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THE ELIMINATION OF RACCOON RABIES FROM WOLFE ISLAND, ONTARIO: ANIMAL DENSITY AND MOVEMENTS

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ABSTRACT: During 1996 to 1998, an average of 52% to 55% of the raccoon (*Procyon lotor*) population on Wolfe Island, Ontario was vaccinated against rabies during proactive trap-vaccinate-release (TVR) operations. However, during 1999, the percent of the population vaccinated declined to 39% and an outbreak (6 cases) of raccoon rabies occurred on the island from December 1999 to January 2000. The raccoon population on Wolfe Island declined dramatically (71% reduction) from 1,067 raccoons (mean density=8.4/km^2 [6.4–12.4, 95% CI]) during 1999 to 305 raccoons (mean density=2.4/km^2 [0.87–4.1, 95% CI]) in the spring of 2000. Raccoon density immediately following the outbreak was significantly lower in cells with rabies cases, suggesting that rabies had a negative effect on population size. However, raccoon density had doubled by 1 yr following the outbreak. Movement of raccoons on Wolfe Island was as great as 24 km. Male raccoons moved greater distances than females. Movements to surrounding islands were also noted for raccoons ear tagged on Wolfe Island which indicates the island could serve as a focus for greater geographic rabies spread. Point infection control (PIC) during 2000, TVR during 2001–02, and the aerial distribution of Vaccinia-Rabies Glycoprotein (V-RG) baits during 2000 and 2003–05 were used to eliminate rabies from Wolfe Island. No cases have been detected since late January 2000 (to February 2007).

Key words: Density, Ontario, *Procyon lotor*, rabid raccoon, rabies, raccoon, raccoon rabies.

INTRODUCTION

The raccoon (*Procyon lotor*) rabies virus variant (hereafter referred to as raccoon rabies) was first reported in Ontario during July 1999 (Wandeler and Salsberg, 1999; Rosatte et al., 2001). From 1999 to 2006, 132 cases were reported in eastern Ontario (Rosatte et al., 2005, 2006). Six of those were found on Wolfe Island during December 1999 and January 2000. The Ontario Ministry of Natural Resources (OMNR) implemented proactive trap-vaccinate-release (TVR) programs on Wolf Island during 1996–2002 in response to the threat of rabies. Data collected during TVR operations on the island provided information on raccoon density and movements prior to, during, and following the raccoon rabies outbreak on Wolfe Island, and are the bases for this analysis.

MATERIALS AND METHODS

During 1996 to 2002 (except 2000), TVR was conducted on Wolfe Island, Ontario (44.2°N, 76.4°W), as part of the OMNR proactive program to prevent raccoon rabies from becoming established in Ontario. Wolfe Island is approximately 127 km^2 in area, about 28 km in length, and varies from about 2 km to 9 km in width (Figs. 1, 2). The Wolfe Island program was part of a larger TVR operation on the mainland (an approximate 500–800 km^2 area) adjacent to Wolfe Island (Fig. 1).

Tomahawk #106 and #108 livetraps (Tomahawk Live-tap Co., Tomahawk, Wisconsin, USA), baited with sardines, were set in each of 10 trapping cells on Wolfe Island during the summer/fall of each year (Fig. 2). Trapping normally consisted of an initial period of 4 nights/wk for 2 wk (75 traps/cell), followed by a trapping period of 8 nights over a 2 wk period (25 traps/cell). However, in 2001–02, trapping effort was increased to 100 traps/cell for 4 nights/wk over a 3 wk period. Each of the 10 trapping cells was 8.5–16 km^2 in area. All

