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COMPARING ONRAB® AND RABORAL V-RG® ORAL RABIES VACCINE FIELD PERFORMANCE IN RACCOONS AND STRIPED SKUNKS, NEW BRUNSWICK, CANADA, AND MAINE, USA

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ABSTRACT: Control of rabies in mesocarnivore reservoirs through oral rabies vaccination (ORV) requires an effective vaccine bait. Oral rabies vaccine performance in the field may be affected by a variety of factors, including vaccine bait density and distribution pattern, habitat, target species population density, and the availability of competing foods. A field study in which these covariates were restricted as much as possible was conducted along the international border of the state of Maine (ME), USA, and the province of New Brunswick (NB), Canada, to compare the performance of two oral rabies vaccines in raccoons (*Procyon lotor*) and striped skunks (*Mephitis mephitis*). RABORAL V-RG® (vaccinia-rabies glycoprotein recombinant oral vaccine in fishmeal-coated sachet) or ONRAB® (adenovirus-rabies glycoprotein recombinant oral vaccine in Ultralite bait matrix) were distributed in ME and NB, respectively, by fixed-wing aircraft at a density of 75 baits/km^2 along parallel flight lines spaced 1.0 km apart. Sera were collected from live-trapped raccoons and skunks 5–7 wk post-ORV and assayed to determine antibody prevalence in each area. Duplicate serum samples were provided blind to two different laboratories for analyses by rabies virus serum neutralization assays (at both laboratories) and a competitive enzyme-linked immunosorbent assay (at one laboratory). There was no significant difference in the proportion of antibody-positive animals determined by the three serologic methods, nor was there a significant difference between ONRAB and RABORAL V-RG in the proportion of antibody-positive striped skunks observed post-ORV. In contrast, the proportion of antibody-positive raccoons was significantly higher in the ONRAB- versus the RABORAL V-RG–baited areas (74% vs. 30%; χ^2=89.977, df=5, P<0.0001). These data support that ONRAB may serve as an effective tool for raccoon rabies control.

Key words: ONRAB, oral rabies vaccine, RABORAL-VRG, raccoon, striped skunk.

INTRODUCTION

Raccoon rabies, caused by the antigenically distinct raccoon rabies virus variant, was first discovered in southern Maine (ME), USA, in 1994 and continued to spread through southern and eastern ME at a rate of approximately 75 km/yr (Maine Centers for Disease Control, 2010). In 2000, raccoon rabies was detected in a striped skunk (*Mephitis mephitis*) near the town of St. Stephen, New Brunswick (NB), Canada, across the border from Calais, ME. Sixty-four additional cases of raccoon rabies in raccoons (*Procyon lotor*) and skunks were reported in NB between 2000 and 2002; no case has been detected since March 2002 (Canadian Food Inspection Agency [CFIA], 2011).

During 2001 and 2002, the NB Department of Health (NBDOH) conducted a wildlife rabies control program consisting of population reduction and trap-vaccinate-release (TVR) (parenteral vaccination with IMRAB® 3 [off-label use, Merial Inc., Athens, Georgia, USA]) components designed according to programs conducted in the province of Ontario (Rosatte et al.,

