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## ELIMINATION OF RABIES FROM RED FOXES IN EASTERN ONTARIO

Charles D. MacInnes,<sup>1,12</sup> Stephen M. Smith,<sup>2</sup> Rowland R. Tinline,<sup>3</sup> Neil R. Ayers,<sup>4</sup> Peter Bachmann,<sup>1</sup> David G. A. Ball,<sup>3</sup> Laurie A. Calder,<sup>5</sup> Sarah J. Crosgrey,<sup>6</sup> Carolyn Fielding,<sup>7</sup> Peggy Hauschildt,<sup>3</sup> Janet M. Honig,<sup>3</sup> David H. Johnston,<sup>8</sup> Kenneth F. Lawson,<sup>9</sup> Christopher P. Nunan,<sup>1</sup> Michael A. Pedde,<sup>1</sup> Bruce Pond,<sup>1</sup> Robert B. Stewart,<sup>10</sup> and Dennis R. Voigt<sup>11</sup>

<sup>1</sup> Rabies Unit, Ontario Ministry of Natural Resources, Box 4840, Peterborough, Ontario K9J 8N8, Canada

<sup>2</sup> Department of Biology, University of Waterloo, Waterloo, Ontario N2L 3G1, Canada

<sup>3</sup> Geographic Information Systems Laboratory, Queen's University, Kingston, Ontario K7L 3N6, Canada

<sup>4</sup> Ontario Ministry of Natural Resources, Aviation Services, 2621 Skead Road, Unit 14A, Postal Bag #500, Garson, Ontario P3L 1W3, Canada

<sup>5</sup> R. R. #4, Sunderland, Ontario L0C 1H0, Canada

<sup>6</sup> R. R. #1, Elmvale, Ontario L0L 1P0, Canada

<sup>7</sup> 20 Heritage Drive, Bath, Ontario K0H 1T0, Canada

<sup>8</sup> 1080 Braemar Lane, Sarnia, Ontario N7V 3B5, Canada

<sup>9</sup> 13260 Keele Street, King City, Ontario L7B 1A4, Canada

<sup>10</sup> 603 Earl Street, Kingston, Ontario K6L 2K6, Canada

<sup>11</sup> 1457 Height Road, R.R. #3, Lindsay, Ontario K9V 4R3, Canada

<sup>12</sup> Corresponding author (e-mail: macinnch@netrover.com)

**ABSTRACT:** The province of Ontario (Canada) reported more laboratory confirmed rabid animals than any other state or province in Canada or the USA from 1958–91, with the exception of 1960–62. More than 95% of those cases occurred in the southern 10% of Ontario ( $\approx 100,000$  km<sup>2</sup>), the region with the highest human population density and greatest agricultural activity. Rabies posed an expensive threat to human health and significant costs to the agricultural economy. The rabies variant originated in arctic foxes: the main vector in southern Ontario was the red fox (*Vulpes vulpes*), with lesser involvement of the striped skunk (*Mephitis mephitis*). The Ontario Ministry of Natural Resources began a 5 yr experiment in 1989 to eliminate terrestrial rabies from a  $\approx 30,000$  km<sup>2</sup> study area in the eastern end of southern Ontario. Baits containing oral rabies vaccine were dropped annually in the study area at a density of 20 baits/km<sup>2</sup> from 1989–95. That continued 2 yr beyond the original 5 yr plan. The experiment was successful in eliminating the arctic fox variant of rabies from the whole area. In the 1980's, an average of 235 rabid foxes per year were reported in the study area. None have been reported since 1993. Cases of fox rabies in other species also disappeared. In 1995, the last bovine and companion animal cases were reported and in 1996 the last rabid skunk occurred. Only bat variants of rabies were present until 1999, when the raccoon variant entered from New York (USA). The success of this experiment led to an expansion of the program to all of southern Ontario in 1994. Persistence of terrestrial rabies, and ease of elimination, appeared to vary geographically, and probably over time. Ecological factors which enhance or reduce the long term survival of rabies in wild foxes are poorly understood.

**Key words:** Arctic fox rabies variant, *Mephitis mephitis*, oral vaccination, rabies, rabies virus, rabies control, rabies elimination, red fox, striped skunk, *Vulpes vulpes*.

### INTRODUCTION

Ontario (Canada) reported more laboratory confirmed rabid wild, domestic, and companion animals than any state or province in the United States or Canada, each year from 1958 to 1991, with the exception of 1960–62. Over 95% of those cases occurred in the agricultural parts of southern Ontario, an area that is <10% of the total province. The affected region has more than 85% of the provincial population

(over 8 million people), a fact which stimulated efforts to control the disease. The main wildlife vector for rabies in southern Ontario was the red fox (*Vulpes vulpes*), accounting for approximately 45% of reported cases; the striped skunk (*Mephitis mephitis*) accounted for approximately another 19% (MacInnes, 1987; Voigt and Tinline, 1982). In 1989, after several years of research on baits (Bachmann et al., 1990), vaccines (Lawson et al., 1987, 1989, 1992) and distribution methods (MacInnes

et al., 1992), we initiated the experiment described in this paper. The objective was to evaluate whether aerial distribution of baits containing oral vaccine could eliminate terrestrial rabies from a treated area.

The arctic fox variant of rabies virus was the only terrestrial form of the disease found in Ontario at the time (Webster et al., 1985; Smith and King, 1996). It entered the province from the north during the 1950's, and by 1959 was firmly established throughout southern Ontario (Johnston and Beauregard, 1969; Tabel et al., 1974). By 1972, rabies had disappeared from northern Ontario (MacInnes, 1988), and from most areas in Quebec and the New England states of the USA. In southern Ontario the disease persisted at high levels with local cyclical patterns (Tinline, 1988). Vaccine-baits were effective in immunizing foxes, but not skunks, in the laboratory (Lawson et al., 1989). Since the red fox was the principal vector and skunk incidence was driven by fox incidence (Tinline, 1988), the trial proceeded on the assumption that elimination of rabies from foxes would be followed by the disappearance of rabies from skunks within 2 yr. Rabies was expected to disappear from other terrestrial wildlife and domestic animals at the same time; variants in bats would not be affected. The vaccine used would not immunize raccoons (*Procyon lotor*) by the oral route, so the raccoon variant would not be prevented from entering Ontario by the program described in this paper.

