

# ORAL RABIES VACCINATION OF RACCOONS AND STRIPED SKUNKS WITH ONRAB® BAITS: MULTIPLE FACTORS INFLUENCE FIELD IMMUNOGENICITY

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**ABSTRACT:** Multiple control methods have been used in North America to manage the spread of rabies caused by the raccoon (*Procyon lotor*) rabies virus variant (RRVV). Recently, oral vaccination with ONRAB® vaccine baits, which contain an adenovirus rabies glycoprotein recombinant, has been made available as an additional tool for rabies control. Our objectives were to estimate rabies antibody prevalence in wild-caught raccoons and striped skunks (*Mephitis mephitis*), and identify factors influencing the probability of being antibody positive at the individual level in these species, following oral rabies vaccination (ORV) campaigns in which ONRAB was distributed aerially in 2007–2009 in southern Québec, Canada. Following the aerial distribution of 43–155 ONRAB baits/km<sup>2</sup>, the annual percentages of antibody-positive raccoons and skunks varied between 35% and 56% and 11% and 17%, respectively. In raccoons, the probability of being antibody positive was positively associated with age and density of ONRAB distributed, and influenced by the number of previous ORV campaigns conducted. Conversely, this probability was negatively associated with estimated abundance of raccoons in the trapping cell and proportion of residential areas near the raccoon capture location. None of the variables examined explained variation in the probability of being antibody positive in skunks. Our results indicate that the ONRAB density applied during ORV campaigns should be adjusted to account for variations in raccoon population density and presence of residential areas to increase the likelihood of creating an effective immunological barrier against RRVV. The high percentage of juvenile raccoons (annual mean = 45 ± 3 [SE]%) and skunks (66 ± 2%) captured during post-ORV monitoring suggests that ORV campaigns should be conducted at least annually to account for the recruitment of naïve individuals into the populations. In Québec, the increasing use of ONRAB coincided with the elimination of rabies caused by RRVV. Nonetheless, our results indicate that improvements to this vaccine bait and/or the distribution techniques are required to increase its efficacy, especially in striped skunks.

**Key words:** Raccoon abundance, bait density, *Mephitis mephitis*, oral rabies vaccination, *Procyon lotor*, raccoon rabies virus variant, antibody prevalence, striped skunk.

## INTRODUCTION

Rabies is a lethal zoonosis of great concern worldwide. In North America, most reported rabies cases now occur in wild mesocarnivores with multiple variants of the rabies virus generally being maintained in different reservoir species. Raccoons (*Procyon lotor*) are the reservoir for

the raccoon rabies virus variant (RRVV), which accounts for the majority of confirmed rabies cases in the United States (Blanton et al., 2011). Several rabies outbreaks caused by RRVV have also been reported in Canada (Rosatte et al., 2006; Rees et al., 2011). Striped skunks (*Mephitis mephitis*), which are generally sympatric with raccoons, are also frequently

found infected with RRVV (Guerra et al., 2003; Blanton et al., 2011). Both species often live in close proximity to humans (e.g., Prange et al., 2004). Thus, control activities aimed at reducing rabies prevalence and spread in eastern North America have mainly targeted raccoons and skunks because of public health risks and associated costs for postexposure prophylaxis (Sterner et al., 2009).

Oral rabies vaccination (ORV), which consists of the distribution of consumable vaccine baits to induce protective immunity in susceptible hosts, is regarded as a cost-effective strategy for controlling rabies in wild mesocarnivores (Slate et al., 2009; Sterner et al., 2009). Several types of vaccine baits are used for the control of rabies in mesocarnivores. The vaccinia-rabies glycoprotein recombinant RABORAL V-RG<sup>®</sup> (Merial, Athens, Georgia, USA) has been used for many years in the United States (e.g., Sattler et al., 2009) and Canada (Rosatte et al., 2008). More recently, the adenovirus rabies glycoprotein recombinant ONRAB<sup>®</sup> (Artemis Technologies, Guelph, Ontario, Canada) has also been mainly applied in Canada (Rosatte et al., 2009a). The success of ORV campaigns using these vaccine baits, which can be estimated by the percentage of live-trapped individuals with detectable rabies virus antibodies, often differs markedly among the regions where they have been used (e.g., Robbins et al., 1998; Boulanger et al., 2008; Ramey et al., 2008; Rosatte et al., 2009a; see Fehlner-Gardiner et al. 2012 for a comparative study). Besides the vaccine bait used, several factors within the individual (e.g., age and sex), population (e.g., animal population density), control operations (e.g., bait density), and landscape (e.g., habitat composition) have been associated with variations in measured rabies antibody prevalences (Robbins et al., 1998; Blackwell et al., 2004; Ramey et al., 2008; Rosatte et al., 2009a; Sattler et al., 2009). An understanding of the degree to which these factors impact antibody prevalence

is required for designing and implementing effective rabies control programs.