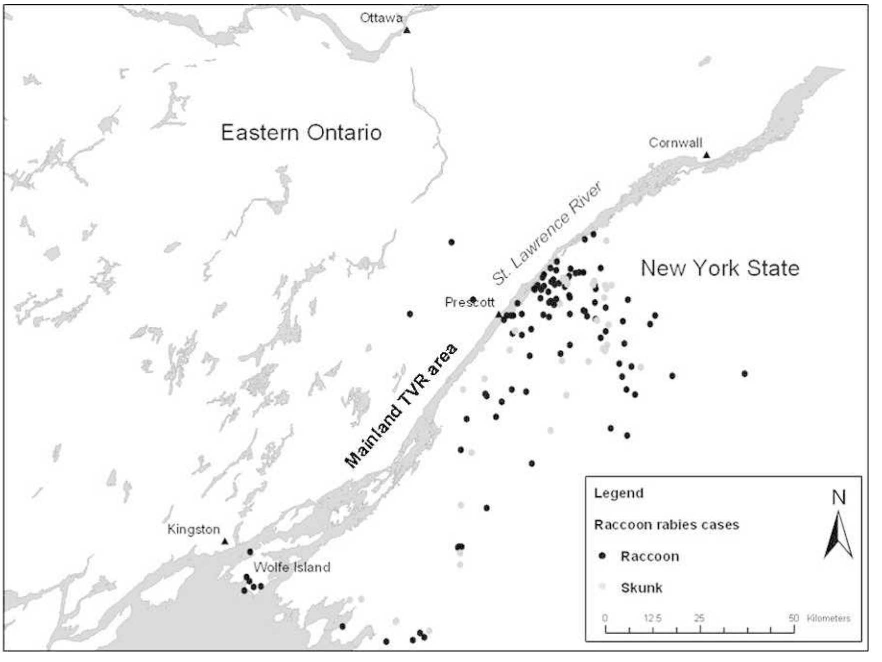

FIGURE 1. Location of raccoon rabies cases during 1999–2000 on Wolfe Island, the mainland of eastern Ontario, and the New York State side of the St. Lawrence River. Thirty-eight cases of rabies in raccoons and skunks occurred in Jefferson County, New York, during 1999; 121 cases of rabies in raccoons and skunks occurred in St. Lawrence County, New York during 1999. Rabies location data were acquired from Cornell University. Rabies case locations are approximate.

captured raccoons and skunks (*Mephitis mephitis*) were ear-tagged for identification (numbered sizes 3 for raccoons and 1 for skunks, National Band and Tag Co., Newport, Kentucky, USA), vaccinated by an injection (1 ml) of Imrab® 3 inactivated rabies vaccine (Merial Inc., Athens, Georgia, USA), and released at the point of capture (Rosatte et al., 1990). The location (Universal Transverse Mercator Coordinates) (UTMC) for each captured animal was determined with a GPS unit (Magellan Trailblazer 300, Magellan Systems Corp., San Dimas, California, USA).

Following the diagnosis of six cases of raccoon rabies in raccoons from Wolfe Island during December 1999 and January 2000 (Fig. 2), a point infection control (PIC) program was implemented during April to June 2000 to remove incubating and clinically suspect raccoons to contain the disease. All live-captured raccoons and skunks were euthanized via an intracardiac injection of 1–2 ml T-61 euthanasia solution (Hoechst Canada, Inc., Regina, Saskatchewan, Canada) following immobilization with 1–2 ml intramuscular injection of ketamine hydrochloride (Ketaset-Rogar/STB Inc., London, Ontario, Canada) (100 mg/ml) and 0.1–0.2 ml of xylazine hydrochloride (Vet-A-Mix, Shenandoah, Iowa). Specimens were submitted to the Canadian Food Inspection Agency, Ottawa Laboratory Fallowfield, Nepean, Ontario, for rabies diagnosis using a fluorescent antibody test (FAT) (Webster and Casey, 1988). Recaptured raccoons and skunks from a previous TVR program were revaccinated with Imrab® 3 and released.