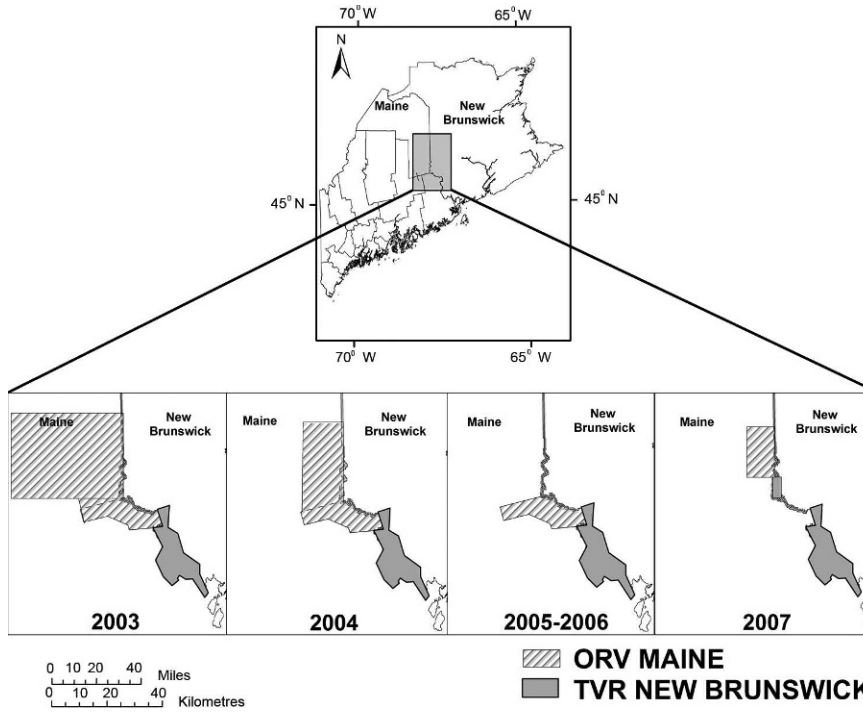

FIGURE 1. Locations of wildlife rabies control programs in New Brunswick, Canada (trap-vaccinate-release [TVR]) and Maine, USA (oral vaccination [ORV]), 2003–07.

2001). From 2003 to 2007, a collaborative approach to control raccoon rabies was implemented by the NBDOH and the US Department of Agriculture, Animal Plant Health and Inspection Services, Wildlife Services (USDA/APHIS/WS). The goal was to achieve a continuous "barrier" of vaccinated raccoons and skunks along the international border to prevent reemergence of raccoon rabies. New Brunswick continued the TVR program and an oral rabies vaccination (ORV) program was implemented in ME, using RABORAL V-RG® (Fig. 1). Although raccoon rabies appeared to be controlled in NB, it continued to spread in ME, with a case detected near the town of Houlton in 2008.

The 2008 ORV zone in ME was placed north of known cases to prevent the spread of raccoon rabies in northern ME and east into NB (Fig. 2). Also in 2008, NBDOH applied for and received an experimental permit from the Veterinary Biologics Section, CFIA, to distribute ONRAB®, an adenovirus-rabies glycoprotein recombinant oral rabies vaccine (NBDOH, 2008). The 2008 ORV programs in ME and NB provided an opportunity to carry out a comparative analysis of the field performance of the two oral rabies vaccine baits with respect to the induction of rabies virus–specific serologic responses in free-ranging raccoons and skunks. Additionally, a comparison of the results of rabies serologic tests conducted in two different laboratories was performed. This project was conducted under the framework established in the North American Rabies Management Plan, which calls for collaboration on key surveillance, control, and research issues to advance rabies control on a continental basis (US Department of Agriculture [USDA], 2008a).

MATERIALS AND METHODS

Study area

The study area is a mixed forest habitat containing hardwoods and softwoods and

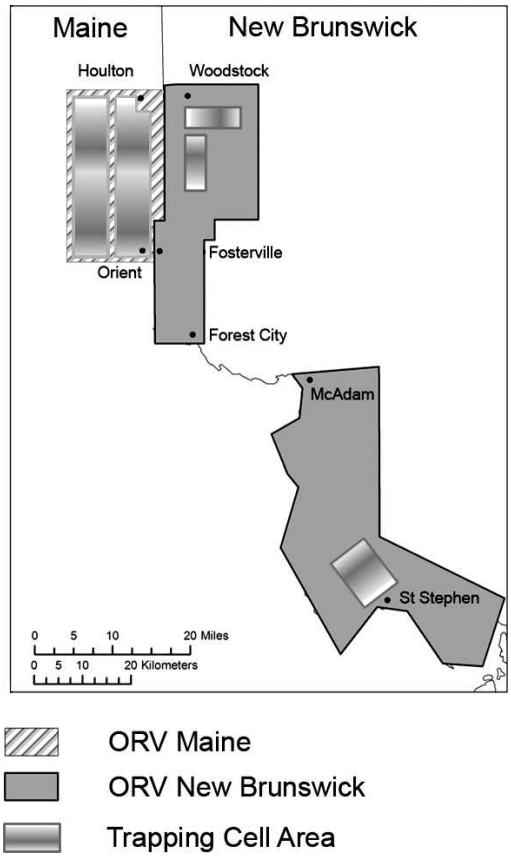

FIGURE 2. Locations of oral rabies vaccination (ORV) programs and areas in which trapping cells were established in New Brunswick, Canada (ONRAB® distribution), and Maine, USA (RABORAL V-RG® distribution), in 2008.

contains agricultural areas with scattered villages and towns. Abandoned farmlands, including old apple orchards that are gradually returning to forest cover, are part of the landscape. Lakes and rivers are found throughout the area.