We report the serologic results obtained in raccoons and skunks over three consecutive years following ORV campaigns in areas relying on the distribution of ONRAB for the control of rabies caused by RRVV in southern Québec, Canada. Our first objective was to estimate the antibody prevalence in each species as a function of the targeted ONRAB bait densities used. The second objective was to assess, at the individual level, the factors that affected the probability of being antibody positive in these two species. According to the factors identified, we then examined the effect of vaccine-bait density variations on the probability of being antibody positive given the average field conditions found in the Québec ORV zone.

## MATERIALS AND METHODS

### Study area

Both ORV campaigns and post-ORV monitoring were conducted in southern Québec (45°14'N, 72°50'W; Fig. 1), Canada, following the first detection of RRVV in 2006 (Rees et al., 2011). The western part of the study area is a flat, rural landscape composed mainly of large agricultural fields interspersed with small patches of deciduous and mixed forest (see Houle et al., 2011 for details). The eastern part of the study area is along the northwestern extent of the Appalachian Mountains and has larger, more contiguous patches of forest and less agriculture.

### Vaccine-bait distribution

In 2007 and 2008, ONRAB baits were distributed aerially with fixed-wing aircraft in mid-August (Fig. 1, Table 1) at a targeted density of 150 baits/km<sup>2</sup> by the Ministère des Ressources Naturelles et de la Faune (MRNF). V-RG baits were also used in 2007 and 2008 in adjacent zones (Fig. 1). Also in 2008, a designated area of 1,382 km<sup>2</sup> east of the ORV zone was baited with ONRAB at a targeted density of 75 baits/km<sup>2</sup> (Fig. 1) to assess the impact of lower bait density on antibody prevalence. In 2009, two modifications to the ORV methods were adopted. First, professional trappers provided additional terrestrial distribution of ONRAB baits by hand in the spring of 2009, targeting areas where

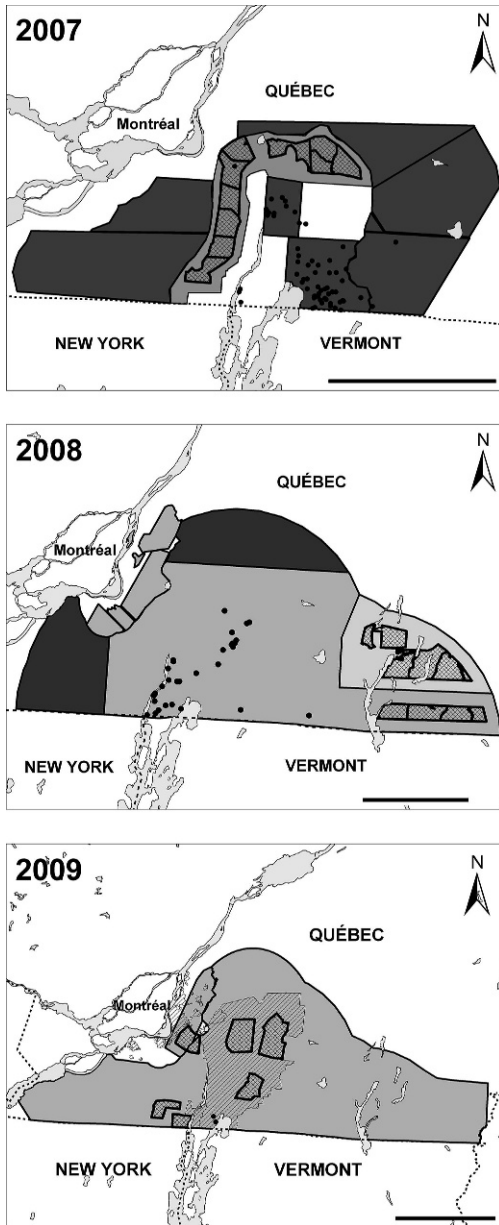


FIGURE 1. Oral rabies vaccination (ORV) zones in 2007, 2008, and 2009 in southern Québec, highlighting the aerial distribution zones for ONRAB® (light grey) and RABORAL V-RG® (dark grey). The hand baiting zone in 2009 is represented by a hashed area. The location of the trapping cells used for post-ORV monitoring is shown by cross-hatched areas surrounded by a thick, black line. The area that received 75 (instead of 150 ONRAB baits/km<sup>2</sup>) in 2008 is shown in lighter grey (east of the ORV zone). Confirmed cases of the raccoon rabies virus variant are indicated by black filled circles