The planning of the control program included three predictions: (1) the 3- to 4-yr cycle of rabies in the treated region would be disrupted ( $H_0$ : the regular rabies cycle would continue uninterrupted); (2) the edges of the treated area would act as barriers to rabies moving in from outside ( $H_0$ : rabies outbreaks beginning outside the area would move into the untreated zone unhindered); (3) rabies would continue to cycle in the rest of southern Ontario ( $H_0$ : rabies cycles outside the treated zone would change in a manner similar those to within the zone).

We began baiting in 1989 and planned to continue the program through at least one cycle of rabies incidence (4 yr). This time frame addressed Anderson's (1991) concern regarding the claims of immediate success of rabies control programs in Europe, where he questioned whether the diminution of rabies cases was an effect of vaccination or a natural decline in the disease. At the end of the 5 yr, the decision to continue the program was to be based on assessment of the success of the program to date.

The objective of this paper is to describe events in eastern Ontario as oral vaccination proceeded, and to evaluate progress of the attempt to eliminate the arctic fox variant of rabies from the whole 30,000 km<sup>2</sup> study area.

## METHODS AND MATERIALS

### Study area and vaccination strategy

The study area of approximately 30,000 km<sup>2</sup> corners at 46°03'N 78°00'W, 44°06'N 77°01'W, 45°34'N, 74°24'W, 45°13'N, 74°18'W included the former counties of Lennox and Addington, Renfrew, Lanark, Frontenac, Leeds, Grenville, Stormont, Dundas, Glengarry, Prescott, Russell, and Carleton. Some of these have been amalgamated and renamed by recent reorganizations, but the 1970 boundaries were not changed, and we used those for analysis purposes. The area had three distinct topographic regions which differed in potential fox habitat. The western portion consisted of low hills of Precambrian Shield rocks, mainly covered by thin soil, with many lakes and small wetlands. This region was mostly forested, although the vegetation was often sparse over large areas of bare rock. It had a low human population density except along the Ottawa River. The region included the Counties of Renfrew, Lanark, and the northern portions of Lennox and Addington, and Frontenac. The central region (Counties of Leeds, Grenville, Carleton, southern Lennox and Addington, and southern Frontenac) consisted of undulating limestone plains with varied drainage and soil types, and 2 to 5% wetlands. There was a mixture of small farms, rural residential properties (<5 ha), suburban housing, and one city (Ottawa). Along the interface between the Precambrian Shield and the limestone plain, there were marginal farms with considerable areas of rough pasture. That was considered the best fox habitat. The

TABLE 1. Quantitative characteristics of the annual vaccine-bait campaigns to eliminate rabies from red foxes in eastern Ontario.

Year	Dates	Area <sup>a</sup> (km <sup>2</sup> )	Number of baits distributed	Flight line spacing <sup>b</sup> (km)	Achieved bait density (baits/km <sup>2</sup> )
1989	2–13 October	14,660	285,010	1 <sup>c</sup>	19.4
1990	11–26 October	31,460 <sup>d</sup>	568,044	1 <sup>c</sup>	18.1
1991	11–26 October	28,805	567,829	1.3	19.7
1992	28 September–4 October	29,590 <sup>e</sup>	580,501	1.3	19.6
1993	27 September–5 October	28,805	543,385	1.3	18.9
1994	3–5 October	17,959	343,017	1.8	19.1
1995	1–4 October	19,664	389,346	2.0	19.8
1996	N/A	0	0	N/A	N/A
1997	36,798	8,850 <sup>f</sup>	157,092	2	17.8
1998	N/A	0	0	N/A	N/A

<sup>a</sup>Area excluding large lakes, towns, and cities (AMCTO, 1995).

<sup>b</sup>Shortest distance between parallel flight lines.

<sup>c</sup>Flight lines over wooded areas, others between concessions.

<sup>d</sup>Some sections baited twice (Fig. 2).

<sup>e</sup>Extra section added (Fig. 2).

<sup>f</sup>Baited to protect the area from re-invasion from the east (see Fig. 2).

eastern portion of the study area (Counties of Prescott, Russell, Stormont, Dundas, and Glengarry) consisted of low, flat, poorly-drained soil that was the flood plain of the Ottawa and St. Lawrence Rivers. Land use there was overwhelmingly agricultural, except for large ( $\approx 10,000$  ha) wooded swamps and peat bogs which were too moist for crop land.

The study area was baited at a rate of 20 baits/km<sup>2</sup> each fall for 5 yr. In 1989, there were not enough baits available to cover the whole area and a rabies outbreak was still present in the middle of the study area. The objective for that season was to contain the outbreak by baiting on either side of the centre. Baiting of the entire study area began in 1990. The baited area varied slightly from year to year (Table 1), in response to a lack of rabies in the peripheral townships, especially in the northwest corner of the region. Baiting continued in 1994 and 1995, beyond the initial 5 yr, after which it was stopped because there were no more cases of fox-strain rabies reported in the whole study area.