The NB study area was located in the southwestern region of the province along the ME border and consisted of two experimental plots (Fig. 2). The north plot was located between Upper Woodstock and Forest City (750 km^2). The upper portion of this plot was approximately 30×15 km; wildlife rabies vaccination activities did not occur in this area prior to 2008. The lower portion was approximately 30×10 km; TVR programs occurred in this area in 2007, specifically between Forest City and Fosterville. The south plot was located between McAdam and St. Stephen (675 km^2). The upper portion of this plot was approximately 30×10 km; TVR programs occurred in this area since 2003. The lower portion was approximately 25×15 km wide; TVR programs occurred in this area since 2001.

The ME study area was in the northeastern region of the state along the NB border (690 km^2), between Houlton and Orient. The plot was approximately 37×19 km; ORV with RABORAL V-RG occurred intermittently in this region during 2003, 2004, and 2007.

Oral rabies vaccines

RABORAL V-RG (Merial) is licensed in the USA for ORV in coyotes (*Canis latrans*) and raccoons (USDA, 2009) and is used under experimental license in gray foxes (*Urocyon cinereoargenteus*). It consists of a plastic sachet containing a suspension of a recombinant, live vaccinia virus vector containing the glycoprotein gene from the Evelyn-Rotkitniki-Abelseth (ERA) rabies virus. The sachet is either enclosed in a solid fishmeal polymer (FP) bait, or is coated with wax and fishmeal crumbs (CS bait).

ONRAB (Artemis Technologies Inc., Guelph, Ontario, Canada) is a live adenovirus recombinant oral vaccine, consisting of a human adenovirus type 5 vector containing the ERA glycoprotein gene. The vaccine is contained in an Ultralite bait (Artemis Technologies), consisting of a plastic blister pack surrounded by a wax- and fat-based matrix containing tetracycline hydrochloride (HCl; Rosatte et al., 2009). ONRAB is used under CFIA experimental permit in Canada for control of rabies in skunks and raccoons.

Bait distribution

Approximately 90,070 ONRAB baits and 48,600 RABORAL-VRG CS baits were distributed in NB and ME, respectively, between August 25 and 26 by fixed-wing aircraft at a density of 75 baits/km^2 and with a flight-line spacing of 1.0 km. Hand baiting at a targeted density of 150 baits/km^2 was carried out within the towns of McAdam, Woodstock, and St. Stephen, NB (ONRAB), and Houlton, ME (RABORAL-VRG FP).

Post-ORV sampling

Live trapping: Animal handling procedures were approved by institutional animal care committees and were in accordance with the guidelines of the Canadian Council on Animal Care (2003) and the American Society of Mammalogists Animal Care and Use Committee Guidelines (Gannon et al., 2007). Post-ORV

sampling began 5–6 wk after bait distribution (29 September–10 October in ME and 6–16 October in NB). Trapping areas were at least 5 km from the international border and excluded urban areas baited at 150 baits/km^2 (Fig. 2). Eight trapping cells in NB were 15–20 km^2 in area, covering 120–160 km^2 in the north and south plots combined. Captured animals with an ear tag from a previous TVR program were released without sampling. In ME the majority of the ORV zone was trapped, split between two trappers. Approximately 100 live traps (No. 106 and No. 108, Tomahawk Live Trap Company, Tomahawk, Wisconsin, USA) were used in each trapping cell, resulting in 1,500 and 960 net trap nights in NB and ME, respectively.

Immobilization protocol: Drugs were used off label but were administered either by or under the supervision of a veterinarian. In NB, captured raccoons and skunks were immobilized by intramuscular (IM) injection with a 1:1 ratio of ketamine HCl (100 mg/ml; Pfizer Animal Health, Montreal, Quebec, Canada) and medetomidine HCl (1 mg/ml; Pfizer Animal Health). Atipamezole HCl (5 mg/ml; Pfizer Animal Health) was administered IM to aid in anesthetic recovery. Analgesia was provided by subcutaneous injection of meloxicam HCl (5 mg/ml; Boehringer Ingelheim Vetmedica, Inc., St. Joseph, Missouri, USA). In ME, immobilization was achieved using a 5:1 ratio of ketamine HCl (100 mg/ml; Fort Dodge Animal Health, Fort Dodge, Iowa, USA) and xylazine HCl (100 mg/ml; Lloyd Laboratories, Shenandoah, Iowa, USA). All animals were released at the site of capture upon recovery from anesthesia.

