SHORT COMMUNICATIONS

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Local Surveillance and Control of Raccoon Rabies Virus in Striped Skunks (*Mephitis mephitis*) in Southwestern New Brunswick, Canada

Michael R. Allan,[1,3,4] James P. Goltz,[1] Paul Turmel,[2] and Tony Cole[2] [1]Government of New Brunswick, Department of Agriculture, Aquaculture, and Fisheries, Animal Health Services Branch, Provincial Veterinary Laboratory, 850 Lincoln Road, PO Box 6000, Building 700A, Fredericton, New Brunswick E3B 5H1, Canada; [2]Government of New Brunswick, Department of Energy and Resource Development, Hugh John Flemming Forestry Centre, 1350 Regent Street, PO Box 6000, Fredericton, New Brunswick E3B 5H1, Canada; [3]Current address: Ontario Ministry of Natural Resources and Forestry, Trent University, DNA Building, 2140 E Bank Drive, Peterborough, Ontario K9L 1Z8, Canada; [4]Corresponding author (email: mike.allan@ontario.ca)

ABSTRACT: Targeted surveillance for raccoon rabies virus was conducted between February and May 2017, near Waweig, New Brunswick, Canada, in response to detection of a rabid striped skunk (*Mephitis mephitis*) on 8 February 2017. A total of six skunks, 11 raccoons (*Procyon lotor*), and two porcupines (*Erethizon dorsatum*) were live-trapped, euthanized, and tested for rabies virus antigens using the direct rapid immunohistochemical test. Of these, only two skunks tested positive for rabies. All three rabid skunks came from the same location, an abandoned barn used as a denning site. Four of five skunks removed from this barn were males. Feeding, aggression, extreme response to noise and light stimuli, and exposure to porcupine quills were observed in two rabid skunks. No additional cases of rabies in wildlife were detected in the area since 8 March 2017. A targeted surveillance approach that removed potentially infected wildlife followed by localized oral rabies vaccine distribution was implemented in this locality.

Key words: Denning, *Mephitis mephitis*, oral rabies vaccination, population reduction, rabies, striped skunk, targeted surveillance.

Raccoon (*Procyon lotor*) rabies in Canada has been controlled by several methods, including population reduction, oral rabies vaccination (ORV), and trap-vaccinate-release (Rosatte et al. 2001, 2009). The main tactic used to control wildlife rabies incursions among mesocarnivores in Canada is ORV in and around affected areas with ONRAB® (Artemis Technologies, Guelph, Ontario, Canada). We describe the use of targeted surveillance, focused population reduction, and ORV with ONRAB distribution as a combined strategy to manage rabies in the village of Waweig, Charlotte County, southwestern New Brunswick (NB), Canada. It includes observations on winter denning behavior and clinical signs of rabies in striped skunks (*Mephitis mephitis*), a species commonly affected by spillover infection with raccoon rabies virus in eastern Canada (Fehlner-Gardiner et al. 2012; Mainguy et al. 2012; Lobo et al. 2018)

On 8 February 2017, a wild striped skunk (case accession no. 2083.17) exhibiting clinical signs consistent with rabies was reported to the NB Department of Energy and Resource Development by a homeowner in Waweig. The skunk was seen wandering in the snow during the day, embedded with porcupine (*Erethizon dorsatum*) quills and showing no fear of humans. The skunk was captured in a baited live trap in an abandoned barn, shot, and submitted for rabies testing. Brain samples (smears of the brain stem, cerebellum, and hippocampus) tested positive for rabies virus antigens with the direct rapid immunohistochemical test at the Provincial Veterinary Laboratory in Fredericton, NB (Rupprecht and Niezgoda 2006). The positive test result was confirmed by fluorescent antibody testing, and the variant was identified as raccoon rabies virus at the Canadian Food Inspection Agency's Centre of Expertise for Rabies in Ottawa, Ontario (Kissling 1975; Nadin-Davis 1998).

