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Oral Rabies Vaccination in Raccoons: Comparison of ONRAB® and RABORAL V-RG® Vaccine-Bait Field Performance in Québec, Canada and Vermont, USA

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ABSTRACT: The control of rabies in raccoons (*Procyon lotor*) and striped skunks (*Mephitis mephitis*) in North America has been conducted mainly through aerial distribution of oral vaccine-baits. The effectiveness of the vaccine-bait used is therefore of prime importance for disease eradication. In a previous field comparison between the ONRAB® bait in the province of New Brunswick, Canada, and RABORAL V-RG® bait in the state of Maine, USA, the ONRAB bait produced a higher percentage of antibody-positive raccoons under nearly identical bait distribution for the two vaccines. The main objective of the present study was to conduct a similar cross-border comparison of these two vaccine-baits using raccoon sera collected during post–oral rabies vaccination monitoring in Québec, Canada, and Vermont, USA, where ONRAB and V-RG, respectively, were distributed aerially at a targeted density of 150 baits/km^2. A comparison of the equivalency of two serologic tests used in Canada and the USA was also conducted using sera from raccoons and striped skunks. Rabies virus neutralization assay (USA) yielded similar results to the competitive enzyme-linked immunosorbent assay (Canada), with agreement between the two tests of 92% for raccoon sera and 96% for skunk sera. With both assays, the percentage of antibody-positive raccoons was greater with ONRAB (51%, $n=265$) than with V-RG (38%, $n=66$). These new results support the conclusion from the previous study, that ONRAB vaccine-baits may be more effective for the control of rabies in raccoons.

Key words: Antibody prevalence, oral rabies vaccination, *Procyon lotor*, raccoon, striped skunk, vaccine-bait.

Oral rabies vaccination (ORV) is the main control strategy used in the USA and Canada against raccoon (*Procyon lotor*) rabies, a zoonosis of great concern for public health (Slate et al., 2009). Oral vaccination is accomplished primarily by aerial distribution of vaccine-baits to induce protective immunity in susceptible hosts. Given the objectives and costs of such programs (Slate et al., 2009), the effectiveness of the vaccine-bait used for rabies control and eradication is of prime importance. Two vaccine baits are currently used for control of rabies in raccoons and skunks in North America: a vaccinia-rabies glycoprotein recombinant, RABORAL V-RG® (Merial, Athens, Georgia, USA) and an adenovirus rabies glycoprotein recombinant, ONRAB® (Artemis Technologies, Guelph, Ontario, Canada). Recently, the field performance of these two vaccine-baits in raccoons and striped skunks (*Mephitis mephitis*) was evaluated through a cross-border comparison study (ONRAB in New Brunswick, Canada, V-RG in Maine, USA; Fehlner-Gardiner et al., 2012). Under similar aerial baiting schemes (75 baits/km^2 and 1 km between flight lines), the percentage of raccoons that were positive for rabies virus antibodies was significantly higher with the use of ONRAB than V-RG; the difference was not significant in skunks. Our objective was to perform a similar comparison of the two vaccine-baits using raccoon sera that were collected from post-ORV monitoring in Québec (ONRAB in Ultralite baits) and Vermont (V-RG in coated sachet baits) in 2008. This project was conducted under the framework established in the North American Rabies Management Plan (US Department of

Agriculture [USDA], 2008) to identify the best available oral vaccine-bait.

In Québec, 701,770 ONRAB baits were distributed 18–22 August 2008 over 7,210 km^2 (45°20′N, 72°45′W; Fig. 1) at a targeted density of 150 baits/km^2, by fixed-wing aircraft at a flight-line spacing of 0.75 km. In Vermont, 375,764 V-RG baits were distributed 31 August–3 September 2008 over 5,417 km^2 (44°50′N, 72°34′W; Fig. 1) using the same approach. Sampling for raccoon and skunk sera began 7 wk after bait distribution in Québec (12–20 October) near the city of Coaticook, a minimum of 5 km from the international border (Fig. 1). Sampling in Vermont started 4 wk after the ORV (30 September–28 October) in an area mainly north of the city of St. Albans, near the USA–Canada border (Fig. 1) where habitat composition and structure were similar to the Québec post-ORV monitoring area. Approximately 80–100 baited live-traps (Québec: Havahart, Woodstream Corporation, Lititz, Pennsylvania, USA; Vermont: Tomahawk, Tomahawk Live Trap Company, Tomahawk, Wisconsin, USA) were used over 10 days in designated trapping cells. In Québec, animals were brought to a central location where they were identified to sex, weighed, anaesthetized, sampled for blood, and had a tooth extracted for age determination. In Vermont, the same process was followed, but at the live-trap location. Sampled animals were ear-tagged, vaccinated with a rabies vaccine (IMRAB3, Merial) and released at site of capture. Blood was stored on ice until centrifugation, after which serum was transferred to vials and stored frozen.