Biologic sampling protocol: Each animal was ear tagged (National Band and Tag Co., Newport, Kentucky, USA). Blood (5 ml) was collected from the carotid, external jugular, or subclavian vein using Vacutainer collection tubes without additives (Becton Dickinson, Mississauga, Ontario, Canada). Blood was stored on ice for a maximum of 15 hr before centrifugation for 15 min at 1,500 × G. Serum was transferred to duplicate vials and stored at −20 C until analyzed.

In ME, a first premolar tooth was removed from adult raccoons and skunks; in NB, a second premolar tooth was removed from adults (raccoon and skunk) and a canine tooth was removed from juveniles (raccoon only). Examination of extracted teeth for tetracycline deposition and age determination was performed at Matson's Laboratory (Milltown, Montana, USA).

Serologic analyses for rabies antibodies: Duplicate serum samples were sent to the Centre of Expertise for Rabies, CFIA, and to the Rabies Laboratory, Wadsworth Center (WC), New York State Department of Health; heat inactivated at 56 C for 30 min; and tested in rabies virus neutralization assays (RVNAs) according to established procedures (Knowles et al., 2009 [CFIA]; Trimarchi et al., 1996 [WC]). In brief, for both RVNAs, serial dilutions of test sera were incubated with 50–100 50% tissue culture infectious dose ($TCID_{50}$) of Challenge Virus Standard (CVS-11, VR959, American Type Culture Collection, Rockville, Maryland, USA) prior to the addition of mouse neuroblastoma cells (MNA, BioWhittaker, Walkersville, Maryland, USA). Following incubation at 37 C, 5% CO_2, for 48 hr, the cells were fixed with cold 75% acetone (CFIA) or methanol:formalin (1:1; WC), and stained with a fluorescently labeled goat anti-rabies ribonucleoprotein polyclonal antibody (CFIA, in-house preparation) or a commercial anti-rabies nucleoprotein monoclonal antibody (mAb) cocktail (WC; DFA 3, Millipore, Billerica, Maine, USA). Antibody titer was calculated using the Spearman-Karber formula (Lorenz and Bogel, 1973; CFIA) or was defined as the highest dilution yielding 100% reduction of virus in the well (WC). Titers were normalized against the World Health Organization (WHO) 2nd International Reference Serum (National Institute of Biological Standards and Control, Potters Bar, Hertfordshire, UK). Results are expressed in IU/ml and considered positive if the titer was $\geq$0.5 IU/ml.

The competitive enzyme-linked immunosorbent assay (cELISA), which measures the ability of a test serum to inhibit the binding of a glycoprotein-specific, peroxidase-labeled, neutralizing mAb to immobilized ERA strain rabies virus, was carried out at CFIA as previously described (Elmgren and Wandeler, 1996). Results are expressed as the percentage inhibition (%I) of binding of the mAb. The cELISA positive threshold values were 25%I and 26%I for raccoon and skunk sera, respectively. The cELISA, as compared to the CFIA RVNA, 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.

Statistical analyses

Statistical analyses were conducted using GraphPad Prism 5 software (GraphPad Software Inc., La Jolla, California, USA). Chi-square analysis was used to test for associations between the proportion of antibody-positive animals detected, the serologic test employed, and the oral vaccine distributed. For comparison of antibody prevalence and age structure in skunks between ME