#### Rabies records

Changes in the prevalence of rabies over time were evaluated using two data sources. The first source was rabies case records routinely compiled by the Canadian Food Inspection Agency (CFIA). The second was fox carcasses collected from fur trappers. The data re-

ported in this paper were obtained from the CFIA central data system (CFIA, Nepean, Ontario, Canada). Diagnosis of rabies in Canada is the responsibility of the CFIA (formerly Agriculture and Agri-Food Canada, Agriculture Canada, and Canada Department of Agriculture), which maintains a network of district offices throughout Canada. Those offices assembled all specimens submitted to the laboratories, either directly from members of the public or through cooperation with provincial agencies. The surveillance system was passive, involving reports from people who encountered suspected rabid animals. No active collections were undertaken by CFIA. CFIA performed laboratory analyses (at the Animal Diseases Research Institute [ADRI], Nepean, Ontario, Canada) and field officers made clinical diagnoses on some farm animals. Originally, specimens were sent for laboratory diagnoses only in cases of suspected exposure of a human or a domestic animal. In 1994, we requested that all suspect rabid animals be submitted for laboratory diagnosis, regardless of human contact, in order to increase the probability that rabid foxes and skunks would be submitted for diagnosis. This study used only laboratory confirmed cases; clinically diagnosed cases were excluded.

The data set was collected according to a standard protocol for submission and reporting of specimens. From 1958–88, the cases were

TABLE 2. The results of a rabies control program in eastern Ontario. These are expressed as the probability of the observed number of quarters free of rabid foxes since their vaccination began in 1989. The expected value is based on quarters free of rabid foxes from 1957 to 1988. There were 128 quarters in that period. Significance was set at  $<0.004$  (see text). The four values in italics failed to meet that criterion.

County	Area (km <sup>2</sup> )	$P^a$	Observed <sup>b,c</sup>	$P^{n,c}$
Ottawa-Carleton	2,696	0.15	12	0.0002
Dundas	1,019	0.44	9	<i>0.0062</i>
Frontenac	3,820	0.3	20	0.0006
Glengarry	1,246	0.5	15	<i>&lt;0.006</i>
Grenville	1,195	0.34	20	<i>&lt;0.0002</i>
Lanark	2,939	0.19	13	0.0004
Leeds	2,195	0.3	20	<i>&lt;0.0002</i>
Lennox and Addington	2,841	0.4	14	0.004
Prescott	1,245	0.57	9	<i>0.03</i>
Renfrew	7,639	0.16	17	<i>&lt;0.0002</i>
Russell	758	0.57	17	<i>&lt;0.0018</i>
Stormont	1,027	0.44	12	<i>&lt;0.0098</i>

<sup>a</sup> The probability of observing a rabies-free quarter in a county from the first rabies occurrence after 1957 until 1988.

<sup>b</sup> The observed number of consecutive rabies-free quarters from the last reported rabies case to the end of 1995.

<sup>c</sup> The probability of seeing the observed run length.

recorded as occurring within 1 of the 12 counties listed previously. From 1989 on, each case had a UTM (Universal Transverse Mercator Code) location assigned. Each record included a date and geographic locator. Quarters (3 mo intervals) were the basic time periods used for this analysis. That ensured adequate sample sizes. County level data were used for analysis because counties were the smallest geographic units in which rabies cases were recorded over the whole time period. In addition to the CFIA surveillance, fox carcasses were purchased from fur trappers. We collected from trappers throughout the study area but the distribution of carcasses was neither random nor uniform. The number of specimens per area was a joint function of fox density and the number and distribution of trappers. Brain samples were submitted to the CFIA-ADRI laboratory for screening for rabies by the fluorescent antibody test (FAT) (Webster and Casey, 1988; Dean et al., 1996). This was done to meet World Health Organization (WHO) guidelines (WHO, 1992).

#### Statistical evaluation

Rabies data recorded by county were clearly not independent in either space or time. Therefore conventional statistical tests such as parametric regression could not be used because their distributions require statistical independence. Diminution of rabies was evaluated by the number of postintervention rabies free quarters in relation to patterns recorded before the bait program began (Table 2). For each county the probability that the county enjoyed the observed sequence of  $n$  rabies-free

quarters by chance alone was computed as follows: the preintervention pattern of rabies was defined by the presence (1) or absence (0) of rabies over a 128-quarter period (32 yr, 1957–88). Based on that 128-quarter sequence, the probability of all first order transitions (0 → 0; 0 → 1; 1 → 1; 1 → 0) was computed. Two binary (0, 1) arrays, each of length 1,000, were then constructed. The first described, on the basis of these transition probabilities, the frequency distributions of 0s or 1s following a “0”. The second tabulated the frequency distribution of 0s or 1s following a “1”. The length of 1,000 was selected to define the distributions to a precision of 0.001. The worst case assumption was that the last quarter immediately prior to the initiation of the experiment was rabies positive (“1”). For that situation an array of length  $n$  was incrementally generated by randomly selecting elements from these two arrays. The array from which an element was drawn at random depended on the prior quarter value (0 or 1). If used to generate an array of length 128, this randomization algorithm created an array that closely mimicked the preintervention pattern of rabies cases. The null hypothesis was that the baiting program had no impact on rabies case frequency. The probability of the *observed* pattern of postintervention rabies-free quarters was computed as the proportion of 5,000 randomly generated arrays of size  $n$  in which the observed pattern occurred. The probability of a type 1 error ( $\alpha$ ) was set at 0.05. Given that the experiment was conducted over 12 nonindependent counties, the probability of the postintervention rabies

free period under the assumption of a true null was conservatively assessed against Bonferroni-corrected  $\alpha$  of 0.004. The Bonferroni correction results from division of the initial  $\alpha = 0.05$ , by the number of experiments, in this case 12 counties (Sokal and Rohlf, 1995:240–242). All tests were two-tailed. Although our intent was to reduce rabies by oral vaccination, we could not rule out the possibility that rabies might increase, either by chance or as a result of the treatment. Transition probabilities were computed in MATLAB® (The MathWorks, 1997); the randomization procedure was conducted in *Resampling Stats*® (Simon, 1995).

