The Rabies Reporter

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The Rabies Reporter, a scientific newsletter about current issues in rabies research and control, is a joint effort of the Rabies Research Unit of the Ontario Ministry of Natural Resources; the Rabies Laboratory at the Animal Diseases Research Institute of the Canadian Food Inspection Agency; the Ontario Ministry of Health; and the Geographic Information Systems Laboratory of Queens University, Kingston. Articles for future issues will be welcomed by the editor. The Rabies Reporter is not refereed, and should not be cited in papers intended for refereed journals. Send contributions, letters and inquiries to:

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FALL 2001 BAITING IN OHIO, WEST VIRGINIA AND PENNSYLVANIA

I would like to start off by saying a heart-felt thank you to everyone that was involved with the Fall 2001 ORV baiting. The professionalism and the compassion that everyone showed during this tragic time in the world's history were very impressive. We did have some difficulties, but with what was going on in the world at that time our problems were minor.

Bob Hale
Ohio Department of Health

This was the start of the "Great Barrier" for raccoon rabies. A United States Department of Agriculture (USDA) Wildlife Services initiative made the multi-state expansion possible. The barrier starts in Erie County, Pennsylvania (joining up to Chautauqua County, New York), goes down along the Ohio / Pennsylvania border and south into West Virginia, stopping southwest of Charleston. This was a very large logistic challenge with numerous states and federal agencies, plus a foreign country.

The pre-bait drop work started on September 7 with the packing of all the equipment needed to support the baiting operation. It was then off to the airport in Columbus to pick up Bradley Hicks with the Texas Department of Health. He was invited to assist and to be a back up to me during the baiting. We departed Columbus the next morning at 6 a.m. to meet with the USDA Wildlife Services staff in

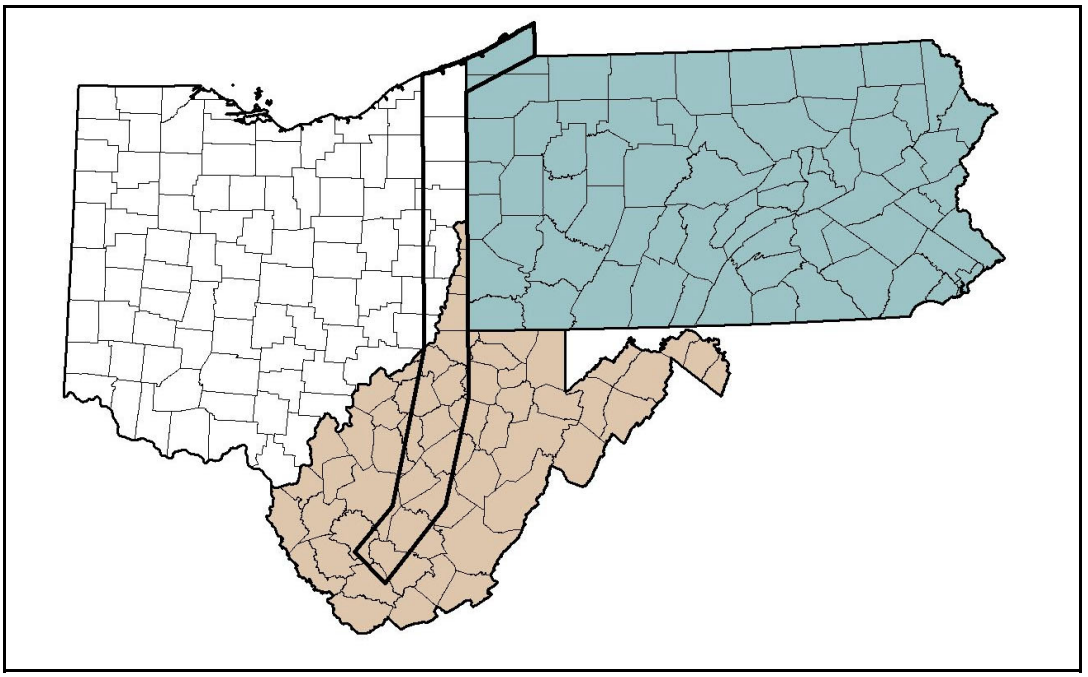

Figure 1. Map of the Ohio, West Virginia, and Pennsylvania bait zones. This project was underway on 11 September. It was completed successfully, a result of real teamwork by all concerned.

Youngstown. We had two work trailers to set-up with electricity, phones, fax machines and computers. The first two bait trailers arrived. We really had our work cut out for us that weekend.

On September 10, ground baiting operations in Ohio started. Local health department personnel "throw" baits into predetermined locations. Eleven Ohio Army National Guard soldiers arrive on site and start loading bait tubs for flights that are scheduled the next day. A helicopter from the Ohio Department of Natural Resources (500NR) arrives and starts with its aerial operations. Staff from Ohio Department of Health (ODH), West Virginia Department of Health (DOHWV), West Virginia Department of Agriculture (DOAWV), and USDA / Wildlife Services from all three states begin arriving. Around noon, OPI arrives at Elser, followed later by OGC and OGA. After exchanging hellos, it is back to work unloading the planes and setting up the flight watch system. The staff goes through safety briefings with the Ontario staff and the planes are loaded for an early morning take off to the north.

September 11, wheels are off the ground on time and 500NR is off to finish its area also. OGC is back to the airport first and reloaded and starts to head south towards Charleston for a media flight. OPI and OGA are just finishing their first flight when the attacks in New York, Washington D.C. and the foiled attempt in Pennsylvania occur. The FAA grounds all aircraft; OPI and OGA make it back to Elser, along with 500NR. OGC is stuck in Charleston, West Virginia and has to spend the night. Needless to say, the media flight is cancelled. Helicopter pilot, Joe Barber returns to Columbus by car. We will transfer those baits to the Otters. Three and a half flights are completed this day.

September 12, Neil Ayers is in Charleston with OGC. After much discussion with the FAA, he gains permission to fly back to Elser but not to drop baits. OGC arrives back at Elser at 5:30 p.m.

The next few days we are grounded due to the FAA not allowing any aircraft to fly under Part 137 (agricultural aviation). Staff from OMNR, USDA, ODH and DOHWV work on operational justification for permission to continue baiting operations.

September 15, early in the morning the FAA releases all Part 137 operations. With this news, we have wheels off at 9:16 a.m., but we are short on crew. Some of the crew flies back-to-back flights. Released crews return from their

homes during late morning. We complete nine flights for the day.

September 16, everyone is in great spirits since we are flying again. Wheels are off the ground at 7 a.m. and the weather is great. The flights are on the third rotation when the FAA orders all Part 137 operations grounded again. Once again, OGC with Neil Ayers and the same crew as before are stuck in Charleston along with OPI. OGA is close enough to return to Elser. This time, Neil gets to the right people at the FAA office and is able to get permission to fly back to Elser but not to drop baits. A day that started on a very high note becomes very low once again. With all of the down time we find something to keep our spirits up…. we fish. Okay, Neil calls it the "cesspool," but it does give us something to do.

September 17, no flights allowed for the day, but ground baiting in Ohio is 95 percent complete. Pennsylvania Department of Health has decided not to aerial bait in their state but opts to ground bait instead. Flight lines are modified, since the original flight lines include Pennsylvania areas. Bradley Hicks can't guarantee that his flight back to Austin will happen. He decides to rent a car and return to Texas; in fact he is ready to rent a U-Haul to get back home.

September 18, FAA releases all Part 137 flights once again; we are in the air at 9:15 a.m. The weather is great and we complete nine flights for the day. Bradley Hicks makes it home.

September 19, flights are off the ground by 7:55 a.m., a little delay due to morning fog. This is the day people are looking forward to: **deep fried turkey day**! When we could not fly we decided to start paying attention to: "What's for lunch!" So, Chef Montoney deep-fries turkey, chicken and French fries for lunch. The last of the bait is delivered for a total of four and one half trailers of baits. In the past, we have had only one trailer on site. Talk about your growing pains! Nine flights are completed for the day.

September 20, flights off at 7:00 a.m. all going north. Fog is in the south so the next set of flights are north also. This completes all the

northern flights. We did get one set of flights to the south completed. Ground baits for Pennsylvania and West Virginia are counted and moved to one of the empty trailers. This is also the day when 18 tubs of baits are pushed off the ramp and over the hill. No one is hurt – only egos. The spilled baits are quickly re-assembled into the tubs. Nine flights are completed for the day.

September 21, weather delays due to fog. Flights are in the air at 9:52 a.m. with all flights going south into West Virginia. The media flight to Charleston is rescheduled for today, also. Andrew had to press that shirt a second time. Eight flights are completed; not too bad with the weather delay.

September 22, with all flights going to the south we are having delays due to morning fog in the Ohio Valley. Crews are put on standby at the hotel and the fog lifts around 12:30. We are able to squeeze in six flights for the day.

September 23, it is a great morning, clear skies and a little on the cool side. The planes take off at 8:30 a.m. Everything is going great. We only have nine flights and we know some bad weather is going to be coming in overnight. We should get them done today. Ten minutes after the planes take off, they are ordered to land because of another FAA notice grounding all Part 137 flights.

Once again we started calling the FAA to petition them for approval to allow us to fly. After many phone calls and a letter faxed to the FBI, we finally get permission to fly and are back in the air at 2:30 p.m. Considering what is going on, getting clearance to fly in six hours is amazing. On the upside, we are able to get the group photo taken and start the cleanup so, when we do finish, a lot of the work is already done. Three flights are completed today.

September 24, as predicted a weather front moves in and we are grounded because of the rain and fog. West Virginia transports their ground baits and stages them for hand baiting. This is the last day on the National Guards orders and I have to leave also. It is very difficult for me to not finish the baiting, but I have to go see my son graduate from U.S. Navy basic training. He just got his orders and will be departing for Japan in November, assigned to the USS Kitty Hawk.

September 25, with a skeleton crew left in Youngstown, flights resume after the weather lifts at 11:30 a.m. with staff again flying back-to-back flights. The final six flights are

Summary of Baits Deployed by the 2001 Fall Bait Operation

Twin Otters	Helicopter	Ground (Hand) Baiting	Area Covered (sq mi)
Flights = 72	13 Identified areas	Ohio = 60,840	Ohio = 3,289
Hours = 195	Baits = 20,028	Pennsylvania = 138,000	Pennsylvania = 724
Baits = 1,339,806		West Virginia = 60,840	West Virginia = 4,330
(Ohio = 576,117			
West Virginia =763,689)			

flown and completed around 5:30 p. m. It is decided to pack everything that night and to do the final cleanup of the area. The Pennsylvania ground baits are delivered and staged in Erie.

September 26, the three otters head for Stratford; USDA personnel head back to their states; and ODH personnel head for Columbus. What should have taken eight days to complete, took two weeks…. but we're finally done!

Fall ORV Stats: Total Baits Distributed = 1,619,514

Bob Hale
Rabies Program Coordinator
Ohio Department of Health
Columbus OH

DON'T SAY ANYTHING BUT… PROGRESS OF ONTARIO'S PROGRAM TO ELIMINATE RACCOON RABIES

If you are superstitious, don't read this article. If you are scientifically inclined, then read on. The question is whether mentioning that there has not been a rabid raccoon since 13 September hexes the control effort by assuring that there will be more. After all, there have been gaps of several weeks in the past, notably from 12 June to 8 August 2000.

Figure 2 shows the historical picture. When winter began in 2000, the action area was around Athens. The first case in a local barn was at the end of the year. As cases began to come in at the beginning of the new year, there were two more from this same barn.