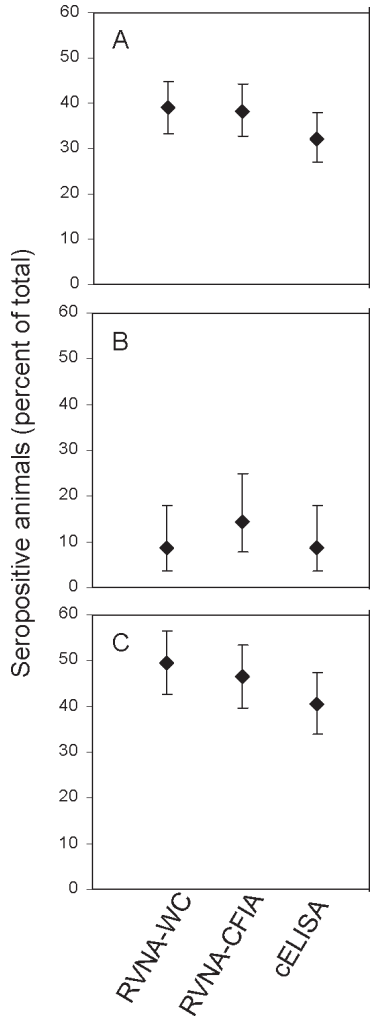

FIGURE 3. Proportion of animals trapped in both New Brunswick, Canada, and Maine, USA, that were positive for rabies virus antibodies, detected using three serologic tests: Canadian Food Inspection Agency (CFIA) rabies virus neutralization assay (RVNA-CFIA), Wadsworth Center rabies virus neutralization assay (RVNA-WC), and CFIA competitive ELISA (cELISA). 95% confidence intervals of the proportions are indicated by the lines above and below each symbol. (A) All trapped animals; (B) skunks (*Mephitis mephitis*) only; (C) raccoons (*Procyon lotor*) only.

and NB, Fisher's exact test was used because of sample sizes <5 in some categories.

RESULTS

Captures

Seventy-six raccoons were captured in NB. Of these, one vaccinated during the 2007 TVR program (identified by an ear tag) and one refractory to anesthesia were not sampled, and one animal did not yield a sufficient volume of serum for all tests. Of the 38 skunks captured, four vaccinated during previous TVR programs were excluded from the study and one animal did not yield sufficient serum for all tests. None of the captured animals retained in the study had evidence of scars or lesions in the ear tissue indicative of a previous ear tag that had been lost. In ME, 125 raccoons and 36 skunks were captured and sampled, with sufficient sera collected for all analyses.

Test performance comparison

To compare the performance of the serologic tests, data from ME and NB samples were combined. All three tests estimated similar proportions of antibody-positive individuals (Fig. 3A), with the RVNA-WC detecting 39.0% of animals antibody positive (95% confidence interval [CI] 33.3–44.9), the RVNA-CFIA 38.2% antibody positive (95% CI 32.6–44.2) and the cELISA 32.2% antibody positive (95% CI 26.9–38.0; Fig. 4A). There was no significant difference in the estimated proportion of antibody-positive animals among the three tests ($\chi^2=3.147$, df=2, $P=0.21$). Similarly, when the percentage of antibody-positive animals estimated by each test was compared within species, there were no significant differences found among the tests for either skunks ($\chi^2=1.628$, df=2, $P=0.44$; Fig. 3B) or raccoons ($\chi^2=3.422$, df=2, $P=0.18$) (Fig. 3C). Agreement between test results was 92.5% for cELISA vs. RVNA-CFIA; 93.3% for cELISA vs. RVNA-WC; and 96.3% for RVNA-CFIA vs. RVNA-WC. For about half those samples in which there was test disagreement, the titer or the cELISA value was close to the respective cut-off values (data not shown). When all three tests were compared, the overall agreement was 91.0%. Of the 21 samples for which there were disagreements, for 14 (67%) the discrepancy was

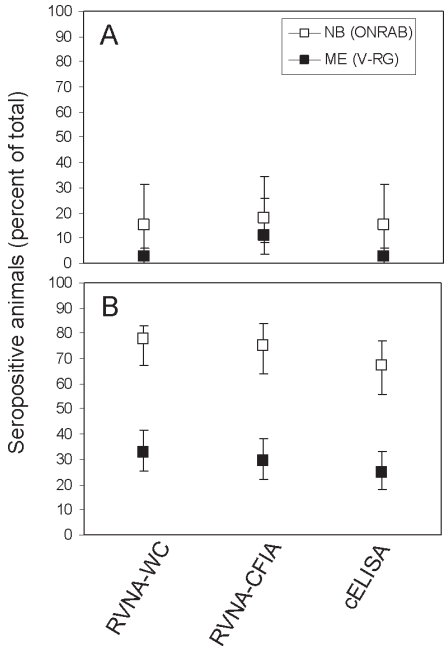

FIGURE 4. Proportion of (A) skunks (*Mephitis mephitis*) and (B) raccoons (*Procyon lotor*) that were positive for rabies virus antibodies as detected by three serologic tests: Canadian Food Inspection Agency (CFIA) rabies virus neutralization assay (RVNA-CFIA), Wadsworth Center rabies virus neutralization assay (RVNA-WC), and CFIA competitive ELISA (cELISA). Samples were gathered from New Brunswick, Canada (open boxes), where ONRAB® baits were distributed, and from Maine, USA (closed boxes), where RABORAL V-RG® baits were distributed. 95% confidence intervals of the proportions are indicated by lines above and below the boxes.

due to a negative cELISA result (data not shown).

