PREVALENCE OF TETRACYCLINE AND RABIES VIRUS ANTIBODY IN RACCOONS, SKUNKS, AND FOXES FOLLOWING AERIAL DISTRIBUTION OF V-RG BAITS TO CONTROL RACCOON RABIES IN ONTARIO, CANADA

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PREVALENCE OF TETRACYCLINE AND RABIES VIRUS ANTIBODY IN RACCOONS, SKUNKS, AND FOXES FOLLOWING AERIAL DISTRIBUTION OF V-RG BAITS TO CONTROL RACCOON RABIES IN ONTARIO, CANADA

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ABSTRACT: More than 3.6 million baits containing a recombinant vaccinia virus–rabies glycoprotein (V-RG) oral rabies vaccine were aerially or hand-distributed during 1999–2006 in an approximate 4,000-9,000 km^2 area of eastern Ontario, Canada, as part of a multitactic approach to control the raccoon variant of rabies. The efficacy of the program was assessed through the collection and testing of >6,900 animals for bait acceptance and rabies virus-specific antibodies. Raccoon acceptance of rabies vaccine baits was significantly greater (71–83%) in areas baited at a density of 150 baits/km^2 compared to areas baited at 75 baits/km^2 (26–58%), and more raccoons consumed vaccine baits in areas baited with a flight line spacing of 0.75 km (45.3% [321/708]) than with a spacing of 1.5 km (33.8% [108/320]). In addition, greater numbers of raccoons consumed vaccine baits during a drop in September (52.7% [213/404]) as opposed to a June bait drop (34.6% [216/624]). Seropositivity rates for raccoons ranged between 7% and 28% in areas baited at 75/km^2 and 10% to 27% in areas baited at 150/km^2 with statistical differences varying among years and treatments. The last case of raccoon-variant rabies reported in Ontario was in September 2005. The control of raccoon rabies in Ontario has resulted in an estimated $6M to $10M Cdn annual savings in rabies-associated costs.

Key words: Oral vaccination, *Procyon lotor*, rabid raccoon, rabies, raccoon, raccoon rabies, rabies control, vaccine baits, V-RG.

INTRODUCTION

The primary terrestrial vectors of rabies in Ontario, Canada, have been red foxes (*Vulpes vulpes*) and striped skunks (*Mephitis mephitis*) for the Arctic variant, and raccoons (*Procyon lotor*) for the raccoon-variant (Johnston and Beauregard, 1969; Rosatte, 1988; Rosatte et al., 2006). The Arctic variant entered Ontario from the north during the 1950s (Johnston and Beauregard, 1969), and the raccoon variant of rabies progressed into Ontario from New York State during 1999 (Wandeler and Salsberg, 1999; Rosatte et al., 1997, 2001, 2005, 2006). The disease has been controlled in Ontario using a variety of tactics, including oral rabies vaccination with baits (ORV), population reduction, and trap–vaccinate–release (Johnston et al., 1988; MacInnes et al., 2001; Rosatte et al., 2001, 2007a, b, c, d). In addition to ORV in Ontario, vaccine baits have been distributed in New York State in adjacent areas along the St. Lawrence and Niagara rivers since 1995 (rivers act as geographic barriers to raccoon movement) to protect Ontario's borders from an invasion or reinvasion of raccoon rabies. This was a cooperative effort between New York State (Cornell University), the United States Department of Agriculture (USDA), and the Ontario Ministry of Natural Resources (OMNR). The Ontario

ORV program is part of a larger campaign to distribute vaccine baits in eastern North America to control raccoon rabies for public health and economic benefits (Slate et al., 2005). An application and experimental design was submitted to the Veterinary Biologics and Biotechnology Section (VBBS) of the Canadian Food Inspection Agency (CFIA), Ottawa, Ontario, during August 1999, for approval to use V-RG baits to control raccoon rabies in Ontario. Approval to use V-RG in Ontario was granted in September 1999. This paper reports on the results of the Ontario portion of the international effort to control raccoon rabies during 1999–2006 via the aerial distribution of a recombinant vaccinia virus–rabies glycoprotein oral vaccine.

MATERIALS AND METHODS

Study area

The study area, located in eastern Ontario (Fig. 1; 44°45′N, 75°50′W) is primarily rural with exposed Precambrian rock, glacial moraines, and plains with sandy deposits and some marshy areas (Wickware and Rubec, 1989). To the south, the St. Lawrence River acts as a partial barrier to animal and disease movement; however, there are numerous islands in the river and some bridges that serve as an aid to animal movement. A total of 132 cases of raccoon-variant rabies were reported in the study area during 1999–2005, with just five cases occurring during 2004–05 (Rosatte et al., 2001, 2006). Ontario has been free of reported raccoon variant rabies since 24 September 2005 (to 1 July 2008).

Vaccine bait manufacture/acquisition

RABORAL V-RG® (Merial Limited, Athens, Georgia, USA) is a registered vaccine bait. The minimal protective dose for licensure of RABORAL V-RG for use in raccoons in the US is $10^{7.0}$ Median tissue culture infective doses ($TCID_{50}$)/ml (Merial Limited, unpubl. data). The Fishmeal Polymer (FP) baits used in this study during 1999 were a component of the RABORAL V-RG package. Other bait types used in this study (Ontario Slim) were not RABORAL V-RG because they were manufactured from bulk V-RG vaccine acquired from Merial Limited. Bulk V-RG (titer of at least $10^{8.0}$ $TCID_{50}$/ml at production) was purchased from Merial Limited, shipped to Ontario in polypropylene carboys, and used to manufacture Ontario Slim (OS) baits (Fig. 2) at Artemis Technologies Inc., Guelph, Ontario, Canada. The matrix of the OS bait consisted of 42% beef tallow (oleo), 28% MICROBOND® wax, 20% icing sugar, 8.5% vegetable oil, 1% marshmallow extract, and 0.5% dark-green, food grade, fat-soluble dye. The OS bait weighed approximately 13 g with dimensions of 35 mm × 35 mm × 11 mm. The polystyrene vaccine container (used during 1999–2002), known as a blister pack, contained 1.8 ml (±0.1 ml) of V-RG vaccine. Polyvinyl chloride was used to manufacture the blister pack during 2003–06. The blister pack was embedded in the matrix so that the lid of the blister pack was visible as an identification label.

About 23,000 RABORAL V-RG Fishmeal Polymer (FP) baits (Fig. 2) were purchased from Merial Limited, and about 31,300 were acquired from Cornell University, Ithaca, New York, USA. RABORAL V-RG FP baits consisted of a vaccine-filled polyethylene sachet inserted into the middle of a bait matrix and weighed approximately 24 g, with dimensions of 30 mm × 30 mm × 20 mm. Coated Sachet (CS) baits (Fig. 2) were also acquired from Merial Limited during 2001–03. The CS was a 57 mm × 22 mm × 3 mm polyethylene package weighing approximately 3– 4 g, which is coated on the exterior with unspecified attractant fish oil. Approximately 1.8±0.1 ml of V-RG is sealed within the package. The matrix of OS and RABORAL V-RG FP baits also contained 100–200 mg of tetracycline hydrochloride as a biomarker to indicate bait acceptance in raccoons, skunks, and foxes. CS baits did not contain a biomarker.

