Canine distemper virus in Lake Baikal seals *(Phoca sibirica)*

L. V. Mamaev, I. K. G. Visser, S. I. Belikov, N. N. Denikina, T. Harder, L. Goatley, B. Rima, B. Edginton, A. D. M. E. Osterhaus, T. Barrett

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The virus epizootic which resulted in significant mortality in Siberian seals *(Phoca sibirica)* in Lake Baikal during 1987/88 was caused by canine distemper virus. Sequence analysis of the virus glycoprotein genes revealed that it was most closely related to recent European field isolates of canine distemper virus. This paper presents evidence that the same virus continued to circulate in seals in Lake Baikal after the initial epizootic. Three out of 45 brain tissue samples collected from seals culled in the spring of 1992 were positive for canine distemper virus-specific nucleic acid by the reverse transcription/polymerase chain reaction and the sequences were closely related to that of the original virus isolated in 1988.

THE morbilliviruses constitute an antigenically related genus within the Paramyxoviridae. They are RNA viruses with unsegmented, negative-sense, single-strand genomes of approximately 16 kb encoding six structural proteins and at least two non-structural proteins (Cattaneo and others 1989, Barrett and others 1991). In addition to canine distemper virus which infects Canidae and Mustelidae, the genus also includes measles virus of humans, rinderpest virus which infects cattle and other large ruminants, and peste des petits ruminants virus which infects sheep, goats and other small ruminants. The virus epizootic in seals in northern Europe during the summer and autumn of 1988 was caused by a morbillivirus closely related to but distinct from canine distemper virus (Mahy and others 1988, Osterhaus and Vedder 1988, Osterhaus and others 1988, Haas and others 1991). A similar disease occurred in seals in Lake Baikal, Siberia, in December 1987, somewhat earlier than the European epizootic (Grachev and others 1989, Likhoshway and others 1989, Osterhaus and others 1989, Titenko and others 1990). There was no obvious epidemiological link between the outbreak in marine seals in Europe and the outbreak in freshwater seals in Lake Baikal several thousand kilometres away. Subsequent studies established that the European and Siberian seal isolates were quite distinct from each other and that the Siberian isolate was very similar to canine distemper virus (Osterhaus and others 1989, Visser and others 1990, Barrett and others 1992).

This virus is known to infect a wide range of carnivore species (Appel 1987) and the live attenuated vaccines currently in use are not attenuated for wildlife species (Carpenter and others 1976); it was therefore possible that the infection in Baikal seals was caused by a vaccine strain of the virus which is widely used in domestic dogs and on mink farms in Siberia. However, sequence analysis of the haemagglutinin (H) gene showed that the Siberian virus was more closely related to recent strains of canine distemper virus isolated in Germany than to any of the vaccine strains (Mamaev and others 1995). This paper provides evidence, based on partial sequence data derived from the phosphoprotein (P) gene, that the same virus continued to circulate in Baikal seals after 1988 and confirms that the vaccines in current use were not responsible for the Lake Baikal epizootic.

L. V. Mamaev, MSc, **S.A. Belikov,** PhD, **N. N. Denikina,** MSc, **B. Edginton,** The Limnological Institute, Ulan-Batorskaya 3, Irkutsk 33, Russia

I. K. G. Visser, PhD, **A. D. M. E. Osterhaus,** DVM, PhD, Seal Rehabilitation and Research Centre, Hoofdstraat 94A, 9968 AG Pieterburen, The Netherlands

L. Goatley, BTech, **T. Barrett,** MSc, PhD, Institute for Animal Health, Pirbright Laboratory, Pirbright, Surrey GU24 0NF

T. Harder, DVM, Institute for Virology, Hannover Veterinary School, D-3000 Hannover 71, Germany

B. Rima, MSc,. PhD, School of Biology and Biochemistry, The Queen's University of Belfast, 97 Lisburn Road, Belfast BT9 7BL, Northern Ireland