Vaccine bait performance comparison

Less than 20% of the skunks sampled from both ME and NB were antibody positive, regardless of the serologic test employed (Fig. 4A and Table 1). Although the proportion of antibody-positive skunks was lower in ME (3–11%, depending on test employed) than in NB (15–18%), the differences were not statistically significant ($P>0.10$ for all three tests).

The proportion of antibody-positive raccoons was higher than that of skunks in both NB and ME (Fig. 4B and Table 1); 67–78% (depending on test) of NB raccoons were antibody positive, and 24.8–32.8% of ME raccoons were antibody positive. There was a significant association between the proportion of antibody-positive raccoons and the type of vaccine bait distributed ($\chi^2=89.977$, df=5, $P<0.0001$).

Biomarker deposition and age analysis

There was no significant difference in the proportion of adult raccoons trapped in NB vs. ME ($\chi^2=0.481$, $P=0.83$), whereas there were significantly more adult skunks trapped in ME ($\chi^2=8.168$, $P=0.004$) (Table 2). There were no significant differences in the proportion of antibody-positive adults vs. juveniles for skunks or raccoons in either area ($P>0.15$ in all cases).

Teeth collected from animals captured in NB were also analyzed for the presence of tetracycline, indicative of ONRAB consumption. Teeth from 71 raccoons were analyzed, of which 21 (30%) were positive for tetracycline deposition (tet^+). Of these 21 tet^+ raccoons, 18 were positive in all three serologic tests, and one was positive in both neutralization tests, but negative in the cELISA. The remaining two tet^+ raccoons were negative in all three serologic tests. Only one of 38 skunks analyzed was found to be tet^+ (3%); this skunk also was positive on all three serologic tests.

DISCUSSION

Vaccine efficacy can be evaluated in the laboratory with controlled immunization and challenge experiments, whereas field assessment of ORV is limited to indirect measures such as estimating what proportion of the target population has consumed a bait or is rabies virus antibody positive. If surveillance levels are adequate, then disease prevalence can provide a measure of the success of a control program (Wandeler, 2000; Slate et al., 2009). However, when the level of surveillance is low or when rabies is not present and ORV is being used proactively to establish an "immune barrier," serologic

TABLE 1. Vaccine performance comparison for two oral rabies vaccines (RABORAL V-RG in Maine [ME], USA, and ONRAB in New Brunswick [NB], Canada) in skunks (*Mephitis mephitis*) and raccoons (*Procyon lotor*).[a]

	Skunks		Raccoons	
Serologic test	NB-ONRAB positive/total (%)	ME V-RG positive/total (%)	NB-ONRAB positive/total (%)	ME V-RG positive/total (%)
RVNA-WC	5/33 (15)	1/36 (3)	57/73 (78)	41/125 (32.8)
RVNA-CFIA	6/33 (18)	4/36 (11)	55/73 (75)	37/125 (29.6)
cELISA	5/33 (15)	1/36 (3)	49/73 (67)	31/125 (24.8)

[a] RVNA = rabies virus neutralization assay; WC = Wadsworth Center, New York, USA; CFIA = Canadian Food Inspection Agency; cELISA = competitive enzyme-linked immunosorbent assay.

response to vaccine is a key measure of ORV performance.

The gold standard for estimation of antibody response to rabies vaccination is the virus neutralization assay (VNA), which measures the ability of serum to inhibit the growth of virus in a permissible cell line. The rapid fluorescent focus inhibition test (Smith et al., 1973) is the main test for human serology, but similar tests are routinely used for analysis of animal sera (Trimarchi et al., 1996; Cliquet et al. 1998; Knowles et al., 2009). Virus neutralization assays are complex procedures requiring highly trained personnel for their performance. Various factors can impact test outcome, including cell density, virus strain and dose, and the presence of nonspecific or cross-reacting materials (Moore and Hanlon, 2010). Calibration of titers against an international antibody standard facilitates the comparison of results derived from different tests, although the threshold value considered positive may vary depending on the study. Periodic interlaboratory collaborations are vital in establishing confidence in results, which are key in evaluating the success of ORV campaigns. Although the RVNAs performed at CFIA and WC differed in various parameters, there was no significant difference between the proportions of antibody-positive animals determined by either test. Despite a completely different test platform, the cELISA determined similar antibody prevalences to both RVNAs. Thus this antibody-binding assay appears to be a useful alternative to the more technically demanding and time-consuming VNAs for the serologic evaluation of ORV campaigns, particularly when sample sizes are large.

