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AERIAL DISTRIBUTION OF ONRAB® BAITS AS A TACTIC TO CONTROL RABIES IN RACCOONS AND STRIPED SKUNKS IN ONTARIO, CANADA

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ABSTRACT: During August 2006 and 2007, baits containing oral rabies vaccine, live adenovirus vector, known as ONRAB®, were aerially distributed in SW Ontario, Canada. Bait acceptance during 2006 was 62 and 74% in raccoons (*Procyon lotor*) in areas baited at 150 baits/km^2 and 75 and 77% in plots baited at 300 baits/km^2. During 2007, bait acceptance for raccoons ranged between 59% and 80%, and 83% and 87%, in areas baited at 75 and 400 baits/km^2, respectively. Bait acceptance by skunks varied among plots (5–24%). Rabies virus-specific seroconversion during 2006 averaged 66 and 81% in raccoons in areas baited at 150 and 300 baits/km^2, respectively. During 2007, seroconversion by raccoons was 76 and 84% in areas baited at 75 and 400 baits/km^2, respectively. Seroconversion by skunks varied among plots (17–51%). Vaccine efficacy, as judged by the percentage of animals that consumed a bait and seroconverted, averaged 79 and 87% during 2006 for raccoons in areas baited at 150 and 300 baits/km^2, respectively, and 81 and 90% in areas baited during 2007 at 75 and 400 baits/km^2, respectively. Because tetracycline marking was poor in skunks, an estimate of vaccine efficacy was not possible. Aerial distribution of ONRAB® vaccine baits seems to be a feasible tactic for controlling rabies in skunks and raccoons.

Key words: *Mephitis mephitis*, Ontario, oral rabies vaccination, *Procyon lotor*, rabies, raccoon, striped skunk, vaccine.

INTRODUCTION

More than 54,800 animals were reported with rabies in Ontario, Canada, from 1954 to 2006 (excluding clinical cases). The majority (66%) of those cases were in red foxes (*Vulpes vulpes*; 46%) and striped skunks (*Mephitis mephitis*; 20%) that were most likely infected with an Arctic variant of rabies virus (Johnston and Beauregard, 1969; MacInnes, 1987; Rosatte, 1988; Nadin-Davis et al., 1994; Canadian Food Inspection Agency, unpubl. data). Fortunately, the Arctic variant of rabies has nearly been eliminated in Ontario as a result of oral rabies vaccination campaigns targeting red foxes (MacInnes, 1987; Johnston et al., 1988; Rosatte et al., 1992, 1993, 2007a; MacInnes et al., 2001). During 1999, another strain of rabies, the raccoon (*Procyon lotor*) variant, was reported in Ontario (Wandeler and Salsberg, 1999; Rosatte et al., 2001). Since then, 132 cases (130 raccoons, two striped skunks) have been confirmed as positive with the raccoon variant of rabies (as of 1 October 2008). The Ontario Ministry of Natural Resources (OMNR) has implemented a successful, multifaceted raccoon rabies control program using point infection control, trap-vaccinate-release (TVR), and oral rabies vaccination, with vaccinia-rabies glycoprotein vaccine-baits, to contain and eliminate the raccoon variant of rabies from the Province (Rosatte et al., 2001). The program has been so successful that only five cases of the raccoon variant of rabies have been reported in Ontario during the past 5 yr, with no cases reported since September 2005 (as of 1 October 2008).

The OMNR has been faced with the challenge of controlling terrestrial wildlife rabies with two rabies variants and three

primary rabies vectors—foxes, skunks, and raccoons. During the past two decades, much research has focused on the development of an oral vaccine that would orally immunize these three species in Ontario (Johnston et al., 1988; Rosatte et al., 1992; MacInnes et al., 2001; Rosatte et al., 2007b). One of the most promising vaccines has been a human adenovirus type 5-rabies glycoprotein recombinant vaccine (AdRG1.3); AdRG1.3 was modified from the first construct (AdRG1.0) in the early to mid-1990s at McMaster University, Hamilton, Ontario, Canada (Yarosh et al., 1996). During 1993, Microbix Biosystems Inc. (Toronto, Ontario, Canada), was commissioned by OMNR to prepare a master seed of virus that OMNR acquired from Microbix in 1999. Subsequent laboratory trials were conducted at the Canadian Food Inspection Agency (CFIA), Nepean, Ontario, Canada, and production vaccine was developed by Artemis Technologies Inc. (Guelph, Ontario, Canada), with assistance from the National Research Council, Biotechnology Research Institute. The trade name for this product is ONRAB®.

Before approval to field test ONRAB® vaccine-baits in Ontario was granted by the CFIA, Veterinary Biologics Section (VBS), extensive laboratory testing of the experimental vaccine had to be completed. The candidate vaccine had to be proven to be safe, pure, stable, effective, and genetically stable (Yarosh et al., 1996; Lutz-Wallace et al., 1995a, b; Randrianarison-Jewtoukoff and Perricaudet, 1995). That research included efficacy experiments in skunks, safety trials in 19 species, and challenge of immunized skunks with Arctic variant rabies virus at CFIA, Ottawa Laboratory Fallowfield (OLF), Nepean, Ontario, Canada (Canadian Food Inspection Agency unpublished data).

Here, we report on field trials designed to determine the efficacy of AdRG1.3, hereafter called ONRAB®, in baits, for immunizing free-ranging skunks and raccoons against rabies in SW Ontario. The objectives of this research were to determine the efficacy of ONRAB® baits to produce a rabies virus-specific serologic response in free-ranging skunks and raccoons and to determine the optimum density of ONRAB® baits and flight line spacing required to reach and subsequently immunize a significant portion (>60%) of a free-ranging skunk and raccoon population against rabies.