Serum samples from Québec were sent to the Centre of Expertise for Rabies at the Canadian Food Inspection Agency (CFIA) to be tested with a competitive enzyme-linked immunosorbent assay (cELISA; Elmgren and Wandeler, 1996). Samples from Vermont were sent to the Rabies Laboratory, Wadsworth Center (WC), New York State Department of Health, to be tested with a rabies virus

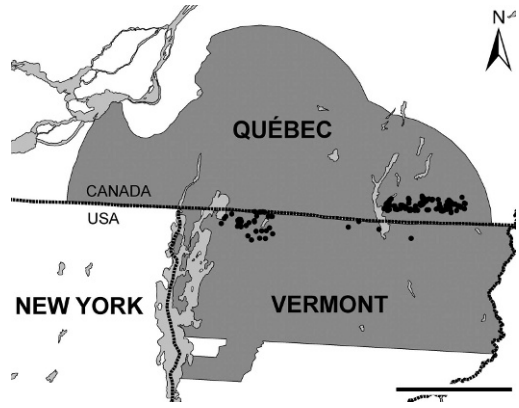

FIGURE 1. Zones (in gray) where aerial oral rabies vaccination (ORV) programs were conducted in the province of Québec (QC), Canada, and the state of Vermont (VT), USA. Black filled circles indicate the spatial distribution of raccoons (*Procyon lotor*) sampled during post-ORV monitoring for rabies antibody prevalence where ONRAB® (QC) and V-RG® (VT) vaccine-baits were used. Striped skunks (*Mephitis mephitis*) captured in QC were from the same sampling area. Bar=50 km.

neutralization assay (RVNA; Trimarchi et al., 1996). In 2010, duplicate serum samples archived from 2008 from Québec and Vermont were sent to WC for RVNA analysis and to CFIA for cELISA analysis, respectively. The sharing of samples allowed for blind comparison of antibody prevalence estimates obtained with both assays. An animal was categorized as antibody positive by RVNA when the titer was ≥0.5 IU/ml, which is considered a conservative threshold, whereas a positive cut-off value of ≥25% (raccoon) and ≥26% (skunk) inhibition was used for the cELISA (Fehlner-Gardiner et al., 2012).

Chi-square tests were used to compare the performance of the two serologic assays, the age and sex ratios between the two areas, and the percentage of antibody-positive raccoons according to the vaccine-bait used. A generalized linear model (GLM) with a binomial error structure was also used to determine whether the individual probability of a raccoon being antibody-positive was influenced by its age class (adult/juvenile), sex, and vaccine-bait used. For this GLM

analysis, all the categorical explanatory variables were simultaneously examined using a backward stepwise model selection procedure until only significant variables remained ($\alpha=0.05$). All analyses were conducted in SAS 9.1 (SAS Institute, Cary, North Carolina, USA).

Sera were available for 265 raccoons and 30 skunks in Québec, and 66 raccoons in Vermont, from areas where 150 baits/km^2 were distributed. Additional serum samples from a zone where 75 baits/km^2 were distributed in Québec were also considered to compare the two assays, but not the field performance of the vaccine-baits. Agreement between RVNA and cELISA was 92.0% ($n=415$) with raccoon sera and 96.0% ($n=50$) with skunk sera, and the percentage of antibody-positive raccoons identified with RVNA (46.3%) and cELISA (52.0%) did not differ statistically ($\chi^2=2.78$, $P=0.10$). Since these two serologic tests are normally used for post-ORV monitoring in USA and Canada, respectively, this suggests that they can be considered equivalent for the purpose of estimating antibody prevalence in the field.

Because no skunk sera were available from Vermont, the comparison between vaccine-baits was performed for raccoons only. The number of female (F) and male (M) raccoons sampled was similar between the two areas (Québec=129 F, 134 M; Vermont=32 F, 34 M; $\chi^2=0.007$, $P=0.93$), but the number of adults (A) and juveniles (J) differed (Québec=157 A, 105 J; Vermont=27 A, 39 J; $\chi^2=7.74$, $P=0.005$). The percentage of antibody-positive raccoons in Vermont was 37.9% ($n=66$) for the two assays, whereas in Québec the percentages were higher whether analyzed by cELISA (50.9%, $\chi^2=3.61$, $P=0.057$) or RVNA (51.5%, $\chi^2=3.92$, $P=0.047$).

Because differing age structure in the raccoons sampled from Vermont and Québec may have affected the observed antibody prevalence, 27 A and 39 J were randomly selected from the Québec sample to obtain a balanced comparison with respect to sample size and age structure. This subsampling for Québec was repeated ($n=5$) without replacement to assess the variation generated by this procedure. The mean percentage of antibody-positive raccoons in Québec was 56.1% (range=51.5–60.6%) by cELISA and 57.6% (54.5–62.1%) by RVNA. In a comparison of the average percentage of antibody-positive individuals between Vermont and the subsample from Québec, a significant difference was found with both cELISA ($\chi^2=4.38$, $P=0.04$) and RVNA ($\chi^2=5.13$, $P=0.02$).

