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ORIGINS OF THE RABIES VIRUSES ASSOCIATED WITH AN OUTBREAK IN NEWFOUNDLAND DURING 2002–2003

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ABSTRACT: After being free of rabies of terrestrial mammals since 1988, an outbreak of rabies occurred on the Island of Newfoundland in December 2002 and continued into the middle of 2003. Twenty-one cases, all due to the arctic fox strain of rabies virus, were reported. To explore the immediate origins of this outbreak, viruses from the Newfoundland epizootic were genetically compared to two other rabies viruses recovered in mid-2002 from Cartwright, a mainland coastal community near the Island. While all Island isolates from the 2002–03 outbreak were genetically very similar, consistent with a single introduction from the mainland, they were phylogenetically quite distant from the two samples from Cartwright. A broader-based study examined the relationships between the Island viruses and arctic fox strain viruses originating from across Canada over a period of 14 yr. This analysis indicated that the Newfoundland outbreak viruses were most similar to a variant identified in Labrador in 2004 but also widely distributed in northern Quebec both before and after the Newfoundland incursion. The eastern coastline of mainland Labrador has harbored a particularly large number of variants during the study period, some of which have not been detected elsewhere. A small number of Greenland isolates included in this study were dispersed within the clades of the Canadian samples rather than forming a discrete cluster, an observation that may underline the relative ease of movement of the rabies arctic lineage between these two countries as a result of animal movements over pack ice.

Key words: Fox, genetic variant, molecular epidemiology, rabies virus.

INTRODUCTION

In Canada several wildlife species, including the striped skunk (*Mephitis mephitis*) (Pybus, 1988), arctic fox (*Alopex lagopus*), red fox (*Vulpes vulpes*), and many bat species (Nadin-Davis et al., 2001), maintain specific geographically localized rabies virus strains; in addition, sporadic incursion of the raccoon (*Procyon lotor*) strain of rabies from the United States has been reported (Wandeler and Salsberg, 1999; MacInnes, 2000). Arctic foxes are known to harbor rabies throughout their circumpolar range including northern Canada, Alaska, Greenland, and northern regions of the former Soviet Union (Tabel et al., 1974; Mork and Prestrud, 2004). In the mid-1950s an outbreak of rabies, which was historically and epidemiologically linked to the disease in the arctic, spread southwards into much of eastern Canada (Tabel et al., 1974); this resulted in an extensive enzootic in the red fox population that persisted in Ontario until the early 1990s, when provincial authorities began a comprehensive oral vaccination program throughout the endemic zone (Rosatte et al., 1993). While this activity appears to have dramatically reduced fox rabies incidence and eliminated it from some regions (MacInnes et al., 2001), this rabies virus strain persists in parts of northern Canada and remains a threat for reinvasion of southern areas (Nadin-Davis et al., 1994, 2006).

In view of the ability of all rabies virus strains to infect a variety of mammalian species in addition to the reservoir host, the typing of rabies virus strains is essential to improve epidemiological knowledge related to outbreaks and their control. Accordingly antigenic and genetic methods that can differentiate these rabies viruses have been developed (Smith, 2002), and these tools are routinely applied to rabies cases in terrestrial animals in Canada. The application of phylogenetic methods to the study of rabies viruses is not only an important

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epidemiological tool to discriminate between viral strains but allows exploration of viral variation within an individual strain. Using such methods the emergence of geographically restricted variants of the arctic fox strain in Canada has been identified (Nadin-Davis et al., 1993, 1994, 2006).

The Province of Newfoundland and Labrador, the most easterly of the Canadian provinces, is made up of two distinct geographic regions, the Island of Newfoundland and the mainland region of Labrador. Rabies is considered to be endemic in red and arctic foxes in Labrador, and, although it is reported in most years, case numbers fluctuate according to fluctuations in fox population densities. In addition to cases due to the arctic fox variant there has been a single report of rabies in Labrador in a little brown bat (*Myotis lucifugus*) in 2004 (details provided at http://www.nr.gov.nl.ca/agric/animal_diseases/rabies/pdf/batrabies.pdf).

While the Island of Newfoundland is normally free of terrestrial rabies, four exceptions have been documented: a single dog from a north coastal community, which might have been infected on the mainland, was diagnosed in 1955; an outbreak of arctic fox strain rabies involving a total of five confirmed cases occurred in 1988; a single case of rabies caused by a bat variant was found in a fox on the south coast of the Island of Newfoundland in 1989; and, last, an outbreak starting during December 2002 and lasting for 5 mo, resulted in 21 cases, involving 17 red foxes, three sheep, and one cat. Additional details on all of these cases are provided at http://www.nr.gov.nl.ca/agric/animal_diseases/rabies/default.stm; the last outbreak is the subject of this article.