the most rabies cases were documented the previous year (Fig. 1). The baits were distributed in preferred raccoon and skunk habitat (e.g., abandoned buildings and river banks). Second, the aerial distribution of ONRAB was modified to focus on forest patches and adjacent edges of agricultural fields, because raccoons and skunks are more likely to use these habitats (Larivière and Messier, 2000; Baldwin et al., 2004; Barding and Nelson, 2008; Beasley and Rhodes, 2010; Boyer et al., 2011). In all years, flight lines were spaced at 0.75 km, and no baits were distributed over residential areas, large bodies of water, or major roads. The logistic details of bait distribution are provided in Table 1.

**Post-ORV monitoring**

Five to six weeks after each aerial ORV campaign, raccoons and skunks were live-trapped for 10 days in 5–10 trapping cells within the ONRAB ORV zone (Fig. 1). Approximately 80 live-capture traps (Havahart models 1079 and 1081, Woodstream Corporation, Lititz, Pennsylvania, USA) were set in each trapping cell by professional trappers, and baited with sardines, marshmallows, and an olfactory lure (ProCoon®, Leurres Forget, Charette, Québec, Canada). Traps were checked daily and each capture location was recorded with the use of a handheld global positioning system (GPS) unit. Raccoons and skunks were anesthetized following methods described by Robert et al. (2012). Each animal was sexed and ear-tagged for identification. A blood sample (5–10 ml) was collected from the proximal jugular vein; the sample was centrifuged, and the serum was preserved at -70 C. A premolar tooth (PM1 or PM2) was extracted for age determination. Meloxicam (Metacam®, 0.2 mg/kg, Boehringer Ingelheim, Burlington, Ontario, Canada) was given to provide analgesia. Cementum aging according to a 100-µm tooth section was later performed by Matson’s Laboratory, Milltown, Montana, USA. Each animal received an intramuscular injection of an inactivated rabies vaccine (IMRAB®3, Merial). Blood samples were not taken from animals already marked with an ear tag to exclude individuals that had been previously vaccinated intramuscularly from the serologic analyses. Animal handling methods for trapping operations complied with the Agreement

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(n=66, 32, and 2 in 2007, 2008, and 2009, respectively). Scale bars=50 km (note variations in scale between maps).

TABLE 1. Number of ONRAB<sup>®</sup> vaccine baits that were distributed per year for the control of the raccoon rabies virus variant in southern Québec, Canada.

Year	Period	Area (km <sup>2</sup> )	Distribution method	Distribution approach	No. ONRAB baits
2007	15–19 August	1,016	Aerial baiting	Uniform	117,585
2008	18–22 August	7,210	Aerial baiting	Uniform	701,770
2009	27 Apr–5 May	2,568	Hand baiting	Targeting habitats	70,090
2009	17–22 August	11,487 <sup>a</sup>	Aerial baiting	Targeting habitats	946,400

<sup>a</sup> ONRAB baits were also hand distributed in the spring of the same year.

on International Humane Trapping Standards (Government of Canada, 1998) and all sampling procedures were approved by the animal care committee of the MRNF.

#### Antibody prevalence in raccoons and striped skunks

Raccoon and skunk serum samples were analyzed for the presence of rabies virus antibodies with the use of a competitive enzyme-linked immunosorbent assay (cELISA) according to established methods (Elmgren and Wandeler, 1996) and as previously used in post-ORV studies conducted in Ontario (Rosatte et al., 2009a, 2011). Results of this assay were expressed as the percentage inhibition of glycoprotein-specific, peroxidase-labeled monoclonal antibodies binding to microtiter plates coated with rabies virus. Inhibition values of  $\geq 25\%$  and  $\geq 26\%$  were considered as positive thresholds for the presence of rabies virus antibodies in raccoons and skunks, respectively. At these threshold values, the cELISA has a sensitivity of 75% and a specificity of 92% for raccoon sera, and a sensitivity of 85% and a specificity of 96% for skunk sera, when compared against a rabies virus neutralization assay with a positive threshold of  $\geq 0.5$  IU/ml (Fehlner-Gardiner et al., 2012).