Vaccinia- Rabies-Glycoprotein (V-RG) baits were aerially distributed (OMNR Twin Otter aircraft) at a density of 75 or 150 baits/km^2 and at a flight line spacing of about 1.5 km on Wolfe Island during 26–27 June 2000, and during August of subsequent years from 2003 to 2005. Bulk oral vaccine was purchased from Merial Inc., Athens, Georgia, and vaccine-baits (1.8 ml V-RG/bait) were manufactured at Artemis Technologies Inc., Guelph, Ontario.

Raccoon population estimates during 1996 to 2002 (excluding 2000) were determined using mark-recapture data (collected during TVR programs) and a Petersen model (Krebs, 1989). Raccoon population estimates were derived for the period April to June 2000, using the Leslie Regression model (catch/unit effort) for exploited populations (Krebs, 1989). A students' *t*-test (Statistica 6.0 software,

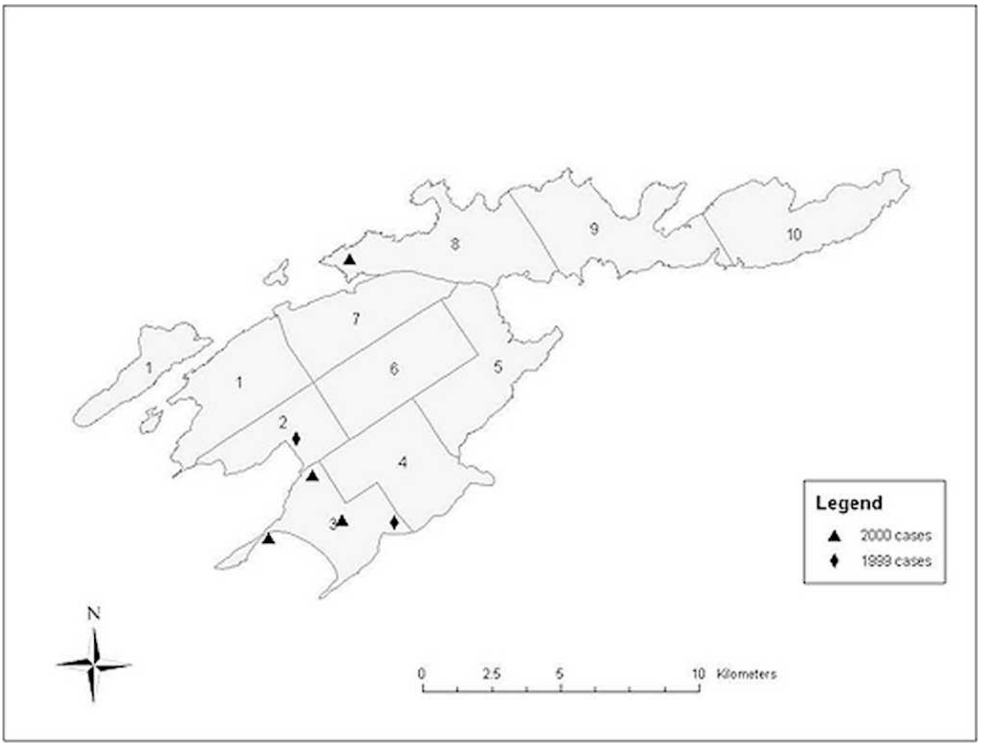

FIGURE 2. Location of trapping cells and raccoon rabies cases on Wolfe Island, Ontario during 1999–2000.

StatSoft, Tulsa, Oklahoma, USA) was used to determine if there were differences in raccoon density among cells and among years (Zar, 1974). Percent vaccinated data were normalized using an arcsine transformation, then among-area differences were compared using a *t*-test (Zar, 1974).

