as juveniles and those $\geq$1 yr as adults. If a tooth was unavailable for aging (n=43), the relative age (juvenile or adult) determined at the time of capture was used. Additionally, a thin section of tooth was examined microscopically by using an ultraviolet filter to detect fluorescence indicative of tetracycline biomarker (Johnston et al. 1999), typically visible 2 d after bait consumption (Hanlon et al. 1989).

Data analysis

Unless otherwise specified, all further analyses and results refer to raccoons because they were

TABLE 1. The number of rabies antibody-positive sera and number of animals tested for each target species by year and trapping period (prior to or after Ontario Rabies Vaccine Bait distribution) tested for rabies virus neutralizing antibodies by using a modified virus neutralization test. Animals were captured in St. Lawrence County, New York, USA, from 2013 to 2015.

		No. positive/no. tested					
		2013		2014		2015	
Common name	Scientific name	Prebait	Postbait	Prebait	Postbait	Prebait	Postbait
Coyote	*Canis latrans*	0	0	0	0	0/1	0
Fisher	*Martes pennant*	0/6	0/2	0/1	0	0/1	0
Gray fox	*Urocyon cinereoargenteus*	0	0	0	0/1	0	0
Raccoon[a]	*Procyon lotor*	183/405	55/108	216/341	140/237	152/304	165/286
Red fox	*Vulpes vulpes*	0	0	0	0	2/5	0
Striped skunk[a]	*Mephitis mephitis*	1/27	9/31	6/19	6/60	4/23	10/40

[a] Sera from 41 raccoons and three striped skunks could not be evaluated at the test cutoff.

the primary target species. We used a generalized linear mixed model implemented in Program R, package lme4 (Bates et al. 2015; R Development Core Team 2017) to examine the RVNA seroprevalence and tetracycline prevalence in populations prior to and after baiting across the 3-yr study. Year, trapping period (prebait or postbait), age, and sex were considered fixed effects, and study cell was considered a random effect. We considered two relationships with seroprevalence and year: a linear effect of year (representing a trend in time, referred to as "trend") and each year individually (to examine annual variability in the effect on seroprevalence, referred to as "year"). We examined models with simple main effects, an additive model, and models with relevant two-way interactions and compared these to the null model. Models were compared by using the second-order Akaike's information criterion (AICc), and models within two AICc of the top model were considered competitive, with model averaging being used when model uncertainty occurred (Burnham and Anderson 2002).

RESULTS

Aerial baiting was conducted in northern New York along the Canadian border with 356,550 ONRAB baits distributed at 75 baits/km^2 in the respective ORV zone from 2013 to 2015 (Fig. 1). Across the study cells, 39,385 baits were distributed, and actual bait densities for the ONRAB treatment area were 74, 78, and 75 baits/km^2 for 2013, 2014, and 2015, respectively.

A total of 1,474 unique raccoons were captured, and 248 were recaptured. All recaptures, with the exception of one raccoon, were recaptured in the same study cell as they were originally captured. The raccoon was a naïve juvenile male when originally sampled, and 2 yr later was RVNA positive when captured approximately 26 km from its original location. The percentage of males (54.1%) and females (45.8%) trapped during the study was nearly the same, and 74% were adults. One coyote, 10 fishers, one gray fox, five red foxes, and 203 striped skunks were also sampled; sera from the one coyote and 36 of the striped skunks tested RVNA positive (Table 1).

The top model for RVNA seroprevalence included two interactions: between year and trapping period and between trapping period and age (Table 2). The postbait RVNA seroprevalence increased each year, whereas the prebait seroprevalence varied annually (Fig. 2). The postbait seroprevalence was higher each year in adults compared with juveniles (Fig. 2). Similarly, the postbait seroprevalence in 2015 compared with the prebait seroprevalence in 2013 was significantly higher in adults but not in juveniles (Fig. 2). The model estimates for postbait seroprevalence of adults and juveniles for 2013, 2014, and 2015 were 57.6% (95% confidence interval [CI]: 47.7–66.8), 61.6%

TABLE 2. Model results for rabies virus neutralizing antibody seroprevalence response (titers >0.125 IU/mL were considered positive) of a raccoon (*Procyon lotor*) population sampled in St. Lawrence County, New York, USA, from 2013 to 2015 prior to and after baiting with Ontario Rabies Vaccine Baits.

