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High-Density Baiting with ONRAB® Rabies Vaccine Baits to Control Arctic-Variant Rabies in Striped Skunks in Ontario, Canada

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ABSTRACT: The Arctic variant of rabies virus has been maintained in striped skunks in small foci in southwestern Ontario, Canada, despite the control of the disease in red foxes. To control the disease in skunks, high-density baiting with ONRAB® oral rabies vaccine baits was conducted by air and by hand distribution of baits in the vicinity of skunk cases. During 2009, antibody prevalences in skunks were higher in areas baited at a density of 300 baits/km^2 and flight-line spacing of 0.25 km than at 0.5-km spacing. Once an area containing Arctic-variant cases was treated with high densities of ONRAB baits, the disease did not reoccur in skunks in those areas. During 2009, only eight skunks were diagnosed with the Arctic variant of rabies virus in Ontario.

Key words: *Mephitis mephitis*, Ontario, oral rabies vaccination, rabies, rabies control, striped skunk, vaccine.

An Arctic variant of rabies virus (RV) has been prevalent in Ontario, Canada since the mid-1950s (Johnston and Beauregard, 1969). Until the late 1980s, red foxes (*Vulpes vulpes*) and striped skunks (*Mephitis mephitis*) accounted for the majority (approximately 60–65%) of rabies cases in Ontario (Rosatte, 1988). As red foxes were considered the primary wildlife RV reservoir species in Ontario, wildlife disease models predicted that the Arctic variant of RV would not be sustained in Ontario if 60–70% of the red fox population were vaccinated (MacInnes et al., 1988). A system was developed to orally immunize foxes by aerially distributing vaccine baits (ERA®) in the rabies enzootic areas of southern Ontario (Black and Lawson, 1980; MacInnes et al., 2001). The disease in foxes was under control in rural areas of Ontario by the mid-1990s (MacInnes et al., 2001) and had been eliminated from metropolitan Toronto by 1996 (Rosatte et al., 2007a) and from eastern Ontario by 1997 using ERA vaccine baits.

After ERA baiting campaigns, rabies disappeared from red fox and skunk populations in most of southern Ontario. For unknown reasons, however, the disease was maintained in striped skunks in small foci in southwestern Ontario (Nadin-Davis et al., 2006; Rosatte et al., 2007b). Previous studies showed that vaccines such as ERA and V-RG were ineffective in orally vaccinating skunks (MacInnes et al., 2001; Rosatte et al., 2008). Therefore, research was initiated to develop an oral vaccine capable of producing immunity in all three of the primary rabies host species (striped skunks, raccoons [*Procyon lotor*], and red foxes) in southern Ontario (Prevec et al., 1990; Charlton et al., 1992; Lutze-Wallace et al., 1995; Rosatte et al., 2009a; Yarosh et al., 1996). Rabies vaccine, live adenovirus vector (AdRG1.3), named ONRAB,® was developed and shown to be safe when administered orally to captive target and nontarget species (Knowles et al., 2009). During 2006 and 2007, the Ontario Ministry of Natural Resources (OMNR) field-tested ONRAB in southwestern Ontario at a variety of densities and seroconversion results indicated that the vaccine was efficacious in raccoons and skunks (Rosatte et al., 2009b). In this paper we report the results of the use of high densities of ONRAB baits around the location of rabid skunk cases to control Arctic-variant RV in striped skunks in southwestern Ontario in 2008–2010.

TABLE 1. Summary of the aerial distribution of rabies vaccine baits in southwestern Ontario during 2008 and 2009.