V-RG stability experiments

The titer of V-RG virus in OS and CS baits was determined over time under field conditions to verify the vaccine potency and stability. Ontario Slim and CS baits containing V-RG were placed outdoors in access-proof, steel mesh cages. Sample baits were retrieved from the cages weekly for 35 days, frozen at −80 C, and then all baits were titrated in a single assay. Day-0 baits were frozen (−80 C) immediately and subsequently thawed for titration with those retrieved at the other time intervals. Day 0 was the first day ±2 of ORV aerial baiting for each year tested (Tables 1 and 2). Titers of day-0 baits were compared with production titers (taken at time of manufacture) to determine if there was virus loss during storage between manu-

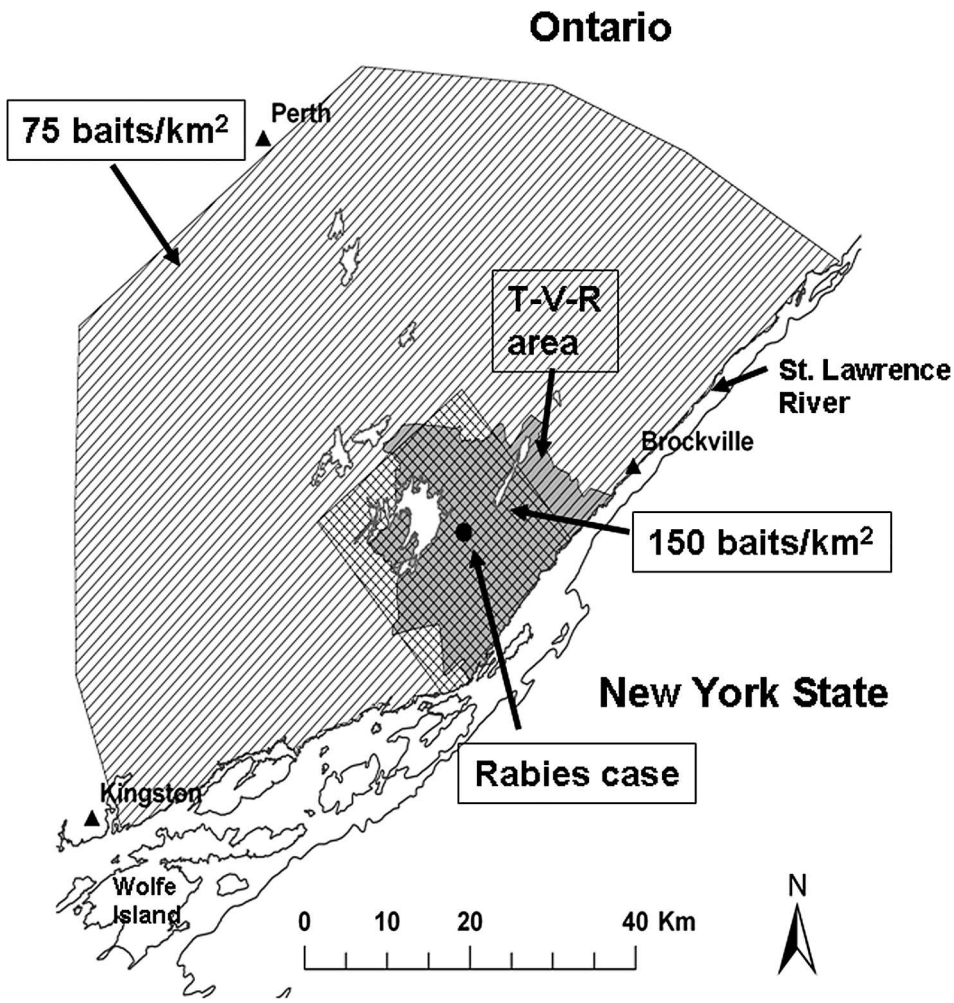

FIGURE 1. Location of V-RG baiting area in eastern Ontario, during 2005. In response to a single case of raccoon-variant rabies during 2005, V-RG baits were aerially distributed at 75 baits/km^2 in a 4,620 km^2 area. In addition, trap–vaccinate–release was implemented within 10 km of the case and V-RG baits were aerially distributed over a 526 km^2 area at a density of 150 baits/km^2. This was the last reported case of raccoon rabies in Ontario that was diagnosed on 23 September 2005 (to 1 July 2008).

facture and distribution. The V-RG titer was determined using the microtiter fluorescent antigen test (FA_{50}) on VERO cells. A standard reference virus of known titer (Std 200/700) was calibrated against the test samples to validate the assay. Titers were calculated by the methods of Spearman–Kärber or Reed and Muench (Lorenz and Bögel, 1973) and reported as geometric mean titer (GMT) $\log_{10}$ $TCID_{50}$/ml. Artemis Technologies, Inc. assayed V-RG using a microtiter cell culture infectious dose 50% ($CCID_{50}$) assay and the GMT of the triplicate results was reported as $\log_{10}$ $CCID_{50}$/ml.

Pre/Post-ORV sample collection

During a Point Infection Control Program (PIC) to contain the first case of raccoon rabies in eastern Ontario during July 1999, blood was collected from raccoons (as detailed in Rosatte et al., 2001), which served as a control sample prior to baiting in September, 1999. In subsequent baiting campaigns, raccoons and skunks were live-trapped in trapping cells (each approximately 10 km^2) within the areas that were baited (OMNR–collected live samples). All trapped raccoons and skunks were processed and released according to Rosatte et al. (2001). A second premolar tooth

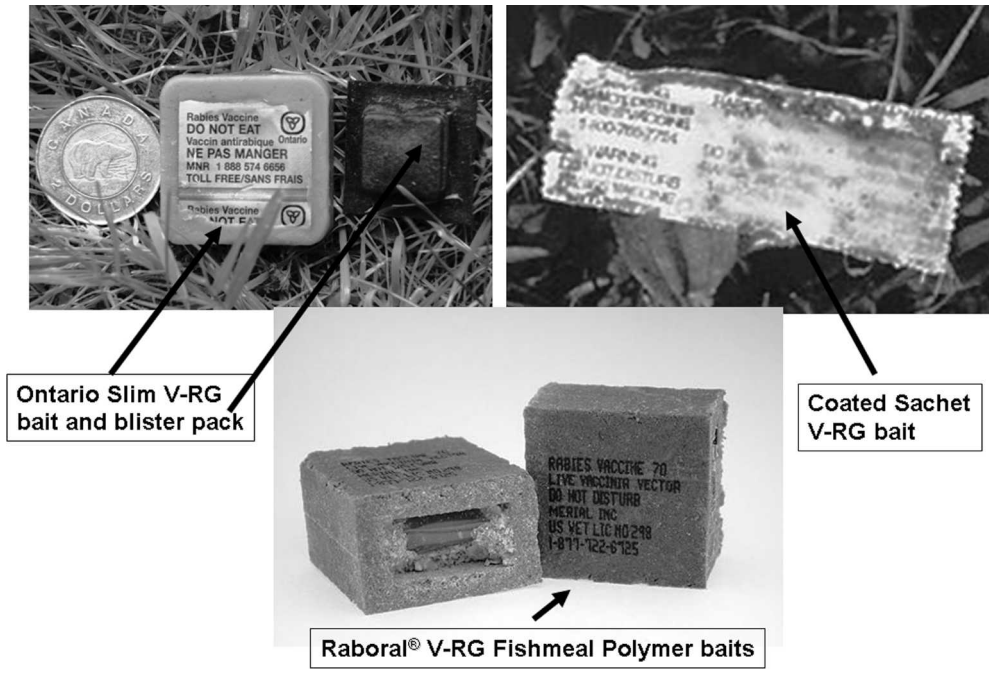

FIGURE 2. Photo of V-RG baits (Ontario Slim [OS], Coated Sachet [CS], Raboral V-RG® Fishmeal Polymer [FP]) used in Ontario, Canada, to control raccoon rabies during 1999–2006 (OS baits were used during 1999–2006, CS during 2001–03, and FP during 1999).

was also extracted from each animal using tooth extraction tools. Canine and premolar teeth were also extracted from a sample of raccoons that had been euthanized during PIC operations in 1999 and 2004. Animals (cadavers) were also collected from licensed Ontario fur trappers and hunters (cadaver samples) in baited and nonbaited areas, during all years, to assess bait acceptance and vaccine efficacy. Canine teeth and chest cavity fluids were extracted from these animals. Results are given for both live (OMNR) and cadaver samples. Second premolar teeth were examined for tetracycline presence in live samples, and canine teeth were used for biomarker determination in cadaver samples.