#### Data analyses

**Weighted bait density and habitat composition in animal home ranges:** Aircraft were equipped with an automated delivery system that recorded the number of ONRAB baits distributed in real time during each flight. The flight lines were recorded with the airplane GPS. This information was used to interpolate a continuous bait density surface across the ORV zone (Fig. 2A) using ARC GIS version 9.3 (Environmental Systems Research Institute, Redlands, California, USA). From this surface, we estimated the vaccine-bait density likely encountered by each sampled animal by first defining an ecologically plausible home range of 12.6 km<sup>2</sup> (Rosatte et al., 2010), as defined

by a 2-km radius centered on the animal capture location (Fig. 2B). We then calculated the mean vaccine-bait density as a weighted average of one-bait increment density polygons within the home range (Fig. 2C), referred to hereafter as “weighted bait density.” We used the mean of all the individual weighted bait densities in a given year to estimate the overall bait density applied in the trapping cells. To consider the possible landscape influences on antibody prevalence, we also characterized the habitat composition of each home range by calculating the proportions of the 12.6-km<sup>2</sup> area that could be classified into five macrohabitat categories: 1) forest, 2) agricultural fields, 3) residential areas (e.g., houses, barns), 4) water (e.g., lakes, rivers) and 5) other, according to habitat maps produced by the MRNF from aerial photographs.

**Raccoon abundance index:** Trapping success of raccoons was used as an index for raccoon abundance in each trapping cell. We only considered raccoons because this species exhibited more statistically informative variation among trapping cells (6–18 individuals/km<sup>2</sup>) than skunks (1–2/km<sup>2</sup>; Jolicoeur et al., 2009). For each cell, trapping success was calculated as the number of unique raccoons captured divided by the total number of trap nights. The calculation for trap nights was adjusted by subtracting the number of recaptured raccoons, the captures of other species, and the number of sprung traps. This corrected value was then multiplied by 100 to produce the raccoon abundance index (RAI).

**Statistical modeling:** We used a generalized linear model with a binomial error structure to model the species-specific probability of a given animal being antibody positive. We tested for the effects of age (juvenile or adult), sex, and the number of days elapsed between the median date of the aerial ORV campaign and blood sampling to account for a possible temporal influence on antibody titers (Sattler et al., 2009). Given the capture location, we



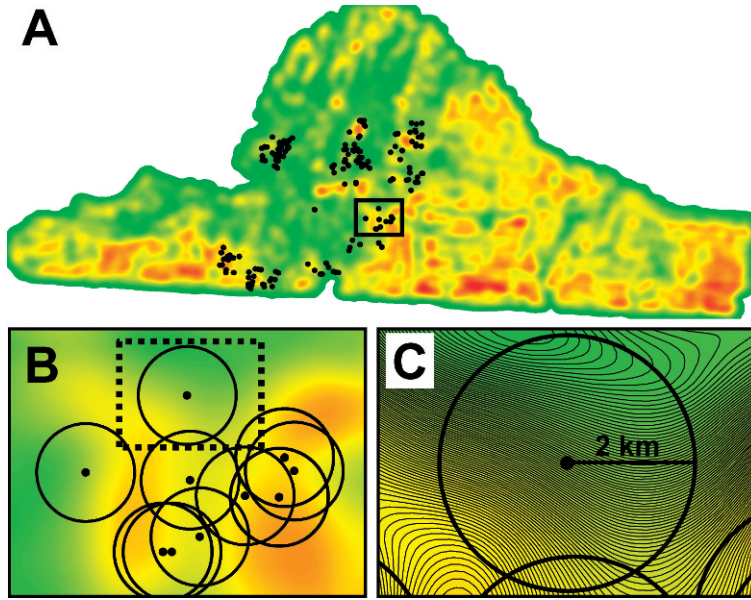


FIGURE 2. (A) Spatial variation in density of ONRAB® baits distributed during the 2009 aerial oral rabies vaccination (ORV) campaign in southern Québec, Canada, and raccoon sample locations (●) for post-ORV monitoring. Vaccine-bait density varied from low (green, minimum = 1 bait/km<sup>2</sup>) to high (red, maximum = 226 baits/km<sup>2</sup>). (B) Two-kilometer buffer zone around each capture location of sampled raccoons to represent a home range for which the average vaccine-bait density is calculated. (C) Fine-scale vaccine-bait density gradient contours used for the derivation of the weighted mean bait density in an animal's plausible home range.

also included covariates for the RAI in the trapping cell in which the animal was caught, the weighted bait density, the proportion of macrohabitat types within an individual's home range (excluding the category 'others' to avoid multicollinearity problems), and the cumulative number of past ORV campaigns likely experienced by an animal given its age and capture location (hereafter referred to as "age-corrected number of ORV campaigns"). We also assessed whether a difference in the serologic results was detectable after the uniform bait distribution strategy (2007 and 2008) was changed to a habitat-targeted strategy in 2009. All covariates were examined simultaneously with the use of a backwards stepwise model selection procedure. The least significant term at each analytical step was dropped based on its *P* value until only significant terms remained ( $\alpha=0.05$ ).