Movements of raccoons on Wolfe Island were determined using the location of recaptured animals (identified by ear tag number) and the UTMC location recorded at the capture/recapture locations. Raccoon capture and recapture locations and distances traveled by each raccoon were calculated and plotted using GIS software (Arcview 3.2). Straight-line distances from points of capture to points of recapture were used in the analysis. Where more than one recapture location was available for an individual animal, the cumulative distance between the points was used to provide an indication of the movement capability of raccoons as well as the potential for infected raccoons to transmit rabies. Data were tested for normality using Levene's test for homogeneity of variance, and were subsequently log-transformed. Movement data were tested for among-year differences using an independent *t*-test (Zar, 1974). When no differences were found, data were pooled and analysed using a two-factor analysis of variance for differences between sex and age (Zar, 1974). Differences in movement prior to and following the population reduction program were tested using an analysis of variance with treatment and sex being the independent variables.

RESULTS

Rabies control operations

From 1996 to 1998, an average of 52–55 % of the raccoon population on Wolfe Island was vaccinated annually against rabies during TVR operations (Table 1). Only 39% of the population was vaccinated in 1999 and six cases of raccoon variant rabies were reported on Wolfe Island from December 1999 to January 2000. A population reduction program was implemented during April to June 2000 and the raccoon population was reduced by 58% (Table 2). At that time, only 21% of the Wolfe Island raccoon population had been

TABLE 1. Estimated raccoon population size, density, and percent of population vaccinated for Wolfe Island, Ontario, 1996–2002.[a]

TVR program date[a]	Trap nights (*n*)	Sample size (*n*)[a]	Raccoon population estimate[b] Mean (95% CI)	Raccoon density/km² Mean (95% CI)	Estimated % of raccoon population vaccinated Mean (95% CI)
July–Sept 1996	3,792	379	547 (446–728)	4.3 (3.5–5.7)	55 (42–68)
Aug–Sept 1997	7,387	641	923 (765–1,199)	7.3 (6.0–9.4)	52 (40–63)
June–Oct 1998	7,363	542	857 (709–1,104)	6.8 (5.6–8.7)	52 (40–63)
June–Nov 1999	6,410	495	1067 (814–1,569)	8.4 (6.4–12.4)	39 (26–51)
Apr–June 2000[a]	7,149	239	305 (111–515)	2.4 (.87–4.1)[c]	21 (12–57)[d]
Sept–Oct 2001	9,991	447	630 (492–912)	5.0 (3.9–7.2)	52 (36–67)
July–Aug 2002	11,786	1,100	609 (565–662)	4.8 (4.5–5.2)	84 (78–90)

[a] There was no trap-vaccinate-release (TVR) program on Wolfe Island during 2000. Instead, a population-reduction program was implemented during April to June and V-RG baits were aerially distributed during the late summer. Sample size includes recaptures.

[b] The study area was 127 km.²

[c] Preparturition density which was statistically lower than density in other years.

[d] Estimated percent of the raccoon population during April–June 2000 that was vaccinated against rabies during the previous year.

vaccinated previously (Table 1). Those animals were re-vaccinated and released. In addition, during April–June 2000, the percent of the raccoons on Wolfe Island that had been vaccinated previously in 1999 in cells with rabies cases was lower (mean=11.9%, SD=14.1), although not significantly, than in cells without rabies cases (mean=25.8%, SD=14.3; t=-1.77, P=0.11) (Table 2). Raccoon population size was also lower, although not significantly, in those cells with rabies (mean=13.3 SD=4.6) than in cells without rabies cases (mean=37.9, SD=11.0; t=-1.4, P=0.19) (Table 2).

The V-RG baits (9,675) were aerially distributed on Wolfe Island during the summer of 2000. Trapping effort during TVR operations was increased during 2001 and 2002 and 52% and 84%, respectively, of the raccoon population was vaccinated against rabies during those 2 yr (Table 2). The V-RG baits were distributed each year on Wolfe Island during

TABLE 2. Rabies vaccination status of raccoons and the percent of the population euthanized on Wolfe Island, Ontario, April to June 2000.[a]