Bait acceptance testing and age determination

Teeth were prepared using established techniques described in Bachmann et al. (1990) and Johnston et al. (1987). The presence of tetracycline (epi-fluorescence) was detected with a compound microscope under ultraviolet incidence light using requisite excitation and barrier filter combinations (Johnston et al., 1987). Bait acceptance was estimated by the number of teeth exhibiting tetracycline fluorescence (Johnston et al., 1987). In canine teeth, age was determined with a similar microscope using polarizing filters under transmitted light to distinguish age zones or annuli (via birefringence) in undecalcified tooth cementum (Johnston et al., 1987). Premolars were decalcified and stained with hematoxylin (post-tetracycline assessment)—microscopy was under transmitted incandescent light. Number of annuli verified the animal's age (Johnston et al., 1987).

Serology testing for rabies virus-specific antibodies

A competitive ELISA (cELISA) was used to determine rabies glycoprotein-specific antibody levels in raccoon and skunk sera as described previously (Elmgren and Wandeler, 1996). Percent inhibition of binding of a horseradish peroxidase (HRP) labeled, glycoprotein-specific monoclonal antibody to microtiter plates coated with whole ERA virus was calculated from the ratio of the optical density of the test sample to that of a control well representing 100% binding. The positive cut-off values for each species were determined by Receiver Operating Characteristics analyses using a neutralizing titer of 0.5 IU/ml as the criterion for classifying a sample as positive (Metz, 1978). Using virus neutralization as the reference standard, the cELISA sensitivity and

TABLE 1. Production, Day-0 (release) and Day-35 titers (geometric mean titer±standard deviation) of V-RG in Ontario Slim (OS) lots and Coated-Sachet (CS) series baits in Ontario, Canada, 2000–06.

Year [a]	Bait type	GMT production[b,c]	GMT day-0[d]	GMT day-35	Sample size[e]	Lot or Series[f]
2000	OS	8.18±0.25	7.75±0.20	7.25±0.20	20	00-07,-08,-09,-15 (60–90)
2001	OS	7.65±0.58	7.25±0.32	4.48±1.28	20	00-07,-16; 01-03,-05 (110–170)
2001	CS	ns	7.90±0.29	6.55±0.33	5	13177
2002	OS	7.80±0.18	6.79±0.46	3.74±1.34	20	01-16,-18; 02-03,-08 (110–225)
2002	CS	ns	7.65±0.14	6.45±0.27	5	13209
2003	OS	7.68±0.10	7.56±0.28	7.23±0.32	20	03-01,-05,-09,-13 (80–110)
2003	CS	ns	7.40±0.29	4.70±0.86	5	13218
2004	OS	7.83±0.26	7.80±0.29	7.51±0.40	20	04-03,-07,-08,-14 (110–120)
2005	OS	7.65±0.13	7.36±0.26	6.84±0.91	20	05-02,-03,-04,-05 (170–180)
2006	OS	7.35±0.17	7.30±0.25	7.16±0.25	20	05-08,-11,-13,-14 (110–120)

[a] NA for 1999.

[b] GMT = geometric mean titer ($\log_{10}$ $TCID_{50}$/ml), ns = no sample.

[c] Production titer is the titer at time of vaccine-bait manufacture (n=4).

[d] Day-0 = the first day of ORV aerial bait distribution in the respective years; September 6 was designated day-0 in 2000; there were no test vaccine baits placed in conjunction with the earlier June baiting in 2000. In 2002 and 2003, equivalent vaccine-bait samples of some lots were also placed at the test site at later dates (i.e., late September in 2002 and early October in 2003) than day-0.

[e] n for day-0 and day-35 samples only.

[f] The number ranges in parentheses beside the lot numbers refer to the number of days between vaccine-bait manufacture (production) and day-0.

specificity were 84 and 85% for raccoon sera and 91 and 95% for skunk sera, respectively.

For determination of antibody levels in chest cavity fluid obtained from trapper-acquired cadavers, a modified cELISA (blocking [b] ELISA) was used due to non-specific reactions generated by these samples in the cELISA. Using clean raccoon sera, test agreement between cELISA and bELISA tests ranged between 90% and 96%. For the bELISA, chest cavity fluid diluted in phosphate-buffered saline (1:70) was incubated on virus-coated plates overnight at 4 C. Following washing of the plates, horseradish peroxidase labeled glycoprotein-specific monoclonal antibody was added, incubated for 1.5 hr at 28 C, and then developed and analyzed as in the cELISA.

Aerial bait distribution

Baits were distributed aerially using Twin Otter (DHC-6) aircraft (De Havilland Canada Ltd., Downsview, Ontario, Canada) outfitted with specially designed baiting machines (MacInnes et al., 2001). Altitude was approximately 150 m above ground level and flight speed was approximately 250–270 km/hr. Flight lines were predetermined using GIS and AUTOCAD® software and loaded into the GPS navigational system. In some baiting campaigns, baits were also distributed by hand in selected urban (green) areas and parks and on some islands. The detailed parameters of the baiting campaigns carried out from 1999 to 2006, including dates, baits types, flight line spacing, and bait density are outlined in Table 2.

Statistical analysis

Data were analyzed using a log-linear analysis (using STATISTICA® software) to examine associations between the categorical variables: sex, maturity, flight line spacing, drop date, and bait acceptance during 2000–04 because bait density and flight line spacing were consistent among these years. Chi-square analysis was also used for post hoc comparisons and to compare differences in serology and age of raccoons from areas receiving OS or CS baits.

RESULTS

V-RG stability experiments

The production GMT (n=4) of OS V-RG for all lots was within requisite for release for ORV (≥7.00 $\log_{10}$ $TCID_{50}$/ml) (Table 1). With the exception of 2002, the day-0 GMT for OS V-RG lots was within range of the production GMT regardless of the number of days between manufacture and day-0 (first day of ORV aerial baiting);

TABLE 2. Summary of V-RG aerial baiting ORV to control raccoon rabies in eastern Ontario, Canada, 1999–2006.

Baiting date	Bait type[a]	Bait density (baits/km^2)[a,b]	Flight-line spacing (km)	Area (km^2)	Number of baits distributed
9 September 1999	OS	70	1.00	400	27,000
9 September 1999	FP	70	1.00	350	23,000
27 September 1999	FP	70	1.00	450	29,700
26–28 June 2000	OS	75	0.75/1.50	5,110	297,720
5–7 September 2000	OS	75	0.75/1.50	8,600	481,924
20–23 August 2001	OS	75	0.75	7,770	473,180
20–23 August 2001	OS	150	0.75	800	99,120
20–23 August 2001	CS	150	0.75	800	101,924
16–23 August 2002	OS	75	0.75	6,000	387,990
18–22 August 2002	OS	150	0.75	900	111,024
18–22 August 2002	CS	150	0.75	900	101,250
18–22 August 2003	OS	75	0.75	7,000	416,664
18–22 August 2003	OS	150	0.75	900	87,264
18–22 August 2003	CS	150	0.75	900	99,900
20–22 August 2004	OS	75	0.75	5,700	378,854
20–22 August 2004	OS	150	0.75	800	95,688
19–21 August 2005	OS	75	0.75	4,620	248,861
19–21 August 2005	OS	150	0.75	526	27,432
19–22 August 2006	OS	67–75	0.75, 1.50[b]	4,000	209,009
Total					3,697,504

[a] Target density in forested and agricultural areas exclusive of urban and built-up areas or water; OS=Ontario Slim; FP=Fishmeal Polymer; CS=Coated Sachet.