A very large effort was mounted to contain this invasion during the period April to October. First, there was the regular TVR. Year end totals for 2001 along the St. Lawrence St. Lawrence River (TVR lasted from July 16-November 2) were: over 720 km^2 completed by fourteen research technicians using the standard 80 plus traditional trapping cells as in previous years (1995-2000). Over 5,000 different raccoons, 300 skunks and 1,000 feral cats were vaccinated with Imrab 3 over the 12,000 total captures. More trapping effort was used here due to the active raccoon rabies outbreak in the region. Again, over 65% of the raccoons in the trapping zone were estimated to have been vaccinated. Holly Simpson supervised the operation based out of the OMNR Brockville office.

The immediate response to the new rabies cases was a combined Point Infection/TVR operation from Athens to Johnstown, from April 2 - July 8. The area started out as 300 km^2, but soon expanded to over 600 km^2. It was treated by 25-35 research technicians and local trappers in and around the most recently confirmed cases. This operation allowed us to remove raccoons, skunks and foxes within a five kilometer radius of all confirmed cases. Over 2,500 raccoons, 257 skunks and 6 foxes were humanely euthanized. Four additional positive cases were confirmed from the testing of these samples. Outside of this depopulation zone, a further five-kilometer-wide belt was treated by TVR. Over 4,000 raccoons, 400 skunks, 20 foxes and 1,000 feral cats were treated during that operation. Again, an average of over 65% of the raccoon population was calculated to have been vaccinated. Mike Allan was in charge of this operation based out of a Ministry of Transport (MTO) location in Crosby, Ontario (between Kingston and Smith Falls).

A total of 653,000 V-RG baits were dropped on the region, by MNR Twin Otters, during August 2001. These baits were placed in eastern Ontario within 50 km of any confirmed raccoon rabies case.

Two bait types were used, the coated sachet and the Ontario slim. These were deployed at two densities - 75 and 150 baits/km^2). During the month of October, seven research technicians collected blood (for the presence of rabies antibodies) and 2nd premolar teeth (for the presence of tetracycline biomarker) from more than 400 raccoons. Mike Allan supervised the project out of the same MTO location.

Baits were hand distributed in urban areas during the period 15 July - 31 October. Over 8,000 baits were hand placed in Brockville, Smith Falls, Kingston, Cornwall, and

Ottawa. V-RG baits were placed at a density of 75 baits/km^2 throughout utility corridors, green space, water course ravines, city & federal park islands and parks. Holly Simpson and Colin Langford supervised and completed the operation.

In addition to these operations around the actual rabies outbreak, the regular preventive TVR operation in the Niagara peninsula was repeated for the eighth consecutive year. Over 680 km^2 were treated by nine research technicians using the same 77 trapping cells as in previous years (1994-2000). Over 4,500 different raccoons, 300 different skunks, and 1,000 feral cats were all vaccinated with "Imrab 3" out of the total 10,000 total captures. These totals are very consistent with other years, and operations in other places. Over 65% of the raccoon population was vaccinated based on our capture/ recapture data. Laura Bruce supervised the OMNR Rabies Unit operation based out of the OMNR Vineland office.

If you study Figure 3 carefully, you will note that all but two of the cases during 2001 occurred within a roughly 40 km x 40 km area. The two outliers were both interesting. The case near Cardinal was at a major truck stop, and close to the Ogdensburg—Prescott bridge. Was this another crossing of the river from New York? That seems unlikely, as there have been very few cases on the New York side in 2001. The other guess is that this might be the dreaded hitchhiking raccoon, which climbed off a truck at the truck stop. That may be a neat idea, but there is no evidence. The second outlier is the case near the St. Lawrence, in the western edge of Brockville. Both of these cases occurred in the established TVR zone, so little extra control effort was applied.

As you can imagine, there is a

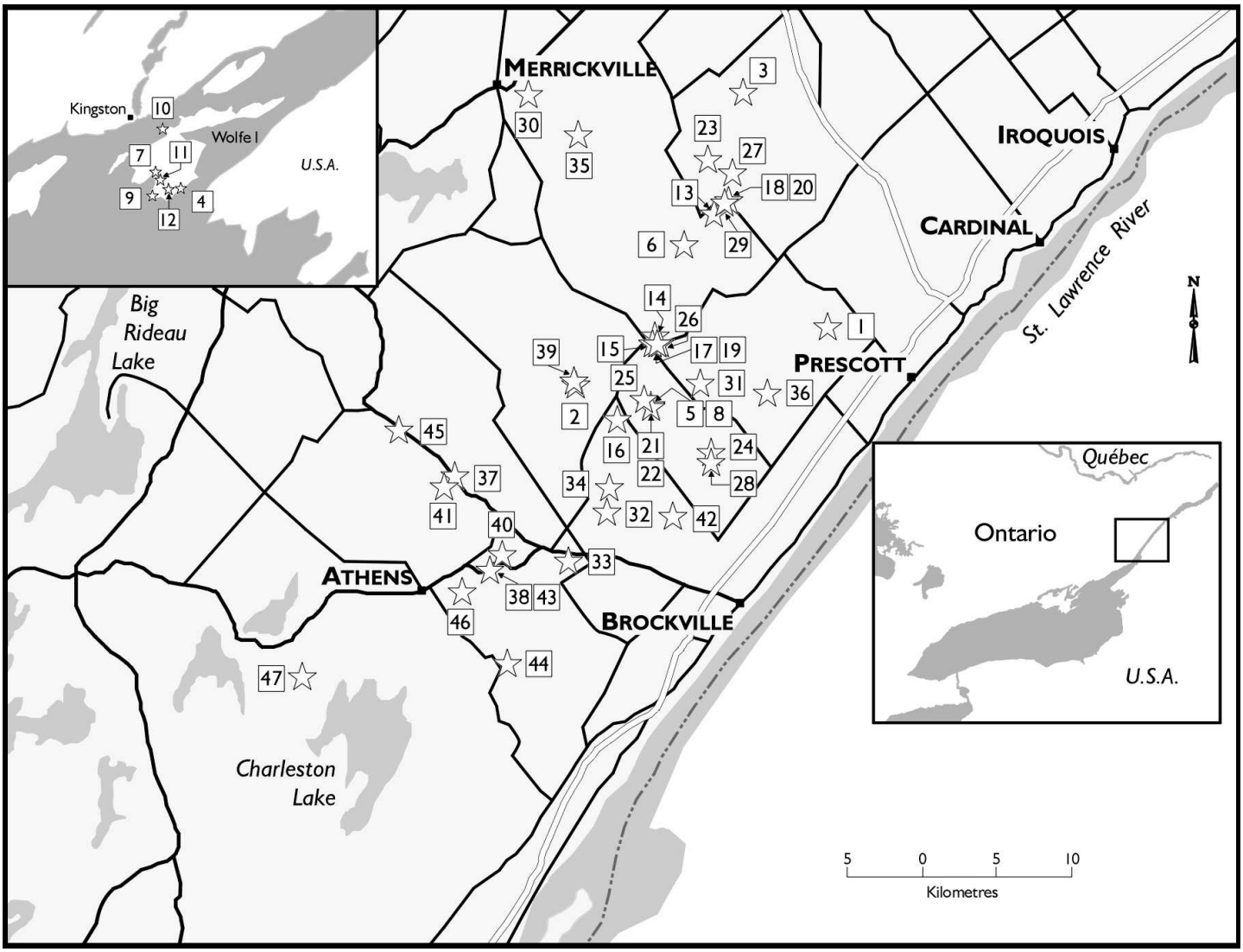

Figure 2. Raccoon rabies cases in eastern Ontario, 14 July 1999 to 31 December 2000.

blizzard of numbers coming from these efforts. Some areas have been depopulated or treated with TVR in successive years. Preliminary analysis indicates that unless over 90% of raccoons are removed, there will be enough left to rebuild the density to almost the level before treatment after a single breeding season. Substantial movements of tagged animals have been observed along the St. Lawrence, just as in the Niagara TVR area in previous years. The whole control effort has not been helped by the fact that raccoons did very well in 2000 and 2001. Densities in the area are among the highest observed in the region since 1995.

There was another interesting feature of the summer. We finally had our first two skunks infected with raccoon rabies (cases 60 and 64 in figure 3). The Ontario statistics are very different from most new invasions of raccoon rabies, in that 84/86 of all the cases have been raccoons. No dogs, cats or livestock have been found with rabies in eastern Ontario.

So, is the outbreak all over?? We really won't be sure for at least another year. This is the first time that such an intensive effort to curb an outbreak has been applied. While we had hoped to wipe out rabies in 1999, the outbreak has proven more stubborn than anticipated. This makes predictions difficult. It will be easier to see what happened when we can look back. Meanwhile, hold your breath and hope that the 13 September case really was the last.

Dennis Donovan and
Charlie MacInnes
Rabies Research Unit
Peterborough

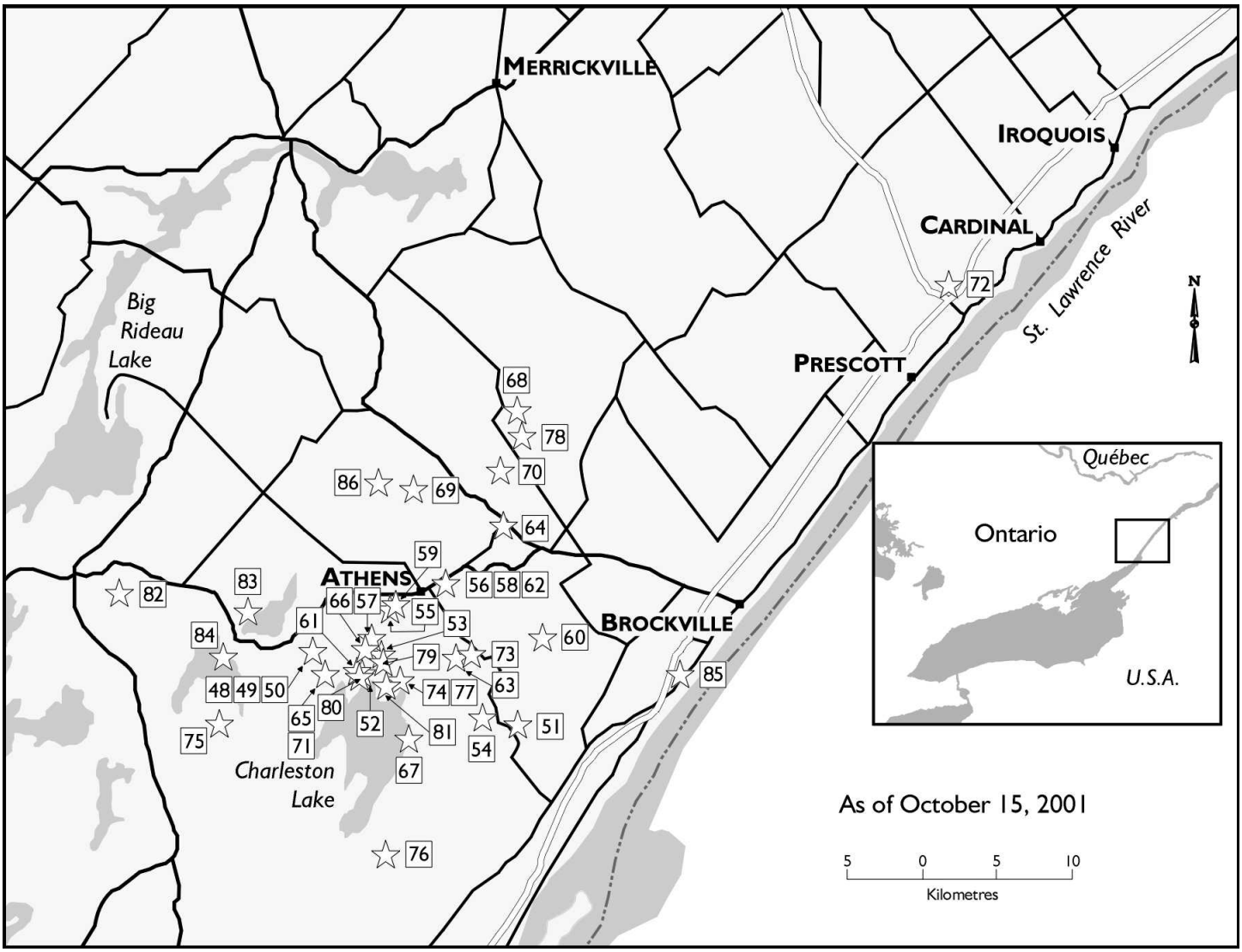

Figure 3. Map of the raccoon rabies cases in eastern Ontario 1 January to 15 October 2001. Note the whole area covered is only about 40 km by 40 km, except for two outlying cases. Comparing the front at the end of 2000 with the outermost cases in 2001 suggests that the front advanced by only about 20 km to the west. The few cases in areas where raccoon rabies was present last year is also noteworthy.