FIGURE 1. Photo of ONRAB® rabies vaccine-baits.

MATERIALS AND METHODS

ONRAB® vaccine-bait production

Virus was prepared at the National Research Council, Biotechnology Research Institute and shipped as bulk vaccine to Artemis Technologies Inc. where ONRAB® vaccine-baits were manufactured during 2006 and 2007. Each bait contained 1.8±0.1 ml of ONRAB® vaccine (titer of not $<10^{9.5}$ cell culture infectious dose 50% [$CCID_{50}$]/ml) in an elongated plastic blister pack that was coated with the attractant-bait matrix (Fig. 1). The bait and vaccine container that constitutes the Ultralite bait, were the fruition of many years of research at the OMNR Rabies Research and Development Unit, Peterborough, Ontario, Canada, and Artemis Technologies Inc. (Bachmann et al., 1990; Rosatte et al., 1998; Johnston et al., 1999; Rosatte and Lawson, 2001). The attractant on the vaccine-baits was composed of partially hydrogenated vegetable shortening (34%), Microbond® wax (International Wax Ltd., Agincourt, Ontario) (30%), stearine (12.5%), icing-sugar (20%), vegetable oil (1%), artificial marshmallow flavor (1%), artificial sweet flavor (1%), and a fat-soluble food dye (0.5%) (khaki green) to camouflage the baits. The bait matrix also contained 100 mg of

tetracycline hydrochloride as a biomarker to evaluate bait ingestion as described in Johnston et al. (1999). Each vaccine-bait weighed approximately 4 g. The body of the blister pack was an elongated oval with dimensions of 30×14×10 mm and a rectangular lip extending to 40×20 mm (Fig. 1). The blister pack contained an identifying label as to the contents of the bait and a toll free phone number where people could obtain information on the baits. Quality control-tested baits were stored at −25 C to −35 C until the date of the air-drop at which time they were transported in a refrigerated truck at −25 C to the drop-site.

Environmental assessment and approval for using ONRAB® vaccine-baits in Ontario

After extensive laboratory testing, a review of the OMNR ONRAB® field trial proposal by CFIA and Health Canada staff, and a favorable environmental assessment, approval to distribute ONRAB® vaccine-baits in Ontario was granted to OMNR by CFIA, VBS, in July 2006.

Field trial study area

The field trial study area lies within ecodistricts 7, 8, and 12 in ecoregion Hurontario located in SW Ontario, as described by Wickware and Rubec (1989) and Bachmann et al. (1990). The area has gently rolling topography overlain with glacial till and is primarily composed of cropland (50%) and pastureland (5–18%) in association with deciduous woodlots (Bachmann et al., 1990). Skunk and fox densities are approximately 1–2/km^2 compared with raccoon density that averages 5–10/km^2 (Rosatte, 2000; Rosatte and Lariviere, 2003). This area was baited in previous years with ERA® baits containing tetracycline and during 2006 and 2007 in proximity to the ONRAB® test plots.

ONRAB® vaccine-bait distribution

The ONRAB® baits were distributed in SW Ontario on 15 August 2006 using two OMNR Twin Otter (DHC-6) fix-winged aircraft at an altitude of approximately 150 m above ground level and airspeed of approximately 240 km/hr (150 knots; Fig. 2). Predetermined flight-lines were uploaded to the onboard computerized navigational system developed by OMNR. The bait distribution machine in the aircraft was controlled by the OES computer and the navigator. The vaccine-baits were aerially distributed in four 256-km^2 plots with each baiting plot measuring 16×16 km (Fig. 2). Baiting density in two plots was 150 baits/km^2 and an additional two plots were baited at 300 baits/km^2. Flight-line spacing in all plots was 0.5 km. During 14–15 August 2007, six plots (each 18×18 km) (1,944-km^2 area) were baited with ONRAB® baits at densities of 75 or 400 baits/km^2, with a flight-line spacing of 0.5 km. The baiting area was just south of the 2006 plots.

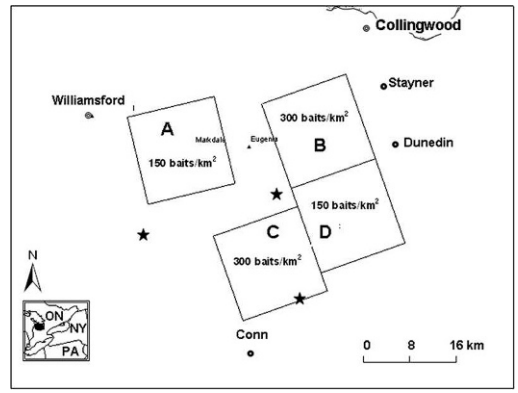

FIGURE 2. Map of SW Ontario, Canada, depicting bait density and the location of plots (A, B, C, and D) baited with ONRAB® vaccine-baits during 15 August 2006. The three stars represent the location of three rabid skunks identified in 2006. The black square in the inset represents the location of the study area in relation to New York (NY), Pennsylvania (PA), and Ontario (ON).

Postbaiting monitoring

Five to six wk after baiting (commencing late September 2006/2007), OMNR technicians live-trapped (Tomahawk 106, 108; 100 live-traps/9-km^2 trapping cell; sardine bait) a sample of skunks and raccoons from each of the baiting plots (Fig. 3). Skunks and raccoons were immobilized using ketamine hydrochloride (5 mg/kg) and medetomidine hydrochloride (50 μg/kg), with antipamazole hydrochloride (0.25 mg/kg) as an antagonist. All animals that were processed were eartagged, a second premolar tooth was removed, and 3–5 ml of blood was collected (femoral or subclavian vein or via cardiac puncture). Skunks and raccoons were released at the point of capture upon recovery from immobilization. The cells were trapped for eight nights to allow for population density estimation (Krebs, 1989).