[b] 1.50 km only on St. Lawrence River islands adjacent to the mainland.

day-0 GMT in 2002 was 6.79±0.46 (Table 1). In some years all (2005) or some (2001 and 2002) of the vaccine-bait lots were from the previous year's production (Table 1). Vaccine lots manufactured in a previous year were retitrated in the year of baiting to assure requisite titers for release. Production GMT and number of days between production and day-0 refer to retest titers in those cases (Table 1). For OS V-RG, GMT ($\log_{10}$ $TCID_{50}$/ml) decline over 35 days ranged between 0.14 to 0.52 in 2000 and in 2003 to 2006; titer decline was significantly greater over the same period in 2001 (2.77) and 2002 (3.05) ($P<0.001$; Table 1). In 2003, and subsequent years, the main constituent of blister pack in the bait was changed to polyvinyl chloride from polystyrene (constituent from 1999 to 2002). We found that increased gaseous (CO_2 and water vapor) transfer in the more permeable polystyrene blister pack elevated the pH of the vaccine suspension, consequently resulting in loss of virus viability (Artemis Technologies, Inc., unpubl. data). However, in 2000, with the polystyrene blister pack, the virus loss was minimal (GMT 0.5) over the 35 days in contrast to 2001 and 2002 with the same blister pack material (Table 1). It is likely that ambient temperature also affected virus titers (Bachmann et al., unpubl. data). The CS V-RG GMT virus loss over the 35-day period was 1.20 (2002), 1.35 (2001), and 2.70 (2003; Table 1).

Animals sampled from nonbaited areas

About 13% (147/1,145) of the raccoons and 7% (2/28) of the skunks sampled from nonbaited areas were positive for tetracycline. About 3% (19/595) of the raccoons and no (0/23) skunks were antibody-positive for rabies in nonbaited areas. In addition, 7% (1/14) of the foxes sampled in

TABLE 3. Bait acceptance (tetracycline+) and serology results for raccoons in areas that were baited with V-RG rabies vaccine in eastern Ontario, Canada, during 1999–2006.[a]

Baiting Date	Bait type	Bait density (/km^2)	Flight spacing (km)	Raccoon teeth Tetra+[b] % (*n*)	Raccoon sera cELISA+[b] % (*n*)	Raccoon teeth Tetra+[c] % (*n*)	Raccoon sera bELISA+[c] % (*n*)
8 September 1999	OS	70	1.0	61 (150/248)	18 (43/236)	ns	ns
27 September 1999	FP	70	1.0	44 (43/98)	14 (13/94)	ns	ns
26–28 June 2000	OS	75	0.75	39 (166/430)	10 (41/403)	ns	ns
26–28 June 2000	OS	75	1.5	26 (50/194)	ns	ns	ns
5–7 September 2000	OS	75	0.75	56 (155/278)	8 (18/238)	44 (314/708)	0.4 (3/699)
5–7 September 2000	OS	75	1.5	46 (58/126)	ns	32 (100/311)	1 (2/308)
20–23 August 2001	OS	75	0.75	51 (134/265)	7 (17/259)	50 (217/435)	1 (6/428)
20–23 August 2001	OS	150	0.75	83 (76/92)	11 (8/72)	55 (6/11)	0 (0/12)
20–23 August 2001	CS	150	0.75	ns	27 (45/169)	16 (32/198)[d]	5 (10/202)
16–23 August 2002	OS	75	0.75	55 (200/362)	7 (25/340)	51 (275/537)	2 (12/531)
16–23 August 2002	OS	150	0.75	80 (92/115)	10 (9/86)	75 (121/161)	6 (9/159)
16–23 August 2002	CS	150	0.75	ns	18 (41/228)	27 (40/146)[d]	5 (7/143)
18–22 August 2003	OS	75	0.75	58 (145/251)	10 (24/231)	55 (113/204)	4 (8/203)
18–22 August 2003	OS	150	0.75	71 (151/213)	23 (39/171)	43 (3/7)	14 (1/7)
18–22 August 2003	CS	150	0.75	ns	16 (17/110)	ns	ns
20–22 August 2004	OS	75	0.75	47 (101/213)	28 (37/133)	59 (209/357)	4 (13/356)
20–22 August 2004	OS	150	0.75	ns	ns	74 (67/90)	7 (6/88)
19–21 August 2005	OS	75	0.75	ns	ns	59 (148/252)	13 (32/253)
19–21 August 2005	OS	150	0.75	ns	ns	72 (41/57)	28 (16/57)
16–18 August 2006	OS	75	0.75	ns	ns	63 (88/140)	5 (7/140)

[a] OS = Ontario Slim bait with V-RG; FP = Fishmeal Polymer bait with V-RG; CS = Coated Sachet bait with V-RG; ns = not applicable because CS baits did not contain tetracycline and/or samples were not collected; Tetra+ = tetracycline-positive; ELISA = enzyme linked immunosorbant assay.

[b] Samples collected by Ontario Ministry of Natural Resources staff (live samples) in preselected plots; sample locations known with certainty; samples collected 5–6 wk post-baiting when antibody is expected to peak; cELISA cut-off was 20%; vaccine efficacy data (ELISA+/Tetra+) are not presented due to stability problems during 2001/02; however, as noted in the results section, vaccine efficacy was 16%, 29% and 56% during 2003/04.

[c] Samples collected from Ontario trappers (cadaver samples); location of samples not known with certainty; samples collected during winter several months post-baiting (i.e., antibody might not be detectable according to antibody/time curve); cadavers collected during 2000/01 might have eaten baits distributed during June and/or September 2000; 3.7% (53/1,439) of trapper-acquired raccoons were both tetracycline and ELISA-positive; bELISA cut-off was 20% inhibition.

[d] These raccoons were collected in areas baited with CS baits that did not have tetracycline in them (i.e.. the tetracycline was probably acquired outside of the baiting area and the animals dispersed into the baiting area).

nonbaited areas were antibody-positive and 37% (7/19) were tetracycline-positive.

ORV aerial-baiting campaigns

1999 Program: Based on the prevalence of tetracycline in second premolar teeth, 61% (150/248) of the raccoons sampled consumed OS baits containing V-RG (Table 3). About 18% of sampled raccoons had rabies virus antibody as determined by a cELISA (Table 3). Based on tetracycline prevalence in second premolar teeth, 44% (43/98) of the raccoons sampled consumed RABORAL V-RG FP baits (Table 3). Raccoon acceptance of FP baits based on tetracycline in canine teeth was 64% (137/215). There were no detectable differences in acceptance of FP baits based on sex and age of raccoons ($P=0.26$). About 14% of sampled raccoons had rabies virus antibody (Table 3). In addition, raccoon acceptance of OS baits was significantly greater (61%) than acceptance of FP baits (44%) based on tetracycline prevalence in premolar teeth ($P< 0.05$) (Table 3).

2000: OS bait aerial distribution cam-

paigns were carried out in June and September, and hand distribution of OS baits was carried out in towns, parks, and islands during late August (Table 2). Greater numbers of raccoons consumed OS V-RG baits during September 2000 than during June 2000 ($P=0.0002$; as assessed by prevalence in second premolar teeth 2.5–9 wk post-baiting; Table 3). Additionally, greater numbers of raccoons consumed baits in areas baited with a flight line spacing of 0.75 km compared to 1.5 km ($P=0.0005$; $P=0.002$ for trapper-sampled raccoons; Table 3). Serology results were low among all sampled animals with ≤10% of raccoons having rabies virus antibody (Table 3).