HOLY FLEDERMAUS, BATMAN WHAT HIT US?

There have already been more rabid bats in Ontario in 2001 than in any previous year since 1980, and the year is not yet done. While it is a record, the margin of record is not large. The 52 bats (so far this year) exceed the 48 for all of 1983, 47 in 1984, and 44 in 1991. The third quarter is always the time when most rabid bats are encountered.

The tables clearly show that rabid bats were widespread, except in the western region. The Niagara Health Unit put out a news release about six rabid bats by late August. That was alarming, since they only had one in 2000, none in 1999, and one in 1998. Meanwhile, Leeds and Grenville, the site of the raccoon rabies cases, has almost as many.

There was a report on 680 news, subsequent to the Niagara release, stating that 25% of all bats have rabies. That is absolutely ridiculous. Derek Pinto, information officer for the Rabies Unit, came up with a figure from the New York Rabies lab which indicates that roughly 4% of bats tested in that lab turn out to be rabies positive. Even this figure is actually misleading, because bats have to be in human living space, or dead, sick, or acting suspiciously before they will be submitted. In 1997, I tried to get an estimate of the number of bats living in southern Ontario during the summer, from Brock Fenton of York University. Brock has studied bats all over the world, but in one of his books he relates starting in Ontario when he was a teenager. Brock demurred, and I can't blame him. So let me give a very, very rough estimate. On the average evening in summer, I can see at least 10 bats around my yard light on an average night. In the 1 km^2 around my home there must be at least ten such yard lights, so that gives 100 bats/km^2. If you expand that to the 100,000 km^2 of southern Ontario, then there are 10,000,000 bats. Now, I suspect that I am greatly underestimating the number of bats around my light, as I can see ten at a given moment. There are probably many more than 10 yard lights in the block I live in, but how typical is that? I am sure the distribution of yard lights is not uniform across southern Ontario. The point, however, is that bats are very numerous. The greatest precision that I will claim for the 10,000,000 estimate is that maybe, just maybe, the true number of bats in southern Ontario is closer to 10,000,000 than to 1,000,000 or 100,000,000. Rick Rosatte estimates a million raccoons in southern Ontario. We may have ten times more bats. Measured against that estimate, rabid bats are very rare events, even when we consider that the reporting rate for rabid bats must be very poor. It also seems true that there is never enough rabies among bats to cause reductions in numbers large enough for us to notice. As has been said in several previous issues, the bats which inhabit southern Ontario have very low potential for increase, so if rabies were killing a large percentage, bats would all but disappear.

There are at least four variants of rabies in Ontario bats. The most commonly encountered is the eastern variant of big brown bat rabies, which is the variant involved in over 90% of our reported rabid bats.

Conclusion: Yes, we had more rabid bats than usual this summer, although only barely more. This is not cause for alarm. But, treat bats with respect. They might have rabies.

Charlie MacInnes
Rabies Research Unit
Peterborough

FOX VARIANT RABIES IN NORTHERN ONTARIO: RAPID CHANGES AND RAPID RESPONSE

The perennial source of fox variant rabies in Ontario is the Hudson Bay coast. Rabies seems to be present all the time in far northern Ontario, or else it may be frequently renewed by incursions from further north. Certainly, the last visible set of cases in Ontario's Hudson Bay lowlands was preceded by a few cases in arctic foxes around Churchill, Manitoba. I certainly do not understand how rabies has persisted among arctic foxes for at least three centuries, but it seems to be up there always. There are so few settlements that not many rabies cases are reported.

A year ago, there were many rabies cases around Cochrane and Iroquois Falls in northern Ontario. This summer there were no cases in that area. The next rabies-prone region south of Cochrane is the Little Clay Belt farmland between Kirkland Lake and New Liskeard. Fox rabies bypassed that, but appeared in the Sudbury area early in 2001. There were two areas of concern, the suburbs and farms to the immediate northwest of Sudbury, and a larger area along the north and west shores of Lake Nipissing. We decided, in late July, to drop baits on the latter area during the September operations. However, there had been no cases in the northwestern area since April, so no baiting was planned. Then, there was a case near Dowling during the last week of August so we changed our plans for baiting. It was a good thing, too. On Thursday 27 September, a rabid fox came into

one of the stables at the race track at Chelmsford. It was a good feeling to be dropping baits over the track less than a week later.

There is the standard problem in these areas: are there rabid foxes in the forested areas where there are few people? The rabies cases are almost all in or near the edges of the farmland. In the Sudbury area, there is a lot more forest than farm, so where is the rabies, really? The reason for this ramble is that we will not know for at least a year whether our bait drop covered a large enough area.

Another detail is that we have not yet had the virus identified to subtype. The last time fox rabies moved in to the west Nipissing and Sudbury areas, it came from the south. This time, that source is lacking, as a result of baiting, so our guess is that this outbreak came from the north.

Charlie MacInnes
Rabies Research Unit
Peterborough

FOX VARIANT RABIES IN SOUTHERN ONTARIO: NO NEWS IS NOT GOOD NEWS

Fox rabies continues to persist, more in skunks than in foxes, in six to eight counties in the western region of Ontario. The number of cases this year is almost the same as a year ago, and very slightly greater than in 1999 or 1998. The number of rabid foxes has been 12 or less in the first 9 months of each of the last 4 years. The number of rabid skunks in the same intervals ranged from 22 to 34.

Simcoe County experienced its first case of terrestrial rabies in several recent years. However, this was less than 3 km inside the western edge of the county, and less than 20 km east of several cases of the past 2 years.

There is one feature of the present situation which is unexpected and hard to explain. The percentage of foxes with rabies antibody dropped sharply in 1998, as is explained in another article in this issue. What is interesting is that the number of rabid foxes has not increased

We certainly hope that an effective skunk vaccine and bait come on stream soon.

Charlie MacInnes
Rabies Research Unit
Peterborough.
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FAREWELL TO MIDHURST, HELLO TO CODRINGTON

The wildlife research animal holding facility has been at Midhurst for almost 40 years. When I first came to MNR, in 1973, there were two compounds of animals in what was called the Wildlife Compound. The Communications Branch maintained a collection of Ontario wild animals under the very loving care of Jim Scott. Many of these animals traveled to the CNE, the Royal, and many county and regional fairs. Animals that had to be kept for legal cases, and rescued wildlife were also housed there. Away at the back of the property, there was a very primitive research compound. Eventually, Jim Scott retired, the Communications animals were moved to a new facility at Springwater Park and Research inherited the front compound. Jim Scott was a good cage designer, so it was a good, but aging facility where a variety of wild species could be kept for research purposes. As research budgets shrank, only rabies kept animals at the compound.

Fast forward a couple of decades. When both Fisheries and Wildlife Research were moved from Maple to Peterborough, a series of changes began. The fish genetics research facility at Maple was, of course, closed, but the need for the work continued. The Fisheries Research group took over a mothballed provincial hatchery at Codrington. The drive from Midhurst to Peterborough took over two hours. Both facilities had staffing problems, especially to feed fish and wildlife on weekends. The natural and sensible action was to move the wildlife compound to the hatchery site at Codrington. This had another huge advantage, as it allowed us to design new facilities specifically designed for research (learned from problems at Midhurst).

Scott Taylor has been in charge at Midhurst since 1996. He was a key person in this move. He designed the new facility, after a lot of consultation., and oversaw the construction. Animals were moved from Midhurst to Codrington in August.

The other hero in the operation was Chris Davies, manager of the Wildlife Research and Development Section. He raised the money to make the move. Before that, it was his managerial view that suggested something needed to be done.

The facility includes fiberglass roofs to cover the animal cages, and secure, locked enclosures for the actual cages. There is also a laboratory where, for example, the animals for serology may be brought to take the blood samples.

The new facility was designed to handle 30% more animals than Midhurst, and it is already bursting at the seams. The second major pressure is that we are finally making real progress on the adenovirus vaccine development. This means that we have to collect the raccoons, foxes and skunks for the efficacy trials, which should start soon. Next summer, Scott and his staff will be collecting a variety of wildlife for safety testing

of the new vaccine.

Hopefully we will have some pictures of the new facility by the time the next issue of Rabies Reporter is ready to go.

Charlie MacInnes
Rabies Research & Development Unit
Peterborough

RABIES REPORTER ENTERS THE ELECTRONIC ERA

The Rabies Reporter is adding a new format. Beginning with this issue, the Rabies Reporter will now be available in . pdf format. This requires Adobe Acrobat Reader (which is available free on the internet).

The Rabies Reporter staff want to encourage everyone who has internet access to subscribe for this method of delivery. This will help reduce our costs which may prevent future subscription costs! If you can imagine the current, photocopying and collating over 400 copies, then folding them and stuffing envelopes, you can imagine why our staff really, really want you to go electronic!

We at Rabies Reporter need to learn more about working in Acrobat, and getting Microsoft Publisher to work with Acrobat. We hope that future issues will have photos, maps and graphs reproduced in colour.

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RABIES SEROLOGY IN FOXES AND RACCOONS IN RELATION TO ORAL VACCINATION

Introduction

The success of aerial vaccine-baiting efforts to control rabies in wild animals is evaluated by 3 criteria: (1) the number of rabid animals (rabies index), (2) immunity to the rabies virus, and (3) bait uptake. Rabies index is generally determined by public submission of suspect animals - it is not part of this discussion. Immunity is evaluated from a blood sample, and bait uptake is determined from tooth or bone tissue of sampled wild animals. Oral rabies vaccines, ERA-BHK-21 (ERA) and Vaccinia-Rabies Glycoprotein (VRG), for foxes and raccoons, respectively, embedded in a bait matrix, are expected to generate an immune response when an animal eats the bait and the vaccine is absorbed into the blood from the oral cavity. After an animal eats a bait, the tetracycline biomarker, which is a component of the bait matrix, is deposited in calcium-based tissues (such as bone and teeth) after absorption into the blood from the small intestine. We can determine bait-uptake of individual animals because the tetracycline deposits in the bones and teeth are visible under ultra-violet light *(Rabies Reporter, November 1998, 9 (3): 8-9).*

The Rabies Research and Development Unit has collected wild red fox *(Vulpes vulpes)* specimens since 1989 from areas treated with ERA rabies vaccine baits. Results of bait ingestion, as evidenced by tetracycline deposition (T+), and rabies immunization or antibody presence (RA+), in wild foxes from areas that received ERA baits between 1989 and 1999 are outlined in Table 1. Bait-uptake from 1989 through 1996 ranged from 33 - 79%. During the same period, rabies antibody, as determined by ELISA (enzyme-linked immunosorbent assay) (University of Toronto (1989-91) *(Barton and Campbell 1988)* and Animal Diseases Research Institute (ADRI) of the Canadian Food Inspection Agency (1992-99) *(Elmgren and Wandeler 1996)*), was also variable but had a smaller range of 24 - 46%. These data suggest more foxes ingested and deposited tetracycline than absorbed vaccine and produced detectable levels of rabies antibody. From 1989 through 1996, the ratio of RA+/T+ was 41 - 73%. Although % T+ in 1997 to 1999 was similar to previous years (66 - 71%), %RA+, and the ratio of RA+/T+, dropped off significantly (5 - 17% and 8 - 25%, respectively). The number of wild raccoons *(Procyon lotor*) testing positive for rabies antibody was also very low after the 1999 bait drop in eastern Ontario (14% and 17% for Fishmeal Polymer and Ontario Slim Baits, respectively) despite relatively high bait acceptances. These values were much lower than those observed for raccoons caught in New York State and Ohio the same year, although bait acceptances among areas were similar. Different labs used different serology tests on different types of blood samples to determine the presence of rabies antibody for animals caught in Ontario and the U.S. The

Table 1. Results of bait (or tetracycline) ingestion (T+) and rabies mmunization or antibody presence (RA+) in wild red foxes *Vulpes vulpes* from areas receiving baits containing ERA-BHK-21 oral rabies vaccine in Ontario from 1989 to 1999.