A phylogenetic approach has been applied to explore the origins of the 2002–03 outbreak. It was suspected that the two cases of rabies reported on the coastal mainland some months prior to the Island outbreak might be epidemiologically related. Because preliminary sequencing studies did not strongly support this conclusion, a more thorough study of the extent of arctic fox rabies virus variation across much of northern Canada was undertaken to identify the likely origins of this outbreak.

MATERIALS AND METHODS

Diagnosis and viral typing

Brain smears of all rabies suspect specimens were tested for the presence of viral antigen using the direct fluorescent antibody (FA) test as described (Webster and Casey, 1988). This included all 21 cases recovered from the Island of Newfoundland in 2002 and 2003 and a collection of 39 specimens recovered from Ontario, Quebec, Labrador, and northern regions of Canada between the years 1990–2004. Antigenic typing of specimens, which discriminates the arctic fox strain from other indigenous Canadian rabies viruses, was performed using a 16 monoclonal antibody panel (Nadin-Davis et al., 2001). This study also included four samples from Greenland, selected from a collection of 35 previously characterized viruses; these originated from different geographical areas and were genetically representative of the range of variation observed in that country.

Genetic characterization of viruses

Total brain RNA was recovered from each specimen using TRIzol reagent according to the supplier's directions (Invitrogen, Burlington, Ontario, Canada). Amplification of rabies viral sequences was performed by targeting either the N or P genes by a standard reverse transcription-polymerase chain reaction (RT-PCR) as described (Nadin-Davis, 1998; Nadin-Davis et al., 2002). The resulting amplicons were purified using the Wizard PCR purification system (Promega, Madison, Wisconsin, USA) and then sequenced using a NEN 4200L automated sequencer (LiCor, Lincoln, Nebraska, USA). Partial N and P gene sequences of 600 nucleotides each, corresponding to bases 188–787 and 1,614–2,213, respectively, of the Pasteur Virus (PV) reference strain (GenBank accession M13215), were determined.

Nucleotide sequences were aligned using CLUSTALX v. 1.8 (Thompson et al., 1997) and then subjected to phylogenetic analysis using the PHYLIP v. 3.63 software package (Felsenstein, 1993) using neighbor joining (NJ), maximum likelihood (ML), and maximum parsimony (MP) methods. Trees were

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TABLE 1. Summary of all rabies diagnostic submissions from the Province of Newfoundland and Labrador over a 22-yr period.

Year	Positives/total submissions		
	Mainland	Island	Entire province
1985	0/0	0/0	0/0
1986	0/3	0/4	0/7
1987	0/4	0/2	0/6
1988	16/32	4/402	20/434
1989	0/13	2/172	2/185
1990	0/6	0/18	0/24
1991	1/9	0/14	1/23
1992	14/33	0/16	14/49
1993	1/3	0/8	1/11
1994	0/2	0/8	0/10
1995	1/7	0/12	1/19
1996	18/40	0/14	18/54
1997	0/3	0/6	0/9
1998	0/7	0/4	0/11
1999	0/0	0/6	0/6
2000	1/10	0/12	1/22
2001	9/31	0/14	9/45
2002	2/5	2/18	4/23
2003	0/5	19/104	19/109
2004	7/30	0/513	7/543[a]
2005	4/7	0/2,357	4/2,364[a]
2006	0/1	0/8	0/9
Total	74/251	27/3,712	101/3,963

[a] Values include 487 and 2,321 survey samples that were examined in 2004 and 2005, respectively, in addition to samples received through the normal submission process.

converted to a graphics format using TREEVIEW (Page, 1996).

RESULTS

Rabies surveillance in the Province of Newfoundland and Labrador

The data of Table 1 summarize both the rabies-positive and total submissions made by the Province of Newfoundland and Labrador to the rabies diagnostic laboratory of the Canadian Food Inspection Agency between the years 1985–2006. Data are divided according to whether the specimens came from the mainland or the Island. Submissions were made for all years except for 1985, although numbers vary substantially from year to year. The large submission numbers in 1988/9 and again in 2004–05 were due to increased surveillance on the Island in response to rabies outbreaks. It is evident that over this 22-yr period peaks of rabies activity have occurred on the mainland in a four-year cycle; peaks were observed in 1988, 1992, 1996, and 2001. This cyclic pattern is less evident in recent times with cases reported for all years since 2001. Most of these cases involved the red fox.