Date	Vaccine bait type	Bait density/ km^2	Flight-line spacing (km)	Number of baits distributed	Target species
11–14 August 2008	ONRAB	300	0.5	516,375	Skunks
15–19 September 2008	ONRAB	20	2.0	431,460	Foxes
17 September 2008	ERA	20	2.0	65,016	Foxes
13–24 September 2009	ONRAB	20	2.0	194,672	Foxes
13–24 September 2009	ERA	20	2.0	196,601	Foxes
13–24 September 2009	ONRAB	300	0.5	572,535	Skunks
13–24 September 2009	ONRAB	300	0.25	172,260	Skunks

During 2008 and 2009, the OMNR attempted to control foci of Arctic-variant RV cases in striped skunks in southwestern Ontario by aerial distribution of high densities of ONRAB oral rabies vaccine baits. In response to isolated cases that occurred after aerial baiting, OMNR staff hand-placed baits during 2009 and 2010. ONRAB and ERA vaccine baits were also distributed at a low density to maintain control of rabies in the red fox population during 2008 and 2009.

ONRAB is a human adenovirus type 5 recombinant virus expressing the rabies glycoprotein gene. Virus was prepared at the National Research Council, Biotechnology Research Institute, Montreal, Quebec, Canada, and shipped as bulk vaccine to Artemis Technologies Inc., Guelph, Ontario, Canada, where ONRAB vaccine baits were manufactured during 2008 and 2009. Each bait contained 1.8 ($\pm$0.1) ml of ONRAB vaccine (titer $\geq 10^{9.5}$ 50% cell culture infectious dose [$CCID_{50}$]/ml]) in an elongated plastic blister pack that was coated with an attractant matrix. The size of the vaccine bait, components of the bait matrix, and vaccine container material were described by Rosatte et al. (2009b).

We used the spatial distribution of rabid animals (wildlife, domestic livestock, and pets) in 2007 and 2008 to determine the placement of ONRAB rabies vaccine baits during the summer of 2008. As ONRAB had yet to be licensed in Ontario, approval to distribute ONRAB was requested and was granted by the Canadian Food Inspection Agency, Canadian Centre for Veterinary Biologics. To control rabies in skunks, 516,375 ultralite (UL) baits containing ONRAB oral rabies vaccine were distributed in three small plots (64 km^2 each in the counties of Bruce, Grey, and Wellington, centered at 43°59′N, 80°58′W) and one large study plot (2,382 km^2 in Perth, Waterloo, and Wellington counties, centered at 43°38′N, 80°44′W). Locations of the small plots were determined by individual cases of rabid skunks and the size and location of the large plot was determined by clusters of rabies cases in skunks. All skunk plots were baited at a density of 300 baits/km^2 and a flight-line spacing of 0.5 km during 11–14 August 2008. Flight-line spacing was defined as the distance between parallel flight lines (Table 1). During 15–19 September 2008 we distributed 431,460 UL baits containing ONRAB oral rabies vaccine over a 25,809-km^2 area, at a density of 20 baits/km^2 and flight-line spacing of 2.0 km to target foxes (essentially the same area was baited during 2008 as during 2009; Fig. 1, Table 1). We distributed baits within a 50-km radius of rabies case locations.

From 13–24 September 2009 we aerially distributed 1.1 million ONRAB and ERA rabies vaccine baits over 27,500 km^2 (Fig. 1; Table 1) at 20 baits/km^2 and 2.0-km flight-line spacing (Fig. 1). This included 196,601 UL baits containing ERA rabies vaccine distributed in the northern portion of the baiting area (10,952 km^2). In addition, 194,672 UL baits containing ONRAB were distributed in the southern