Model tested[a]	K[b]	ΔAICc[c]	ω[d]	LL[e]
Year×period+period ×age	9	0	0.98	−128.34
Year×period+age	8	9.02	0.01	−134.38
Year+period+age	6	10.41	0.01	−137.89
Year×period+sex+age	9	11.64	0	−134.17
Year+period+sex+age	7	12.71	0	−137.67
Period+age	4	18.45	0	−144.47
Year+age	5	20.33	0	−144.16
Period+sex+age	5	20.36	0	−144.18
Trend+period+age	5	20.37	0	−144.18
Trend×period+age	6	22.35	0	−143.86
Trend+period+sex+age	6	22.46	0	−143.92
Trend×period+sex+age	7	24.6	0	−143.61
Trend+age	4	32.78	0	−151.64
Age	3	33.57	0	−153.22
Sex+age	4	35.71	0	−153.1
Year	4	78.57	0	−174.53
Year+period	5	79.91	0	−173.95
Year×period	7	80.72	0	−171.67
Year+sex	5	81.07	0	−174.53
Year+period+sex	6	82.52	0	−173.95
Year×period+sex	8	83.61	0	−171.67
Trend	3	96.56	0	−171.67
Trend+period	4	97.12	0	−183.81
Period	3	98.32	0	−185.6
Trend+sex	4	98.94	0	−184.72
Intercept only	2	99.37	0	−187.26
Trend×period	5	99.58	0	−183.79
Trend+period+sex	5	99.6	0	−183.8
Period+sex	4	100.67	0	−185.58
Sex	3	101.64	0	−187.26
Trend×period+sex	6	102.18	0	−183.78

[a] period = trapping period (prebait or postbait); trend = linear effect of year.

[b] K = number of parameters.

[c] The second-order Akaike's information criterion (AICc) for the top model is 273.51. All models include a parameter for the random effect of cell.

[d] ω = AICc model weight.

[e] LL = log likelihood of the model.

(95% CI: 54.6–68.3), and 63.7% (95% CI: 57.0–69.9), respectively. Sex was not an important factor affecting RVNA seroprevalence.

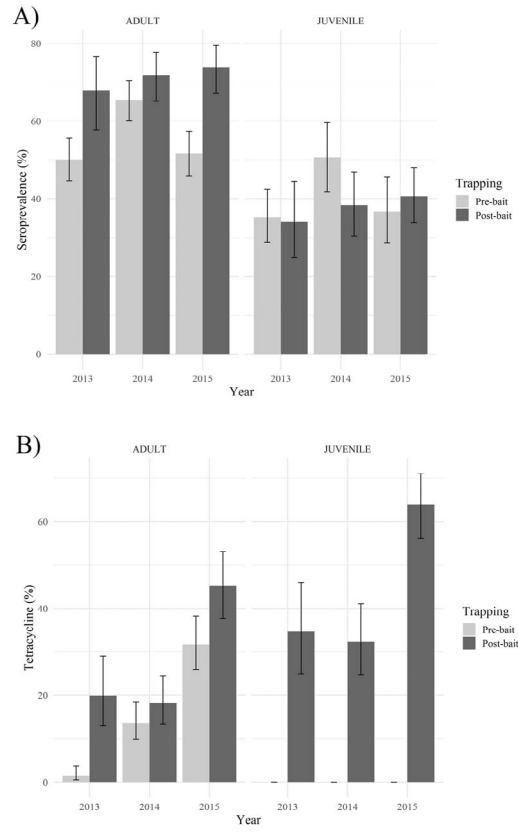

FIGURE 2. The estimated seroprevalence of rabies virus neutralizing antibodies (A) and biomarker (tetracycline) prevalence (B) by age class of raccoons (*Procyon lotor*) captured in St. Lawrence County, New York, USA, prior to baiting with Ontario Rabies Vaccine Baits compared with postbait distribution each year in 2013–15. The 95% confidence intervals are shown as vertical bars.