#### Vaccine-baits

The bait matrix was a fat-wax mixture made of oleo stock (58%) (1989–90, Canada Packers, Ltd., Toronto, Ontario, 1991–95, Canamera Foods Ltd., Toronto); Microbond® (32%), a strengthening wax (International Wax Ltd., Agincourt, Ontario); either mineral oil (9%) (1989–91, Daminco Inc., Mississauga, Ontario) or unrefined fish-liver oil (9%) (1992–96, Swimco Canada Inc., Georgetown, Ontario); chicken essence (1%) (International Flavours and Fragrances (Canada) Ltd., Concord, Ontario); and tetracycline HCl (100 mg/bait) (1989–90, Novopharm Ltd., Toronto; 1991–93, Ward Robertson Ltd. Toronto Canada; 1994–95, Gurvey and Berry Company Inc., Toronto). The baits measured  $35 \times 35 \times 15$ – $17$  mm, were pale yellow-brown in color, and weighed 16–19 g each. A yellow information label (1989, Partners Labels, Scarborough, Ontario; 1990, Evergreen Labels, Richmond Hill, Ontario; 1991–95, Talbot Marketing Ltd., Kitchener, Ontario) was affixed on one side (Fig. 1).

The vaccine was Connaught Laboratories' ERA® strain of live rabies virus, propagated in BHK-21 cells, at a titre of  $10^{7.3 \pm 0.3}$  Tissue Culture Infectious Doses per ml (Lawson et al., 1989). A volume of 1.8 ml was contained in a  $17 \times 17 \times 7$  mm blister-pack formed of  $14 \times 10^{-4}$  mm (7.5 mil) polyvinyl chloride (Pureplast Inc., Cambridge, Ontario). The lid was a laminate of paper, plastic, and aluminum foil, with a warning label printed on the paper side (Vins Plastic, Bradford, Ontario). This blister-pack was embedded in the middle of the bait. The baits were manufactured by Connaught Laboratories Ltd. (Toronto) in 1989 and 1990, and by Langford Laboratories Ltd. and its successors, Langford-Cyanamid Inc and Ayerst Veterinary Services Ltd (Guelph, Ontario) from 1991 to 1995. The production machinery was designed and built for this project (Inoform Ltd, Pickering, Ontario). Extensive laboratory



FIGURE 1. Photograph of the bait used in an experiment to eliminate fox rabies from eastern Ontario. The rabies vaccine was contained in a plastic blister-pack embedded within the fat-wax matrix. The top bait has matrix removed to show the vaccine package.

testing of these vaccine-baits indicated that the vaccine was effective in immunizing foxes but not skunks or raccoons (Lawson et al., 1989).

#### Flight planning and navigation

The baits were dropped from DeHavilland DHC-6-300 Twin Otter aircraft (DeHavilland of Canada Ltd., Downsview, Ontario). The planned flight altitude was 150 m, but the actual height above ground varied from 70 to  $>300$  m, depending on topography and weather. The flight speed of the aircraft was usually 270 km/hr, but ground speeds varied from 230 to 310 km/hr depending on wind velocity and direction.

Initially the layout of flight lines was based upon earlier field experiments (Bachmann et al., 1990). Flight lines ran parallel to the forested areas at the back of survey lots, putting the lines midway between the concession roads, usually 1.3 km apart. Over unbroken forest, flight lines were 1.0 km apart in 1989. The

lines were hand-drawn on map sheets used by navigators to direct the pilots. When this method was applied across 124 townships in eastern Ontario and flown at the faster speed of the Twin Otter, excess flying time and navigational problems were encountered. The roads in adjacent townships did not line up. Therefore flight lines that followed the road grids had many short, angled segments that were difficult to follow at 270 km/hr and low altitudes. Analysis of National Topographic Series (NTS, Ottawa, Ontario) 1:50,000 maps revealed that straight, parallel, flight lines ignoring the survey fabric covered as much of the ideal fox habitat area as complex lines matching the surveys. Field experiments revealed that straight, parallel flight lines and wider spacing did not result in lower acceptance of baits by foxes. Thus, from 1991 onward, flights followed relatively straight lines and were only bent to achieve efficient coverage of complex land shapes. The spacing between flight lines was increased to 2 km in 1995 to further increase flight efficiency.

In 1989 the latitude and longitude of end-points of lines were read from NTS 1:50,000 maps and entered manually into the LORAN-C navigation system (ARNAV Inc., Puyallup, Washington, USA) of each aircraft. By 1991 we prepared digital lines with AutoCAD® (Autodesk Canada Inc., Markham, Ontario) and listed the coordinates to be entered into the unit. In 1994, the navigation system was upgraded from Loran to Global Positioning System (GPS) manufactured by the same company. In 1995 we developed our own CAD/GIS software based on AutoCAD which uploaded digital waypoints directly into memory of the GPS unit.

#### Bait delivery

Machinery installed in the aircraft was designed for this project by Packaging Machine Designs Ltd. (Pickering, Ontario) and Redford Robotics (Richmond Hill, Ontario). The system consisted of a moving conveyor belt and a rotating drum positioned over a metal chute leading to an open camera hatch at the rear of the aircraft cabin. When baits on the conveyor belt reached the rotating drum, a series of protruding pins picked them off the conveyor and dropped them down the chute. The navigator read the ground speed from the cockpit navigation unit and used a calibration table to set the drum-rotation speed to achieve a target bait density. The navigator, who had a clear view forward and to the side of the aircraft, stopped the bait machine to avoid baiting areas of human activity or areas where baits would be wasted, such as bodies of water (MacInnes et

al., 1992). There is concern for the safety of humans exposed to ERA vaccine, which is a modified live rabies virus. Therefore we sought to drop baits where they would not readily be found by humans. The console recorded the number of baits dropped. The achieved bait densities are shown in Table 1.