Year	N	T+ (%)	RA+ (%)	%RA+/T+	Bait[2] type	Bait[3] attractant	Bait colour	Biomarker[4] (mg)	Blister[5] pack	Vaccine[6] potency (base)	Vaccine[7] potency (field)	Sero-assay[8]	Lab	Units	RA+ value
1989	736	243 (33)	177 (24)	73	OR	C	White	TetB (100)	PolyVC	7.25 - Co	Yes	i-ELISA	U of Toronto	titration[9]	≥ 1:16
1990	1248	699 (56)	474 (38)	68	OR	C	White	TetB (100)	PolyVC	7.35 - Co	Yes	i-ELISA	U of Toronto	titration	≥ 1:16
1991	3046	1462 (48)	853 (28)	58	OR	C	White	TetHCl (100)	PolyVC	7.44 - La	Yes	i-ELISA	U of Toronto	titration	≥ 1:16
1992	605	387 (64)	212 (35)	55	OR	CC	White	TetHCl (126)	PolyVC	7.36 - Cy	No	i-ELISA	U of Toronto	titration	≥ 1:16
1993	944	614 (65)	387 (41)	63	ORm	CC	White	TetHCl (100)	PolyVC	7.30 - Cy	No	c-ELISA	ADRI	%inhibition	≥ 20%
1994	795	620 (78)	254 (32)	41	ORm	CC VS*	White	TetHCl (100)	PolyVC	7.28 -Cy	No	c-ELISA	ADRI	%inhibition	≥ 20%
1995	603	440 (73)	229 (38)	52	ORm	CC VS*	White	TetHCl (100)	PolyVC	7.26 - Ay	Yes	c-ELISA	ADRI	%inhibition	≥ 20%
1996	833	658 (79)	383 (46)	58	ORm	CC VS*	White	TetHCl (100)	PolyVC	7.30 - Ay	Yes	c-ELISA	ADRI	%inhibition	≥ 20%
1997	1036	715 (69)	176 (17)	25	ORm	CC VS	White	TetHCl (100)	PolyVC	7.43 - Ay	No	c-ELISA	ADRI	%inhibition	≥ 20%
1998	553	393 (71)	44 (8)	11	ORm	VS	White	TetHCl (100)	PolyVC	7.24 - Ar	No	c-ELISA	ADRI	%inhibition	≥ 20%
1999	337	222 (66)	17 (5)	8	ORm ORm OS	VS VS VS	White Green Green	TetHCl (100)	PolyVC PolySt(f)	7.36 - Ar	Yes	c-ELISA	ADRI	%inhibition	≥ 20%

[1] N = number of red foxes sampled

[2] OR = Ontario regular bait (20 g), ORm = Ontario regular bait -modified-(16 g), OS = Ontario slim bait (13 g)

[3] C = Chicken, CC = Chicken-Cod, VS = Vanilla sugar, VS* = Vanilla sugar (distributed on experimental plots only)

[4] TetB = Tetracycline Base, TetHCl = Tetracycline Hydrochloride

[5] PolyVC = Polyvinylchloride, PolySt(f) = Polystyrene(flocked)

[6] Mean base production titer of vaccine, log10 TCID (Tissue culture infective doses)/ml and vaccine-bait manufacturer: Co = Connaught Labs, La = Langford, Cy = Cyanamid-Langford,
Ay = Ayerst, Ar = Artemis

[7] Record of field assay of vaccine potency

[8] ELISA = enzyme-linked immunosorbent assay, i-ELISA = indirect ELISA, c-ELISA = competitive ELISA

[9] Titration = ELISA optical density readings converted to titration units (Barton 1986)

Rabies Research and Development Unit analyzed all Ontario and New York raccoon teeth for the presence of tetracycline, however, Ohio had their own testing lab. These observations led us to investigate a number of possible sources of error that could help explain the sudden drop in rabies antibody in fox populations after 1996 and the current low prevalence of rabies antibody in wild raccoons.

Table 2. Protocol for testing fresh and necropsy blood of same individual captive and wild red foxes and raccoons.

Blood type	Sero-assay	Sero-assay Laboratory
fresh (serum)	c-ELISA	ADRI
necropsy	c-ELISA	ADRI
fresh (serum)	b-ELISA*	ADRI
necropsy	b-ELISA*	ADRI
fresh (serum)	serum neutralizing	ADRI
fresh (serum)	serum neutralizing	other laboratory

*b-ELISA = blocking ELISA, a modified version of the c-ELISA (competitive ELISA)

Theories and Hypotheses

1. Some aspect of the rabies vaccine bait or the bait delivery system changed after 1996, and as a result, the vaccine in the bait did not reach the oral cavity of the target animals, or the vaccine was not potent enough to generate an immune response.

2. The sero-assay (ELISA), or some component of the testing procedure, changed after 1996 and was subsequently not as sensitive.

3. The poor quality of blood samples taken from carcasses at the time of necropsy makes it difficult to detect rabies antibody using the ELISA test. Necropsy blood samples for assays generally consist of coagulated blood or scrapings from body cavities of skinned or unskinned carcasses, most of which were frozen for 3-4 weeks. Fresh blood samples should give more accurate results.

Annual differences in the rabies vaccine bait are summarized in Table 1. In general, the low levels of rabies antibody observed in 1997 through 1999 do not appear to be correlated with any specific bait characteristic(s). Since the method of bait delivery to wild foxes and raccoons was the same each year, we ruled out theory #1, for now, as a factor contributing to antibody reduction over time. The production vaccine titer was checked each year before bait distribution and values were acceptable in all cases. Field vaccine potency was not checked each year and unfortunately 1997 and 1998 were two of those years. It should also be noted that three different types of baits (ORW[1], ORG[2], and OSG[3]) containing different vaccine blister packs were dropped in southern Ontario in 1999 and their possible effects on seroconversion are unknown.

Although poor-quality (necropsy) blood samples were used in all years, it is possible some aspect of its collection, storage, or processing unknown to us had changed. To address theories 2 and 3, ADRI will perform a series of analyses on both fresh sera and necropsy blood samples using different serology tests. Fresh sera samples will also be analyzed for rabies antibody by a second lab (possibly NY Dept. of Health) and their results will be compared with those from ADRI.

Experimental Design

Goals

1. To examine the effect of sero-assay on rabies antibody results.

2. To examine the effect of blood quality on rabies antibody results.

3. To examine the effect of laboratory on rabies antibody results (both labs will have to agree on the technique to be used).

Blood samples from wild raccoons, and both wild and captive red foxes, were used to examine the above theories. A captive raccoon blood experiment is in the design stage and will not be discussed here.

Blood sampling

Captive fox experiment (MNR animal research protocol #00-63A)

The purpose of the captive fox experiment was to simulate blood sampling from wild animals, but under controlled conditions, which are not always possible when collecting specimens from trappers and hunters. Thirty captive adult red foxes of the red and silver phenotype and of varying ages and sex were used in the experiment. The foxes were housed in cages and the experiment was done at our Animal Research Facility at Midhurst, Ontario.

Before the experiment, all foxes were bled (10ml) via cardiac puncture on 5 October 2000. Animals were always anaesthetized during blood extractions. On 10 October 2000, foxes were divided into 3 treatment groups of 10 animals each. Foxes in the first group were each fed one of the regular OSG baits containing 2ml of ERA oral rabies vaccine in a blister pack embedded in the bait matrix. Individuals in this group were viewed to confirm bait ingestion and evaluate vaccine spill-

age. Foxes in the second group were administered 1ml of rabies vaccine (IMRAB3) by intra-muscular injection. Foxes in the last group were control animals. They had never eaten a bait containing oral rabies vaccine nor had they ever been vaccinated against rabies. All foxes were starved the morning of the treatment and fed immediately after treatment. Eight of the 10 foxes in group 1 ingested baits on the first day and before 15:00. The remaining two foxes ate their baits on the second day (11 October 2000). There was no detected spillage of vaccine and so it was assumed the vaccine was ingested in all cases.

On 16 November 2000, all foxes were anaesthetized, bled (10 ml), and then euthanized. Blood was collected again 5-10 minutes after death (5-7 ml) and then all carcasses were stored at 4°C for 4 days. On 20 November 2000, fox carcasses were skinned by a licensed trapper then frozen at -20°C. On 11 December 2000, fox carcasses were thawed for one day and then processed in the usual manner. Two upper canine teeth were removed from each animal and a blood sample (8-10 ml) was collected from the heart or chest cavity. On one occasion, it was necessary to collect blood from the abdominal cavity. Blood collected from live animals or shortly after death was considered 'fresh' blood, while the blood material taken in the lab from carcasses frozen for 3 weeks was called 'necropsy' blood. The necropsy blood was the same as the poor quality blood samples taken from carcasses in previous years during the routine necropsy procedure. All blood samples were refrigerated at 4°C for 48 hours.

Wild fox and raccoon survey (MNR animal research protocol #00-63B*)*

The MNR Wildlife Rabies Control Program (WRCP) has enlisted licensed trappers and hunters since its inception (1968) to supply specimens to evaluate the success of the baiting program, monitor rabies incidence, and gather demographic information such as age structure, sex ratio, and reproductive status. Fur trapping and hunting is a wide-ranging and regulated activity in Ontario. In our efforts to enlist trappers and hunters as co-operators, we attempted not to affect the 'normal' activities of the trapping and hunting effort to avoid any biases caused by our involvement.

Selected cooperating trappers and hunters were trained in blood sampling techniques by WRCP staff and provided with the necessary needles and monovette tubes for blood collection. Blood samples were collected from foxes and raccoons trapped or hunted in Ontario between 15 October 2000 - 15 February 2001, from areas that received oral rabies vaccine baits (ERA vaccine baits for foxes and VRG vaccine for raccoons) in June and September 2000. Blood samples were also collected from animals in areas that had never been baited (controls). Co-operators were asked to collect 5-10ml of blood directly from the heart of foxes and raccoons as close to the time after death as possible, but within 30 minutes. Blood samples were refrigerated at 4°C and retrieved by WRCP staff within 48 hours. Blood tubes were identified with a species reference, capture date, trap/hunt location, and cooperator name. Skinned and unskinned carcasses corresponding to the blood samples were collected from co-operators at regular 2 to 4 week intervals. Specimens were usually stored frozen by cooperators until pick-up. Each carcass had an identification tag attached to it with the same information as the blood tube. This way we were sure the blood samples collected earlier corresponded to the carcasses picked up later. At the lab, carcasses were stored at -20°C until processing. The necropsy procedure was the same as for captive animals. Specimens were thawed for 1 day, blood material was collected from the heart or chest cavity, and 2 upper canine teeth were extracted. If blood could not be collected from the heart or chest cavity, it had to be taken from the abdominal cavity or from the collection bag it arrived in. These latter blood samples could contain traces of urine, gastric enzymes, hormones, and/or fat.