The top model for tetracycline response was the same as for the RVNA analysis (Table 3). The postbait tetracycline prevalence among adults was higher than the prebait prevalence for all years, with model estimates of 1.4% (95% CI: 0.5–3.8) prior to ONRAB baiting in 2013 compared with 51.3% (95% CI: 43.9–58.5) after baiting in 2015 (Fig. 2). The postbait tetracycline prevalence increased each year among adults, but the biggest change in juveniles was observed in 2015 (Fig. 2).

During the study, 11 raccoons were euthanized and submitted for rabies diagnostic testing. Three were rabid, and raccoon rabies

TABLE 3. Model results for biomarker (tetracycline) prevalence response of a raccoon (*Procyon lotor*) population sampled in St. Lawrence County, New York, USA, from 2013 to 2015 prior to and after baiting with Ontario Rabies Vaccine Baits.

Model tested[a]	K[b]	ΔAICc[c]	ω[d]	LL[e]
Year×period[e]+period ×age	9	0	1	−80.92
Year×period	7	53.94	0	−110.85
Year×period+age	8	55.3	0	−110.09
Year×period+sex	8	56.83	0	−110.85
Year×period+sex+age	9	58.32	0	−110.07
Trend[f]×period	5	71.63	0	−122.38
Trend×period+age	6	72.12	0	−121.32
Trend×period+sex	6	74.24	0	−122.38
Trend×period+sex+age	7	74.82	0	−121.29
Trend+period	4	83.27	0	−129.45
Year+period	5	83.91	0	−128.53
Trend+period+age	5	84.52	0	−128.83
Year+period+age	6	85.59	0	−128.05
Trend+period+sex	5	85.72	0	−129.43
Year+period+sex	6	86.49	0	−128.5
Trend+period+sex+age	6	87.04	0	−128.78
Year+period+sex+age	7	88.25	0	−128.01
Trend+age	4	164.11	0	−169.87
Year+age	5	166.6	0	−169.87
Trend	3	179.75	0	−178.89
Trend+sex	4	181.7	0	−178.67
Year	4	181.94	0	−178.79
Year+sex	5	184	0	−178.57
Period	3	241.43	0	−209.72
Period+sex	4	243.75	0	−209.69
Period+age	4	243.81	0	−209.72
Period+sex+age	5	246.25	0	−209.69
Age	3	388.69	0	−283.35
Sex+age	4	390.67	0	−283.15
Intercept only	2	395.82	0	−288.06
Sex	3	397.89	0	−287.96

[a] period = trapping period (prebait or postbait); trend = linear effect of year.

[b] K = number of parameters.

[c] The second-order Akaike information criterion (AICc) for the top model is 184.57. All models include a parameter for the random effect of cell.

[d] ω = AICc model weight.

[e] LL = Log likelihood of the model.

was confirmed as the variant for those (Fig. 1). All three were euthanized in 2013 because they were exhibiting abnormal behavior; two were collected during prebait and one during postbait trapping.

DISCUSSION

Raccoon rabies has been detected in Ontario and Quebec provinces, Canada, as a result of infected raccoon movements across the St. Lawrence River (Nadin-Davis et al. 2006; Stevenson et al. 2016). Although raccoon rabies activity in St. Lawrence County has been relatively continuous, it is not uniform throughout, as evident in our study cells. Specifically, the number of rabies cases reported in 2013 was much higher in cell PBG10 compared with PBG09 (Fig. 1). This local epizootic likely impacted the prebait seroprevalence in 2013 within the cell, as has been reported previously (Carey and McLean 1983). As a result, the overall impact of ONRAB baiting observed during the study may have been diminished. Though both study cells had been baited with V-RG for 14 consecutive years prior to the study, animals were not trapped prior to bait distribution or routinely after baiting was conducted; consequently, no data are available to examine trends in serologic response, age structure, or temporal patterns. Despite long-term baiting, an increase in RVNA seroprevalence was observed after ORV baiting with ONRAB. In a study conducted in Virginia using V-RG, no difference was observed after 3 yr when V-RG was applied at 75 baits/km^2 to an area that had previously been baited for 12 yr (Pedersen et al. 2019). Because the RVNA seroprevalence in our study still indicated an increasing trend after 3 yr of baiting with ONRAB, it is possible that the seroprevalence would have continued to increase if sampling had continued (i.e., no saturation effect was observed). However, additional sampling would have been necessary to confirm this pattern. It also suggested that application of ONRAB may boost RVNA seroprevalence in raccoons beyond levels achieved with V-RG (Gilbert et al. 2018). The V-RG baiting prior to the study obscured the relationship between the serologic response and the tetracycline prevalence caused by consuming ONRAB baits. Assuming the tetracycline prevalence in our study was indicative of the response that