N gene comparison

Initial studies targeted 23 rabies isolates including all 21 collected from the Island of Newfoundland in 2002–03 and two cases from the community of Cartwright on the Labrador mainland recovered in 2002. The locations of these cases is shown in Figure 1. A 600-base segment of the rabies virus N gene was amplified for all cases and was sequenced and aligned. Based on this comparison (data not shown), all 21 Island isolates were closely related; compared with the Island index case (02N9337), 16 of these samples had an identical sequence, three samples (03N1834, 03N4057, and 03N4923) differed at just one position, and one sample (03N4056) differed at two positions. In contrast, the two Cartwright specimens, which were identical to each other except at one position, exhibited five (02N2909) or six (02N3548) nucleotide differences, respectively, from the Island's index case. The relatively large difference between the Island's 2002 index case and the two Cartwright samples did not support the conclusion that these cases were directly related epidemiologically. Consequently, alternative sources for this outbreak were sought.

Over the last several years a database of N gene sequences of arctic fox strain rabies viruses recovered from throughout northern Canada has been developed in support of ongoing surveillance of arctic fox rabies in Ontario. An attempt was therefore made to employ this database, together with the Newfoundland sequences, in a phylogenetic analysis aimed at identifying other closely related rabies viruses. However, the high level of se-

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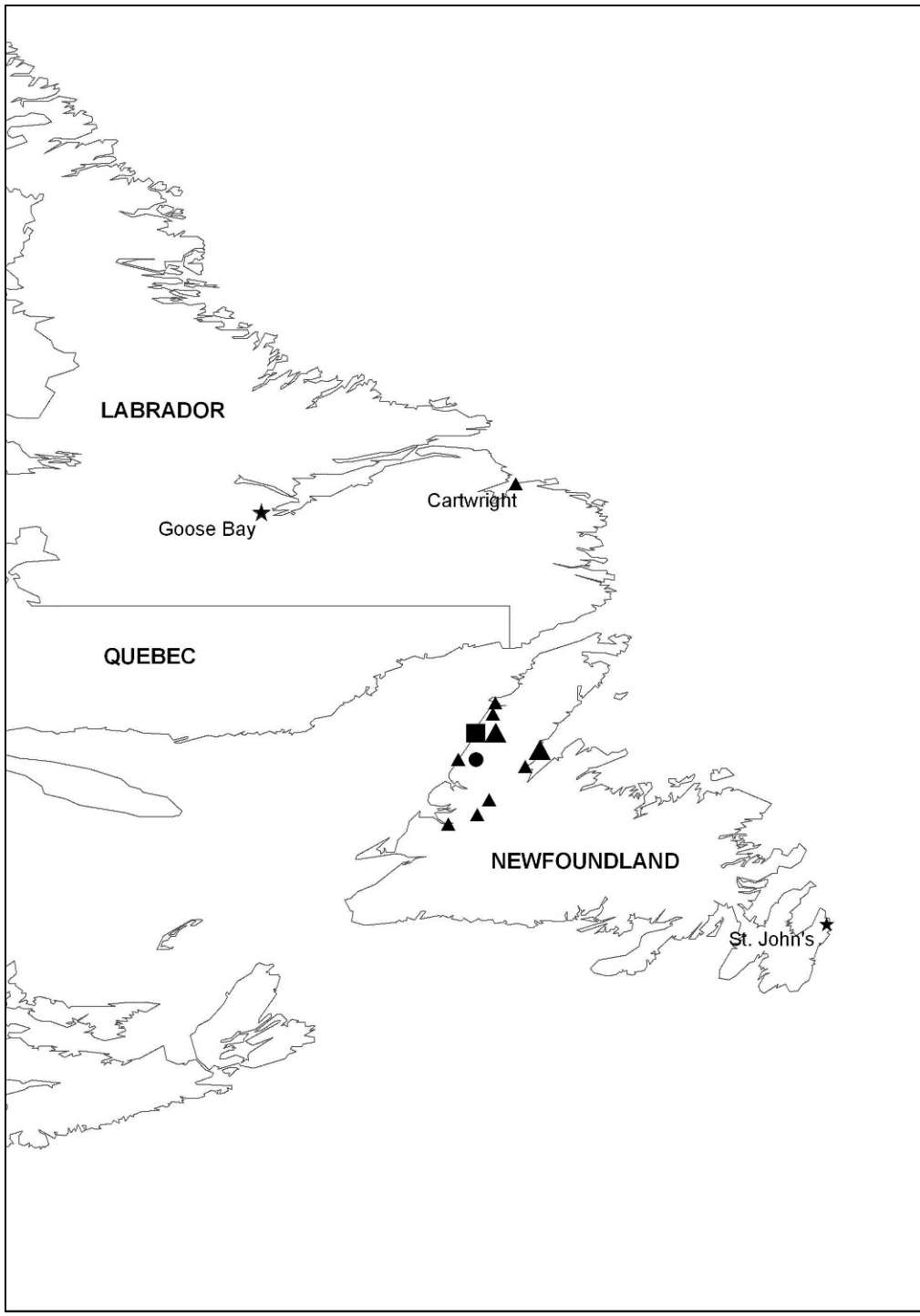