The final model was used to calculate the probability of an animal being antibody positive according to variations in weighted bait densities, with the other explanatory variables kept constant. This allowed a determination of the theoretical number of baits per square kilometer required to obtain a given individual probability of being antibody positive. For this

purpose, we chose a value of 65% probability of a given animal being antibody positive, to be similar to the 63% estimated population-level antibody prevalence needed to prevent the perpetuation of rabies associated with RRV in Massachusetts (Robbins et al., 1998). Because age was determined for most sampled animals, we also examined the age structure of the two mesocarnivore species, and then evaluated the antibody prevalence that could be obtained under the average conditions in southern Québec for a given vaccine-bait density when accounting for age-specific variation in the odds of being antibody positive.

All statistical analyses were conducted with the use of SAS version 9.1 (SAS Institute, Cary, North Carolina, USA) and all tests were two tailed. Dispersion parameter values were close to 1 in the modeling of the binary response variable. In multivariate analyses, multicollinearity (Graham 2003) was tested for among the continuous variables, but none was found.

**RESULTS**

A total of 1,765,755 ONRAB baits were distributed aerially in southern Québec

TABLE 2. Annual percentages (range among trapping cells) of raccoons and striped skunks positive for rabies virus antibodies following oral rabies vaccination campaigns with ONRAB<sup>®</sup> vaccine baits in southern Québec, Canada, given the aerial bait distribution strategy, and targeted and weighted bait densities (raccoon/striped skunk).

Year	Distribution approach	Targeted bait density	Weighted bait density	Raccoon	Striped skunk, <sup>a</sup> % (range)
2007	Uniform	150	152/155	56.2% (33.3–74.7)	15.4 (0.0–27.3)
2008	Uniform	150	117/119	50.9% (46.7–54.7)	16.7 (0.0–25.0)
2008	Uniform	75	52/43	34.7% (25.0–51.4)	12.5 (0.0–50.0)
2009	Targeting habitats	– <sup>b</sup>	67/60	48.5% (23.8–67.7)	11.0 (6.7–25.0)

<sup>a</sup> Because the sample size for skunks was often low within a given trapping cell, the range of values provided must be interpreted with caution. For instance, one antibody-positive striped skunk out of two sampled yielded the 50.0% upper value in one trapping cell in 2008 that was baited at 75 baits/km<sup>2</sup>.

<sup>b</sup> Because of the modification to the aerial bait distribution strategy, no targeted density was used, but an attempt was made to distribute 150 baits/km<sup>2</sup> in forested areas.

between 2007 and 2009 (Table 1) and 1,644 raccoons and 258 skunks were live trapped and tested for post-ORV monitoring. The RAI among trapping cells varied from 7.4 to 18.7 individuals/100 trap nights. From 2007 to 2009, annual antibody prevalence in raccoons ranged from 35 to 56% after the distribution of 52 to 152 ONRAB<sup>®</sup> baits/km<sup>2</sup> (weighted bait densities), and in skunks it was 11 to 17% after the distribution of 43 to 155 baits/km<sup>2</sup> (Table 2). Reducing the targeted vaccine-bait density in a designated area in 2008 from 150 to 75 baits/km<sup>2</sup>, with the use of a uniform bait distribution strategy, decreased the antibody prevalence observed by 16 and 4 percentage points in raccoons and skunks, respectively (Table 2). The habitat-targeted distribution of baits in 2009, which generated lower weighted bait densities compared to a uniform distribution, yielded similar antibody prevalences to those observed in 2007 and 2008 (Tables 2 and 3).

At the individual level, the probability of a given raccoon being antibody positive was greater in adults than juveniles, increased with weighted bait density, and was influenced by the age-corrected number of ORV campaigns (Table 3). There was, however, no significant difference among individuals sampled in an area that had 2–4 previous ORV campaigns (multiple comparison tests,  $P \geq 0.35$  in all cases),

which all differed when compared to a single one ( $P < 0.0001$ , except for 2 ORV with  $P = 0.054$ ). Conversely, the probability of a raccoon being antibody positive decreased with an increase in the RAI and in the proportion of residential areas within the home range (Table 3). In skunks, none of the modeled factors significantly affected the probability of being antibody-positive ( $P \geq 0.10$  in all cases).