Laboratory procedures and sero-assay

After 48 hours at 4^{o}C refrigeration, all blood samples (fresh and necropsy) were centrifuged in the lab at 3,000 rpm for 12 minutes (4^{o}C). Centrifugation of fresh blood samples usually produced a well-defined straw-coloured sera layer on top of a dark layer containing red blood cells and fibrin. Blood samples taken from carcasses that had been frozen always contained ruptured red blood cells. These blood samples did not produce well-defined sera and blood cell / platelet layers after centrifugation and as a result it was difficult to extract pure sera samples from them. For both fresh and necropsy blood, an attempt was always made to extract the best 2-4ml serum sample for analysis. For all specimens, there was a clear serum sample from fresh blood that corresponded with a partially separated or unseparated blood sample from necropsy blood. Tooth samples will be sectioned with an Isomet saw and analyzed under an ultra-violet/polarizing light microscope to evaluate tetracycline deposits and determine the age of the animal.

Table 2 outlines the sero-assays that will be conducted by two laboratories to determine rabies antibody and rabies serum neutralization.

1. ADRI will test fresh sera us-

ing 3 different serology tests (c-ELISA, b-ELISA, neutr)

2. ADRI will test necropsy samples using 2 different serology tests (c-ELISA, b-ELISA)

3. Two different labs will each test fresh sera using the neutralization test.

---

[1] ORW (Ontario Regular White bait) - 3.5 x 3.5 x 1.6cm wax-oleo cube, with a vanilla-sugar attractant, weighing 16-20g and containing 100mg tetracycline hydrochloride; the polyvinylchloride blister pack (2.8 x 2.8 x 0.8cm), containing 2ml vaccine, was completely embedded in the bait matrix.

[2] ORG (Ontario Regular Green bait) - same as ORW except green food-grade dye (5%) was added for colour.

[3] OSG (Ontario Slim Green bait) - 3.5 x 3.5 x 1.1cm wax-oleo cube, with a vanilla-sugar attractant, weighing 13g and containing 100mg tetracycline hydrochloride; the blister pack was the same as in ORW and ORG except it was made out of flocked polystyrene and the paper side of the laminated aluminum seal (which contained the warning label) was exposed while the rest of the blister pack was embedded in the bait matrix.

Acknowledgements: Thanks to the following members of the Rabies Research and Development Unit, OMNR, for procedural input and technical assistance: Kim Bennett, Andrew Bruce, Mark Gibson, Kathryn MacDonald, Heather Logue, Andrew Silver, Holly Simpson, Beverly Stevenson, Scott Taylor, and to Don Dodds who skinned the test animals.

Results will be presented in a future issue of *The Rabies Reporter*.

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1. Barton, L.D. 1986. Determination of rabies-specific antibodies in wildlife sera. Masters Thesis. Department of Microbiology, University of Toronto. pp. 39i.
2. Barton, L.D. and J.B. Campbell. 1988. Measurement of rabies-specific antibodies in carnivores by an enzyme-linked immunosorbent assay. Journal of Wildlife Diseases, 24: 246-258
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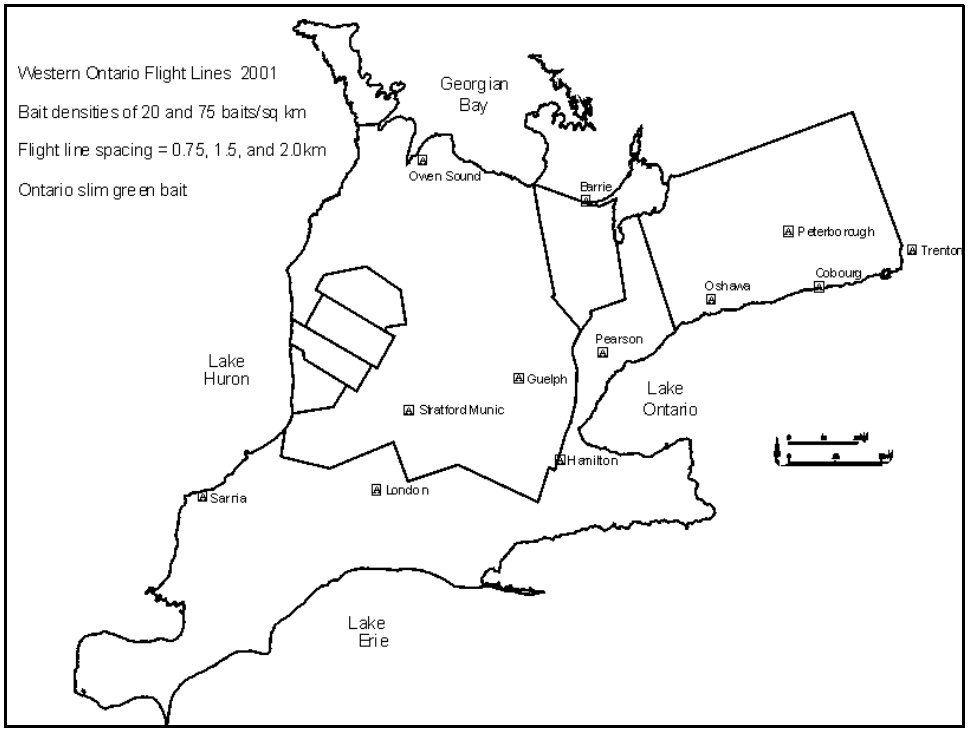
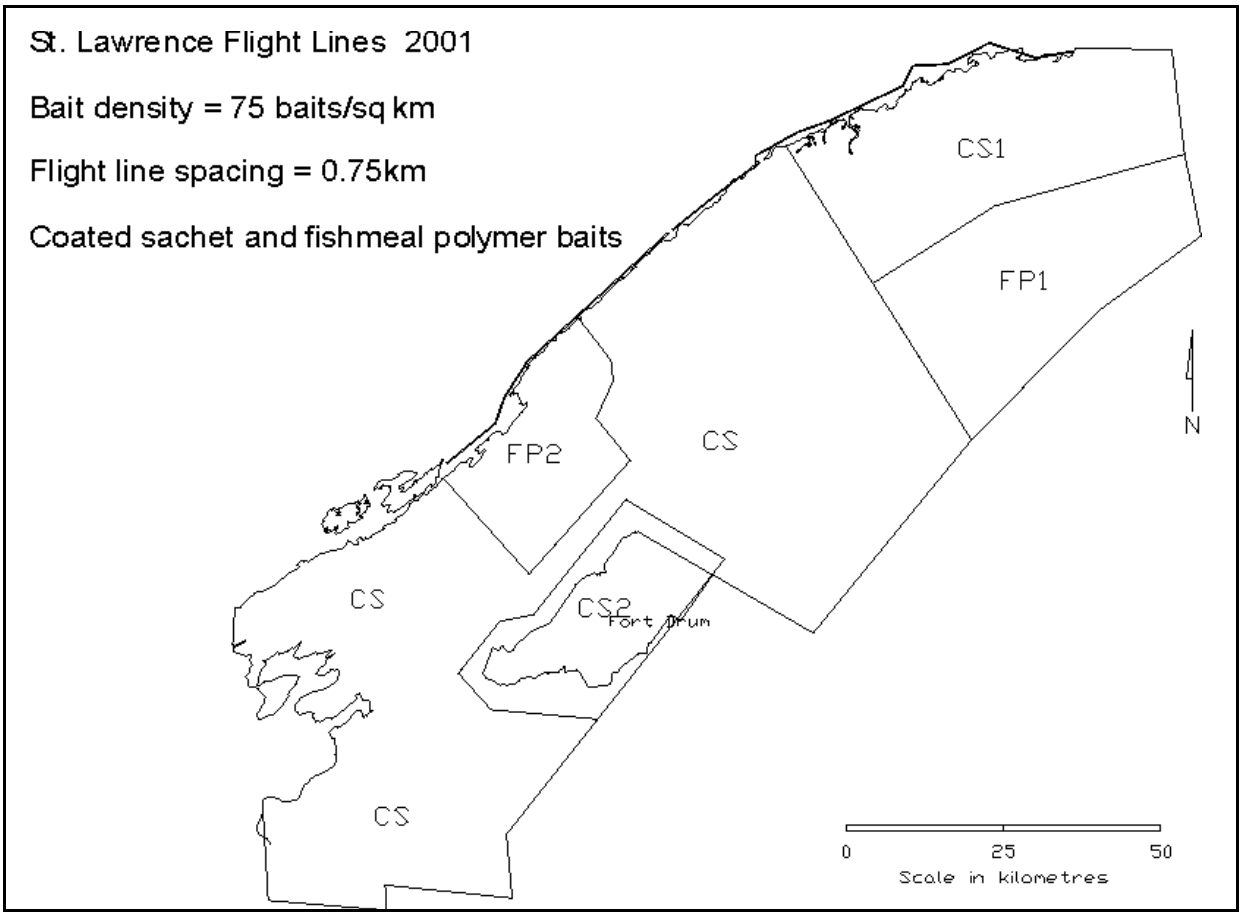
ADRI
Nepean

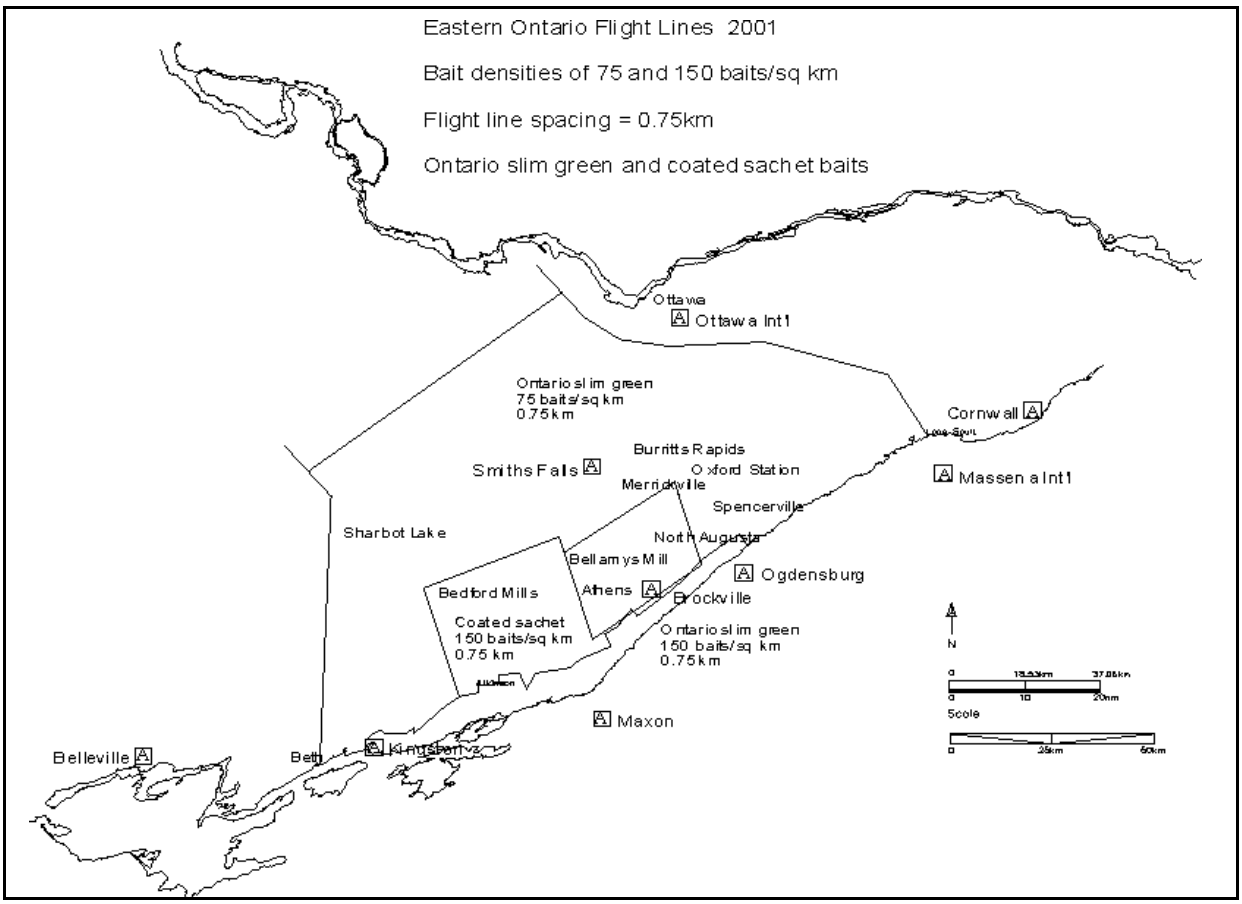
FLIGHT OPERATIONS IN 2001



The areas of Ontario and New York which received air-dropped baits are shown in the maps on the following pages. This was a busy year. The New York and Vermont bait drops started in early August. They were followed by the eastern Ontario raccoon program. The fox programs followed the Ohio drops in September.