All premolar teeth were hand-ground with a grind-stone, polished with emery paper, mounted on slides, examined for tetracycline fluorescence, and aged using a compound

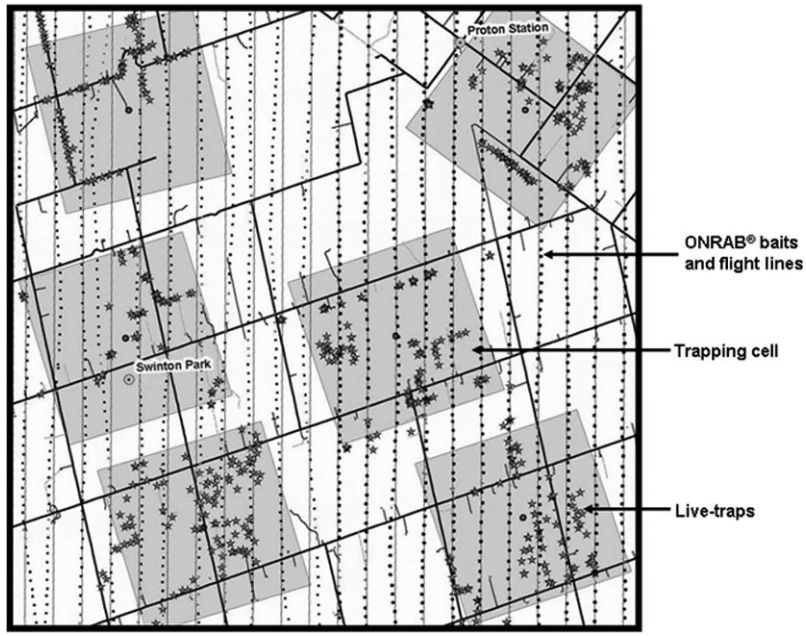

FIGURE 3. Location of flight lines, ONRAB® bait dispersion, postbaiting sampling plots, and live-trap placement in plot A during 2007. Baiting occurred during 14–15 August 2007, and live-trap placement for surveillance sampling of raccoons occurred during late September–early October 2007. Bait acceptance by raccoons was 80% in this plot with a bait density of 75/km^2.

microscope and ultraviolet (UV) light and requisite filter combinations according to Johnston et al. (1999). Blood samples were centrifuged, sera were collected and stored frozen (−20 C) until assay. Sera samples were assayed for the presence of rabies virus glycoprotein-specific antibodies by competitive enzyme-linked immunosorbent assay (cELISA), as described previously (Elmgren and Wandeler, 1996). The results are expressed as a percentage of inhibition of binding of a glycoprotein-specific, peroxidase-labeled monoclonal antibody to microtiter plates coated with ERA® virus. Inhibition values of 14% or greater (skunks) and 16 and 17% or greater during 2006 and 2007, respectively (raccoons), were considered positive for detection of glycoprotein-specific antibodies in serum. These cut-off values were determined previously by Receiver Operating Characteristics analysis of results obtained in cELISA and serum neutralization assays. For analysis of skunk serum, the cELISA has a sensitivity of 98% and a specificity of 94% compared with the serum neutralization assay, in which a sample was considered positive if the titer was ≥0.5 international units/ml. For raccoon serum, the test sensitivity and specificity is 76 and 91%, respectively. Blood and tooth (canine) samples were collected from raccoons and skunks in SW Ontario during 2005 to obtain information on pretrial levels of tetracycline and rabies antibody in those species. Sera were tested using a blocked ELISA (bELISA).

Statistical analyses

Because of the categoric nature of the data, traditional two-way chi-square tests were performed first to test for associations among the variables. A log-linear analysis was also used to test for associations between several categorical variables. Any significant two- or three-way interactions were further examined using chi-square tests of independence. For the majority of the raccoon data analyses (seroconversion and vaccine efficacy), there was no association detected between baiting plots and cELISA values, so data were pooled to examine the overall association of seroconversion, vaccine efficacy, and bait density. For cases in which there were significant plot effects, plots were analyzed separately. The software package Statistica (StatSoft, Tulsa, Oklahoma) was used for all analyses.

TABLE 1. Percentage of raccoons sampled from plots baited at 75, 150, 300, and 400 ONRAB® baits/km^2 during 2006 and 2007 in SW Ontario, Canada, that were tetracycline positive.[a]

Plot and bait density (baits/km^2)	Yr	Tetra+ (*n*)	Tetra− (*n*)	Sample size (*n*)	Tetra+ (%)
A, 150	2006	156	54	210	74.3
D, 150	2006	120	74	194	61.9
C, 300	2006	164	49	213	77.0
B, 300	2006	153	51	204	75.0
A, 75	2007	94	23	117	80.3
D, 75	2007	69	49	118	58.5
E, 75	2007	73	45	118	61.9
B, 400	2007	88	13	101	87.1
C, 400	2007	81	17	98	82.7
F, 400	2007	99	15	114	86.8
Total		1,097	390	1,487	

[a] Tetra+ = tetracycline positive; Tetra− = tetracycline negative; Tetra+ (%) = percentage of raccoon premolar teeth that were tetracycline positive and is an indication of the percentage of the raccoon population that consumed ONRAB® baits. Tooth sections suitable for analysis were available for 1,487 raccoons.