FIGURE 1. Map of the Province of Newfoundland and Labrador showing the locations of all Island rabies cases in 2002–03 and those in Cartwright in 2002. Symbols indicate the infected species as follows: ▲ red fox, ● cat, ■ sheep. Symbol sizes indicate the number of animals diagnosed in each location; smaller symbols represent single cases, and larger symbols represent three or more. Locations of the two main communities of St. John's and Goose Bay are also indicated.

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quence conservation of the rabies virus N gene precluded the generation of a robust phylogeny. Selected viruses were therefore recharacterized using an alternative less conserved target sequence.

Viral P gene analysis

Fifty selected isolates of the arctic fox strain of rabies (see Table 2) were employed for amplification and sequencing of a portion of the viral P gene, and these data were subjected to phylogenetic analysis using an NJ algorithm (Fig. 2). As expected, four Ontario specimens, representing the four geographically restricted variants found in the province in the early 1990s, segregated separately from all other arctic fox strain specimens. The remaining 46 specimens formed one strongly supported cluster (bootstrap value of 90.7) that was further subdivided into four clades (designated A–D). Our prior N gene studies (data not shown) resulted in a similar prediction, and in particular supported the distinctness of the two specimens comprising clade D from the unclassified samples. Figure 3 shows a map of Canada illustrating the locations of samples as grouped according to their phylogenetic relationships (Fig. 2).

Clade A, which was not strongly supported by the bootstrap value (bt=55.7), was nevertheless geographically clustered; it comprised three samples recovered in 1996 from across Labrador as well as four samples recovered during 2001–04 from coastal communities in Labrador. The 20 viruses of clade B included all seven cases from the Island of Newfoundland that were included in this analysis as well as one isolate from northern Ontario, nine samples from communities across northern Quebec, and single samples from such geographically distant locations as the St. Lawrence region close to the city of Montreal, Quebec, and coastal communities in Labrador and Nunavut. These specimens were recovered from 2000–04. Clade C was comprised of five samples retrieved in 2000 and 2002 from more southerly regions of Canada including Ontario and Quebec and included the two Cartwright specimens of Labrador as well as a single sample (V879) that originated from the southern tip of Greenland in 1994 that formed an outlier to this cluster. The two specimens that constituted clade D were both recovered from Nunavut in 1993. Eleven remaining samples, most of which were recovered in the 1990s from the North West Territories (NWT), Nunavut, Quebec, and Ontario, as well as three Greenland samples, did not exhibit any particular clustering pattern beyond their association with the main clade.

Comparison of these sequence data using maximum parsimony (MP) and maximum likelihood (ML) analyses generated trees (data not shown) that were quite similar in structure to that generated by the NJ analysis. Cluster A was poorly supported (bt=47.2) by MP but more strongly supported (bt=78) by ML; cluster B received solid support by both MP (bt=86) and ML methods (bt=88); cluster C was also strongly supported (bt=94.3 by MP and bt=97 by ML); cluster D was poorly supported by MP (bt=55.3) but quite strongly supported by ML (bt=88).

DISCUSSION

The principal goal of this study was to investigate the immediate source of a rabies outbreak on the Island of Newfoundland that began late in 2002 and continued through the first half of 2003. A rabies control program, instigated in 2003, may have contributed significantly to the termination of this outbreak since no terrestrial rabies has been reported on the Island since mid-2003 despite intensive surveillance in the years 2004–05.

Based on rabies case reports that identified the disease in two red foxes in the community of Cartwright on the Labrador mainland, a possible scenario for introduction was the transmission of diseased foxes from the mainland to the Island via the ice floes that frequently

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form in this part of the ocean in late fall. Genetic analysis of all viruses from the Island supported the conclusion that the Island outbreak was likely due to a single incursion, especially given the observation that most variation was seen in the later samples taken when the virus had already been circulating in the area for several months and had had sufficient time to acquire mutations. However, the much greater genetic variation between the viruses of the Island and those of Cartwright did not readily support a close epidemiological connection between them. Consequently a much broader study involving a large collection of rabies viruses retrieved from across northern Canada was undertaken to identify a more likely source for the Newfoundland outbreak. While partial N gene sequences did support certain groupings (data not shown), the limited bootstrap support obtained prompted us to confirm these findings by a more robust analysis. Since phylogenetic associations of lyssaviruses are generally independent of the genomic region characterized (Kissi et al., 1995) the more variable P gene (see Nadin-Davis et al., 2002) was targeted to take advantage of the more informative analysis that such a dataset could generate.