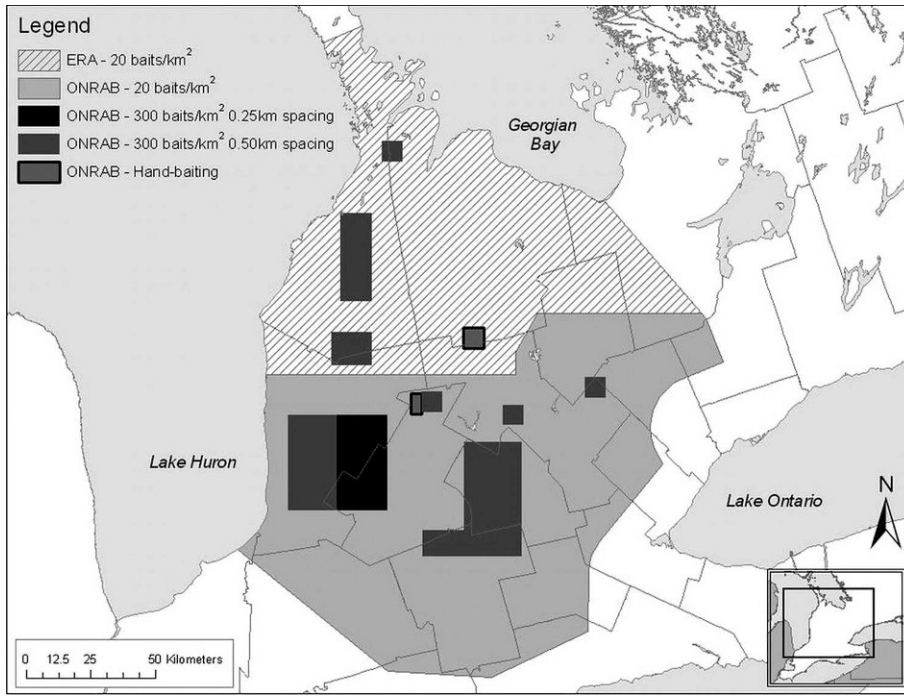

FIGURE 1. Map of southwestern Ontario, Canada depicting bait density and locations of plots baited with ONRAB rabies vaccine baits during 2009. Rabid skunk (*Mephitis mephitis*) cases were located within the plots baited at 300 baits/km^2 with 0.50-km flight-line spacing, but not in the plots at 0.25-km spacing.

portion (16,539 km^2) of the baiting area (Fig. 1). In areas where skunk cases had occurred during fall 2008 through summer 2009, 744,795 ONRAB baits were aerially distributed at 300 baits/km^2 and at flight-line spacing of 0.5 km (Fig. 1; Table 1). These were called rabies management plots. As before, the locations of small plots (64 km^2–8×8 km) were based on single cases of rabid skunks and sizes and locations of larger plots (204 km^2, 420 km^2, and 1,150 km^2) were determined by clusters of rabies cases in skunks. There was also one large experimental plot (1,422 km^2) designed to assess the effect of flight-line spacing (0.25 km versus 0.50 km) on seroconversion in skunks (Fig. 1). Adjacent experimental plots were selected to avoid bias due to differences in habitat, skunk densities, or edge effects. Surveillance operations involving live trapping and serum collection from skunks were conducted 5–6 wk postbaiting. The same methods were used in 2008 and 2009. Differences in serology values for a flight-line spacing of 0.25 and 0.5 km were compared using contingency tables in Statistica 6.0 (StatSoft, 1999).

In response to two rabid skunk cases in southwestern Ontario in late September 2009 after the scheduled 2009 aerial baiting program, we distributed 30,000 ONRAB baits. Baits were hand-distributed at a density of 300/km^2 in a 64-km^2 area within 4 km of each case during 1–2 October 2009. One bait was placed every 10 m along three transects/km^2 of habitat. In addition, we deployed 99,000 ONRAB baits in a 304-km^2 area during March and May 2010 in response to seven cases of Arctic-variant rabies during December 2009 (one skunk and one cow) and April and May 2010 (five skunks). The same methods (hand-baiting at 300 baits/km^2) were used as in October 2009 except that a helicopter was used to distribute about

TABLE 2. Comparison of competitive enzyme-linked immunosorbent assay (cELISA) values (percentage of sera with given inhibition values) for skunk (*Mephitis mephitis*) sera collected in areas aerially baited at a density of 300 ONRAB oral rabies vaccine baits/km^2 and at a flight-line spacing of 0.25 km or 0.5 km in southwestern Ontario, September 2008 and September/October 2009.