Correspondence to Dr T. Barrett

Materials and methods

The virus isolated from Siberian seals in 1988 was originally referred to as phocid distemper virus 2 (PDV-2) to distinguish it from the European seal virus which is known as phocid distemper virus 1 (PDV-1) (Visser and others 1990). A reverse transcription-polymerase chain reaction (RT/PCR) assay, previously developed for the analysis of dolphin and porpoise morbilliviruses (Barrett and others 1993), was used to analyse the RNA extracted from the samples of seal brain collected in 1992. The assay is based on PCR primers derived from highly conserved regions of the P protein gene and amplifies a 429 nucleotide DNA fragment. The cDNA synthesis reactions were carried out on total RNA, derived either from virus grown in tissue culture or from brain tissue sampled post mortem, using random hexanucleotide primers in a total volume of 20 µl. The PCR reactions were carried out on 5 µl of the cDNA in a total volume of 50 µl. In addition, RNA was prepared from several canine distemper vaccines and field virus isolates grown in Vero cells. These were the Rockborn and Onderstepoort vaccine strains of canine distemper virus, the vaccine in use around Lake Baikal, two recent German field isolates of canine distemper virus from a dog and a ferret (Harder and others 1993), a recent field isolate from Belfast and the Siberian seal virus (PDV-2) which was isolated in 1988 from an infected seal brain by co-cultivation with Vero cells (Visser and others 1990). Total RNA was extracted directly from brain samples collected from young seals during the annual cull in 1992 and RNA was also prepared from the original brain tissue from which PDV-2 was isolated. All the RNA samples were extracted by the method of Chomczynski and Sacchi (1987). The PCR-derived DNA of the expected size was purified on low-melting-point agarose and cloned into either pT7 Blue (Applied Biosystems) or pGEMT (Promega) PCR cloning vectors as described by the suppliers, and the resulting plasmids were sequenced by using the M13 universal forward and reverse primers as described by Murphy and Kavanagh (1988) and by direct sequencing of the PCR product with labelled primers (Murray 1989). Forty-five brain and serum samples (J1 to J45) were collected during the spring cull in 1992.

Results

Three of the seal brain samples (J1, J13 and J14) were positive in the RT/PCR reaction. The sequence data from these three positive samples were very similar to those of PDV-2 (Fig 1). Two of the three RT/PCR-positive animals (J1 and J14) had canine distemper virus-specific antibodies by ELISA and J14 also had a neutralising antibody titre of 135. All three were young animals with body-weights of 25 kg or less; of the 45 serum samples tested, six others showed high ELISA and neutralising titres (Mamaev and others 1995).

The Onderstepoort, Rockborn and Siberian vaccine viruses had almost identical nucleotide sequences in this region of the P gene.