From a phylogenetic analysis of 50 rabies viruses of the arctic lineage there was strong support for a close epidemiological relationship between the Island cases and those cases recovered from Labrador and Quebec in 2002–04 (see clade B of Fig 2). Notably the Cartwright samples, which clustered most strongly with samples recovered from northern Ontario and southern Quebec in 2000 (clade C), were members of a separate lineage that had probably traveled eastwards along the north shore of the St. Lawrence River into Labrador. A third distinct group (clade A) comprising samples from Quebec and Labrador (1996–2004) was also identified, while other samples, mainly from the far north, did not form any specific groupings except for the two samples from Nunavut that constitute clade D. The four Greenland samples included in these analyses were recovered from different regions of the country. V888GLD came from Nuuk on the southwestern coast, and the closely related V893GLD was from region 3 a little farther north (precise location not recorded); these locations are relatively near Baffin Island, at latitudes similar to those of Iqaluit and Igloolik, respectively. V864GLD originated from Thule, a community in the far north of Greenland close to Ellesmere Island, while V879GLD, the outlier to group C, was from the southern tip of Greenland. By placing V879GLD in a separate branch of the phylogenetic tree depicted in Figure 2, a north–south segregation is suggested, but otherwise no clear regional pattern of variant distribution is evident. The lack of any clear distinction between the Canadian and Greenland viruses supports the notion of a complex pattern of movement of animals and rabies virus throughout the region. These observations are in accord with those made by Mansfield et al. (2006), who divided arctic fox strain isolates based on N gene phylogeny into two main clades, Arctic 1 and Arctic 2. Indeed, the Arctic 1 lineage they described corresponds to the Ontario branch of this strain as represented by isolates ON.T1–ON.T4 in this study; these same specimens are designated RABN1578, RABN2756, RABN0783, and RABN9196 in the study by Mansfield et al. The Arctic 2 lineage was further subdivided by Mansfield et al. (2006) into two branches: 2a constituting primarily Russian and Alaskan samples and 2b comprising predominantly Canadian and Greenland isolates. Although direct comparison between these two data sets is not possible due to the targeting of two different portions of the genome, two common samples present in both analyses (93N1090 and 92N4055 in this study) support the conclusion that all samples investigated in the present study group within the Arctic 2b clade.

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TABLE 2. List of all rabies specimens genetically characterized in these studies.

Submission no.	Species[a]	Year	Country	Province[b]/Territory	Town of origin	GenBank accession no. N gene	GenBank accession no. P gene	Variant type[c]
91N5406	Wolf	1991	Canada	ON	Timmins		EF178174	U
92N4055	Dog	1992	Canada	ON	Peawanuk		EF178175	U
93N0398	AFX	1993	Canada	NU	Resolute Bay		EF178176	U
93N0625	AFX	1993	Canada	NU	Iqaluit		EF178177	U
93N0782	AFX	1993	Canada	NU	Cape Dorset		EF178178	U
93N1090	Dog	1993	Canada	NU	Igloolik		AF369270	D
93N1395	AFX	1993	Canada	NU	Resolute Bay		EF178179	D
96N1804	AFX	1996	Canada	QC	Puvirnituq		EF178180	U
96N1855	RFX	1996	Canada	NL	Mary's Harbour		EF178181	A
96N2026	RFX	1996	Canada	NL	Churchill Falls		EF178182	A
96N2393	RFX	1996	Canada	NL	Nain		EF178183	A
96N2397	Otter	1996	Canada	QC	Schefferville		EF178184	U
00L0408	RFX	2000	Canada	ON	Fort Severn		EF178185	B
00L0417	RFX	2000	Canada	NT	Paulatuk		EF178186	U
00L0528	RFX	2000	Canada	NT	Tuktoyaktuk		EF178187	B
00N1512	RFX	2000	Canada	ON	North Bay		EF178188	C
00N3420	Dog	2000	Canada	QC	Dorval		EF178189	C
00N4337	RFX	2000	Canada	QC	Mirabel		EF178190	B
00N4820	RFX	2000	Canada	ON	Cochrane		EF178191	C
01N1650	RFX	2001	Canada	NL	Rigolet		EF178192	A
02N2258	AFX	2002	Canada	QC	Inukjuak		EF178193	B
02N2909	RFX	2002	Canada	NL	Cartwright	EF178151	EF178194	C
02N2980	RFX	2002	Canada	QC	Salluit		EF178195	B
02N3548	RFX	2002	Canada	NL	Cartwright	EF178152	EF178196	C
02N9337	RFX	2002	Canada	NF	Port Saunders	EF178153	EF178197	B
02N9406	RFX	2002	Canada	NF	St. Paul's	EF178154		B
03N0132	Cat	2003	Canada	NF	St. Paul's	EF178155		B
03N0352	RFX	2003	Canada	NF	Sop's Arm, White Bay	EF178156		B
03N1166	Dog	2003	Canada	QC	Puvirnituq		EF178198	B
03N1204	RFX	2003	Canada	QC	Akulivik		EF178199	B
03N1497	RFX	2003	Canada	QC	Puvirnituq		EF178200	B
03N1834	RFX	2003	Canada	NF	Sop's Arm, White Bay	EF178157	EF178201	B
03N1883	RFX	2003	Canada	NF	Pynn's Brook	EF178158		B
03N2570	RFX	2003	Canada	NF	Bellburns	EF178159		B