RESULTS

Use of ONRAB® vaccine-baits for the immunization of captive raccoons and skunks against rabies

Efficacy trials using AdRG1.3 were conducted on raccoons during 1992–2001 at CFIA, OLF (Canadian Food Inspection Agency, unpubl. data). In addition, during 2003 to 2006, captive skunks were offered Ultralite baits containing ONRAB® at various titers at CFIA, OLF, to determine the titer needed to immunize skunks against rabies as well as to determine the efficacy of Ultralite baits to deliver the ONRAB® vaccine to skunks (Canadian Food Inspection Agency, unpubl. data). From these experiments, it was determined that a titer of not $<10^{9.5}$ $CCID_{50}$/ml would be adequate for use in the field to immunize skunks (and probably raccoons). Laboratory trials were also conducted to determine the safety of ONRAB® in target as well as nontarget animals should they contact the vaccine-baits. No adverse reaction was noted after oral inoculation of high concentrations (ca. 5 times higher than contained in a bait) of ONRAB® in target/nontarget animals and any shed virus was of short duration and low titer (Canadian Food Inspection Agency, unpubl. data).

Pretrial levels of tetracycline and rabies virus glycoprotein-specific antibodies

Approximately 31% (20/64) of the raccoons collected in SW Ontario during 2005 were positive for tetracycline. All (64) sera samples were bELISA negative. In addition, 30% (6/20) of the skunk teeth were tetracycline positive; only one of 20 sera samples was bELISA positive. That sample was tetracycline negative.

Use of ONRAB® vaccine-baits for the immunization of free-ranging raccoons and skunks against rabies-bait acceptance in raccoons

In total, 195,885 ONRAB® baits were aerially distributed in the four plots in SW Ontario on 15 August 2006 (Fig. 2), and 841 raccoons were captured and processed in the four baiting plots during 18–29 September 2006. However, due to variations in the amount of sera available, missing sex and age data for some raccoons, and quality of tooth sections, some of the analyses used data from fewer raccoons (see footnotes in tables).

There was an association between plot and bait acceptance (percentage of raccoons that were tetracycline positive) at 150 baits/km^2 (62 and 74%) ($\chi^2=7.19$, $P=0.0073$, $n=404$; Table 1), but no association was noted at the higher bait density

TABLE 2. Percentage of adult and juvenile raccoons sampled from plots baited at 75, 150, 300, and 400 ONRAB® baits/km^2 during 2006 and 2007 in SW Ontario, Canada, that were tetracycline positive.[a]

Raccoon age	Yr	Tetra+ (*n*)	Tetra− (*n*)	Sample size (*n*)	Tetra+ (%)
Adult	2006	293	136	429	68.3
Juvenile	2006	297	85	382	77.7
Adult	2007	255	76	331	77.0
Juvenile	2007	249	86	335	74.3
Total		1,094	383	1,477	

[a] Tetra+ = tetracycline positive; Tetra− = tetracycline negative; Tetra+ (%) = percentage of raccoon premolar teeth that were tetracycline positive and is an indication of the percentage of the adult and juvenile raccoon population that consumed ONRAB® baits. 2006 data are from areas baited at 150 or 300 baits/km^2, and 2007 data are from areas baited at 75 or 400 baits/km^2. Ages were available for 1,477 raccoons.

(300/km^2; 75 and 77%). There was no association detected between bait acceptance, plots, and sex and age of raccoons at the two bait densities. However, when plots were combined, bait acceptance was higher (78%) in juvenile raccoons than in adults (68%) (χ^2=9.11, P=0.0026, n=811; Table 2).

During 14–15 August 2007, 357,345 ONRAB® baits were aerially distributed in six plots in SW Ontario. In total, 696 raccoons were processed from the baiting plots during late September/early October 2007. There was a significant variation in bait acceptance among plots at 75 baits/km^2 (χ^2=14.67, P=0.0007, n=353) but not at 400 baits/km^2 (P=0.599; Table 1). There was also a significant association between bait acceptance and bait density, with higher acceptance occurring in plots baited at 400/km^2 (χ^2=31.75, P=0.00001, n=666; Table 1). In addition, no differences were noted in bait acceptance by juvenile and adult raccoons (P=0.4149; Table 2).

Seroconversion in raccoons

No association was detected between plots and raccoons that were ELISA positive within each density, so 2006 data were pooled. There was a significant overall association between the number of raccoons that were cELISA positive and bait density (χ^2=21.79, P=0.0000, n=841), with higher seroconversion in raccoons from plots that received 300 baits/km^2 (81%) than those sampled from plots baited at 150 baits/km^2 (66%; Table 3). There was also a significant association between raccoon serologic values and raccoon age when all plots were combined (χ^2=33.06, P=0.0000, n=805), with cELISA values higher (82%) in adult raccoons than in juveniles (64%; Table 4). There was no association between raccoon serology and the sex of

TABLE 3. Seroconversion in raccoons sampled from plots baited at 75, 150, 300, and 400 ONRAB® baits/km^2 during 2006 and 2007 in SW Ontario, Canada.[a]

Bait density (baits/km^2)	ELISA+ (*n*)	ELISA− (*n*)	Sample size (*n*)	ELISA+ (%)
75	274	89	363	75.5
150	271	137	408	66.4
300	349	84	433	80.6
400	280	52	332	84.3
Total	1174	362	1,536	

[a] ELISA+ = ELISA positive; ELISA− = ELISA negative; ELISA+ (%) = percentage of raccoon sera samples that were ELISA positive and is an indication of the level of seroconversion in the raccoon population. Sera were available for 1,536 raccoons.

TABLE 4. Seroconversion in adult and juvenile raccoons sampled from plots baited at 75, 150, 300, and 400 ONRAB® baits/km^2 during 2006 and 2007 in SW Ontario, Canada.[a]

Raccoon age	Yr	ELISA+ (*n*)	ELISA− (*n*)	Sample size (*n*)	ELISA+ (%)
Adult	2006	348	77	425	81.9
Juvenile	2006	243	137	380	63.9
Adult	2007	315	38	353	89.2
Juvenile	2007	239	103	342	69.9
Total		1,145	355	1,500	

[a] ELISA+ = ELISA positive; ELISA− = ELISA negative; ELISA+ (%) = percentage of raccoon sera samples that were ELISA positive and is an indication of the level of seroconversion in the adult and juvenile raccoon population. The 2006 data are from areas baited at 150 or 300 baits/km^2, and the 2007 data are from areas baited at 75 or 400 baits/km^2. Sera from aged animals were available for 1,500 raccoons.

raccoons when all plots were combined (χ^2=0.08, P=0.7727, n=841).