When analyses were conducted at the individual level with a GLM, sex ($\chi^2=0.24$, df=322, $P=0.63$) and age ($\chi^2=1.77$, df=325, $P=0.18$) did not influence the probability of a raccoon being antibody positive for cELISA, whereas the vaccine-bait used did so marginally ($\chi^2=3.65$, df=329, $P=0.056$). For RVNA, only vaccine-bait type was significantly associated with the probability of being positive ($\chi^2=3.97$, df=328, $P=0.046$), as age ($\chi^2=0.35$, df=321, $P=0.55$) and sex ($\chi^2=0.47$, df=325, $P=0.49$) were removed from the model.

This field performance comparison between ONRAB and V-RG was carried out retrospectively using archived sera. Although the targeted bait densities and flight spacing were equivalent in the Vermont and Québec ORV zones, there are several variables that were not controlled, which may have influenced the antibody prevalence observed. For example, ORV campaigns in Vermont had been conducted annually since 1997, whereas 2008 was the first year in which ORV occurred in the Québec area sampled. Since the number of cumulative ORV campaigns has been shown to be positively associated with antibody prevalence in raccoons (Sattler et al., 2009), prevalence in Vermont may have been expected to be higher than that in Québec. In addition, bait density could have been slightly higher in Vermont due to the hand distribution of 1,550 additional V-RG baits in Franklin County where most of the raccoons were sampled; no additional

baits were distributed in the area sampled in Québec. Raccoon density in the two areas may have also influenced antibody prevalence. In Québec, raccoon density in the area sampled was estimated to be 6–18 raccoons/km^2. In the same year, raccoon density indexes conducted by the Wildlife Services of the Animal and Plant Health Inspection Service, USDA, was 3–10 raccoons/km^2 in Vermont. The density estimation methods used in each area were different, however, so these estimates may not be comparable. Nonetheless, differences in raccoon densities could have lead to a bias because less vaccine-baits per individual in Québec than Vermont would decrease the opportunity for raccoons to find baits. Despite these differences that should have favored a higher antibody prevalence in Vermont compared with Québec, the opposite was found in that ONRAB generated a significantly higher percentage of antibody-positive raccoons than did V-RG. Because some raccoons were captured close to the international border, this could have influenced the results of this comparison if animal movements between countries occurred, which constitutes another possible limitation of this study. Whether the different bait designs and attractants used affected this comparison is also unknown.

Higher antibody prevalence in Québec was consistent with the findings of the ONRAB/V-RG comparison conducted in New Brunswick and Maine (Fehlner-Gardiner et al., 2012). The antibody prevalence determined in Vermont in 2008 was higher than that observed in Maine the same year using V-RG (38% vs. 30%), an observation not unexpected since the baiting density in Vermont was twice that used in Maine. However, in a comparison of the ONRAB-baited areas, Québec had a lower antibody prevalence than New Brunswick (51% vs. 74%) despite higher bait density, indicating that other factors also affected field performance. Two possible explanations for the lower antibody prevalence in Québec may be related to raccoon density (<5/km^2 in New Brunswick vs. 6–18/km^2 in Québec), or habitats that may have influenced the availability of competing food alternatives to the ONRAB vaccine-baits.

Our results indicate that for the same targeted vaccine-bait density, the use of ONRAB resulted in higher rabies antibody prevalence in raccoons than V-RG. This suggests that ONRAB may constitute a better option for the oral vaccination of raccoons against rabies.

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LITERATURE CITED

Elmgren LD, Wandeler A. 1996. Competitive ELISA for the detection of rabies virus-neutralizing antibodies. In: *Laboratory techniques in rabies*, 4th Ed., Meslin F-X, Kaplan M, Koprowski H, editors. World Health Organization, Geneva, Switzerland, pp. 200–208.

Fehlner-Gardiner C, Rudd R, Donovan D, Slate D, Kempf L, Badcock J. 2012. Comparing ONRAB® and RABORAL V-RG® oral rabies vaccine field performance in raccoons and striped skunks in New Brunswick, Canada and Maine, USA. *J Wildl Dis* 48:157–167.

Sattler AC, Krogwold RA, Wittum TE, Rupprecht CE, Algeo TP, Slate D, Smith KA, Hale RL, Nohrenberg GA, Lovell CD, et al. 2009. Influence of oral rabies vaccine bait density on rabies seroprevalence in wild raccoons. *Vaccine* 27:7187–7193.

Slate D, Algeo TP, Nelson KM, Chipman RB, Donovan D, Blanton JD, Niezgoda M, Rupprecht CE. 2009. Oral rabies vaccination in North America: Opportunities, complexities, and challenges. *PLoS Negl Trop Dis* 3 (e549): 1–9.

Trimarchi CV, Rudd RJ, Safford M. 1996. An in vitro virus neutralization test for rabies antibody. In: *Laboratory techniques in rabies*, 4th Ed., Meslin F-X, Kaplan MM, Koprowski H, editors. World Health Organization, Geneva, Switzerland, pp. 193–199.

US Department of Agriculture. 2008. *North American Rabies Management Plan*, http://www.aphis.usda.gov/wildlife_damage/oral_rabies/downloads/Final%20NARMP%209-30-2008%20(ENGLISH).pdf. Accessed August 2012.

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