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TABLE 2. Continued.

Submission no.	Species[a]	Year	Country	Province[b]/Territory	Town of origin	GenBank accession no. N gene	GenBank accession no. P gene	Variant type[c]
03N2586	RFX	2003	Canada	NF	Daniel's Harbour	EF178160		B
03N2596	RFX	2003	Canada	NF	Daniel's Harbour	EF178161		B
03N2634	RFX	2003	Canada	NF	Daniel's Harbour	EF178162		B
03N2718	Ovine	2003	Canada	NF	Daniel's Harbour	EF178163		B
03N2792	RFX	2003	Canada	NF	Cormack	EF178164		B
03N2795	Ovine	2003	Canada	NF	Daniel's Harbour	EF178165		B
03N3361	RFX	2003	Canada	NF	St. Paul's	EF178166		B
03N3362	RFX	2003	Canada	NF	Corner Brook	EF178167		B
03N3363	RFX	2003	Canada	NF	Cormack	EF178168		B
03N3532	Ovine	2003	Canada	NF	Daniel's Harbour	EF178169	EF178202	B
03N4056	RFX	2003	Canada	NF	White Bay South	EF178170	EF178203	B
03N4057	RFX	2003	Canada	NF	White Bay South	EF178171	EF178204	B
03N4058	RFX	2003	Canada	NF	White Bay South	EF178172	EF178205	B
03N4923	RFX	2003	Canada	NF	White Bay South	EF178173	EF178206	B
04N0573	Wolf	2004	Canada	QC	Radisson		EF178207	B
04N3365	RFX	2004	Canada	NL	Nain		EF178208	A
04N3719	RFX	2004	Canada	QC	Kuujjuaq		EF178209	B
04N3889	RFX	2004	Canada	NL	Davis Inlet		EF178210	A
04N4249	RFX	2004	Canada	QC	Kuujjuaq		EF178211	B
04N4250	RFX	2004	Canada	QC	Kuujjuaq		EF178212	B
04N4320	RFX	2004	Canada	NL	Davis Inlet		EF178213	B
04N5133	Dog	2004	Canada	NL	Davis Inlet		EF178214	A
ON.T1	Skunk	1991	Canada	ON	Westmeath		AF369265	—
ON.T2	Skunk	1991	Canada	ON	Etobicoke		AF369266	—
ON.T3	RFX	1991	Canada	ON	Wallaceburg		AF369267	—
ON.T4	RFX	1990	Canada	ON	Flesherton		AF369268	—
V864GLD	Fox	1990	Greenland		Thule		DQ275551	U
V879GLD	—	1994	Greenland		—		DQ275552	C
V888GKD	Fox	2001	Greenland		Nuuk		DQ275553	U
V893GLD	Fox	2001	Greenland		—		DQ275554	U
99N3306R	Raccoon	1999	Canada	ON	Prescott		AY854603	—

[a] Foxes are denoted as either AFX (arctic fox), RFX (red fox), or fox when the species was undetermined.

[b] The mainland of Labrador is denoted NL, while the Island of Newfoundland is denoted NF to permit discrimination between the two areas.

[c] Variant type, as defined in Figure 3, is indicated as either A, B, C, D, or U (unclassified).
Dashes indicate that the information was not recorded.