For the 2007 data, there was a significant association detected between bait density and raccoons that were seropositive (χ^2=8.41, P=0.0037, n=695), with 84 and 76% of the raccoons being seropositive at bait densities of 400 and 75 baits/km^2, respectively (Table 3). Greater numbers of adult raccoons (89%) were seropositive than juveniles (70%, P=0.00001; Table 4).

Vaccine efficacy in raccoons

Vaccine efficacy is defined as the percentage of bait ingesting animals (verified by tetracycline-positive teeth) that demonstrated antibody (cELISA positives). These analyses used only raccoons that showed evidence of ingesting baits in the current year of second premolar teeth sections. No association was detected between plots and vaccine efficacy, so data were pooled. There was a significant overall association between vaccine efficacy and bait density (χ^2=7.88, P=0.0050, n=590), with higher seroconversion in raccoons from 2006 plots that received 300 baits/km^2 (87%) than in those baited at 150 baits/km^2 (79%; Table 5). There was also a significant association between antibody prevalence and raccoon age when all 2006 plots were combined (χ^2=26.88, P=0.0000, n=587), with seroconversion higher in adult raccoons (91%) than juveniles (75%; Table 6). There was no association between cELISA values and sex of raccoons when all plots (2006) were combined (χ^2=0.13, P=0.7127, n=590).

A significant association was detected between bait density and vaccine efficacy for the 2007 data with higher seroconversion in raccoons from plots baited at 400 baits/km^2 (90%) than in plots baited at 75 baits/km^2 (81%, P=0.006; Table 5). There was also an association between raccoon age and antibody prevalence, with greater

TABLE 5. ONRAB® vaccine efficacy in raccoons in SW Ontario, Canada, that ate ONRAB baits in areas baited at 75, 150, 300, and 400 ONRAB® baits/km^2 during 2006 and 2007.[a]

Bait density (baits/km^2)	ELISA+ (*n*)	ELISA− (*n*)	Sample size (*n*)	ELISA+ (%)
75	192	44	236	81.4
150	215	58	273	78.8
300	277	40	317	87.4
400	240	27	267	89.9
Total	924	169	1,093	

[a] Raccoons that ate ONRAB baits as determined by the presence of tetracycline in second premolar teeth; ELISA+ = ELISA positive; ELISA[minus] = ELISA negative; ELISA+ (%) = percentage of raccoon sera samples from raccoons that consumed a bait that were ELISA positive and is an indication of vaccine efficacy in raccoons.

TABLE 6. ONRAB® vaccine efficacy in adult and juvenile raccoons in SW Ontario, Canada, that ate ONRAB® baits in areas baited at 75, 150, 300, and 400 ONRAB® baits/km^2 during 2006 and 2007.[a]

Raccoon age	Yr	ELISA+ (*n*)	ELISA− (*n*)	Sample size (*n*)	ELISA+ (%)
Adult	2006	265	25	290	91.4
Juvenile	2006	224	73	297	75.4
Adult	2007	236	19	255	92.5
Juvenile	2007	196	52	248	79.0
Total		921	169	1,090	

[a] Raccoons that ate ONRAB baits as determined by the presence of tetracycline in second premolar teeth; ELISA+ = ELISA positive; ELISA− = ELISA negative; ELISA+ (%) = percentage of raccoon sera samples from raccoons that consumed a bait that were ELISA positive and is an indication of vaccine efficacy in adult and juvenile raccoons. The 2006 data are from areas baited at 150 or 300 baits/km^2, and 2007 data are from areas baited at 75 or 400 baits/km^2.

numbers of adult raccoons (93%) than juveniles (79%) being cELISA positive (P=0.0001; Table 6).

Bait acceptance in striped skunks

Bait acceptance and seroconversion of skunks of known age was based on results obtained from 426 skunks captured in the four plots during the ONRAB® postbaiting surveillance program between 18 and 29 September 2006. Bait acceptance by skunks (based on tetracycline deposition in second premolars) was significantly different among plots baited at 150 baits/km^2 (14 and 5%; χ^2=4.46, P=0.034, n=222) and 300 baits/km^2 (24 and 12%; χ^2=5.08, P=0.0249, n=204). Although the greatest level of bait acceptance by skunks (24%) was observed in plot B baited at 300 baits/km^2, the second greatest bait acceptance (14%) was found in plot A baited at 150 baits/km^2. Bait acceptance in juvenile skunks (based on the detection of tetracycline in second premolars) was twice (19% [39/203]) that observed in adults (9% [19/223]; χ^2=10.33, P=0.001). Tetracycline results were the same for male and female skunks, with an acceptance rate of 13% for both sexes.

Seroconversion in striped skunks

There were significant differences in skunk antibody prevalence (% ELISA+) detected among plots baited at 150 baits/km^2 (49 and 17%; χ^2=24.51, P=0.00) and 300 baits/km^2 (51 and 37% (χ^2=3.82, P=0.05). Although the greatest prevalence of cELISA-positive skunks (51%) was observed in plot B baited at 300 baits/km^2, the second highest level (49%) was found in skunks from plot A baited at 150 baits/km^2. The lowest antibody prevalence was detected in skunks from plot D (17%), which was baited at 150 baits/km^2. The percentage of cELISA-positive sera from juvenile skunks (42% [85/203]) was not significantly higher than the antibody prevalence for adults (37% [82/223]; χ^2=1.16, P=0.28, n=426). Antibody prevalence for male and female skunks was also similar at 39 and 41%, respectively.