We investigated the abandoned barn, referred to as the Waweig barn, on 20 February 2017 and found decomposed remains of two raccoons, scats, and tracks of raccoons, skunks, and porcupines. Fresh tunneling into old hay stored in the barn revealed what appeared to be a wildlife denning location. The apparent perpetuation of rabies in this area prompted a focused removal of target

TABLE 1. Rabies virus antigen (RVA) diagnostic test results for wild striped skunks (*Mephitis mephitis*), raccoons (*Procyon lotor*), and a porcupine (*Erethizon dorsatum*) removed during a targeted surveillance operation by the Department of Agriculture, Aquaculture, and Fisheries Rabies Program, in Waweig, New Brunswick, Canada, February–May 2017.

Species	No. tested	Diagnostic test performed[a]	No.		Location in latitude, longitude
			RVA positive	RVA negative	
Striped skunk	5	dRIT; dFA[b]	3	3	45°13′14″N, −67°07′48″W
Striped skunk	1	dRIT; dFA[b]	0	0	45°14′20″N, −67°07′19″W
Raccoon	5	dRIT	0	5	45°14′38″N, −67°07′12″W
Raccoon	2	dRIT	0	2	45°14′38″N, −67°08′10″W
Raccoon	2	dRIT	0	2	45°14′06″N, −67°07′22″W
Raccoon	1	dRIT	0	1	45°14′31″N, −67°07′12″W
Raccoon	1	dRIT	0	1	45°13′44″N, −67°07′48″W
Porcupine	1	dRIT; dFA[c]	0	1	45°13′44″N, −67°07′48″W
Porcupine	1	dRIT; dFA[c]	0	1	45°13′4″N, −67°07′48″W

[a] dRIT = direct rapid immunohistochemical test; dFA = direct immunofluorescence assay.

[b] dFA was performed on presumably rabid skunks to confirm dRIT results, and rabies virus typing was also conducted.

[c] Both porcupines demonstrated inconclusive results with dRIT.

species (mainly raccoons and skunks) to determine the extent of infection, elimination of animals that may be incubating rabies virus, and ORV distribution to boost immunity in target wildlife species.

Between 17 February 2017 and 28 February 2017, 39 live traps baited with sardines were placed in abandoned buildings along a 4-km section of Upper Waweig Road, where the index case had occurred. A trapping effort of 310 trap nights yielded 40 captures, including four striped skunks, eight raccoons, 20 porcupines, two domestic cats (*Felis catus*), five red squirrels (*Tamiasciurus hudsonicus*), and one Norway rat (*Rattus norvegicus*). All captured raccoons and skunks and two porcupines, with evidence they may have been in contact with the index case (missing quills), were euthanized with a .22 caliber rifle shot to the heart. All nontarget animals were released unharmed. The direct rapid immunohistochemical test was conducted on three skunks and two porcupines from the Waweig barn and on eight raccoons and one skunk from other trapping locations along the Waweig Road. One skunk (case accession 2237.17) captured in the Waweig barn on 23 February 2017 was diagnosed as rabid, but all other animals tested negative (Table 1).

From 1 March 2017 to 1 May 2017, surveillance was continued in the Waweig barn and a neighboring farm building. A total effort of 243 trap nights resulted in the capture of one more raccoon and one additional skunk from the Waweig barn and two raccoons from a neighboring farm building. The skunk (case accession 3100.17) was captured on 8 March 2017 and tested positive for rabies virus antigens in the brain. All raccoons tested negative.

In April and August 2017, 500 ONRAB ORV baits (125 baits/km^2) and 900 ONRAB ORV baits (225 baits/km^2) were hand distributed in and around the Waweig barn and along Upper Waweig Road, respectively, to vaccinate resident and transient raccoons and skunks. In August 2017, ORV baits were distributed aerially at a density of 75 baits/km^2 over 5,700 km^2 in Charlotte and York counties, including the village of Waweig.