A lot of people helped make these drops happen. Special thanks are due to the senior Air Service and Fire Management personnel, who got us a second aircraft, and to all the waterfowl people along the Hudson and James Bay coasts, who cheerfully helped coordinate their priorities with ours. It was teamwork at its best.


St. Lawrence Flight Lines 2001
Bait density = 75 baits/sq km
Flight line spacing = 0.75km
Coated sachet and fishmeal polymer baits
CS1
FP1
FP2
CS
CS
CS2
Fort Drum
CS
N
0
25
50
Scale in kilometres

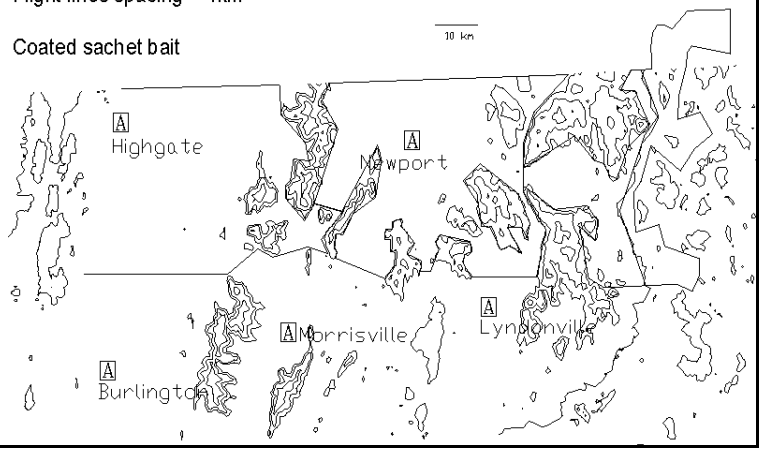

Eastern Ontario Flight Lines 2001
Bait densities of 75 and 150 baits/sq km
Flight line spacing = 0.75km
Ontario slim green and coated sachet baits
Ottawa
Ottawa Int1
Ontario slim green
75 baits/sq km
0.75 km
Cornwall
Massena Int1
Burritts Rapids
Oxford Station
Smiths Falls
Merrickville
Spencerville
Sharbot Lake
North Augusta
Bellamys Mill
Ogdensburg
Bedford Mills
Athens
Brockville
Coated sachet
150 baits/sq km
0.75 km
Ontario slim green
150 baits/sq km
0.75 km
Maxon
Belleville
N
Scale

Vermont & New Hampshire Flight Lines 2001

Bait density = 70 baits/sq km

Flight lines spacing = 1km

Coated sachet bait

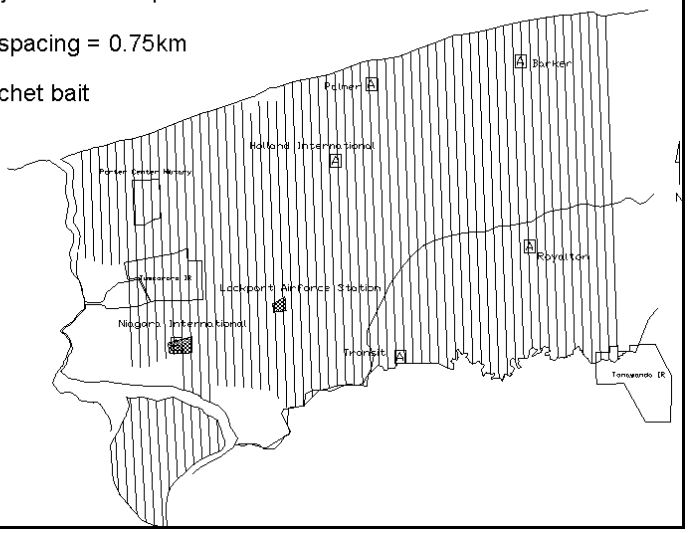


Niagara Flight Lines 2001

Bait density = 75 baits/sq km

Flight line spacing = 0.75km

Coated sachet bait

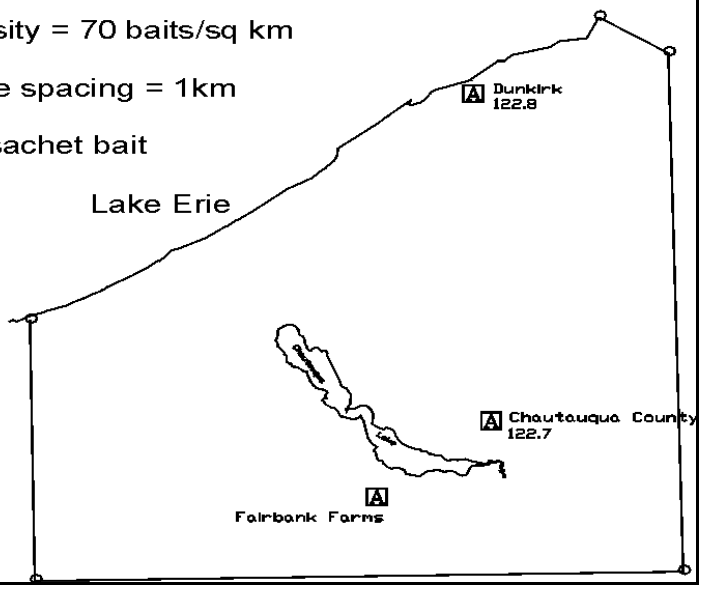


Dunkirk Flights Lines 2001

Bait density = 70 baits/sq km

Flight line spacing = 1km

Coated sachet bait



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Animal Rabies Report: July to September 2001

Animal Type	Fox			Raccoon			Skunk			Other Wildlife			Bat			Dog			Cat			Livestock			Totals		
County or Region	#	Cumulative		#	Cumulative		#	Cumulative		#	Cumulative		#	Cumulative		#	Cumulative		#	Cumulative		#	Cumulative		#	Cumulative	
		01	**00**		**01**	**00**		**01**	**00**		**01**	**00**		**01**	**00**		**01**	**00**		**01**	**00**		**01**	**00**		**01**	**00**
Eastern																											
Stormont, Dundas & Glengarry													1	1	1										1	1	1
Prescott																									0	0	0
Russell																									0	0	0
Ottawa-Carleton													3	5	6										3	5	6
Renfrew														1											0	1	0
Lanark																									0	0	0
Leeds and Grenville				11	39	31		2					5	6	3										16	47	34
Frontenac						4							1	3	3										1	3	7
Lennox & Addington														3											0	3	0
Central																											
Hastings															2										0	0	2
Prince Edward													1	1											1	1	0
Northumberland															1										0	0	1
Victoria														1											0	1	0
Haliburton																									0	0	0
Peterborough							1	4	1				2	2											3	6	1
Durham														1	1										0	1	1
York Region													1	1											1	1	0
Toronto																									0	0	0
Simcoe							1	1					3	3	2							1	1		5	5	2

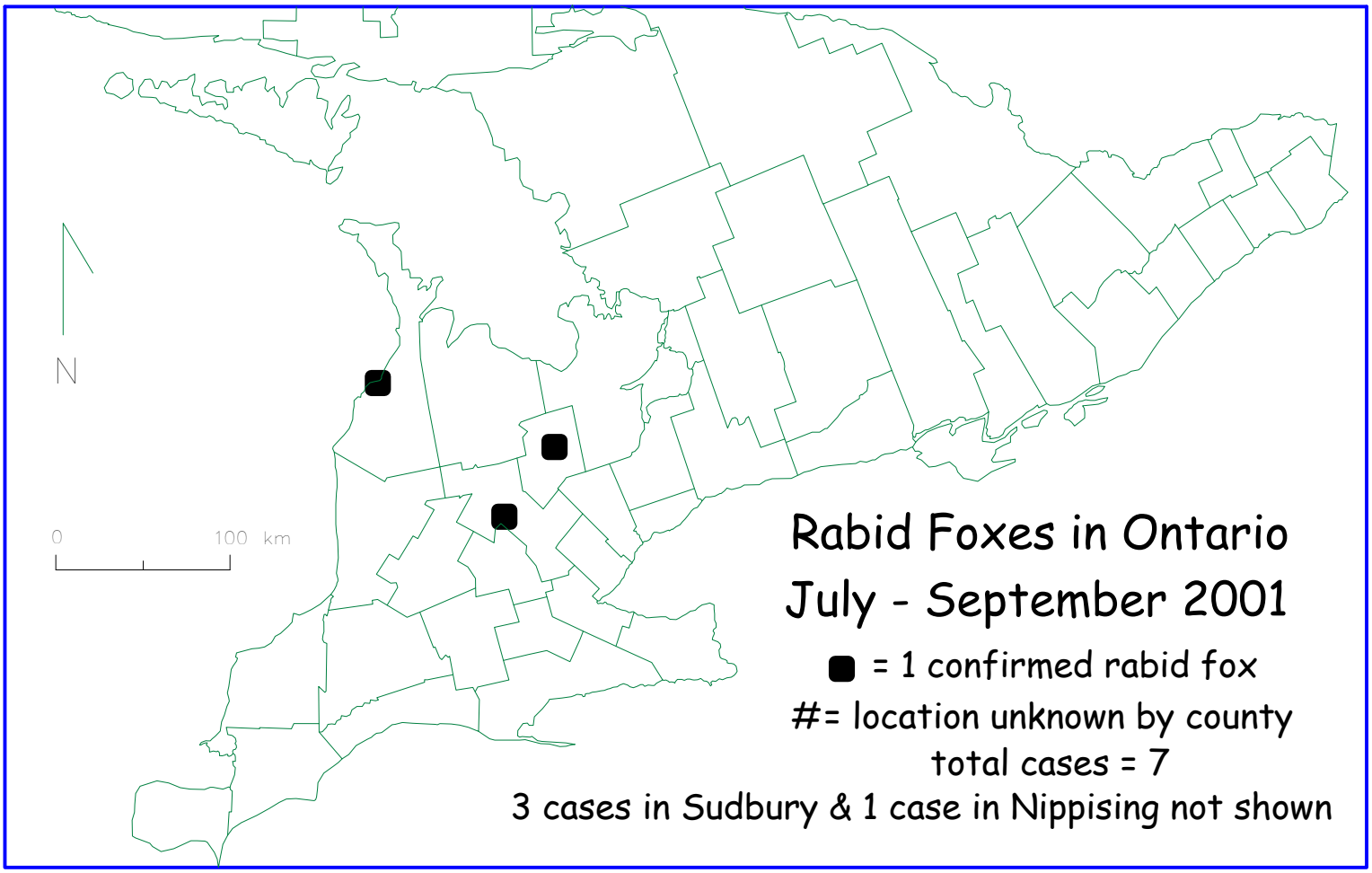
Animal Rabies Report: July to September 2001