Year	Flight-line spacing (km)	Inhibition value <16% (*n*)	Inhibition value ≥16 and <26% (*n*)	Inhibition value ≥26% (*n*)	Inhibition value ≥16% (*n*)
2008	0.5	40 (66/163)	32 (52/163)	28 (45/163)	60 (97/163)
2009	0.5	64 (36/56)	16 (9/56)	20 (11/56)	36 (20/56)
2009	0.25	38 (28/73)	27 (20/73)	34 (25/73)	62 (45/73)

44,000 ONRAB baits. Baiting was delayed because skunks den during the winter in Ontario.

To assess the ability of ONRAB to elicit an immune response, skunks were live-trapped, processed, and sera sampled 5–6 wk postbaiting during the 2008 and 2009 operations as described by Rosatte et al. (2009b). A competitive enzyme-linked immunosorbent assay (cELISA) was used to detect rabies antibody in skunk sera (Elmgren and Wandeler, 1996). The cELISA detects antibodies that are capable of binding RV glycoprotein and is a measure of the humoral immune response to vaccination. Results are expressed as the percentage inhibition of binding of the monoclonal antibodies. Values <16 indicate that the animal did not produce detectable antibody; values ≥26 indicate the production of glycoprotein-specific antibodies. Values ≥16% and <26% are classified as "suspect positive," indicating the detection of binding activity in the assay that may or may not be attributable to specific antibodies. At the 26% cutoff value, the cELISA has a sensitivity of 85% and a specificity of 96% as compared with a virus neutralization assay with a positive cutoff value of 0.5 IU/ml. Appropriate cutoff values were determined by receiver operating characteristics analysis.

Postbaiting surveillance in the high-density, experimental baiting plot took place from 22 September to 3 October 2008. We live-trapped and collected sera from 205 striped skunks (169 new captures and 36 recaptures) and 163 serum samples were screened for rabies antibody. Twenty-eight percent (45/163) were strong positive (cELISA ≥26%; Table 2) and 60% (97/163) were suspect positive (cELISA ≥16%). Postbaiting surveillance also was conducted in the experimental high-density ONRAB plots (flight-line spacing comparison study) 18–30 October 2009. Of 184 captured striped skunks (136 new captures and 48 recaptures), serum was collected from 129. Of the sera collected from areas baited at 300 baits/km^2 and 0.25-km flight-line spacing, 34% (25/73) were strong positive and 62% (45/73) were suspect positive (Table 2). In the suspect-positive category, seroconversion in skunks was significantly greater in areas baited using parallel flight lines spaced 0.25 km apart (P=0.003) compared with 0.5-km spacing. However, the differences were marginally insignificant in the strong-positive category (P=0.067). Seroconversion rates in 2008 (flight-line spacing 0.5 km) and 2009 (flight-line spacing 0.25 km) were not significantly different (P=0.302).

Although baiting occurred at very high densities (300 baits/km^2) and more than 1 million ONRAB baits were distributed over 27,000 km^2, only 24 people reported finding ONRAB baits during 2009. Of those, 12 baits were contacted by dogs and there were no human contacts with vaccine. No adverse reactions to the bait or vaccine were reported.

The cost to distribute ONRAB oral rabies vaccine baits using Twin Otter aircraft at 300 baits/km^2 averaged $606 Canadian dollars (Cdn)/km^2 in Ontario during 2009. That cost included the

vaccine baits (89% of total cost), aircraft, staff salaries, supplies, and equipment. The costs of baiting at 300 baits/km^2 at flight-line spacings of 0.5 km and 0.25 km were $603 Cdn and $618 Cdn/km^2, respectively. The cost of using a helicopter during May 2010 for baiting at 300 baits/km^2 was $639.99 Cdn/km^2.

During 2009 there were 18 cases of Arctic-variant RV infection reported in Ontario (eight striped skunks, five cattle, three sheep, and two red foxes). The number of reported cases was down significantly from 2008, when there were 41 cases of Arctic-variant RV infection reported (25 skunks, seven cattle, three dogs, two cats, two horses, two red foxes; Fig. 2). In addition, during 2008, 18.9% (25/132) of skunks submitted for testing were rabid with Arctic-variant RV compared with 9% (8/86) in 2009.