In summary, three of five striped skunks captured from the Waweig barn were rabid. All rabid skunks were males. Clinical signs of rabies were observed in two of the three rabid skunks. The clinically rabid index case was lured to a baited live trap and was observed eating sardines in the trap. The second rabid skunk (case accession 2237.17) with clinical signs was found alive in a trap, together with a

dead, nonrabid male skunk. The rabid skunk exhibited bouts of extreme aggression in response to noise (created while moving a clear plastic sheet to prevent handlers from being sprayed) and light (from a flashlight used inside the barn) stimuli. This live rabid skunk aggressively attacked the dead skunk inside the trap. The head of the dead skunk sustained severe trauma, including a fractured jaw, and the legs were fractured and chewed, either during the initial confrontation or during observation. No clinical signs were observed in the third rabid skunk (case accession 3100.17).

Clinical signs of rabies in captive and free-ranging skunks vary, with some animals demonstrating aggression, including biting and fighting with conspecifics, whereas others die without showing any abnormal behavior (Gough and Niemeyer 1975; Greenwood et al. 1997). The aggressive behavior of the rabid skunk (i.e., in the trap with the dead male skunk) may be related to rabies rather than territorial aggression. Male skunks generally avoid each other and are not considered territorial (Theimer et al. 2016). However, the capture of two free-ranging male skunks together within a single live trap was unusual. Wounds were observed on both of these skunks, resulting from conflict before or during capture.

As observed in the index case, exposure to porcupine quills may be suggestive of rabies in wildlife (Rosatte et al. 2006). Phobic spasms and hyperactive gag reflex have been observed in humans with rabies (Hemachudha et al. 2002), which could be associated with difficulty in swallowing, but the index case was observed ingesting sardines in the trap. Clinical signs observed in two of the three rabid skunks appeared to be between the prodromal and excitative stages of rabies.

Four of five skunks removed from the Waweig barn were males. The presence of multiple male skunks in this barn suggests that male skunks may den communally in winter for the benefits of thermoregulation or, possibly, that they were visiting the barn in search of females (Theimer et al. 2016, 2017). Female striped skunks often den communally, and a male may den with one or more females. However, males are reported to avoid each other and possibly compete for den sites containing multiple females (Sunquist 1974; Houseknecht and Tester 1978). The tunnels in the hay may have enabled multiple male skunks to live near each other without sharing immediate space. Our observations suggest that inactive barns stored with hay may offer winter denning opportunities and the potential for communal denning of single or multiple species. Activities associated with breeding and denning at these sites provide opportunities for intra- and interspecies contact and rabies virus transmission, as evidenced during outbreaks in Alberta and eastern Ontario (Rosatte 1984; Rosatte et al. 2006). From 2015 to 2017 in Charlotte and York counties, wildlife rabies surveillance data showed that 70% of raccoons and 45% of striped skunks with rabies were found between January and April. Similar findings were reported in eastern Ontario (Rosatte et al. 2006), suggesting that rabies virus transmission during the denning and breeding period is significant. Detection of three rabid skunks in a single barn suggests that communal denning sites may be areas of high risk in rabies endemic areas.

Animal health surveillance is a tool to monitor disease trends, to facilitate the control of disease or infection, and to provide data for use in risk analysis for animal or public health purposes (World Organization for Animal Health 2017). The targeted surveillance response to the detection of a rabid striped skunk at Waweig in the winter of 2017 was successful in detecting two additional rabid skunks at the same barn within a month of detection of the index case. Because the standard diagnostic laboratory test procedure for rabies in animals requires brain tissue, all animals that were tested for rabies virus antigens were euthanized, which effected limited population reduction in the geographic area where the index case was detected. The ONRAB oral rabies vaccine baits had been distributed by air at 75 baits/km^2 and flight line spacing of 1 km throughout

most of Charlotte County, including the village of Waweig, during August 2015 and 2016. Local detection of rabies at this site prompted ORV by hand in April and August 2017, in addition to vaccine baits that were distributed by air during 2017. No additional rabies cases have been detected since March 2017.

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