From our model for raccoons, we predicted that 178 baits/km<sup>2</sup> would be required to achieve a probability of being antibody positive of 65% in adults when ONRAB is the vaccine bait used in a naïve area (Fig. 3). In juveniles, 206 baits/km<sup>2</sup> were required, on average, to reach the same probability (Fig. 3). In adult raccoons sampled in areas with  $\geq 2$  ORV campaigns, the required density for the same probability was 50 baits/km<sup>2</sup> (Fig. 3).

The annual percentage of live-trapped juveniles was always higher in skunks (range=61–68%) than in raccoons (range=42–51%; Fig. 4). Given the overall age structure in raccoons (55.4% adults, 44.6% juveniles, Fig. 4) and the age-specific predicted probabilities of being antibody positive (Fig. 3), a single distribution event of 125 ONRAB baits/km<sup>2</sup> in an area where only naïve raccoons would be found is predicted to achieve an antibody prevalence of 48.0% under

TABLE 3. Generalized linear model of the effects of age class (adult vs. juvenile), sex (female vs. male), aerial ONRAB® weighted bait density, type of aerial oral rabies vaccination (ORV) strategy (habitat targeted vs. uniform), number of ORV campaigns that an individual likely experienced (relative to four consecutive ORV campaigns), raccoon abundance index, number of days elapsed between the median date of the aerial ORV and blood sampling, and proportions of different macrohabitat categories (forest, agricultural, residential, water) within the hypothetical home range on the probability of individual raccoons from southern Québec, Canada, testing positive for rabies virus antibodies.

Variables	$\beta$	SE	$\chi^2$	df	P
Age class	0.29	0.11	6.38	1,484	0.01
Weighted bait density	0.011	0.001	66.4	1,484	<0.001
Raccoon abundance index	-0.06	0.02	11.2	1,484	<0.001
Proportion of residential areas	-6.28	0.93	50.0	1,484	<0.001
Number of ORV campaigns			43.4	1,484	<0.001
1	-1.38	0.35	15.5	1,484	<0.001
2	-0.41	0.60	0.46	1,484	0.50
3	0.11	0.41	0.07	1,484	0.79
Not retained in the model					
Type of aerial ORV strategy	-0.06	0.23	0.07	1,436	0.79
Proportion of forest	-0.26	1.03	0.06	1,437	0.80
Days since ORV	-0.01	0.01	1.12	1,438	0.29
Proportion of water	-1.59	0.98	2.67	1,439	0.10
Proportion of agricultural fields	-0.27	0.23	1.35	1,440	0.24
Sex	-0.20	0.11	3.23	1,441	0.07

similar conditions as those observed in southern Québec. Antibody prevalence would increase to 64.3% for  $\geq 2$  ORV campaigns. This is assuming that raccoons within the same age class have a similar

probability of seroconversion when exposed to the vaccine contained in the ONRAB bait, which remains questionable.

**DISCUSSION**

The antibody prevalence obtained with ONRAB vaccine baits in southern Québec

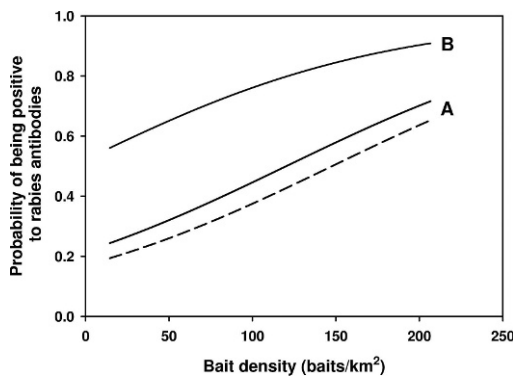


FIGURE 3. Predicted individual probability of a raccoon being positive for rabies virus antibodies given the density of ONRAB® vaccine baits distributed in the home range after a single aerial oral rabies vaccination (ORV) campaign in adults (solid line) and juveniles (dashed line; A), and  $\geq 2$  consecutive ORV campaigns (adults only; B), for average values of the raccoon abundance index and the proportion of residential areas within a raccoon plausible home range in southern Québec, Canada.