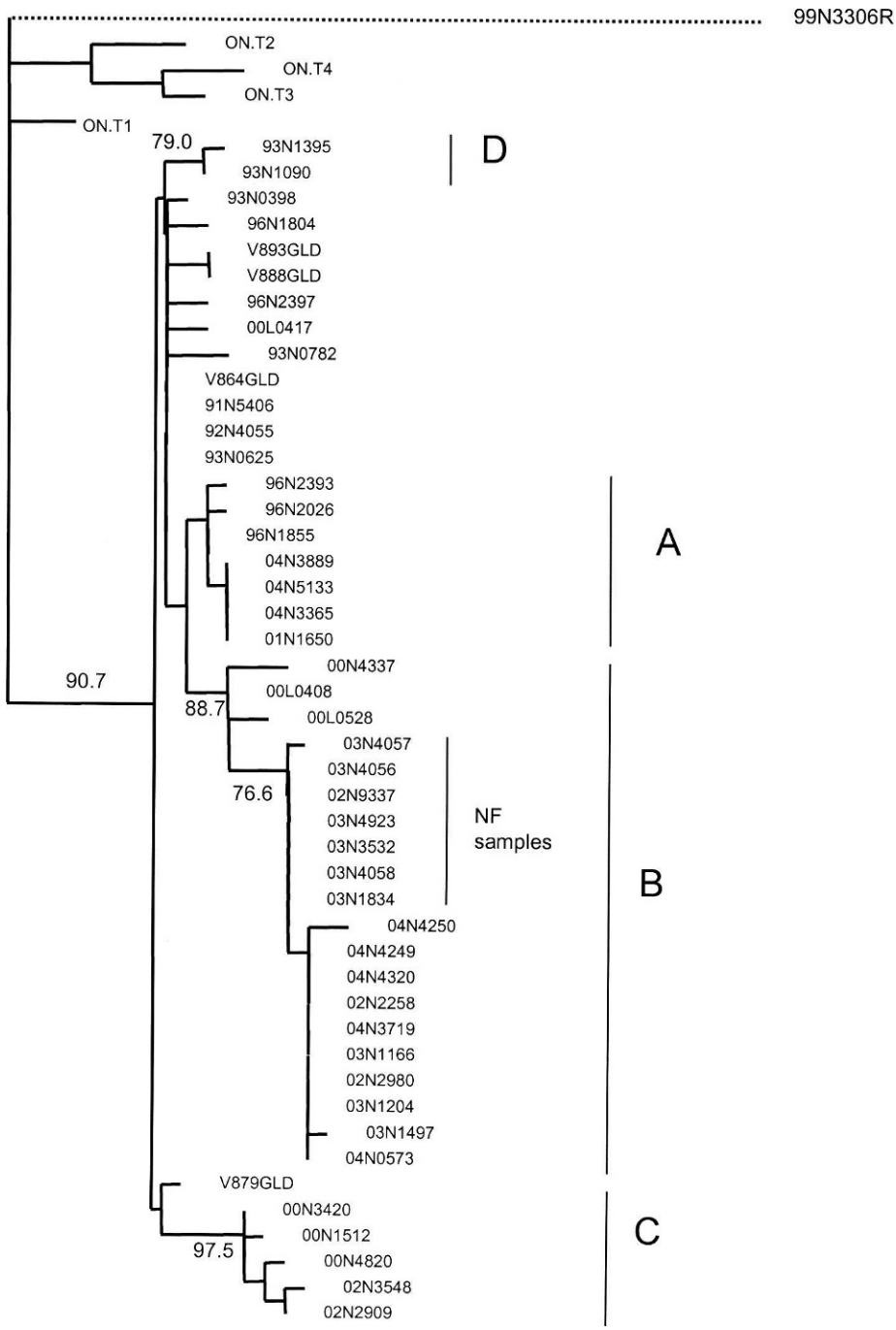


FIGURE 2. Phylogeny of 50 rabies specimens recovered from Canada and Greenland. These partial P gene sequences were aligned using CLUSTALX and then subjected to a NJ phylogenetic analysis using PHYLIP software employing 1,000 bootstrap replicates of the data. Bootstrap values >70% are illustrated to the left of major branches. The branch connecting the raccoon specimen 99N3306R, used as an outgroup in the analysis, is not to scale.

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FIGURE 3. Map of Canada showing the geographical distribution of samples employed in these studies according to their phylogenetic groupings thus: ▲, clade A; ●, clade B; ■, clade C; ♦, clade D; ★, unclassified. Standard size symbols represent single specimens, intermediate size symbols represent 3–5 specimens, and the large circle represents >10 specimens. Province/territory names are shown by their two-letter abbreviations: AB, Alberta; BC, British Colombia; MB, Manitoba; NB, New Brunswick; NL, Newfoundland and Labrador; NS, Nova Scotia; NT, North West Territories; NU, Nunavut; ON, Ontario; PE, Prince Edward Island; QC, Quebec; SK, Saskatchewan; YT, Yukon Territory.