The bait distribution strategies used in the present study were selected to target raccoons because they are the reservoir species for raccoon rabies. Although both ONRAB (Yarosh et al., 1996) and RABORAL V-RG (Grosenbaugh et al., 2007) given by direct instillation into the

TABLE 2. Prevalence of antibody to raccoon rabies virus by age for skunks (*Mephitis mephitis*) and raccoons (*Procyon lotor*) captured in Maine (ME), USA, and New Brunswick (NB), Canada, following oral rabies vaccine with RABORAL V-RG (Maine) and ONRAB (New Brunswick).[a]

	Skunks				Raccoons					
	Adult		Juvenile		Adult		Juvenile		Unknown	
Area	*n* (%)	Pos (%)	*n* (%)	Pos (%)	*n* (%)	Pos (%)	*n* (%)	Pos (%)	*n* (%)	Pos (%)
NB	17 (52)	4 (23)	16 (48)	0 (0)	42 (58)	36 (86)	31 (43)	20 (65)	na	na
ME	31 (86)	1 (3)	5 (14)	0 (0)	68 (54.4)	25 (37)	56 (44.8)	20 (36)	1 (0.8)	0 (0)

[a] Age was determined by examination of extracted teeth or estimated by field observation; juvenile is defined as young of the year. Pos = positive; na = not applicable.

oral cavity (DIOC) have been shown to be immunogenic in skunks, the bait density and flight line spacing used here likely did not favor bait acceptance in this species because skunks have smaller home ranges than raccoons (Rosatte, 2000; Rosatte and Lariviere, 2003). As such, it was not surprising that antibody prevalence in skunks was low in both ME and NB. Furthermore, the higher antibody prevalence observed in skunks in the ONRAB study area was not significant. In contrast, a significantly larger proportion of raccoons in the ONRAB-baited plots were antibody positive (74%) as compared to the RABORAL V-RG–baited plot (30%).

The two products used in this study differed in bait size, composition, and attractants utilized, which may have impacted bait acceptance by target species, or the release of vaccine into the oral cavity following bait consumption. The absence of a biomarker in the RABORAL V-RG CS bait precluded a determination of whether the lower antibody prevalence observed in this study was the result of insufficient immunogenicity or insufficient bait uptake. However, results of laboratory studies in which a V-RG suspension was administered by DIOC support that this vaccine is immunogenic in raccoons (Rupprecht et al., 1986, 1988). Although 74% of raccoons from the ONRAB-baited area were antibody positive, only 30% had evidence of tetracycline deposition. These data indicate that tetracycline deposition may not be a good correlate of vaccine bait consumption and suggest that incorporation of this biomarker into the bait is unnecessary. Previous studies have shown that the bioavailable concentration of tetracycline within a bait can be less than half of the stated concentration at manufacture (Johnston et al., 2005) and that deposition within premolar teeth is less reliable than that in canines (Rosatte et al., 2008). Nevertheless, that 90% of tet^+ raccoons were also antibody positive supports the conclusion that the ONRAB baits consumed were immunogenic in this species.

Previous ORV field trials in Ontario found that larger proportions of adult raccoons consumed baits than juveniles (Rosatte et al., 2008) or that adults had higher antibody prevalence than juveniles (Rosatte et al., 2009). An age bias in raccoon captures was not evident in our study; we found no significant difference in the proportion of adult animals captured in NB vs. ME, or in the percentage of antibody-positive adults vs. juveniles in either area that could account for the higher antibody prevalence observed in NB raccoons.

Comparison of vaccine bait performance in ORV programs is confounded by variations in ecologic and technical parameters inherent to these field studies (Wandeler, 2000). Results from previous studies with ONRAB in Ontario (Rosatte et al., 2009) and V-RG in the USA (Hanlon et al., 1998; Robbins et al., 1998; Sattler et al., 2009; Slate et al., 2009) are difficult to compare because of differences in habitats, target population densities, and bait distribution strategies, as well as the use of different serologic tests with varying positive threshold values. We controlled covariates as much as possible to facilitate comparison of the two vaccine baits. All samples were subjected to the same serologic analyses using the same positive threshold values (0.5 IU/ml or its equivalent in the cELISA, consistent with the value accepted by most regulatory agencies as evidence of adequate response to vaccination in pets; Briggs and Schweitzer, 2001). In the sampled areas, baits were distributed over the same 2-day period in late August, using the same target densities and flight line spacing. The study plots consisted of similar habitats separated only by the international border. As such, competition for bait consumption by nontarget species was expected to be similar across the plots, as were the environmental conditions to which the baits were exposed and the availability of food sources that may have impacted bait acceptance by target species.