Trapping cell number	Rabies cases/cell	Raccoon sample size (*n*)[a]	Estimated raccoon population size Mean (95%CI)	Estimated % of 2000 raccoon population previously vaccinated during 1999 Mean (95%CI)	Estimated % of 2000 raccoon population euthanized Mean (95%CI)
1	0	15	15.3 (13.1–17.6)	20 (17.1–23)	78.4 (68.2–91.6)
2	1	5	6.1 (0–13.2)[b]	0[b]	82 (37.9–100)
3	4	10	11.9 (0–23.7)[b]	8.4 (4.2–100)[b]	75.6 (38–100)
4	0	17	18.7 (10.9–26.4)	21.4 (15.2–36.7)	69.5 (49.2–100)
5	0	37	48.4 (16.6–80.2)	10.3 (5–24.1)	66.1 (40–100)
6	0	15	15.3 (8.4–22.3)	52.3 (35.9–100)	45.8 (31.4–83.3)
7	0	21	21.0 (17.4–24.5)	38 (32.7–46)	62 (53.1–74.7)
8	1	23	21.8 (16.9–22.6)[b]	27.5 (26.5–35.6)[b]	78 (75.2–100)
9	0	34	53.5 (10–117.6)	20.6 (9.4–100)	43 (19.6–100)
10	0	62	93.4 (17.6–167.2)	18.2 (10.2–96.6)	48.2 (26.9–100)
All	6	239	305.4 (111–515.3)	20.6 (12.2–56.8)	57.6 (34.2–100)

[a] Raccoons that were previously vaccinated against rabies, as evidenced by ear tags, were released upon capture; non-ear tagged raccoons were euthanized. Sample size includes both euthanized, and vaccinated and released, raccoons.

[b] Mean raccoon population size and % previously vaccinated was lower than in cells without rabies cases.

TABLE 3. Estimated raccoon density in trapping cells on Wolfe Island during June to November 1999 and April to June 2000, with respect to cell distance from rabies cases that occurred during December 1999 to January 2000.[a]

Trapping cell no.	Distance of cell perimeter from rabies cases (km)	Estimated raccoon density/km^2 during 1999 (95%CI)	Raccoon density/km^2 during 2000 (95%CI)[b]	Reduction in raccoon population density from 1999–2000 %
1	1	4.5 (2.5–24.2)	1.2 (1.0–1.4)	73
2	0	1.3 (0.8–3.1)	0.7 (0–1.6)	42
3	0	8.7 (4.1–95.8)	1.1 (0–2.2)	87
4	0.1	7.2 (4.6–21.9)	1.6 (0.91–2.2)	78
5	>5 <9	12.5 (7.6–39.7)	3.7 (1.3–6.2)	70
6	1	6.0 (4.3–11.2)	1.1 (0.6–1.6)	82
7	1	9.8 (5.5–33.3)	1.9 (1.6–2.2)	81
8	0	4.6 (2.1–300)	1.5 (1.2–1.6)	67
9	>7 <13	5.6 (2.8–356)	3.3 (0.6–7.4)	41
10	>15 <22	18.9 (6.1–36.9)	6.7 (1.3–11.9)	65
All		8.4 (6.4–12.4)[c]	2.4 (0.9–4.1)	71

[a] Four cases of raccoon rabies were reported in cell 3, and one case each in cells 2 and 8 during December 1999 and January 2000.

[b] Raccoon density is a preparturition estimate and does not include animals that were euthanized during April to June 2000.

[c] Raccoon density was significantly higher in 1999 than following the outbreak in 2000.

2003–05 (about 44,000 in total); however, TVR was discontinued after 2002.