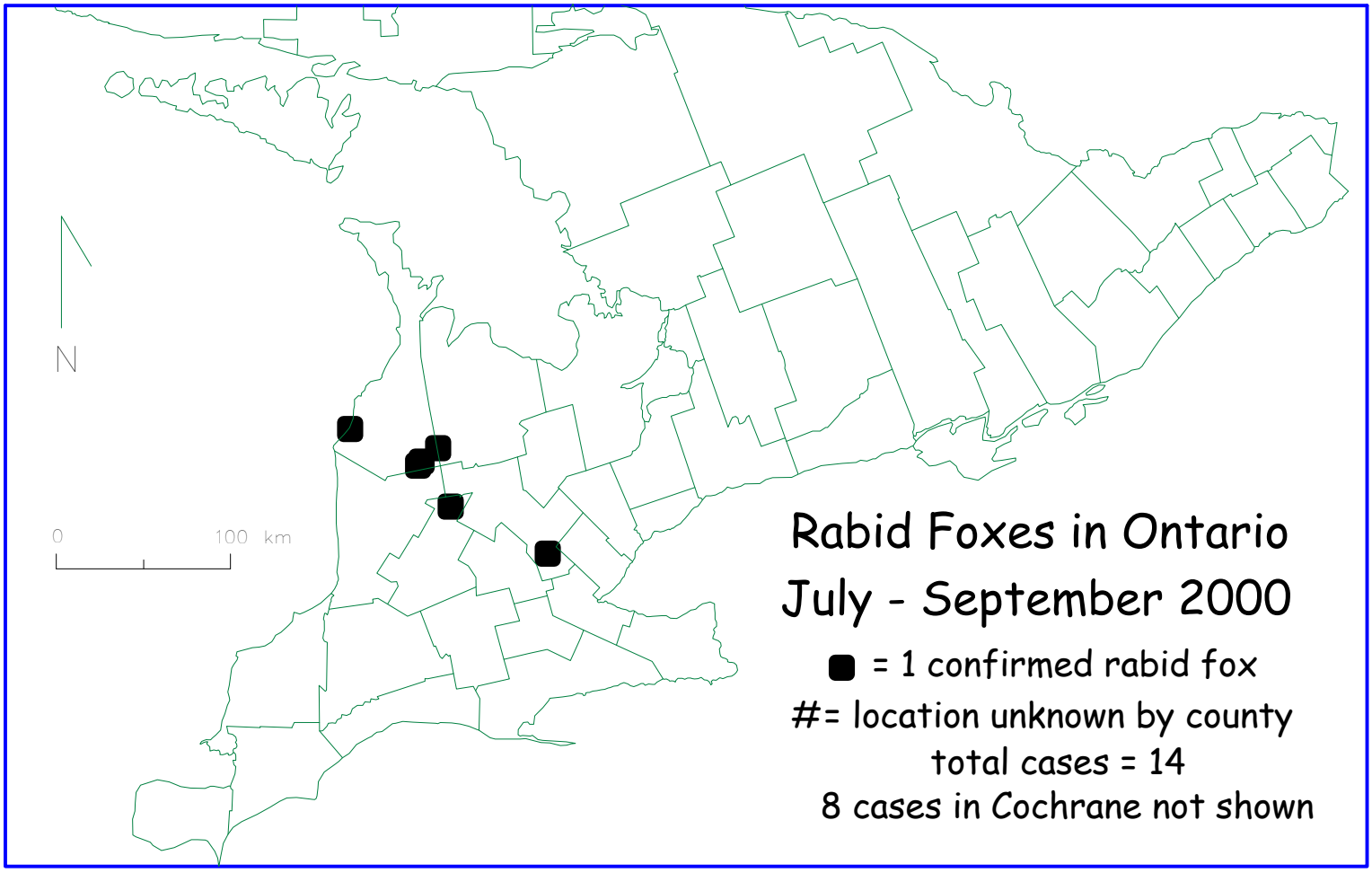
Animal Type	Fox			Raccoon			Skunk			Other Wildlife			Bat			Dog			Cat			Livestock			Totals		
County or Region	#	Cumulative		#	Cumulative		#	Cumulative		#	Cumulative		#	Cumulative		#	Cumulative		#	Cumulative		#	Cumulative		#	Cumulative	
		01	**00**		**01**	**00**		**01**	**00**		**01**	**00**		**01**	**00**		**01**	**00**		**01**	**00**		**01**	**00**		**01**	**00**
Western																											
Peel															1										0	0	1
Halton															1										0	0	1
Dufferin	1	1						1	4													3	8	1	4	10	5
Wellington	1	1	2				2	5	2				1	1	1	1	1						1	1	5	9	6
Waterloo								3	1													1	1		1	4	1
Perth			1				1	3	2																1	3	3
Grey							4	6	12									1				1	2	1	5	8	14
Bruce			4				2	5	6									1					1	2	2	6	13
Huron								3	5																0	3	5
Southern																											
Wentworth													1	1											1	1	0
Haldimand-Norfolk													1	1											1	1	0
Brant															1										0	0	1
Niagara													9	10	1										9	10	1
Elgin													4	4											4	4	0
Oxford																									0	0	0
Middlesex													4	5	4										4	5	4
Lambton													2	2											2	2	0
Kent													1	1	2										1	1	2
Essex																									0	0	0

Animal Rabies Report: July to September 2001

Animal Type	Fox			Raccoon			Skunk			Other Wildlife			Bat			Dog			Cat			Livestock			Totals		
County or Region	#	Cumulative		#	Cumulative		#	Cumulative		#	Cumulative		#	Cumulative		#	Cumulative		#	Cumulative		#	Cumulative		#	Cumulative	
		01	**00**		**01**	**00**		**01**	**00**		**01**	**00**		**01**	**00**		**01**	**00**		**01**	**00**		**01**	**00**		**01**	**00**
Northern																											
Muskoka																									0	0	0
Parry Sound																									0	0	0
Nipissing	1	3															1						1		1	5	0
Sudbury	3	14						1											1	1					4	16	0
Cochrane		2	14						1		2						1	3					3		0	8	18
Timiskaming											1														0	1	0
Algoma																									0	0	0
Thunder Bay																									0	0	0
Rainy River																									0	0	0
Kenora																									0	0	0
Regional Totals																											
Eastern				11	39	35		2					10	19	13										**21**	**60**	**48**
Central							2	5	1				7	9	6							1	1		**10**	**15**	**7**
Western	3	3	7				9	26	32				1	1	3	1	1	2				5	13	5	**19**	**44**	**49**
Southern													22	24	8										**22**	**24**	**8**
Northern	4	19	14					1	1		3						2	3	1	1			4		**5**	**30**	**18**
TOTALS	**7**	**22**	**21**	**11**	**39**	**35**	**11**	**34**	**34**	**0**	**3**	**0**	**40**	**53**	**30**	**1**	**3**	**5**	**1**	**1**	**0**	**6**	**18**	**5**	**77**	**173**	**130**

Notes for this quarter: Livestock this quarter - 2 horses (1 in Waterloo and 1 in Simcoe), all the rest were cattle.
Bat - 1 red bat and 1 hoary bat in Niagara, 1 little brown bat in Simcoe, all other rabid bats this quarter were big brown bats