would have been observed in an ORV naïve area, the increase in tetracycline prevalence from <1% to 54.2% after 3 yr of baiting suggests ONRAB would have resulted in a higher antibody prevalence if ORV had not occurred previously. Even though a tetracycline biomarker can be used to assess bait consumption, it does not always signify concurrent antibody development (Rosatte et al. 2008) and can vary, depending on the tissues selected for analysis (Algeo et al. 2013). Partial bait consumption can result in tetracycline detection but may be insufficient for development of an antibody response (Brown et al. 2012). In our study, the tetracycline prevalence doubled in adults and juveniles from 2014 to 2015, but a corresponding increase in seroprevalence was not observed. Because ORV baits with no biomarker were distributed for 14 yr prior to our study, the cumulative effect of tetracycline may have been more pronounced compared with the serologic effect of bait consumption. Disparities between serologic and tetracycline prevalence patterns could also be attributed to a variety of factors, such as individual heterogeneity and environmental or sampling variation.

In another study in which ONRAB was applied in northern New York, Vermont, and New Hampshire, the RVNA seroprevalence was 27% across study cells prior to baiting with ONRAB (but with previous V-RG baiting) and averaged 69% after 3 yr of ONRAB baiting (Gilbert et al. 2018). In rural West Virginia, seroprevalence (on the basis of the rapid fluorescent focus inhibition test) increased from 9.6% in an ORV naïve area prior to baiting to 49.2% after baiting with ONRAB for a single year (Slate et al. 2014). Without accounting for the different serologic test used in West Virginia, our results indicated a more modest increase in seroprevalence (45.2% prior to baiting and 57.7% after 3 yr of baiting), but the trend and amplitude was still higher than the average of 30% often observed after baiting with V-RG (Slate et al. 2009). The prebait seroprevalence in 2013 was higher than usual due to elevated circulation of rabies virus in the county (Fig. 1) and because ORV baiting had occurred for 14 consecutive years prior to the study. The impact of our study is perhaps more apparent when focusing on adults, since 73.8% (95% CI: 67.2–79.5) of adult raccoons were seropositive after 3 yr of baiting (Fig. 2). However, because the average life span of raccoons is approximately 2–3 yr (Hadidian et al. 2010), juveniles are assumed to have the most potential to affect the disease dynamics of the population. After 3 yr of ONRAB baiting, the RVNA seroprevalence in the juvenile cohorts only increased from 35.3% (95% CI: 28.7–42.4) to 40.6 (95% CI: 33.7–47.9). Targeting juvenile raccoons for ORV uptake is essential for obtaining the long-term goal of raccoon rabies elimination.

Skunks can also be infected with the raccoon rabies virus variant (Guerra et al. 2003). Safe and effective vaccines and baits for skunks are needed for ultimate rabies elimination. Although our study was not designed specifically to evaluate skunks, we had the opportunity to test 203 skunks, and the RVNA seroprevalence increased from 4% (95% CI: 1–18) prior to ONRAB baiting in 2013 to 25% (95% CI: 14–40) after baiting in 2015. This is similar to a study conducted in Maine and New Brunswick where 3% (n=36) and 15% (n=33) of skunks developed RVNA (on the basis of competitive enzyme-linked immunosorbent assay) after baiting with V-RG and ONRAB, respectively (Fehlner-Gardiner et al. 2012). In another 3-yr study conducted in the northeastern US where ONRAB baits were distributed, the RVNA seroprevalence in skunks increased from 0% (n=4) to 17% (n=12) in naïve study sites and from 0 (n=14) to 18% (n=55) in areas previously baited with V-RG (Gilbert et al. 2018). These results suggest that ONRAB may be a more effective bait for skunks than V-RG, but additional studies designed to evaluate skunks are recommended.

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