Vaccine efficacy in striped skunks

Only 14% (58/426) of second premolar skunk teeth were positive for tetracycline, yet 39% (167/426) of the sera from all plots were cELISA positive. This suggests that more skunks were immunized than ate baits (or at least, ingested the tetracycline in the matrix). Because vaccine efficacy is defined as the percentage of cELISA positive of tetracycline positives, any calculations using the existing data would prove meaningless and inconclusive. These discrepancies suggest there could be a problem with tetracycline marking, tetracycline detection in skunk teeth, or bait matrix ingestion by skunks; consequently, the bait acceptance result of

14% is likely an underestimate of the true value.

Vaccine-bait contacts

There were only two public inquiry calls after the 2006 ONRAB® bait-drop. OMNR staff was notified of a dog finding four ONRAB® baits on 18 August 2006. A resident found a single bait on 15 August 2006. There were no reports of vaccine contact in either incident. After the 2007 baiting program, one person contacted a bait, and one dog chewed a bait. No adverse reactions were noted in either case.

DISCUSSION

The most feasible tactic for immunizing terrestrial wildlife rabies vectors over large geographic areas is through the aerial distribution of oral rabies vaccine baits (Rosatte et al., 2007b). Although ERA® in Ontario Slim baits have proven to be effective in controlling the Arctic variant rabies in red foxes in Ontario (Rosatte et al., 1992, 1993, 2007a, b; MacInnes et al., 2001), OMNR staff have had to rely on labor-intensive tactics such as population reduction and TVR to control the disease in striped skunks and raccoons because ERA® is not effective in these species (Rosatte et al., 2001). This study focussed on determining the feasibility of the aerial distribution of baits containing ONRAB® oral rabies vaccine to immunize raccoons and striped skunks against rabies. The vaccine used employs a human adenovirus type 5 vector into which has been inserted a DNA copy of the ERA® virus glycoprotein gene. Adenoviruses belong to the family *Adenoviridae* are nonenveloped DNA viruses, and they are commonly found in mammals, including humans (Randrianarison-Jewtoukoff and Perricaudet, 1995). Adenoviruses are distributed worldwide and infections with human adenovirus serotype 5 do not result in serious disease (Rowe et al., 1955; Andiman and Miller, 1982; Charlton et al., 1992; Russell, 1998). Human adenoviruses are currently being utilized in vaccine development due to their genetic stability and ability to be grown to high titers in a variety of cell types (Prevec et al., 1990), and they are being considered to serve as vectors for human vaccination and gene therapy (Bonnekoh et al., 1998; Molinier-Frenkel et al., 2000; Flotte, 2004). There are no perceived environmental or public heath impacts of distributing ONRAB® in Ontario, or elsewhere, because the vaccine has been extensively safety tested and as shown in this study there were very few human or companion animal contacts with the vaccine and no adverse reactions noted.

Bait acceptance, immunity, and vaccine efficacy results for ONRAB® in raccoons were exceptional. Higher seroconversion values for adult raccoons were expected as adult immune systems would probably be more developed than in young animals (Black and Lawson, 1980; Rosatte et al., 2007b). Variation among bait acceptance/serologic results is a function of second premolar teeth being used to assess bait acceptance, and it is known that tetracycline does not always fluoresce in these teeth compared with canine teeth. That is, in some cases, antibody prevalence results were higher than bait acceptance results. This could also be a function of background antibody found in raccoon populations that may be acquired through contact with the rabies virus or contact with vaccine from previous baiting campaigns.

Higher vaccine efficacy values for raccoons from high-density plots may be a function of multiple bait ingestions, although we have no way of proving that theory in adult animals. It must be emphasized that high densities (300 and 400/km^2) of baits were used in some of the baiting plots. These densities of baits may be used in point infection control operations to contain a single or few cases of rabies. However, they would not be cost effective over large geographic areas

where bait densities of 75–150 baits/km^2 would be more appropriate. Of prime importance is that ONRAB® was proven to produce a significant immune response in raccoons when tested in the field. Future research will focus on refining bait density and flight-line spacing values to evaluate whether the control of rabies using ONRAB® in raccoons (and skunks) over large areas is cost-effective and feasible (Kemere et al., 2002; Recuenco et al., 2007).

The rabies seroconversion values reported in this study for free-ranging striped skunks, although not as high as for raccoons, were still, to our knowledge, the highest reported in the literature. We are confident the serologic response was due to contact with ONRAB® because pretrial (2005) levels of rabies virus glycoprotein-specific antibodies in skunk (as well as raccoon) sera samples were negligible. There were between plot effects for both skunk bait acceptance and antibody prevalence (as well as variation in raccoon bait acceptance during 2007). The exact cause of this variation is unknown but may be related to the number of baits dropped per flight line, bait dispersion, habitat characteristics, the position of baiting lines within the plots, as well as the placement of live-traps with respect to the dispersion of vaccine baits. This is evidenced where trap placement was close to baiting lines and bait acceptance was high (Fig. 3). If traps are located distant from baits, bait acceptance would be expected to be lower.

Another unknown in this study is the actual level of skunk acceptance of ONRAB® baits that occurred postbaiting due to challenges with biomarker data. Given that experiments using ERA® in sponge and blister pack baits at densities of 17–25 baits/km^2 during 1984–1987, in SW Ontario, yielded acceptance values by skunks (using canine teeth) of 14–38% (Bachmann et al., 1990), and acceptance of ERA® baits during 2005 was 30% (canine teeth), it was expected that skunk acceptance values at bait densities of 150 and 300/km^2 would be even higher than the earlier results (Bachmann et al., 1990). Future research will focus on our ability to assess bait acceptance in free-ranging skunks.