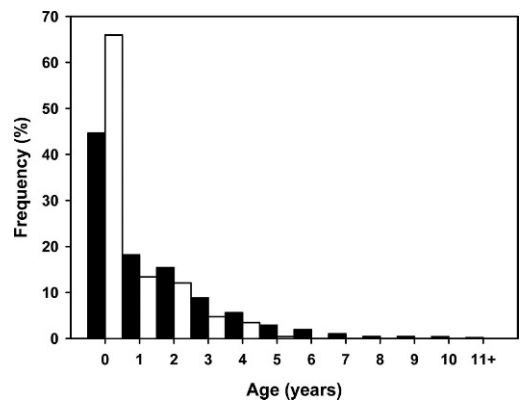


FIGURE 4. Age structure of wild-caught raccoons (black bars,  $n=1,551$ ) and striped skunks (white bars,  $n=232$ ) during post-oral rabies vaccination studies conducted in 2007, 2008, and 2009 (all years pooled) in southern Québec, Canada. Individuals aged  $\geq 11$  yr old are pooled.

differed markedly between raccoons and skunks, with raccoons having higher prevalences in all years examined. Analyses conducted at the individual level also identified several factors to consider in the design of programs using ONRAB against RRVV. The dominant factors included vaccine-bait density and raccoon abundance, proportion of residential areas in the baiting zone, and the history of ORV campaigns. In addition, our study indicated that targeting the habitats in which raccoons are generally found in ORV programs can allow for an optimization of the vaccine baits used within a given area.

In skunks, antibody prevalence was consistently low (generally <20%), and we were unable to identify any factors that could explain the variance observed in the individual probability of being antibody positive. As a potential explanation for this observation, Jojola et al. (2007) identified that baits with a meat-flavored matrix were more likely to be consumed by captive skunks than baits with a sweet-flavored matrix, such as the artificial marshmallow flavoring used in the ONRAB. The low number of skunks observed in proximity of ONRAB baits in field experiments conducted in the Québec ORV zone (Boyer et al., 2011) supports that the current bait formulation may not be as attractive for skunks as it is for raccoons. The smaller jaws of skunks may also not permit them to chew the bait in a fashion that is optimal for vaccine delivery, as previously speculated for the V-RG coated-sachet bait (Grosenbaugh et al., 2007). If ONRAB was changed to suit skunks better, bait formulations would still need to remain effective for raccoons. Species-specific distribution techniques, such as reducing flight-line spacing and increasing bait densities in problematic areas, may be another way to optimize bait uptake in skunks (Rosatte et al., 2011).

Antibody prevalence in raccoons often exceeded 50%. At the individual level, the multivariate analysis indicated that the

probability of a given raccoon being antibody positive was partially explained by the number of baits distributed per square kilometer. However, our model predicts that for a first ORV campaign, an increase of 5 percentage points (i.e., from 65% to 70%) in the probability of being antibody positive in adult raccoons would require a 12% increase in the density of ONRAB baits distributed (from 178 to 200/km<sup>2</sup>), a pattern consistent with previous observations by Sattler et al. (2009) with V-RG. Given that the cost of ORV is largely dependent on the number of baits distributed (Sterner et al., 2009), managers aim toward cost effectiveness by using the minimum bait density required for achieving successful rabies control. The overall antibody prevalence necessary to eliminate RRVV from an enzootic area remains unknown, although results from trap–vaccinate–release (TVR) programs carried out during the raccoon rabies epizootic in Ontario, Canada, suggest prevalences greater than 70% may be required (Rosatte et al., 2009b); making it difficult to determine what bait density should be used.

In our multivariate analysis, there was a negative relationship between the individual probability of being antibody positive and the RAI. An increase of one unique raccoon captured in a given trapping cell per 100 trap nights was accompanied, on average, by a decrease of 6% in the odds of a raccoon of being antibody positive. These data are consistent with the observations of Ramey et al. (2008), who reported that low antibody prevalence was observed in an area where insufficient bait densities were applied in presence of high raccoon densities. Therefore, knowledge of raccoon densities is of prime importance for distributing a sufficient density of baits to immunize the population at risk. Density estimation can be logistically challenging in a landscape designated for ORV control because these areas are often large, inherently having sufficient variation in raccoon densities to