Despite the fact that many parameters were similar between the ME and NB

study plots, other factors could impact the interpretation of the antibody prevalence data, such as rabies control programs in previous years. The number of cumulative annual or semiannual ORV campaigns using RABORAL V-RG has been associated with gradual increases in antibody prevalence in raccoons (Robbins et al., 1998; Sattler et al., 2009). Our NB test plots were naïve with respect to ORV, but TVR programs had been carried out in previous years. Animals that had been vaccinated as part of a TVR program were not sampled to eliminate this bias. The ME plot had been baited with RABORAL V-RG in 2003, 2004, and 2007, but it was not possible to ascertain if the animals analyzed in 2008 had consumed baits in previous years. Thus some of the 25 antibody-positive adult raccoons captured in ME may have been immunized prior to the 2008 study.

Trapping plans differed in ME and NB as well: the total area trapped was larger in ME than in NB; however, the trapping effort was more intensive in NB (1,500 vs. 960 trap nights) and commenced 1 wk later than in ME. Whereas skunk captures were similar in ME and NB, fewer raccoons were captured in NB, which might suggest lower raccoon population density that could increase the opportunity for ONRAB consumption by individuals. However, raccoon density indexing conducted by USDA/APHIS/WS in northern ME between 2002 and 2008 using WS National Rabies Management Program standard protocol (USDA, 2008b) and estimates from the NB south plot TVR programs from 2001 to 2007 (NBDOH, unpubl. data) both estimated an average of 4–5 raccoons per km^2, with higher densities in the vicinity of towns and residential areas. Thus, the lower number of raccoon captures was most likely the result of the much smaller trapping area in NB. The later sampling in NB may have affected trapping success because of effects of declining temperatures on animal movements. Effects of earlier sampling in ME on the probability of detecting circulating antibody in sampled animals were also considered. However, previous serologic assessments following ORV with V-RG–containing baits showed that maximal antibody prevalence was detectable at 4–6 wk (Hanlon et al., 1998) and at 5 wk postbaiting in raccoons (Sattler et al., 2009). Similar antibody response kinetics were observed in captive raccoons offered an experimental VRG bait (Brown et al., 2011). Together these data suggest that the earlier sampling in ME was still within the optimal time frame for detection of a humoral immune response in raccoons.

Although the targeted bait density was equivalent, estimation of the achieved bait density by division of the number of aerially distributed baits by the total study area showed that achieved bait density was slightly higher in ME than in NB (70/km^2 vs. 63/km^2). It is not clear if this difference would have any impact on seroconversion rates. Taken together, if the noncontrolled variables in this study had any significant impact on response to vaccination, these would have been expected to favor the RABORAL V-RG study area.

Oral rabies vaccination with RABORAL V-RG has been in use in regions of the eastern USA enzootic for raccoon rabies since the mid-1990s (Slate et al., 2009). Raccoon rabies has been contained east of the Appalachian ridge, likely because of a combination of advantageous landscape barriers and the ORV programs. Control programs combining V-RG ORV with alternative strategies (i.e., population reduction and TVR) appear to have been effective in eliminating raccoon rabies from eastern Ontario (Rosatte et al., 2008) and in restoring a contingency action zone to raccoon rabies–free status in northeastern Ohio (Slate et al., 2009). Although the threshold herd immunity required for rabies elimination in raccoons is unknown, studies suggest that an antibody prevalence of 70% is required for urban dog populations (WHO, 1992), and recent modeling studies suggest that lower thresholds may be adequate to eliminate rabies in red foxes (*Vulpes vulpes*)

in Europe (Thulke and Eisinger, 2008). The antibody prevalence in raccoons achieved in this study using ONRAB suggests that this vaccine bait may prove effective not only for the prevention of raccoon rabies spread out of an enzootic zone, but also for disease elimination. Studies in epizootic areas, such as those currently underway in southern Quebec (Gouvernement du Québec, 2010), are required to test this hypothesis.

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