2001: Aerial baiting with OS and CS baits was carried out in late August using bait densities of 75 and 150 baits/km^2. In addition, hand baiting was carried out on 15 July and 31 October (see Table 2). Raccoon acceptance of OS baits was 83% (76/92) in areas baited at 150 baits/km^2 and 51% (134/265) at 75 baits/km^2 ($P<0.001$; Table 3). The percentage of seropositive raccoons in those areas was 11% and 7%, respectively ($P=0.09$; Table 3). The percentage of seropositive raccoons in areas baited with CS baits (150/km^2) was significantly greater (27% [45/169]) than in areas baited with OS baits (11%; $P=0.01$; Table 3). During additional surveillance operations (cadaver samples), 50% (217/435) and 55% (6/11) of the raccoons sampled from areas baited at 75 and 150 OS baits/km^2, respectively, were tetracycline-positive using canine teeth. Serology values for those animals were very low (Table 3).

During live-capture surveillance operations (6–9 weeks post-baiting), 24% (11/46) of the skunks sampled were tetracycline-positive in areas baited at 75 OS/km^2. Only three skunks were sampled in areas baited at 150 OS/km^2—one of those was positive for tetracycline.

2002: From 16–23 August, aerial baiting with OS and CS baits was carried out using bait densities of 75 and 150 baits/km^2 and hand baiting was carried out in cities, towns, parks, and islands of eastern Ontario (see Table 2). About 80% of the raccoons (92/115) trapped in areas baited at an OS bait density of 150/km^2 contacted baits (Table 3). This was significantly higher than the percentage of raccoons (55% [200/362]) that were tetracycline-positive from areas baited at 75 baits/km^2 ($P<0.001$). No statistically significant differences were detected in serology results for raccoons sampled from areas baited with OS (10%) or CS (18%) baits at a density of 150 baits/km^2 ($P=0.078$). A significant association was detected between the age of raccoons that were positive for tetracycline and bait density ($P<0.001$). More adult than juvenile raccoons were positive for tetracycline in areas baited at 75 baits/km^2, whereas the inverse was true in areas baited at 150 baits/km^2. Biomarker results from raccoon cadaver samples were similar to OMNR-acquired live samples with higher acceptance rates occurring in the higher-density baited areas ($P<0.001$); however, serology values for cadaver samples were very low (Table 3).

About 27% (10/37) of the skunks trapped in areas baited with an OS bait density of 150/km^2 contacted baits as evidenced by the presence of tetracycline in second premolar tooth sections. About 15% (2/13) of the skunks sampled in areas baited at 75 baits/km^2 were tetracycline-positive (Table 4). No differences were found in skunk tetracycline positivity between the two bait densities ($P=0.39$). Skunk bait acceptance and serology results were low for cadaver samples as well (Table 4). However, results for foxes were more promising than in skunks (Tables 4 and 5).

In 2002, it was determined that the blister pack material (polystyrene) led to an increase in pH of the vaccine with a resultant loss in potency (see Table 1 stability results for 2001/02). This might have contributed to the low antibody

TABLE 4. Bait acceptance (tetracycline+) and serology results for striped skunks in areas that were baited with V-RG rabies vaccine in eastern Ontario, Canada, during 1999–2005.[a]

Baiting date	Bait type	Bait density (/km^2)	Flight spacing (km)	Tetra+ % (n)[b]	RNA+ cELISA[b] % (n)	Tetra+ % (n)[c]	RNA+ bELISA % (n) [c]
8 September 1999	OS	70	1.0	22 (17/79)	2 (1/61)	ns	ns
27 September 1999	FP	70	1.0	33 (12/36)	5 (1/22)	ns	ns
5–7 September 2000	OS	75	0.75	ns	ns	35 (23/65)	0 (0/75)
20–23 August 2001	OS	75	0.75	24 (11/46)	ns	ns	ns
20–23 August 2001	OS	150	0.75	33 (1/3)	ns	ns	ns
16–23 August 2002	OS	75	0.75	15 (2/13)	ns	19 (6/32)	3 (1/30)
16–23 August 2002	OS	150	0.75	27 (10/37)	ns	29 (2/7)	0 (0/7)
18–22 August 2003	OS	75	0.75	20 (3/15)	ns	ns	ns
18–22 August 2003	OS	150	0.75	54 (7/13)	ns	ns	ns
20–22 August 2004	OS	75	0.75	21 (15/72)	16 (9/58)	ns	ns
19–21 August 2005	OS	75	0.75	ns	ns	41 (13/32)	ns
19–21 August 2005	OS	150	0.75	ns	ns	50 (13/26)	ns

[a] OS = Ontario Slim bait with V-RG; FP = Fishmeal Polymer bait with V-RG; CS = Coated Sachet bait with V-RG; ns = not applicable because CS baits did not contain tetracycline and/or no samples were collected; Tetra+ = tetracycline-positive; ELISA = enzyme linked immunosorbant assay.

[b] Samples collected by Ontario Ministry of Natural Resources staff in preselected plots; sample locations known with certainty; samples collected 5–6 wk post-baiting when antibody is expected to peak; cELISA cut-off was 20% inhibition during 1999 and 14% during 2004.

[c] Samples collected from Ontario trappers; bELISA cut-off was 20% inhibition; location of samples not known with certainty; samples collected during winter several months post-baiting (i.e., antibody might not be detectable according to antibody/time curve); none (0/31) of the tetracycline-positive skunks sampled during 2000 and 2002 were ELISA-positive.

prevalence observed during 2001 and 2002; however, it does not explain the low prevalence during 2000.

2003: Aerial baiting with OS and CS baits was carried out from 18–22 August 2003. In addition, OS baits were hand-distributed in urban areas as well as parks and islands from August to October 2003. Raccoon acceptance of baits was 58%

TABLE 5. Bait acceptance (tetracycline+) and serology results for red foxes in areas that were baited with V-RG rabies vaccine in eastern Ontario during 2000-2005.[a]

Baiting date	Bait type	Bait density (/km^2)	Flight line spacing (km)	Tetra+ % (n)[b]	RNA+ bELISA % (n)[b]
5–7 September 2000	OS	75	0.75	78 (14/18)	6 (1/17)
5–7 September 2000	OS	75	1.5	69 (20/29)	7 (2/29)
16–23 August 2002	OS	75	0.75	71 (17/24)	17 (4/24)
16–23 August 2002	OS	150	0.75	67 (6/9)	11 (1/9)
16–23 August 2002	CS	150	0.75	50 (3/6) [c]	20 (1/5)
16–23 August 2004	OS	75	0.75	75 (6/8)	13 (1/8)
19–21 August 2005	OS	75	0.75	86 (6/7)	ns

[a] OS = Ontario Slim Bait with V-RG; FP = Fishmeal Polymer bait with V-RG; CS = Coated Sachet bait with V-RG; ns = not applicable because CS baits did not contain tetracycline and/or no samples were collected; Tetra+ = tetracycline-positive; ELISA = enzyme-linked immunosorbant assay.

[b] Samples collected from Ontario trappers; bELISA cutoff was 20% inhibition; location of samples not known with certainty; samples collected during winter several months post-baiting (i.e., antibody might not be detectable according to antibody/time curve). 14% (9/65) trapper-acquired foxes were tetracycline-positive and ELISA-positive during 2000–04.

[c] Due to dispersing foxes because CS baits did not contain biomarker.

(145/251) and 71% (151/213) in areas baited at 75 and 150 OS baits/km^2, respectively (P=0.003; Table 3). Trapper-acquired raccoon cadaver samples had similar results at lower bait densities (Table 3). About 10% and 23% of the raccoons from those respective areas were positive for rabies virus antibody (P=0.0003; Table 3). Of the raccoons that ate OS baits (as evidenced by tetracycline deposits), 16% (23/145) and 29% (44/151) were positive for rabies virus antibody in areas baited at 75 and 150 OS/km^2, respectively. Only 16% (17/110) of the raccoons sampled in areas baited with CS baits at a density of 150/km^2 were rabies virus antibody-positive (Table 3). That was significantly lower than the percent of raccoons that were rabies virus antibody-positive in areas baited with 150 OS baits/km^2 (P=0.02).