Baits were also placed by hand, by naturalist volunteers, in green spaces within metropolitan Ottawa (120 km<sup>2</sup> = 0.4% of the study area, population about 700,000), from 1989 to 1993. Baits were thrown into vegetation at approximately 30 m intervals, from trails, stream banks, or roadsides. These baits were placed in regions where there was fox habitat, but where density of housing made it impossible to achieve adequate coverage from the air. The objective was to achieve 20 baits/km<sup>2</sup> over the whole city area, but that led to higher rates of placement in green space, as no baits were placed in residential areas. Smaller cities, with populations less than 50,000, were not baited. These covered <50 km<sup>2</sup>.

#### RESULTS

The number of rabid foxes declined sharply in the study area after baiting began, from 203 in 1989 to 4 in 1993 and none in subsequent years (Table 3, Fig. 2). In addition, there have been no rabid foxes from trappers' submissions since 1991 (Table 4). As expected, rabies cases also declined in other terrestrial wildlife species and domestic animals (Table 3, Fig. 3). Since 1996, there have been no reported cases of the arctic fox variant of rabies virus in the study area.

The 3 to 4 yr rabies cycle was interrupted and outbreaks expected in 1990–91, 1994–95, and 1999–2000 did not occur. This verified the first prediction of the control program.

As expected bat rabies cases persisted throughout the area in undiminished numbers (Table 3). Raccoon variant rabies virus was first detected in 1999, in an area adjacent to many cases on the New York side of the St. Lawrence River.

A barrier effect (prediction 2) is evident in Figure 2. In 1992, 1993, and 1994 a concentration of rabies cases developed in Hastings and Prince Edward counties just outside the southwestern edge of the study

TABLE 3. Summary of laboratory confirmed rabies cases<sup>a</sup> among selected species in the eastern Ontario study area, reported as mean ( $\pm$ SD).

Year	Fox	Skunk	Bat	Dog	Cat	Livestock	Total
1960–69 <sup>b</sup>	139.8 (75.2)	36.3 (19.6)	5.6 (3.5)	19.2 (10.3)	12.4 (8.0)	83.8 (44.1)	297.1 (147.2)
1970–79 <sup>b</sup>	205.4 (129.4)	43.8 (26.7)	6.6 (6.1)	16.5 (8.8)	8.4 (4.4)	88.7 (62.0)	369.4 (222.2)
1980–88 <sup>b</sup>	235 (197.7)	76.8 (46.5)	8.4 (4.2)	16.1 (13.1)	14.2 (11.2)	42.1 (27.6)	392.7 (277.2)
1989	203	30	2	18	15	63	331
1990	147	41	3	12	6	42	251
1991	45	44	11	6	8	12	126
1992	9	12	4	0	1	10	36
1993	4	3	6	0	0	1	14
1994	0	1	5	0	0	3	9
1995	0	0	7	1	0	1	9
1996	0	1	11	0	0	0	12
1997	0	0	10	0	0	0	10
1998	0	0	9	0	0	0	9
1999	0	0	8	0	0	0	17 <sup>c</sup>

<sup>a</sup> As reported by the Canadian Food Inspection Agency (formerly Agriculture and Agri-Food Canada).

<sup>b</sup> Average annual number of cases.

<sup>c</sup> Includes 9 raccoons infected with the raccoon variant of rabies virus.

area. Only 1 case was reported 10 km inside the baited zone.

Rabies cases continued to fluctuate within the range observed from 1958–89 in the rest of southern Ontario until 1995, after the bait program was extended in 1994 (Fig. 2). That verified prediction 3.

There was strong evidence of fox-rabies elimination for 8 of the 12 counties, ( $P < 0.004$ , Table 2). That was indicated by the actual number of rabies-free quarters observed in each county to the end of 1995 when baiting ceased. In the other four counties (Dundas, Glengarry, Prescott, and Stormont) the pattern was similar in supporting the conclusion of rabies elimination; however, the results were not statistically significant when assessed against the Bonferroni criterion. For those four counties, the preintervention number of rabies cases was low and sporadic or the duration of the postintervention period was brief. These counties are all close to the intense rabies outbreak in Quebec, shown in Figure 2. That made it difficult to demonstrate unequivocally the efficacy of the baiting program. These were four of

the smallest counties (Table 2). Nevertheless, only the results for Prescott did not indicate rabies was controlled. For the other three counties the probabilities were close to significant.

## DISCUSSION

The arctic fox variant of rabies virus was eliminated from eastern Ontario by a series of seven consecutive vaccine-bait campaigns, conducted 1/yr. That meant a reduction in terrestrial rabies cases within the study area from an average of 357 cases annually in the 1980's to zero. The length of the rabies-free period overcame Anderson's (1991) concerns about premature declaration of success (Table 3). The last known case of arctic fox strain rabies occurred in November 1996, so the area remained free of terrestrial rabies for >4 yr.

Rabies elimination was achieved with a single bait drop/yr. In Europe, 2 or 3 bait distributions/yr were considered essential, following World Health Organization (WHO, 1992) guidelines. The first successful vaccine-bait projects in Switzerland