influence ORV efficacy given fixed bait densities. For raccoon rabies control, an apparently valid approach is to concentrate bait distribution in habitats empirically known to be associated with high raccoon densities (Houle et al., 2011), as this has been shown to increase the proportion of immunized raccoons (Robbins et al., 1998). In agriculturally fragmented landscapes, raccoons actively select forest cover (e.g., Beasley and Rhodes, 2010). In these landscapes dominated by cornfields with a few forested patches, which are associated with the highest risks of raccoon rabies (Houle et al., 2011; Rees et al., 2011), transect experiments showed that contact rates of wildlife with baits were highest for ONRAB baits placed in forested habitats rather than along the edge or within adjacent agricultural fields. This pattern occurred especially in the fall and spring when raccoons accounted for half of the wildlife species observed near these baits (Boyer et al., 2011). We therefore propose that targeting residual forest patches, and other types of habitats that are often associated with forested areas (e.g., streams and ponds, abandoned fields) constitutes an adequate approach for vaccine-bait distribution. Interestingly, the habitat-targeted ORV strategy adopted in 2009 did not significantly decrease the antibody prevalence in raccoons while allowing an increase of 22% in the size of the area treated using a similar number of baits (i.e., from 9,430 km<sup>2</sup> in 2008 (when also considering the areas that were treated with V-RG) to 11,487 km<sup>2</sup> in 2009). Thus, such a targeted approach to bait distribution can optimize the efficiency of large-scale ORV programs.

The number of cumulative ORV campaigns that a raccoon has potentially experienced based on its capture location and age also had a positive effect on the probability of being antibody positive. Our statistical model indicates that the required number of baits distributed aerially to obtain a 65% probability of being antibody positive in an adult raccoon would be reduced by 72% after  $\geq 2$  ORV

campaigns when compared to a first ORV campaign (i.e., from 178 to 50 baits/km<sup>2</sup>). Similar field studies utilizing annual or semiannual ORV campaigns with V-RG baits in Massachusetts and Ohio reported that the number of cumulative campaigns was associated with slight gradual increase in antibody prevalence in raccoons (Robbins et al., 1998; Sattler et al., 2009). This indicates that the number of vaccine baits required within a specific ORV zone can be potentially reduced following the first year of application.

A factor that negatively impacted the probability of being antibody positive was the presence of residential areas within a raccoon's home range. Residential food sources are known to attract raccoons (Prange et al., 2004) and, therefore, a vaccine bait may potentially be less attractive in a resource-rich residential environment than in a rural environment. Recently, Bigler et al. (pers. comm.) reported that following an ORV program in New York state, the antibody prevalence in raccoons was negatively correlated with human population density, which may indirectly provide some support to the potential role of alternative food sources in urbanized areas for increasing competition for bait uptake, although it must also be considered that probably fewer baits have been distributed in areas highly populated by humans because of logistic constraints. Either way, the presence of residential areas may reduce the efficacy of ORV campaigns. To mitigate this effect, other control activities such as TVR (Rosatte et al., 2001) could be used in conjunction with ORV near urbanized areas to protect human populations better, especially during an epizootic.

Our age-structure analysis indicated that, as was found in Ontario (Rosatte, 2000), juveniles composed a significant proportion of trapped raccoon and skunk populations. It is possible that the estimated abundance of juveniles may be upwardly biased because inexperienced individuals may be more likely to enter a baited live trap

than adults. However, we hypothesize that juveniles still constitute a large proportion of the population because longevity is generally short in these mesocarnivores (Gehrt, 2005; Rosatte et al., 2010). The observed distribution frequency of ages in both species in our study, if not altered by the potential avoidance of baited traps by experienced individuals, would provide some support for this view. Nevertheless, the renewed presence of many juveniles each year supports implementation of ORV campaigns at least once per year.

Our results suggest that the distribution of ONRAB baits for raccoon rabies control should be optimized according to the spatial heterogeneity influencing the distribution of raccoons in the landscape (see also Boyer et al., 2011). Baiting campaigns should especially take into account raccoon densities, which in turn are often related to the availability of their preferred habitats (see Houle et al., 2011). Accounting for these sources of variation should increase the proportion of individuals within a population that are immunized by ORV campaigns, while also minimizing costs (i.e., the number of vaccine baits used). This in turn should increase the success for containing and eliminating a rabies epizootic in raccoons if vaccination programs are continued over time with adequate frequency and vaccine-bait densities. The control strategies employed in Québec following the first detected RRVV-associated case (Canac-Marquis et al. 2007; Guérin et al. 2008) combined with the increasing use of ONRAB in ORV campaigns since 2007 appear to have been successful in this respect, as the last case was detected in a skunk in April 2009 close to the international border with Vermont (as of March 2012). Our study also highlighted the need for further research on oral vaccine delivery efficacy and immunogenicity in striped skunks. The multitactic approach used in Canada with the current available vaccine baits seems, however, to have been sufficient for eliminating this rabies variant.

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