In this inaugural ONRAB® field trial, excellent bait acceptance and seroconversion were obtained, particularly in raccoons, in which >60% of the populations studied exhibited a specific immune response against rabies. However, using 300–400 baits/km^2 over large geographic areas for the control of rabies in striped skunks and raccoons is probably cost-prohibitive. One strategy to contain an outbreak would be to distribute baits at high densities in a localized area based on knowledge of vector ecology (Rosatte, 2000; Rosatte and Lariviere, 2003; Rosatte et al., 2005, 2006, 2007c, d). Low-density baiting could then be used outside of that area to provide a buffer zone of vaccinated animals should certain cases not be contained in the area of high-density baiting. Such a staggered approach to baiting would be expected to increase the probability of success in containing outbreaks while ensuring control costs are reasonable.

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LITERATURE CITED

Andiman, W., and G. Miller. 1982. Persistent infection with adenovirus type 5 and 6 in lymphoid cells from humans and woolly monkey. Journal of Wildlife Diseases 145: 83–88.

Bachmann, P., R. Bramwell, S. Fraser, D. Gilmore, D. Johnston, K. Lawson, C. Macinnes, F. Matejka, H. Miles, M. Pedde, and D. Voigt. 1990. Wild Carnivore acceptance of baits for delivery of liquid rabies vaccine. Journal of Wildlife Diseases 26: 486–501.

Bonnekoh, B., B. Greenhalgh, S. Chen, A. Block, S. Rich, T. Krieg, S. Woo, and D. Roop. 1998. Ex vivo and in vivo adenovirus-mediated gene therapy strategies induce a systemic anti-tumor immune defence in the B16 melanoma model. Journal of Investigative Dermatology 110: 867–871.

Black, J., and K. Lawson. 1980. The safety and efficacy of immunizing foxes (*Vulpes vulpes*) using bait containing attenuated rabies virus vaccine. Comparative Medicine 44: 169–176.

Charlton, K., M. Artois, L. Prevec, J. Campbell, G. Casey, A. Wandeler, and J. Armstrong. 1992. Oral rabies vaccination of skunks and foxes with a recombinant human adenovirus vaccine. Archives of Virology 123: 169–179.

Elmgren, L. D., and A. Wandeler. 1996. Competitive ELISA for the detection of rabies virus-neutralizing antibodies. *In* Laboratory techniques in rabies, 4th Edition, F.-X. Meslin, M. Kaplan and H. Koprowski (eds.). World Health Organization, Geneva, Switzerland, pp. 200–208.

Flotte, T. R. 2004. Immune responses to recombinant adeno-associated virus vectors: Putting preclinical findings into perspective. Human Gene Therapy 15: 716.

Johnston, D. H., and M. Beauregard. 1969. Rabies epidemiology in Ontario. Bulletin of the Wildlife Disease Association 5: 357–370.

———, D. Voigt, C. Macinnes, P. Bachamnn, K. Lawson, and C. Rupprecht. 1988. An aerial baiting system for the distribution of attenuated or recombinant rabies vaccine for foxes, raccoons, and skunks. Reviews of Infectious Diseases 10: S660–S664.

———, D. Joachim, P. Bachmann, K. Kardong, R. Stewart, L. Dix, M. Strickland, and I. Watt. 1999. Aging furbearers using tooth structure and biomarkers. *In* Wild Furbearer Management and Conservation in North America, M. Novak, J. Baker, M. Obbard and B. Malloch (eds.). CD Edition, Ontario Fur Managers Federation, Sault Ste Marie, Ontario, Canada, pp. 228–243.

Kemere, P., M. Liddel, P. Evangelou, D. Slate, and S. Osmek. 2002. Economic analysis of a large scale oral vaccination program to control raccoon rabies. *In* Human Conflicts with Wildlife: Economic Considerations, L. Clark (ed.). National Wildlife Research Center, Fort Collins, Colorado, pp. 109–116.

Krebs, C. J. 1989. Ecological methodology. Harper and Rowe Publishers Inc., New York, New York, 654 pp.

Lutz-Wallace, C. A., T. Sapp, M. Sidhu, and A. Wandeler. 1995a. In vitro assessments of the genetic stability of a live recombinant human adenovirus vaccine against rabies. Canadian Journal of Veterinary Research 59: 157–160.

———, A. Wandeler, L. Prevec, M. Sidhu, T. Sapp, and J. Armstrong. 1995b. Characterization of human adenovirus 5: Rabies glycoprotein recombinant vaccine re-isolated from orally vaccinated skunks. Biologicals 23: 271–277.

Macinnes, C. D. 1987. Rabies. *In* Wild furbearer management and conservation in North America, M. Novak, J. Baker, M. Obbard and B. Mallock (eds.). Ontario Trappers Association, North Bay, Ontario, Canada, pp. 910–929.

———, S. Smith, R. Tinline, N. Ayers, P. Bachmann, D. Ball, L. Calder, S. Crosgery, C. Fielding, P. Hauschildt, J. Honig, D. Johnston, K. Lawson, C. Nunan, M. Pedde, B. Pond, R. Stewart, and D. Voigt. 2001. Elimination of rabies from red foxes in eastern Ontario. Journal of Wildlife Diseases 7: 119–132.

Molinier-Frenkel, V., C. Le Boulaire, F. Le Gal, H. Gahery-Segard, T. Tursz, J. Guillet, and F. Farace. 2000. Longitudinal follow-up of cellular and humoral immunity induced by recombinant

adenovirus-mediated gene therapy in cancer patients. Human Gene Therapy 11: 1911–1920.