Raccoon density

The raccoon population on Wolfe Island declined dramatically (71% reduction) from 1,067 raccoons (mean density=8.4/km^2 [6.4–12.4, 95% CI]) during 1999 to 305 raccoons (mean density=2.4/km^2 [0.87–4.1, 95% CI]) following the rabies outbreak that occurred in December 1999 and January 2000 (Tables 1, 3). Raccoon density on Wolfe Island before the rabies outbreak (1996–99) was significantly higher (mean=6.7/km^2, SD=1.7) than density in 2000 following the outbreak (mean=2.4/km^2, SD=1.8; t=4.1, P<0.001) (Table 1). In fact, raccoon density in cells with rabies cases (1.1 raccoon/km^2) was on average 78% lower following the rabies outbreak than prior to the outbreak (4.9/km^2). In addition, following the raccoon rabies outbreak (in 2000), raccoon density was significantly lower (mean=1.3/km^2, SD=0.39) in trapping cells that were within 1 km of rabies cases or that had rabies cases, than in cells that were >5 km and <22 km from the rabies case locations (mean=4.6 km^2, SD=1.9; Table 3; t=−4.78, P<0.001). It should be noted that although the 2000 population estimate was 305 raccoons, that figure includes the 197 raccoons that were euthanized during the April to June population reduction program. However, if one assumes a 1:1 ratio of males to females, a 95% pregnancy rate, and a mean litter size of four, the postparturition population estimate for 2000 only would have been on average 312 raccoons. Our estimates indicate that raccoon density had doubled after 1 yr following the population reduction program and rabies outbreak (Table 1).

Raccoon movements

Movements were calculated for 579 different raccoons that were recaptured during 1996 to 2002 on Wolfe Island. Raccoons were found to be mobile on Wolfe Island, with significant distances (≤ 24 km) traversed (Fig. 3, Table 4). Movements among islands were also noted (three raccoons moved from Wolfe Island to two islands about 1 km offshore) (Fig. 3). There were no significant differ-

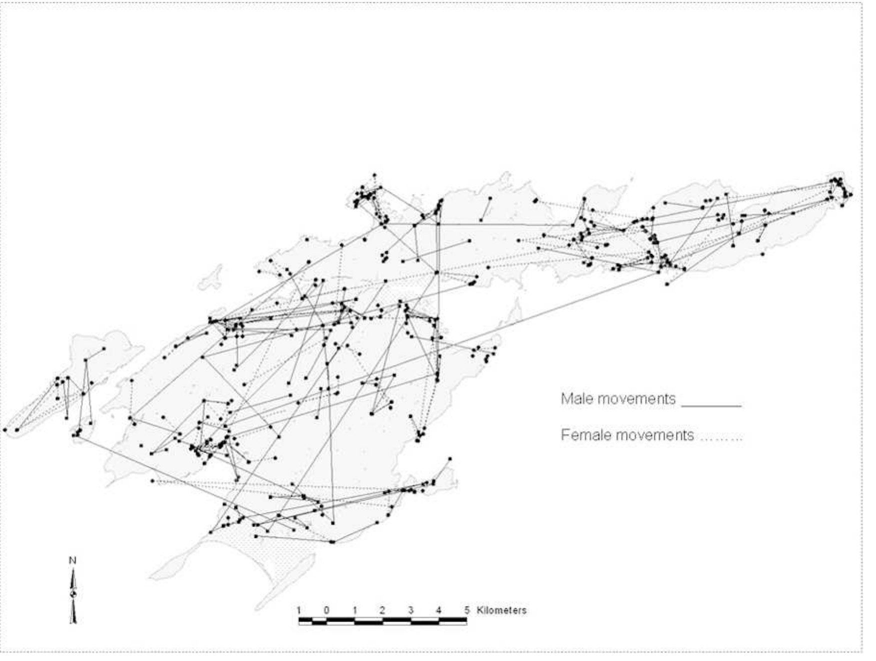

FIGURE 3. Movement of raccoons on Wolfe Island, Ontario during 2001–02.

ences in distances moved between adult and juvenile raccoons either before (F=0.00023, P=0.99) or following (F=1.51, P=0.22) the population reduction program. No within-sex differences could be detected for male or female distances moved before and following the population reduction program (F=2.14, P=0.88). However, male raccoons traveled, on average, greater distances than female raccoons, both before (F=26.4, P<0.0001) and after (F=10.08, P=0.007) the population reduction program (Table 4). Male raccoons moved on average 2.7 km and 2.6 km, respectively, before and after the population reduction program; females moved on average 1.1 km and 1.6 km, respectively, before and after the reduction program.