The Arctic variant of RV has been reported in Ontario for more than 5 decades. During the 1980s more than 2,000 rabies cases were reported each year and more than 2,000 people received postexposure prophylaxis annually (Rosatte, 1988). Costs associated with rabies-related activities averaged $6 million Cdn/yr in Ontario during this period. In view of the public health, animal welfare, and financial costs of rabies in Ontario, it was economically justifiable to develop methods to control and eventually eliminate the Arctic variant of RV.

Since 1989, large-scale aerial distribution operations using ERA oral rabies vaccine baits have controlled Arctic-variant RV in foxes in Ontario (MacInnes et al., 2001; Rosatte et al., 2007a,b). As predicted, this variant disappeared from most of southern Ontario with control in foxes (MacInnes et al., 1988). However, residual foci of this variant of rabies in skunks has been maintained in southwestern Ontario, with spillover back to foxes as well as to pets and domestic stock (Nadin-Davis et al., 2006). Clearly, additional research was needed to develop a tactic to control the disease in skunks and

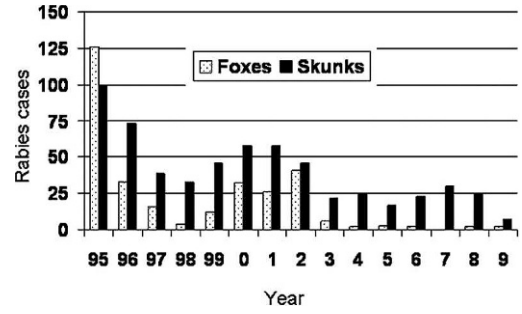

FIGURE 2. Numbers of rabies cases in striped skunks (*Mephitis mephitis*) and red foxes (*Vulpes vulpes*) in Ontario, Canada 1995–2009.

prevent its continued maintenance in southern Ontario.

Because of the sedentary nature of skunks, high densities of baits containing oral rabies vaccine are required to immunize a substantial portion of the skunk population even though skunk densities in southwestern Ontario are about 1 to 2/km^2. Mean home ranges of skunks in Ontario range from <1 km^2 in urban habitats to 1–3 km^2 in rural areas (Rosatte and Lariviere, 2003). This suggests that more vaccine baits are required per unit area of baitable habitat so that each skunk will find at least one bait in its home range. Our study focused on determining the feasibility of the aerial distribution of baits containing ONRAB oral rabies vaccine at high density to immunize and control Arctic-variant RV in striped skunks in Ontario. ONRAB has been extensively safety tested in the laboratory (Knowles et al., 2009) and there were very few human or companion-animal contacts with the vaccine after several million doses were distributed in Ontario during 2006–2010.

Serology results from the 2009 field study high-density plot (300 baits/km^2) showed an improved immune response in skunks at narrower flight-line spacing for the cELISA cutoff value of $\geq$16% inhibition. The higher cutoff value of 26% inhibition reflected a marginally insignificant difference in antibody prevalence between flight-line spacings of 0.25 and

0.5 km. Because the study plots were adjacent to one another in areas of similar habitat and skunk density, we believe our comparison of flight-line spacing during 2009 was valid. In addition, the adjacent placement of the study plots, narrow window between baiting periods and surveillance sample collections, and buffer between the surveillance sample areas negated any possible confounding effects due to immigrating and emigrating animals.

The OMNR has used high-density baiting with ONRAB to control rabies in skunks, first experimentally in 2006 and 2007, and then as a management practice during 2008–2010. Only eight rabid skunks were reported in Ontario during 2009. Once an area with Arctic-variant RV cases was treated with high-density ONRAB baits, the disease was not detected again in skunks in those areas (to August 2010). Currently, rabies management strategies in Ontario consist of low-density baiting (20 baits/km^2) for controlling rabies in red foxes, intermediate bait densities (75 to 100/km^2) for responding to raccoon-variant RV cases in raccoons, and high-density baiting (300/km^2) for the control of rabies in striped skunks. These strategies are designed to maximize the likelihood of rabies control success yet minimize costs.

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