About 20% (3/15) and 54% (7/13) of the skunks sampled in areas baited at 75 and 150 OS baits/km^2, respectively, were tetracycline-positive (P=0.067; Table 4).

2004: During August 2004, OS V-RG baits were distributed in eastern Ontario, including baits in urban areas and islands (Table 2). Bait density was 75 and 150/km^2 and flight line spacing was 0.75 km (Table 2). About 47% (101/213) of the raccoons (204 canine teeth; nine second premolars) sampled from areas baited at 75 baits/km^2 6–9 wk post-baiting were positive for tetracycline (Table 3). A significant interaction was detected between the age of raccoons and tetracycline-positive raccoons—greater numbers of adults (65% [50/77]) were tetracycline-positive than juveniles (39% [51/131]; P<0.001). In addition, 21% (15/72) of the skunks sampled also were tetracycline-positive using canine teeth (Table 4). Based on a cELISA, 28% (37/133) of the raccoon sera and 16% (9/58) of the skunk sera were positive for rabies virus antibody in areas baited at 75/km^2 (Tables 3 and 4). Of the raccoons that were tetracycline-positive and for which a blood sample was collected, 56% (35/63) were seropositive. No significant associations were found among age and sex of raccoons and rabies virus antibody (P<0.07). Greater numbers of raccoon cadavers from high bait density areas were tetracycline-positive than those collected from low bait density areas (P=0.006; Table 3). However, serology results from those areas were not different (P=0.18; Table 3). About 75% of sampled foxes were biomarker-positive; however, serology values were low (Table 5).

2005/06: OS baits were aerially-distributed in campaigns in August 2005/06 (Table 2). The OS baits were additionally hand-distributed in urban areas and islands. Based on cadaver samples during 2005, bait acceptance was significantly greater in high-bait density areas compared to low-density areas but serology results were low (P=0.05; Table 3). However, results were not different for skunk samples (P=0.45; Table 4). Six of seven foxes were biomarker-positive during 2005 operations (Table 5).

Among-year comparisons 2001–03

Among-year differences in bait acceptance were compared only for 2001–03 because bait density, flight line spacing, and bait type were the same for those years. At a density of 75 baits/km^2, bait acceptance for adult raccoons was higher than for juveniles for 2 of 3 yr (2002/03; 61 and 71% for adults vs. 49 and 45% for juveniles; Table 6). Comparisons between sexes from 2001–03 demonstrated that bait acceptance by males was higher than that for females over the 3-yr period (Table 6). Analysis of age classes in 2001 and 2003 revealed no significant differences in bait acceptance between adult and juvenile raccoons captured in areas baited at 150 baits/km^2 (Table 7). However, in 2002, there was an association between tetracycline marking and age (P=0.029) with adult raccoons showing 92% (35/38) acceptance when compared to juvenile raccoons at 75%

TABLE 6. Tetracycline marking in second premolar teeth from adult, juvenile, male, and female raccoons sampled in areas baited at 75 V-RG baits/km^2 in eastern Ontario, Canada, during 2001–03.[a]

Year	Age	Tetra+	Tetra-	Total	% pos	χ^2 value	df	*P* value
2001	Adult	96	89	185	52	0.72	1	0.3953
	Juvenile	36	42	78	46			
2002	Adult	118	77	195	61	4.72	1	0.0299
	Juvenile	81	84	165	49			
2003	Adult	85	34	119	71	17.78	1	<0.001
	Juvenile	59	72	131	45			
2001	Male	80	62	142	56	4.67	1	0.0308
	Female	52	69	121	43			
2002	Male	107	66	173	62	5.82	1	0.0159
	Female	92	95	187	49			
2003	Male	81	45	126	64	4.65	1	0.0311
	Female	63	61	124	51			

[a] Tetra+ = tetracycline-positive; Tetra- = tetracycline-negative; % pos = the percent of raccoon teeth that were positive for tetracycline; df = degrees of freedom.

(57/76; Table 7). Analysis between sex classes demonstrated no significant differences in tetracycline marking between male and female raccoons at this density (Table 7). An analysis was conducted between baiting densities to determine if baiting density had an effect on tetracycline marking in raccoons. In all years, the number of tetracycline-positive raccoons was significantly higher in areas baited at 150 baits/km^2 as opposed to 75 baits/km^2 ($P<0.003$; Table 3). Among-year serology comparisons were not conducted due to the possibility that low results during some years might have been attributed to vaccine stability problems noted previously.

Bait contacts

During 1999–2006 there were 130 public enquiries regarding V-RG baits with baits being contacted by 50 dogs and 42 people. The contacts varied from people who had found baits but did not contact vaccine, people who reported dogs

TABLE 7. Tetracycline marking in second premolar teeth from adult, juvenile, male, and female raccoons sampled in areas baited at 150 V-RG baits/km^2 in eastern Ontario, Canada, during 2001–03.[a]

Year	Age	Tetra+	Tetra-	Total	% pos	χ^2 value	df	*P* value
2001	Adult	40	7	47	85	0.36	1	0.5506
	Juvenile	37	9	46	80			
2002	Adult	35	3	38	92	4.76	1	0.0291
	Juvenile	57	19	76	75			
2003	Adult	78	26	104	75	1.66	1	0.1973
	Juvenile	73	36	109	67			
2001	Male	38	9	47	81	0.25	1	0.6155
	Female	39	7	46	85			
2002	Male	49	12	61	80	0.01	1	0.9136
	Female	43	10	53	81			
2003	Male	81	31	112	72	0.23	1	0.6287
	Female	70	31	101	69			

[a] Tetra+ = tetracycline-positive; Tetra- = tetracycline-negative; % pos = the percent of raccoon teeth that were positive for tetracycline; df = degrees of freedom.

chewing baits, and people who contacted vaccine after handling chewed baits. In cases where humans contacted vaccine, they were immediately referred to a physician; however, no further medical action was required in any of the cases. No adverse effects were noted in the six individuals who reported contacting vaccine. In 2000, a dog that ate baits became ill with diarrhea and vomiting that lasted for 2–3 days. The condition cleared up completely after that. There were no reports of ill effects in other cases of dogs ingesting baits.

Baiting costs

The estimated cost for aerially distributing V-RG baits included costs for vaccine purchase, bait manufacture, aircraft distribution, and post-baiting assessment, and is shown in Table 8. The average cost to distribute baits at 75 baits/km^2 was \$147.39/km^2 (\$140.72–\$151.79 Cdn). The high bait density (150 baits/km^2) cost averaged \$273.53/km^2 (\$262.22–\$281.73 Cdn) (Table 8).

DISCUSSION

More than three million V-RG baits were distributed in eastern Ontario, Canada, during 1999–2006. This was the first time that RABORAL V-RG as well as V-RG in OS and CS baits were used in Canada for the control of raccoon rabies. There were a minimal number of vaccine-bait contacts and no human reactions to vaccine were reported. This is encouraging in terms of human and domestic pet safety.

Raccoon acceptance of vaccine baits (based on tetracycline prevalence) in eastern Ontario in this study varied between 26% and 83%, depending on bait density, bait type, flight line spacing, type of tooth used for detection of tetracycline, and the time of year for baiting. This was comparable to the 59% raccoon acceptance of baits in south-central Ontario as reported in Rosatte

TABLE 8. Costs for distribution of V-RG baits and post-baiting assessment costs in eastern Ontario, Canada, during 2002–05.