DISCUSSION

Rabies control operations

The raccoon rabies virus variant was first reported in July 1999 on the mainland of Eastern Ontario, about 90 km northeast

TABLE 4. Raccoon movements on Wolfe Island, Ontario, during 1996–2002, before and after a population reduction program.[a]

	Sample size (n)		Mean distance moved (m)		95% CI (m)		Range of movements (m)	
Age/Sex[b]	Before	After	Before	After	Before	After	Before	After
JF	66	72	1,100	1,604	359	623	1–9,038	5–18,219
JM	52	74	2,514[c]	2,810[c]	876	885	113–13,839	3–20,718
AF	99	107	1,188	1,574	522	453	17–24,323	5–15,873
AM	52	57	2,840[c]	2,383[c]	991	608	30–14,314	4–13,624

[a] Population reduction program occurred April–June, 2000.

[b] JF = juvenile female, JM = juvenile male, AF = adult female, AM = adult male.

[c] Males moved greater distances than females.

of Wolfe Island (Wandeler and Salsberg, 1999; Rosatte et al., 2001). The source of the mainland Ontario outbreak was most likely due to rabies-infected raccoons dispersing from the Ogdensburg, New York area, because a major epizootic occurred in that area during 1998 and 1999 (Fig. 1). This claim is supported by the similar genetic association of raccoon rabies virus samples from Ontario and New York (Nadin-Davis et al., 2006). Raccoon rabies was reported on Wolfe Island in December 1999 and January 2000. The most likely source of infected raccoon(s) on Wolfe Island was from the Cape Vincent, New York area, which had raccoon rabies cases during 1999 and is about 1 km from Wolfe Island across the St. Lawrence River. This is supported by the genetic distinctness of the virus on Wolfe Island from other Ontario samples (Nadin-Davis et al., 2006). There is also regular ferry service to and from Wolfe Island from the US and raccoons ear tagged in Ontario have been captured in New York. The average incubation period for raccoon rabies is about 30–40 days (Tinline et al., 2002); thus a rabies-infected raccoon from New York likely arrived on Wolfe Island sometime during the summer or fall of 1999. Only 39% of the Wolfe Island raccoon population had been vaccinated during TVR operations in 1999 (due to insufficient trapping effort), a level generally too low to control a point-source infection (Rosatte et al., 2001). In fact, the two cells that accounted for 83% of the rabies cases had the fewest vaccinated raccoons and the lowest raccoon density, pointing toward an insufficient raccoon population vaccination rate to prevent a rabies outbreak. Population reduction during the spring of 2000 and oral rabies vaccination (ORV) with V-RG baits during the summer likely prevented further spread of the disease. Trap-vaccinate-release was continued in 2001 and 2002 (84% vaccination rate), but was replaced by ORV during 2003–05. The island has been free of reported cases of raccoon rabies from 19 January 2000 to 8 February 2007.

Interestingly, the population of raccoons on Wolfe Island during the spring of 2000 had declined by 71% of the 1999 estimated population size, but only six cases of raccoon rabies were detected during December 1999 and January 2000. Either the level of vaccination was sufficient to prevent further infections, or more likely, many rabid animals went unnoticed by the public or government field employees because they died in winter dens or in other hidden areas. There were also unconfirmed reports of dead raccoons being found by residents on Wolfe Island that were not submitted for rabies diagnosis due to their deteriorated condition. It is believed that only 5% to 10% of rabid animals in a given area are actually reported (Braunschweig, 1980; MacDonald, 1980; Bacon, 1981). Another possible explanation for the low number of rabies cases is that animals incubating rabies were euthanized during the population reduction program before clinical rabies developed. Alternately, the disease simply might have "burned out" due to the limited number of unvaccinated raccoons in the population, the small area of the island, or isolation of the island from the mainland.