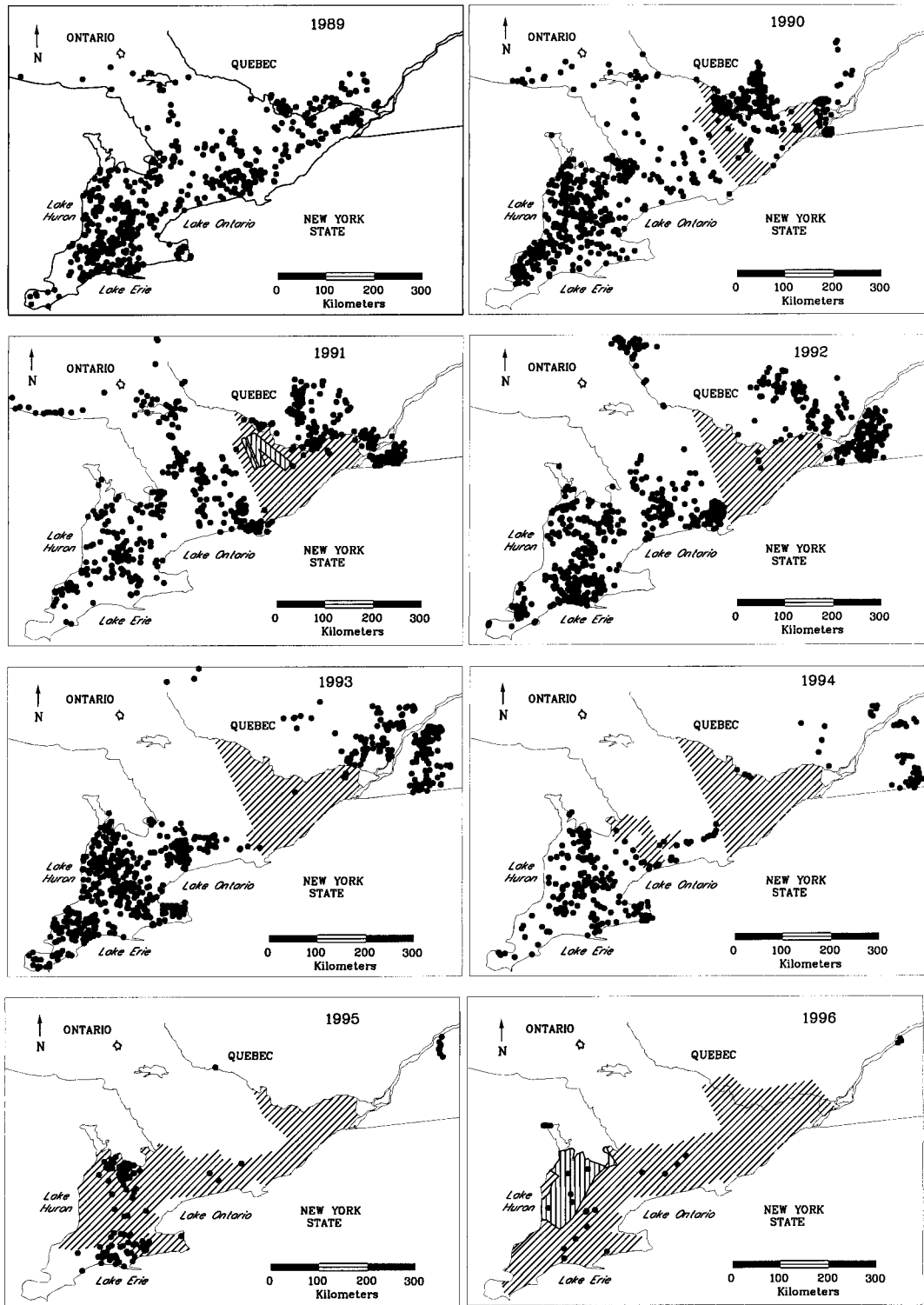


FIGURE 2. The locations of rabid foxes in southern Ontario and western Quebec from 1989 to 1996. The shaded areas show where baits were dropped during the previous fall. Each  $\odot$  represents one rabid fox.

TABLE 4. Prevalence of rabies among foxes obtained by fur trappers within the study area<sup>a</sup>, during a control program to eliminate the Arctic fox variant of rabies virus from eastern Ontario.

Year	FAT <sup>b</sup> positive	FAT <sup>b</sup> negative	Percent positive
1989	13	719	1.81
1990	14	859	1.63
1991	0	1,267	0
1992	0	558	0
1993	0	864	0
1994	0	112	0
1995	0	240	0

<sup>a</sup> Specimens were collected each winter from October to March and are listed for the year in which the collection began.

<sup>b</sup> Fluorescent antibody test, see methods.

(Steck et al., 1982; Wandeler, 1991) used spring and fall campaigns. However, Aubert et al. (1994) suggested that the total number of campaigns is more important than their frequency. Rabies was eliminated in France after two campaigns in one region, but after four campaigns in other regions there were still a few cases (Aubert et al., 1994). Progress was much slower in Germany, where co-ordination between states appeared to be a major problem (Schlüter and Müller, 1995; Stöhr and Meslin, 1996). Rabies was still present in a few areas of western Germany in 1999, after vaccination campaigns that began as early as 1983, and were conducted at least 2/yr. An area of 108,000 km<sup>2</sup> in former East Germany was treated with >10 campaigns, 2/yr, using baits air-dropped at 18–20/km<sup>2</sup>, yet rabies appeared to persist at low levels (Tischendorf et al., 1998).

We conducted seven bait campaigns in eastern Ontario. Baiting might have stopped 1 yr sooner if we had been certain that the remaining rabies cases in skunks and livestock did not indicate the presence of undetected rabid foxes. The last rabid skunk occurred 1 yr after the last bait campaign, yet fox variant rabies did not return in the area surrounding that case. The puzzling feature of the later stages of rabies elimination in eastern Ontario was that the last cases were mostly cattle, and

occurred for >1 yr after the last reported vector case (except for one skunk). In some cases there had not been a rabid fox or skunk detected in the same county with a bovine case for >2 yr. There are at least three competing explanations for this: (1) they represented spillover from undetected rabid foxes or skunks; (2) these animals had exceptionally long incubation periods; or (3) the individual animals were imported from a rabies-infected region while incubating rabies. Baiting of all except the borders of the region could have been terminated after 1993 if the last reported fox case signalled the end of the outbreak. We believe that only 1%–10% of actual rabid foxes and skunks are submitted to the laboratory (MacInnes, 1987).