Year	Baits dropped	Total area (km^2)	Live trap	Carcass[a] collection	Tooth assay[b]	Blood[c] assay	FAT[d]	Total specimen cost	Specimen cost per km^2	Distribution cost[e] per km^2 (75/km^2)	Distribution cost[e] per km^2 (150/km^2)
2002	600,264	8,000	\$40,000	\$42,200	\$19,602	\$15,940	\$14,640	\$132,382	\$16.55	\$140.72	\$262.22
2003	603,826	8,800	\$40,000	\$11,585	\$10,683	\$7,120	\$3,900	\$73,288	\$8.33	\$146.96	\$276.89
2004	474,542	6,500	\$40,000	\$19,737	\$5,870	\$4,000	\$6,450	\$76,057	\$11.70	\$151.79	\$281.73
2005	276,048	5,146	\$40,000	\$20,515	\$6,098	\$4,000	\$6,450	\$77,063	\$14.98	\$150.08	\$273.26
Mean			\$40,000	\$23,515	\$10,563	\$7,765	\$7,875	\$89,698	\$12.89	\$147.39	\$273.53

[a] Cost per carcass was \$43.30 (in 2002) plus a 3% increase per year.

[b] Cost per tooth ranges between \$12.90 (canine) and \$14.70 (premolar) plus a 3% increase per year.

[c] ELISA \$10.00 per sample.

[d] Fluorescent Antibody Test (FAT) \$15.00 per sample.

[e] Distribution costs include [staff salary, aircraft, vehicle, travel, supplies & equipment, service, bait machine, and preparation] + [cost of bait @ \$2.25 each]; the cost of the bait portion is 88% to 94% of the distribution cost.

and Lawson (2001). In New Jersey, tetracycline was found in 73% of sampled raccoons following distribution of FP V-RG baits at a density of 64/km^2 (Roscoe et al., 1998). In our study, baiting campaigns carried out in 2000 showed significantly higher bait acceptance in September as compared to June at both the high (150/km^2) and low (75/km^2) bait densities. This result was not surprising because juveniles with insufficiently developed body size or dentition would be expected to have more difficulty than adults in chewing the baits adequately to contact vaccine. This effect could be minimized by delaying distribution of baits to later in the year when juveniles are more fully developed. In Ontario, this date is about mid-August. However, our results from baiting campaigns in years subsequent to 2000 (all carried out in mid- to late August) showed that in general, bait acceptance was higher in adult raccoons than juveniles, suggesting other factors also might influence bait acceptance. These could include difference in foraging behaviors or traveling range of adults vs. juveniles (Rosatte, 2000), factors that also might have influenced the observed higher bait acceptance in males vs. females in areas of low bait density. Also, in our study, raccoon acceptance of baits was significantly greater in areas baited at 150 baits/km^2 as opposed to 75 baits/km^2. Higher bait acceptance is important especially because oral vaccines are not 100% effective (based on serology and challenge studies; e.g., Rupprecht et al., 1986, 1988), making it necessary to reach more raccoons with vaccine baits in order to maximize immunity against rabies in the field.

We view the bait acceptance values for raccoons in this study as minimal estimates. Previous work from our laboratory indicates that tetracycline marking in raccoon canine teeth might be more efficient than in first or second premolars, as much as 1.6 times (unpubl. data). This is probably due to size differences among the teeth, as well as different tooth extraction and sectioning techniques. However, additional research is needed due to highly variable correction factor estimations among different populations of raccoons in Ontario. It has also been shown in our laboratory that the prevalence of tetracycline in premolar and canine teeth from the same animal can be different (Sobey et al., unpubl.). As such, bait acceptance based on second premolars might be underestimates of the actual value. It is also possible that tetracycline deposition did not occur in every animal that consumed bait. In fact, Johnston et al. (2005) found that about 40% of the tetracycline in a sample of baits used for raccoon rabies control in the US was unavailable for absorption. This means that bait acceptance using canine teeth might also represent an underestimate of actual acceptance values.

Knowledge of background tetracycline levels prior to baiting also is an asset in assessing post-baiting bait uptake (Nunan et al., 1994). If background biomarker levels are high, an overestimate of actual bait acceptance will occur. About 13% of the raccoons and 7% of the skunks sampled from nonbaited areas were positive for tetracycline. This could represent animals dispersing from baited areas or tetracycline acquired from the environment. However, significantly greater numbers of raccoons and skunks were biomarker-positive in baited areas than in nonbaited areas. The high percentage of foxes that were tetracycline-positive in nonbaited areas is most likely attributable to the wide-ranging habits of foxes (Voigt, 1987). Animal movement potential is very important when trying to interpret bait acceptance data. In this study, there were biomarker-positive foxes and raccoons sampled from CS baited areas (i.e., there was no biomarker in the CS baits). These animals may have eaten baits in other areas and dispersed into the CS areas. This is not unexpected because red foxes in Ontario are capable of dispersing >100 km (Voigt, 1987), and in one study

in southern Ontario, 35% of raccoons were documented as moving greater than 20 km but less than 50 km (Rosatte, 2000). Striped skunks are much more sedentary with movements in Ontario being generally <5 km (Rosatte and Lariviere, 2003).

Another consideration when evaluating bait acceptance is raccoon density. If raccoon density is exceptionally high, additional baits might be needed to reach a substantial portion of the raccoon population. For example, in Toronto, Ontario, where raccoon density averaged about 13–20/km^2 (Rosatte, 2000), 400 baits/km^2 yielded bait acceptance values of 82% in raccoons (Rosatte and Lawson, 2001). In contrast, in the areas of eastern Ontario where V-RG baiting was carried out, average raccoon density varied between 4.5 and 7.2/km^2 during 1999–2005 (Rosatte 2000; Rosatte et al., 2001, 2007b), and comparable bait acceptance levels could be achieved at a bait density of 150/km^2. However, there were landscape differences (urban vs. rural) between those areas that might have had some impact on acceptance values due to raccoon dispersion across the landscape and varying available sustenance.

It is clear that a substantial portion of the studied raccoon populations could be enticed to consume baits containing V-RG. However, that did not equate to a similar percentage of the population developing a detectable immune response against rabies virus. The serology results from this study were lower than expected based on published reports, with seroconversion in 7–28% of sampled raccoons, depending on year, bait type, bait density and flight line spacing. These data are inconsistent with results from laboratory efficacy trials, where 80 to 100% of raccoons orally immunized with V-RG seroconverted and were subsequently protected following challenge with rabies virus (Rupprecht et al., 1986, 1988, 1995). There has been a great deal of variation in the reported field efficacy of V-RG in raccoons and it is noted that a variety of serologic tests have been utilized, which will affect the comparison of the results among study areas. Robbins et al. (1998) found that 37, 67, and 77% of the sampled raccoons in three treatment areas in Massachusetts had rabies virus antibody. Roscoe et al. (1998) found that 61% of sampled raccoons were seropositive following distribution of V-RG baits in New Jersey. About 52% of a sample of raccoons on Parramore Island, Virginia, where V-RG had been distributed at a density of 1,000 baits/km^2 during 1990, was seropositive for rabies virus antibody (Hanlon et al., 1998). Even at this extremely high bait density, seroconversion was significantly lower than the bait acceptance of 84% determined by tetracycline deposition (Hanlon et al., 1998). Thus, the seroconversion rates observed in the laboratory are not necessarily reflective of the results obtained from field trials.

It is not entirely clear why the Ontario baiting campaigns yielded such low seropositive rates given the reported success of V-RG in laboratory trials (Rupprecht et al., 1986, 1988). However, because the sensitivity and specificity of the cELISA used in our study were greater or equal to 84% for raccoon and skunk sera when compared with a conventional virus neutralization assay, the observed poor correlation between seropositivity and V-RG bait acceptance was not due to use of an inadequate serologic test. Problems with vaccine stability identified in OS baits distributed in 2001/02 and CS baits distributed in 2003 likely contributed to low seropositive rates in these campaigns. Our results also suggest there might have been some problems in delivering the vaccine from the bait to the sites in the oral cavity required for the development of an immune response. Even after vaccine stability problems had been corrected, vaccine efficacy was only 56% during 2004. Additional research is needed to ensure that vaccine contact via bait consumption is maximized in the oral cavity.