Raccoon density

The seasonal behavior of raccoons should be considered when estimating raccoon density using trapping data. In Ontario, raccoons den during periods of inclement weather (i.e., winter) and are not as easily captured as during the spring, summer, and fall (Rosatte, 2000). However, in this study, trapping occurred during spring to fall when raccoons are active and easily captured. Thus, the timing of trapping should not have been a confounding factor in the analysis.

Raccoon densities on Wolfe island averaged 4.3–8.4/km^2 during 1996–2002 (excluding 2000). These densities are comparable to those found in other studies on raccoons in rural habitats of Ontario (Rosatte, 2000). The raccoon population on Wolfe Island during 1999 was at its highest density (8.4/km^2), doubling in size since 1996. Density estimates on the mainland of Ontario, adjacent to Wolfe Island averaged 6.8 (6.1–7.7) raccoons/km^2 during 1999. High raccoon density probably facilitated rabies spread on Wolfe Island during 1999. Lower raccoon density and population size in cells with rabies cases suggests that the disease might have caused significant raccoon mortality. Overall, the raccoon population on Wolfe Island decreased by 71% between the summer/fall of 1999 and the spring of 2000 (rabies outbreak occurred during December 1999 and January 2000) with a mortality estimate of about 78% for cells with rabies cases. Although some of the mortality could have been due to other causes (distemper, parvovirus, trapping, shooting, road-kills, winter severity), normal annual mortality for raccoons in rabies-free areas of Ontario has been estimated at about 50% (Rosatte, 2000). This suggests that rabies had an additive mortality effect on the population. This is supported by raccoon density on the mainland, which increased from 6.8 (6.1–7.7) during 1999, to 8.7 (8.4–9.0) and 8.1 (7.8–8.4) during 2000 and 2001, respectively. Rabies was not present in that area of the Ontario mainland. Also, rabies mortality might have been higher on Wolfe island had vaccination not occurred. It is also interesting to note the rapid recovery of the raccoon population following the rabies outbreak in December 1999/January 2000, and population reduction program during the spring of 2000. In fact, the population had doubled about a year later (2001), indicating the high resiliency of raccoon populations (Rosatte, 2000).

Raccoon movements

Raccoon movements in this study were constrained by the size and shape of Wolfe Island. However, they are comparable to those reported by Totton et al. (2004) and Rosatte et al. (2005; 2006), but less than those reported by Rosatte (2000) for raccoons in rural habitats of mainland Ontario. Although island size and shape limited raccoon movements, some individuals did move in excess of 20 km (see Fig. 3), suggesting that rabies would have spread through the high-density raccoon population on the island in the absence of management. Among-island movements of raccoons observed in this study support the supposition that rabies-infected raccoons might have crossed the St. Lawrence River from New York to Ontario. Among-island movements of raccoons also suggests that Wolfe Island could serve as a focus for the geographic spread of rabies.

Management implications

An average vaccination level of 39% from TVR in a wild raccoon population might have been insufficient to prevent an outbreak of raccoon rabies. Although proactive rabies control programs are advantageous, careful analyses are required to determine if the level of vaccination is sufficient to prevent and contain an outbreak of rabies. On the mainland of eastern Ontario, a rabies vaccination level of 69% to 82% during 2001–03, apparently prevented point-source cases from spreading in each of those years. In this study, a raccoon density of 8.4/km^2 was sufficient to facilitate an outbreak of raccoon rabies. Therefore, one potential option is to maintain raccoon density below this "threshold" through population reduction. Additional multivariate analyses are in order to more comprehensively evaluate the effect of threshold densities on rabies spread. Alternately, TVR or ORV could be integrated into control strategies to maintain an immunity level above 60%. This study demonstrates that control tactics such as TVR, ORV, and population reduction must be ready for immediate implementation to be most effective. When more than a single tactic is integrated into a rabies control strategy, the relative contribution of each towards the success or failure of the program is often confounded.

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