What defined the enzootic region in southern Ontario in contrast to surrounding areas where this form of rabies did not persist? Two major movements of rabies occurred across southern Quebec in 1956–65 and 1972–74 (Lagacé, 1998). Most of Quebec was free of the arctic fox variant of rabies from 1974–90. The fox variant persisted in a <50-km-wide strip along the Ottawa River adjacent to the Ontario border, and a similar strip in New York along the St. Lawrence River. There is no explanation for fox rabies persistence across all of southern Ontario but not in southern Quebec or northern New England. Quebec authorities did not treat the severe outbreak shown in Figures 2 and 3, yet it diminished by 1995. There have been few rabid foxes and skunks reported since. Similarly, the 1990's outbreak died out in northern New York and Vermont, and was much diminished in New Hampshire and Maine by 2000. Tinline (1988) speculated that areas such as southeast Quebec were too small or had too low a probability of re-invasion for rabies to persist. Northern New York, Vermont, New Hampshire and Maine are more mountainous than southern Ontario. Perhaps that results in several smaller fox populations quite isolated by natural barriers. Such units should have a higher probability that rabies will become

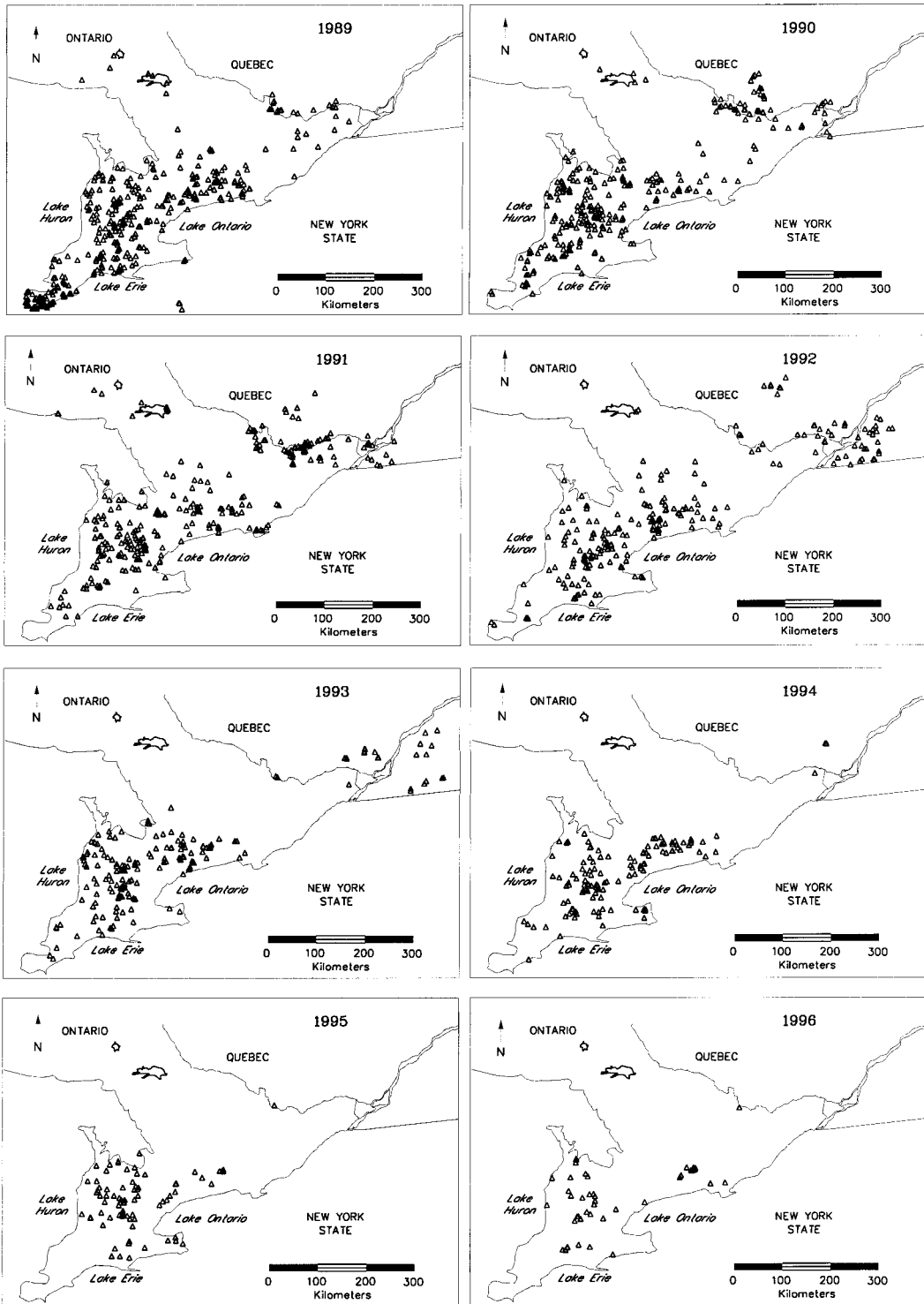


FIGURE 3. The locations of rabid skunks in southern Ontario from 1989 to 1996. Each  $\Delta$  shows the location of one rabid skunk.