The timing of sample collection for

serology also might have had an impact on the detection of seropositive raccoons. One can assume that bait contact by raccoons is rapid because 83% of rabies vaccine baits placed in Ohio, and 80% in Ontario, disappeared within 1 and 2 wk of placement, respectively (Bachmann et al., 1990; Blackwell et al., 2004). In addition, it has been shown that rabies virus antibody can be detected in raccoons and skunks as early as 5 days post-vaccination (IM) (Rosatte et al., 1990). Hanlon et al. (1998) showed that detection of a rabies virus-specific serologic response in raccoons was optimal 4–6 wk following field distribution of V-RG baits, although responses (>0.5 IU/ml) could be detected between 2 and 12 wk. In the present study, sampling in most campaigns began 5 to 6 wk post-baiting, although in some years the sampling period ranged from 2 to 9 wk post-baiting. Therefore, it is possible that some seropositive animals were missed if they were sampled before or after the peak immune response. That is, cadaver samples were collected during winter several months post-baiting when antibody might not be as detectable as in those samples collected by OMNR staff 5–6 wk post-baiting. This was evidenced by the lower serology results for cadaver samples in Table 3. Also, animals collected as cadaver samples might have dispersed previously either into or out of the baiting area.

Although the focus of the V-RG vaccination campaigns was the control of rabies in raccoons, we took the opportunity to look at bait acceptance and seroconversion in striped skunks and red foxes as well. Fox acceptance of V-RG baits was good. This was anticipated because baits were distributed at densities greater than those usually used to target foxes (20/km^2; MacInnes et al., 2001). Low serology results for foxes in this study were unexpected because V-RG has been proven to effectively immunize foxes against rabies as reviewed by Rosatte et al. (2007b). In all likelihood, low seropositive rates for foxes in this study were due to vaccine stability problems noted previously. The observed poor bait acceptance among striped skunks was anticipated because skunks are not as wide-ranging as raccoons (Rosatte, 2000; Rosatte and Lariviere, 2003). Thus the bait densities employed were inadequate to reach a good proportion of the skunk population. Post-baiting assessments of seroconversion were conducted following just three of the campaigns; rabies specific antibody was found in only 2–16% of the skunks sampled. The OS, FP, and CS baits used in this study were designed to deliver vaccine to raccoons, an animal with larger body size and dentition than skunks, likely accounting for the low seropositivity rates in skunks. This is consistent with the results of Grosenbaugh et al. (2007) who found that four of six skunks administered V-RG by direct oral instillation were protected from challenge, whereas those consuming coated sachet baits containing V-RG had low survival rates following rabies challenge. Results from a recent study of rabies vaccine uptake by captive striped skunks have also suggested modification of rabies vaccine baits, including reduction in size, would allow skunks to more easily puncture the vaccine container (Jojola et al., 2007).

Our data suggest that as an ORV tactic, a density of between 75 and 150 baits/km^2, a flight line spacing of 0.75 km, and a baiting time of mid-August should be adequate to reach a substantial portion of the raccoon population in eastern Ontario. Costs for ORV using V-RG baits in this study varied between \$147.00/km^2 and \$273.00/km^2 (Cdn) with bait density being the primary cost determinant. This is comparable to the costs incurred in Ohio during 1997–2000, where costs for baiting at densities of 79–93 baits/km^2 ranged between \$102.00 and \$261.00/km^2 (US) (mean \$153.00/km^2; about \$180.00/ km^2 Cdn during 2006) (Foroutan et al., 2002). It is estimated that rabies prevention costs in the US are about \$230 million to \$1 billion (US) annually (Rupprecht et al.,

1995; Recuenco et al., 2007). Because the raccoon rabies enzootic area covers an approximate 1 million km^2 area in eastern North America (Rosatte et al., 2001), rabies control costs in the $150.00 to $300.00/km^2 range are reasonable and worth the investment with substantial benefits to be gained if the disease is controlled (Kemere et al., 2002). For example, the costs for the V-RG baiting program, as well as TVR and PIC during 1999–2005 in Ontario averaged about $2 million/yr Cdn. The estimated cost if raccoon rabies became epizootic in Ontario is conservatively an additional $8 million–$12 million Cdn annually due to increased costs for human postexposure treatments (2,000–4,000), rabid animal diagnoses (1,500–2,000 cases) as well as costs to investigate suspect rabid animal incidents (Rosatte et al., 2001). Thus the annual savings to Ontario during 1999–2005 is estimated at about $6 million–$10 million Cdn/yr.

V-RG has been used extensively for the oral immunization of raccoons against rabies in the United States (Rupprecht et al., 1986, 1988, 2004; Hanlon et al., 1993, 1998; Slate et al., 2005), in Europe for the control of rabies in foxes (Aubert et al., 1994), and in Texas for the control of rabies in coyotes (*Canis latrans*) and gray foxes (*Urocyon cineroargenteus*) (Fearneyhough et al., 1998). Although V-RG has proved effective for control of rabies in foxes and coyotes, it is less clear whether V-RG has been effective in the field for raccoon rabies control. Despite the widespread use of V-RG in the eastern US, raccoon rabies had become enzootic over an approximately 1 million km^2 area by the late 1990s (Jenkins and Winkler, 1987; Winkler and Jenkins, 1991). In contrast, the eastern Ontario raccoon rabies outbreaks that began in 1999 appear to have been well-controlled with no new cases detected since September 2005, despite high raccoon densities (Wandeler and Salsberg, 1999; Rosatte et al., 2001, 2005, 2006).

The strategy for controlling the raccoon variant of rabies in Ontario included the use of three different tactics; population reduction (PR), trap–vaccinate–release (TVR), and oral rabies vaccination with V-RG baits (ORV). The first line of defense within 10 km of any case of raccoon rabies was PR and Trap-Vaccinate release (Rosatte et al., 2001). This tactic was effective in removing animals that were clinically rabid or incubating rabies for which vaccination is not effective. Trap-vaccinate-release was effective because a significant portion of the vector population could be vaccinated and the intramuscularly injected vaccine was extremely effective (Rosatte et al., 1992, 1993, 2001). The second line of defense, TVR, was also used proactively and reactively along the St. Lawrence River as well as in the Niagara region of Ontario (Rosatte et al., 2007b). The ORV with V-RG baits was used as the third line of defense on the perimeter of PR and TVR areas because the vaccine used orally was not as effective as the IM injected vaccine used in TVR, but ORV was cost effective for covering large geographic areas. When we used PR, TVR, and ORV to contain a case(s) of raccoon rabies, we called this point infection control. This multi-tactic approach to raccoon rabies control in Ontario, each tactic with its own advantages, was probably a wise strategy because if containment failed, the disease could have moved very rapidly across southern Ontario (100,000 km^2 area) due to high raccoon population densities (Robbins et al., 1998; Rosatte, 2000; Rosatte et al., 1991, 2001, 2007b).

It is not clear what the relative contribution of each tactic (PR, TVR, ORV, or PIC) was to the overall efficacy of the Ontario rabies control program; however, if PR and TVR were not employed, in all likelihood, rabies would have spread rapidly across the province. What is known is that the raccoon variant of rabies has not been reported in Ontario since September 2005 (to 1 July 2008) and appears to be

under control. Bait acceptance and serologic results from the present ORV study suggest that distribution of V-RG in the field as the sole tactic for raccoon rabies control in Ontario likely would not have been effective. It is evident that additional research is needed to increase knowledge regarding the factors influencing the success or failure of wildlife oral rabies vaccination programs. Given the high raccoon densities in Ontario and the continued threat of movement of raccoon rabies from enzootic regions of the US as well as eastern Canada, the data presented here also indicate that investigation of new or improved vaccines for the oral immunization of raccoons is warranted.

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