NADIN-DAVIS, S. A., G. A. CASEY, AND A. WANDELER. 1994. A molecular epidemiological study of rabies virus in central Ontario and western Quebec. Journal of General Virology 75: 2575–2583.

PREVEC, L., J. CAMPBELL, B. CHRISTIE, L. BELBECK, AND F. GRAHAM. 1990. A recombinant human adenovirus vaccine against rabies. Journal of Infectious Diseases 161: 27–30.

RANDRIANARISON-JEWTOUKOFF, V., AND M. PERRICAUDET. 1995. Recombinant adenoviruses as vaccines. Biologicals 23: 145–157.

RECUENCO, S., B. CHERRY, AND M. EIDSON. 2007. Potential cost savings with terrestrial rabies control. Biomedcentral (BMC) Public Health 7: 47–56.

ROSATTE, R. C. 1988. Rabies in Canada—History, epidemiology and control. Canadian Veterinary Journal 29: 362–365.

———. 2000. Management of raccoons (*Procyon lotor*) in Ontario, Canada: Do human intervention and disease have significant impact on raccoon populations? Mammalia 64: 369–390.

———, AND K. LAWSON. 2001. Acceptance of baits for delivery of oral rabies vaccine to raccoons. Journal of Wildlife Diseases 37: 730–739.

———, AND S. LARIVIERE. 2003. Skunks (*Genera Mephitis*, *Spilogale* and *Conepatus*). *In* Wild mammals of North America biology, management and conservation, G. Feldhamer, B. Thompson and J. Chapman (eds.). The Johns Hopkins University Press, Baltimore, Maryland, pp. 692–707.

———, M. POWER, C. MACINNES, AND J. CAMPBELL. 1992. Trap-vaccinate-release and oral vaccination for rabies control in urban skunks, raccoons and foxes. Journal of Wildlife Diseases 28: 562–571.

———, C. MACINNES, M. POWER, D. JOHNSTON, P. BACHMANN, C. NUNAN, C. WANNOP, M. PEDDE, AND L. CALDER. 1993. Tactics for the control of wildlife rabies in Ontario Canada. Reviews of the Science and Technical Office of International Epizootics 12: 95–98.

———, K. LAWSON, AND C. MACINNES. 1998. Development of baits to deliver oral rabies vaccine to raccoons in Ontario. Journal of Wildlife Diseases 34: 647–652.

———, D. DONOVAN, M. ALLAN, L. HOWES, A. SILVER, K. BENNETT, C. MACINNES, C. DAVIES, A. WANDELER, AND B. RADFORD. 2001. Emergency response to raccoon rabies introduction into Ontario. Journal of Wildlife Diseases 37: 265–279.

———, M. ALLAN, R. WARREN, P. NEAVE, T. BABIN, L. BUCHANAN, D. DONOVAN, K. SOBEY, C. DAVIES, F. MULDOON, AND A. WANDELER. 2005. Movements of two rabid raccoons, *Procyon lotor*, in eastern Ontario. Canadian Field-Naturalist 119: 453–454.

———, K. SOBEY, D. DONOVAN, L. BRUCE, M. ALLAN, A. SILVER, K. BENNETT, M. GIBSON, H. SIMPSON, C. DAVIES, A. WANDELER, AND F. MULDOON. 2006. Behaviour, movements, and demographics of rabid raccoons in Ontario, Canada: Management implications. Journal of Wildlife Diseases 42: 589–605.

———, M. POWER, D. DONOVAN, C. DAVIES, M. ALLAN, P. BACHMANN, B. STEVENSON, A. WANDELER, AND F. MULDOON. 2007a. The elimination of the Arctic variant of rabies in red foxes in metropolitan Toronto, Ontario, Canada. Emerging Infectious Diseases 13: 25–27.

———, R. TINLINE, AND D. JOHNSTON. 2007b. Rabies control in wild carnivores. *In* Rabies, 2nd Edition, A. Jackson and W. Wunner (eds.). Academic Press, San Diego, California, pp. 595–634.

———, E. MACDONALD, K. SOBEY, D. DONOVAN, L. BRUCE, M. ALLAN, A. SILVER, K. BENNETT, L. BROWN, K. MACDONALD, M. GIBSON, T. BUCHANAN, B. STEVENSON, C. DAVIES, A. WANDELER, AND F. MULDOON. 2007c. The elimination of raccoon rabies from Wolfe Island, Ontario: Animal density and movements. Journal of Wildlife Diseases 43: 242–250.

———, K. SOBEY, D. DONOVAN, M. ALLAN, L. BRUCE, T. BUCHANAN, AND C. DAVIES. 2007d. Raccoon density and movements after population reduction to control rabies. Journal of Wildlife Management 71: 2373–2378.

ROWE, W. P., R. HUEBNER, J. HARTLEY, T. WARD, AND R. PARROTT. 1955. Studies of the adenoidal-pharyngeal-conjunctival (APC) group of viruses. American Journal of Hygiene 61: 197–218.

RUSSELL, W. C. 1998. Adenoviruses. *In* Toply and Wilson's Microbiology and microbial infections, 9th Edition, L. Collier, A. Balows and M. Sussman (eds.). Edward Arnold, London, England, pp. 281–290.

YAROSH, O., A. WANDELER, F. GRAHAM, J. CAMPBELL, AND L. PREVEC. 1996. Human adenovirus type 5 vectors expressing rabies glycoprotein. Vaccine 14: 1257–1264.

WANDELER, A., AND E. SALSBERG. 1999. Raccoon rabies in eastern Ontario. Canadian Veterinary Journal 40: 731.

WICKWARE, G. M., AND C. RUBEC. 1989. Ecoregions of Ontario. Ecological Land Classification. Series (26) Sustainable Development Branch, Environment Canada, Ottawa, Ontario, Canada, 37 pp.

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