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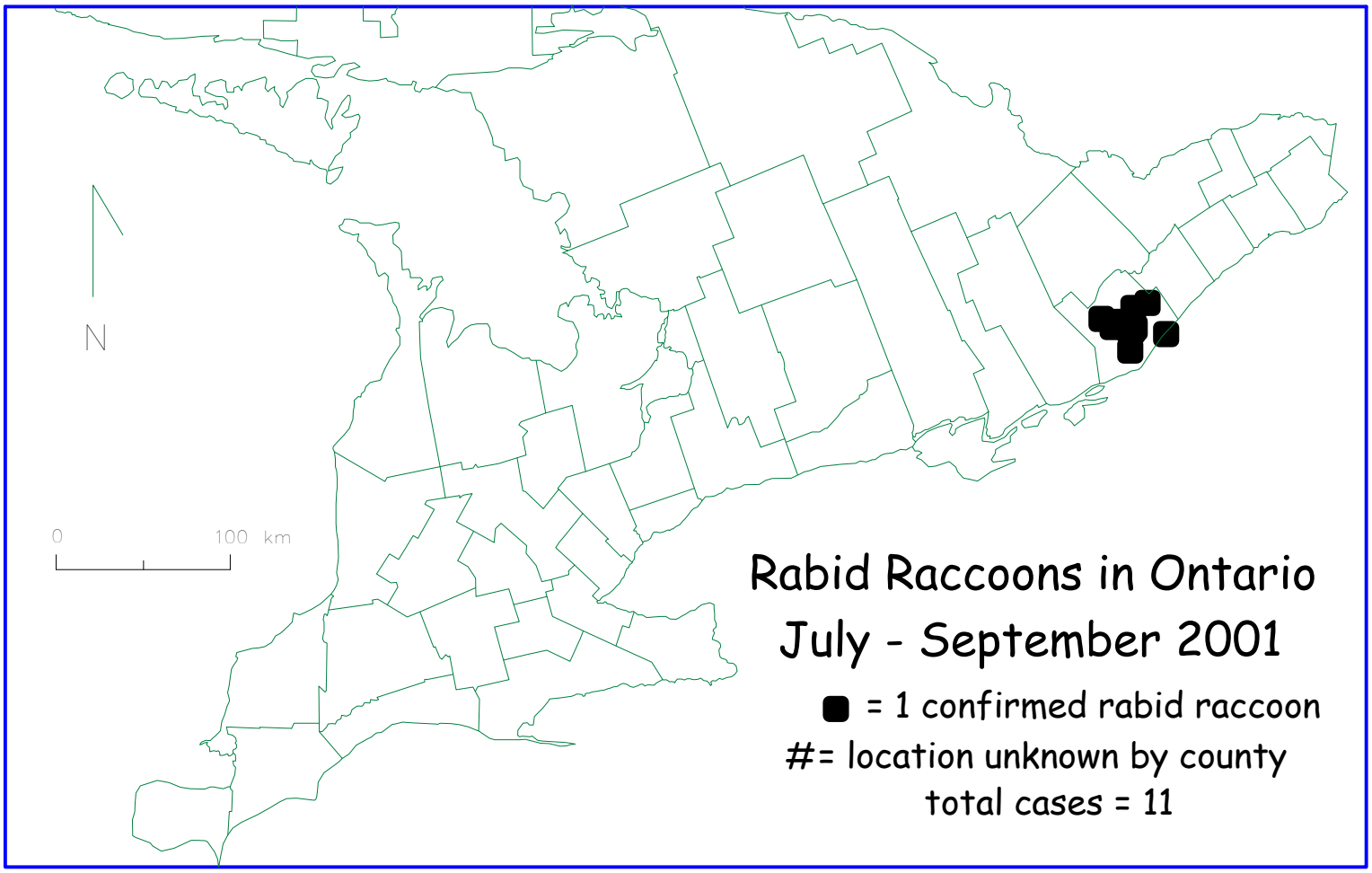
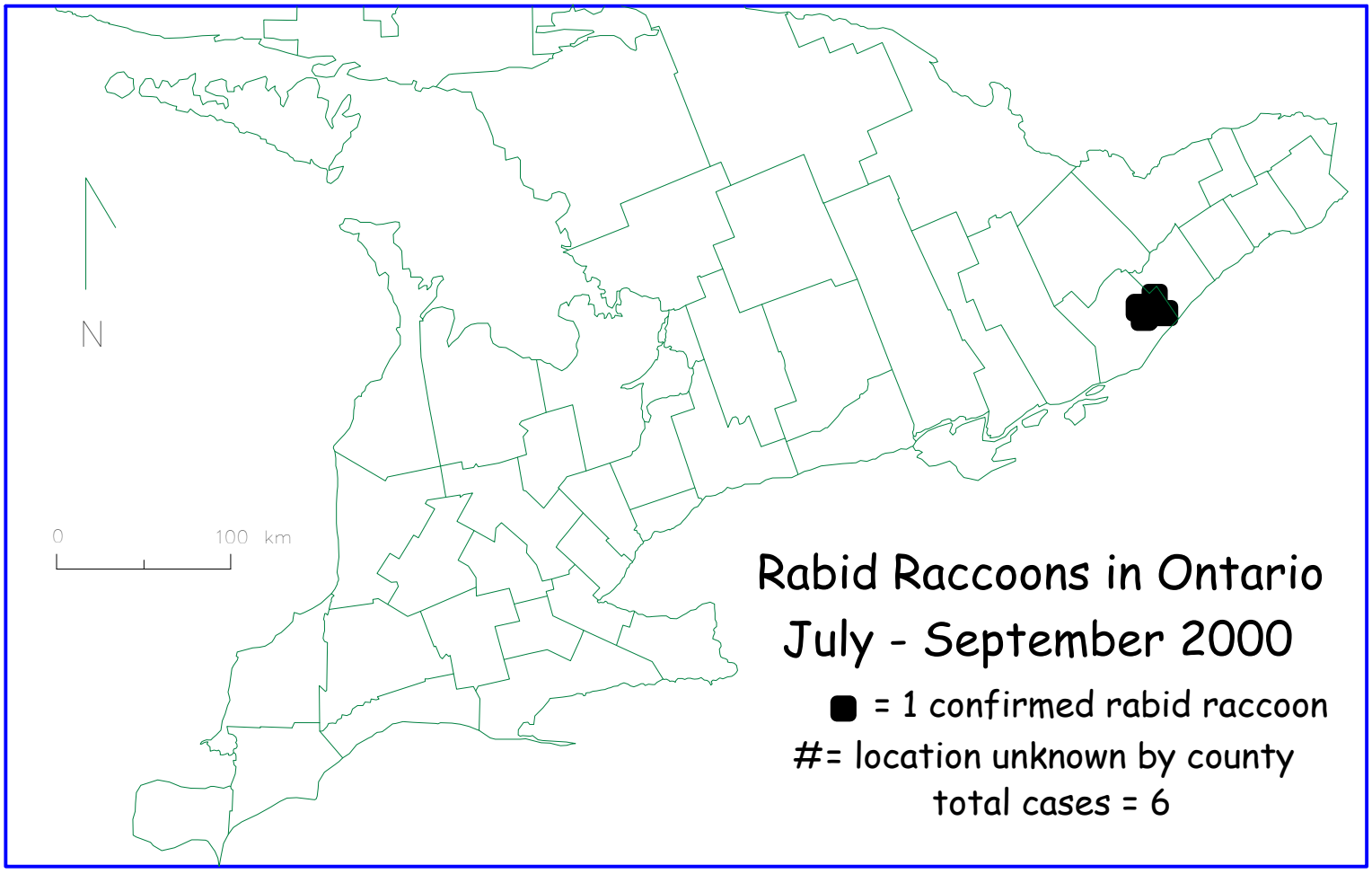
Data Source: Animal Diseases Research Institute, Nepean, Ontario





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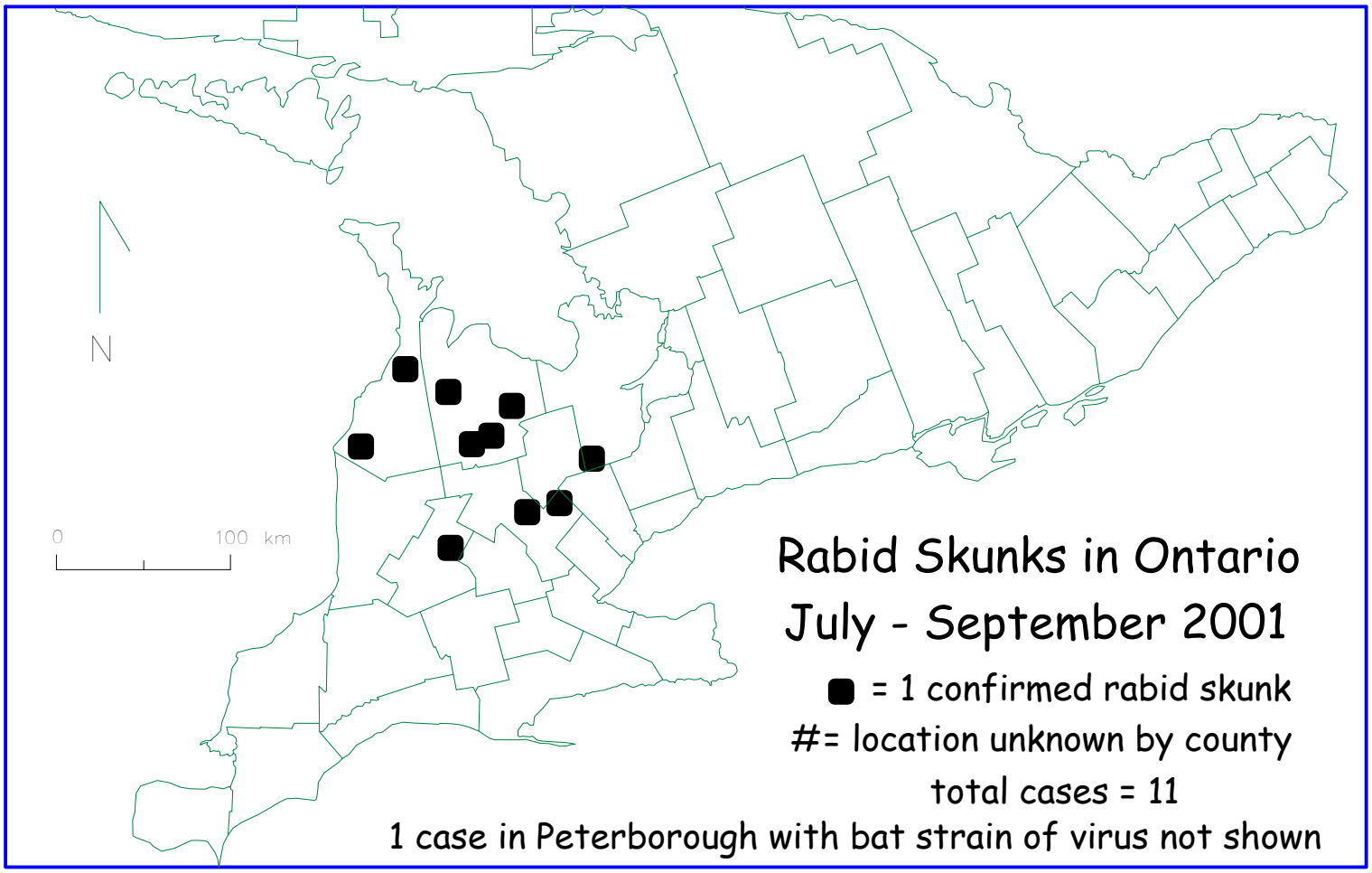

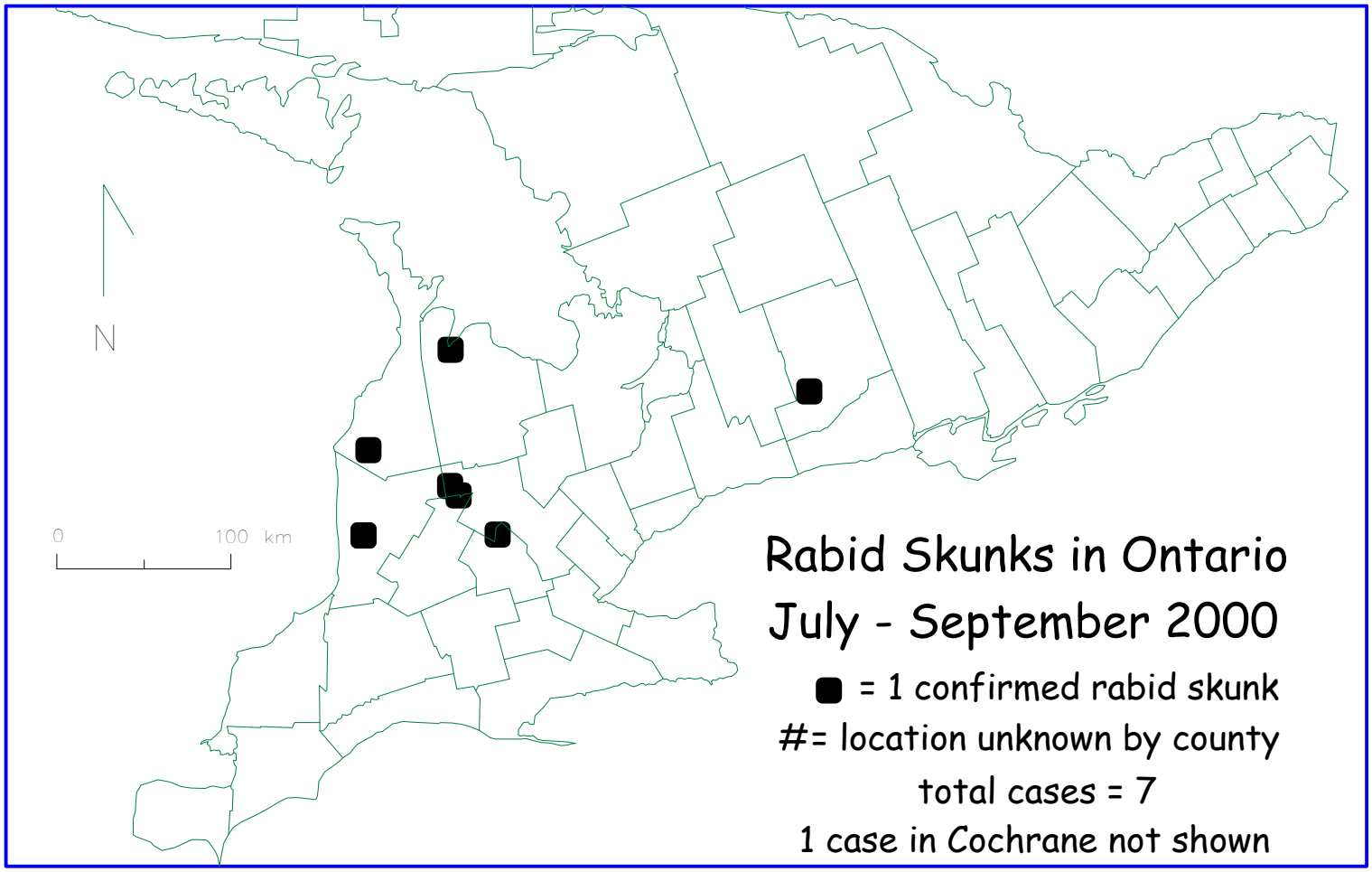

GIS Lab, Queen's University
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