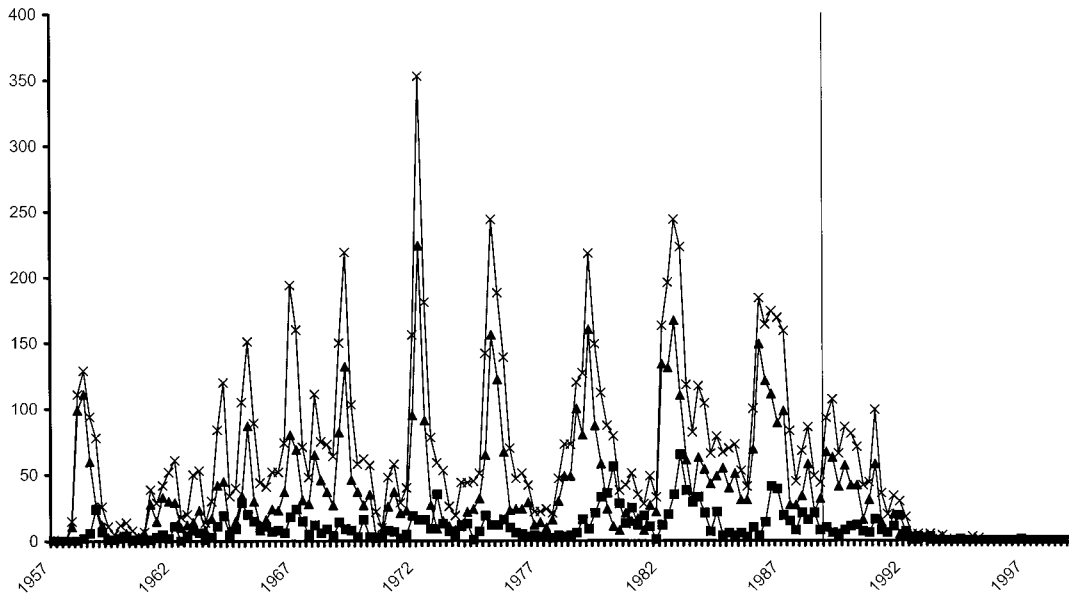


FIGURE 4. Rabies cases in eastern Ontario, 1954 to 1999. The vertical line shows when the bait campaigns began. Note the regularity of the cycles from 1970 to 1989. If these had continued outbreaks were expected in 1990–91, 1994–95, and 1999–2000, but these did not occur. (X denotes total rabies cases,  $\Delta$  shows rabid foxes,  $\blacksquare$  shows rabid skunks).

extinct by chance. Reinfection from adjacent areas would be reduced by the terrain.

The ease or difficulty of complete elimination of rabies from large areas seems to be related to fox ecology. Direct evidence is fragmentary or lacking. Arctic fox variant rabies virus died out naturally from much of the area originally invaded in the 1950's, including southern Quebec, northern New York and New England, and New Brunswick (Tabel et al., 1974). However, it persisted in agricultural southern Ontario. Perhaps, even in the latter area, persistence of the disease was more precarious than, say, of the red fox rabies virus variant in Germany. Although the latter variant crossed from Germany into France in 1965, by the late 1980's the disease had not reached the Atlantic, Channel and North Sea coasts. The front stalled for unknown reasons. Ecological factors were probably important yet considerable analysis did not give clear answers (Ball, 1985; Macdonald and Voigt, 1985; Sayers et al., 1985). Oral vaccination eliminated rabies

promptly from France (Aubert et al., 1994; Müller, 1997), but rabies has persisted at low levels in isolated pockets in Germany (Tischendorf et al., 1998). Attempts to analyze the landscape ecology of rabies have failed to produce universally applicable insights to these differences (Bögel et al., 1974, 1976, 1981; Carey, 1982; Carey et al., 1978; Jackson and Schneider, 1984; MacInnes, 1987; Steck and Wandeler, 1980). There is no simple relationship between fox density and the persistence of rabies (Moegle et al., 1974; MacInnes, 1987). Voigt and Tinline (1982) commented wryly that "the fox density at which rabies is epizootic in Ontario is the density at which Europeans claim rabies will disappear (Bögel et al., 1974, 1976)."

A number of practical considerations affect the ease of elimination of rabies. For example, coverage with baits of the outer suburbs of large urban complexes is difficult because of the density of housing. Baits should be dropped out of sight of humans to enhance human safety. Urban green spaces can include excellent fox hab-

itat. No information is available on how such areas were covered in Germany. In France baits were dropped from helicopters and hand-baiting was used where houses were too frequent to allow thorough coverage from the air. As cited above coordination between regional governments may be problematic; in Ontario almost the entire rabies outbreak was within the jurisdiction that undertook control. Quebec willingly cooperated when Ontario asked to drop baits.

Vaccine-baits were dropped in western Quebec along the Ottawa River in 1995 to prevent re-invasion of the experimental zone. In 1997 and 1998 we again dropped baits in the southwestern portion of Quebec south of the St. Lawrence River in an attempt to contain and then eliminate the last focus of infection in Quebec. The 1997 drop included parts of the study area.

Although baiting was stopped in eastern Ontario, monitoring of rabies incidents and research on spread continues. Long term monitoring of rabies incidence is important because (1) there is still the potential for fox variant rabies to re-enter from Quebec or from the adjacent USA; (2) the virus may persist unobserved in the study area since arctic fox rabies remained in New Hampshire and Maine in 2000, research on control strategies for containing isolated outbreaks must continue; and (3) there continue to be cases of fox variant rabies in northern Ontario, >500 km north of the areas mapped in Figure 2.

The role of skunks in maintaining Arctic fox rabies in eastern Ontario was obscure. Tinline (1988) indicated that skunks probably could not maintain fox rabies for extended periods. The prompt disappearance of rabies from skunks within 1 yr after the last rabid fox occurred in the study area supported that view. However, skunk to skunk transmission of this rabies variant in the wild almost certainly occurred. If skunks could maintain rabies for 1 to 2 yr after an outbreak had reduced the fox population, then skunks would significantly reduce the probability of local extinction of

the virus by keeping it alive while the fox numbers recovered. The ratio of rabid foxes to rabid skunks was greater, during 1958–88, in eastern Ontario than in the rest of the area which was enzootic for arctic fox variant rabies. The choice of eastern Ontario as the site of this first experiment may have fortuitously provided a skunk density favorable to elimination of fox rabies.

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