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Because of the low human population density, and hence limited contact between wildlife and humans in northern Canada, reported cases in the region undoubtedly constitute a very small proportion of the total. In such situations, surveillance data alone are insufficient to identify the emergence and movement of rabies epizootics and the sources of specific outbreaks. As demonstrated in this report, only through the use of molecular epidemiology can viral relationships be identified and then used to trace the likely origins and movements of particular viruses associated with outbreaks. The data presented here (Table 2) suggest that the variant primarily responsible for the outbreak in mainland Labrador in 1996 was the clade A variant, and this variant was still circulating in 2004 while clade C viruses emerged in the area in 2002. However, clade B viruses, responsible for the Island cases, were circulating most extensively in Quebec in 2002 just prior to the outbreak. Only one case due to this variant was identified on mainland Labrador in 2004 subsequent to the Island outbreak. It is possible that this variant was circulating in southern Labrador and/or eastern Quebec in 2002 but was not identified in these locations due to limited surveillance. The apparent pattern of spread from the location of the Island's index case suggested that the introduction had been recent and close to the index case. However, it is uncommon to see foxes arrive on the Island of Newfoundland from the mainland late in the year (November–December) and at such a southern location. The formation of ice floes and bridges between the Island and the mainland usually occurs in late winter and early spring in more northern areas, and these disappear during the summer. This raises the possibility that an introduction from the mainland occurred in early 2002 and persisted unobserved until December 2002.

Annual case reports from the province clearly illustrated that up until 2002 a pronounced periodicity, with a 4-yr cycle, in rabies outbreaks on the mainland was apparent (Fig. 1). Periodicity in rabies outbreaks has been reported previously. Using case prevalence data, Tinline and MacInnes (2004) described regional variations in disease incidence and cycling patterns in Ontario, possibly as a function of species distribution and density; of particular note was the existence of a strong 4-yr periodicity in eastern Ontario, similar to that described for Labrador in this report. The periodicity of rabies in red fox populations in agricultural and urbanized areas is well described and appears to be the result of density dependent transmission. Initial high population densities are reduced by a high disease-induced mortality with a rapid recovery thereafter to restart the cycle (Anderson et al., 1981). It is not known if similar mechanisms are the cause of disease cycles in northern Canada. It is possible that annual variation in case submission to the two Canadian rabies laboratories reflects an undulating incidence of rabies in both species of foxes found in the arctic. However, populations of arctic and red foxes in arctic and subarctic habitats can fluctuate greatly as a consequence of annual fluctuations in prey (rodents, lagomorphs) availability (Tannerfeldt and Angerbjorn, 1996; Englund, 1970). The number of animals submitted for diagnosis therefore may reflect annual population levels rather than or in addition to the incidence of rabies.

The mechanisms that allow for the maintenance of the arctic strain of rabies virus and that generate genetic variants of this strain are poorly understood. It is believed that the arctic fox, which inhabits all circumpolar countries, is the primary reservoir host of the arctic rabies virus lineage. Due to inconsistencies in their primary food source, arctic foxes do, under certain circumstances, range over much greater distances than their normal territory (about 20 km from the home den); both seasonal migrations in Alaska

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(Eberhardt et al., 1983) and long-range migrations in Canada and elsewhere (Garrott and Eberhardt, 1987) have been documented. Such events provide the opportunity for long-range spread of rabies, particularly between countries of the circumpolar region where animal movements are often facilitated by the formation of pack ice. Indeed, the lack of a clear phylogenetic distinction between the arctic rabies viruses originating from Canada and Greenland suggests ongoing movement of animals and the viruses they spread between these two jurisdictions. The relative importance of the red fox in arctic rabies maintenance, given its overlap in range with the arctic fox, is worthy of further study, especially since red foxes continue to spread the disease to many regions of southeastern Canada (Nadin-Davis et al., 2006, and this report). One could speculate that if interspecies transmission occurs frequently, then both species may contribute to the long-term maintenance of this disease. Further studies on the emergence of distinct variants of the arctic lineage and their spatial movements within arctic and red foxes may provide an excellent tool to better understand arctic rabies epidemiology and to predict how climate change and the resulting alterations in fox ecology might impact the spread of this disease. In this regard, the large number of viral variants found in viruses recovered from eastern Canada, especially along the eastern coastline of Labrador, identify this region as a useful area for future studies.

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