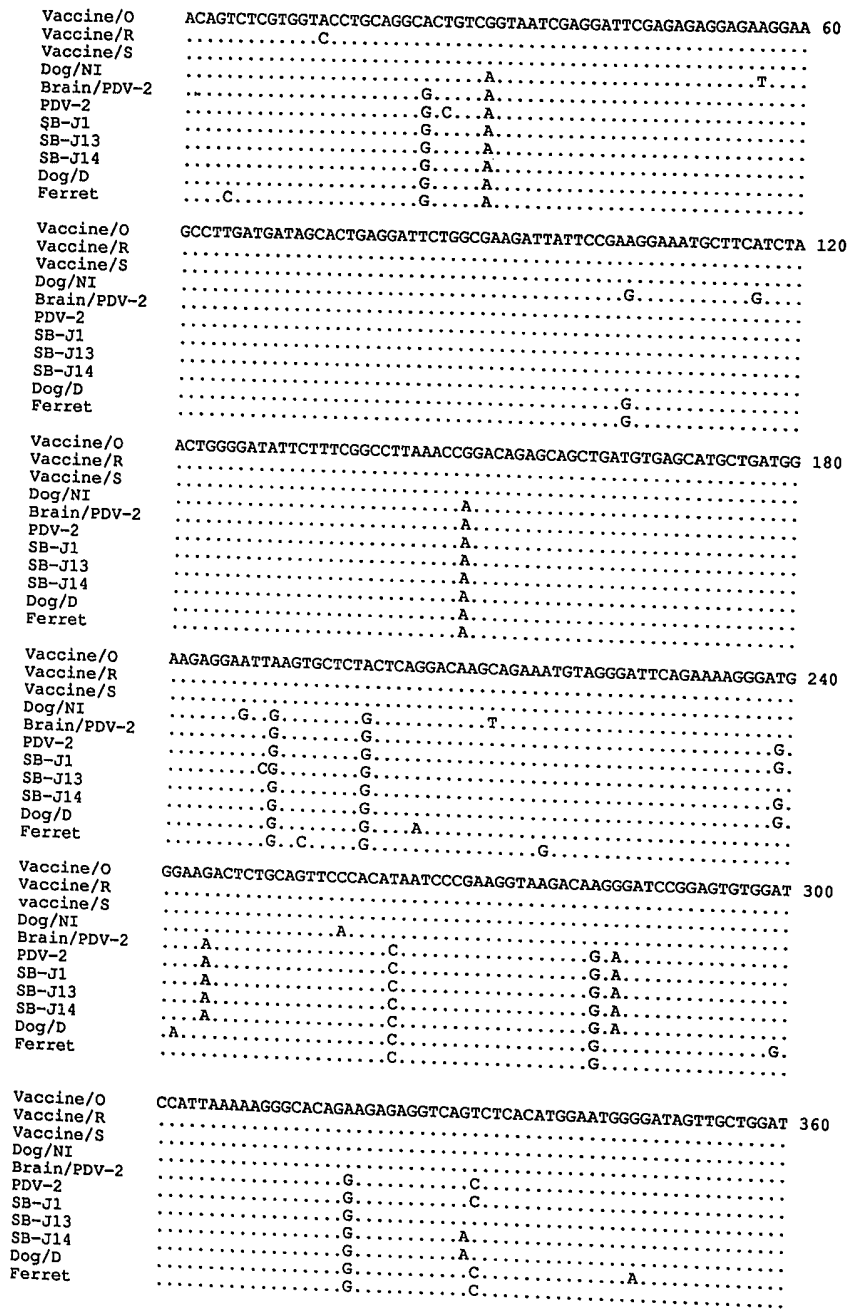

FIG 1: Alignment of the nucleotide sequences (mRNA sense) of the different isolates of canine distemper virus (CDV) described in the text. Vaccine/O: (Onderstepoort vaccine strain); Vaccine/R: CDV (Rockborn vaccine strain); Vaccine/S: (CDV vaccine in use around Lake Baikal); PDV-2 (Visser and others 1990); Brain/PDV-2: (sequence of DNA derived from RNA extracted from the seal brain used to isolate PDV-2); Brain/J1, Brain/J13, Brain/J14 (sequence of DNA derived from RNA extracted from seal brain samples obtained in May 1992); Dog/D (German isolate); Ferret (German isolate); Dog/NI (Belfast isolate). The primer sequences were not included in the alignments

The current Siberian vaccine was identical to Onderstepoort vaccine and differed from the Rockborn vaccine by only one nucleotide transversion (A to C) at position 14. However, the field viruses were quite different from the vaccines. PDV-2 shared sequence changes from the vaccines with German field isolates from a dog and a ferret and these formed a related group. The amplified DNA from the brain tissue (brain/PDV-2) used to isolate PDV-2 showed only one nucleotide change compared with the virus isolate (a nucleotide transition, C to T, at position 26). The sequences derived from the seal brain samples J13 and J14 differed by two nucleotides from the 1988 brain sequence and the sequences derived from J1 differed by three nucleotides. All the viruses were compared by using the PHYLIP DNADIST and FITCH phylogenetic programs (Felsenstein 1989) which confirmed that the Siberian viruses were more closely related to the German dog and ferret isolates of canine distemper virus than to the vaccine viruses, in agreement with sequence data obtained for the haemagglutinin gene of these three viruses (Mamaev and others 1995). The dog isolate from Belfast was distinct from the German and Siberian viruses and more closely related to the vaccine virus (Fig 2). All these isolates of canine distemper virus are quite distinct from PDV-1 and the other known morbilliviruses.

Discussion

These sequence studies clearly show that the Siberian morbillivirus isolate (PDV-2) most closely resembles the recent European isolates of canine distemper virus from a dog and a ferret. The variation in sequence from the two vaccine strains (Onderstepoort and Rockborn) is probably due to evolutionary changes in the virus since the isolation of the vaccine strains in the 1950s. The adaptation of the virus to growth in tissue culture and embryonated eggs to produce the attenuated vaccine is unlikely to have changed the sequence to any great extent because the P gene of the vaccine strain of rinderpest is 99·5 per cent similar to its virulent parent virus (Baron and others 1993). In contrast there are sequence differences between different geographical isolates of measles virus (Taylor and others 1990) and the same is true for rinderpest virus isolates of different geographical origin

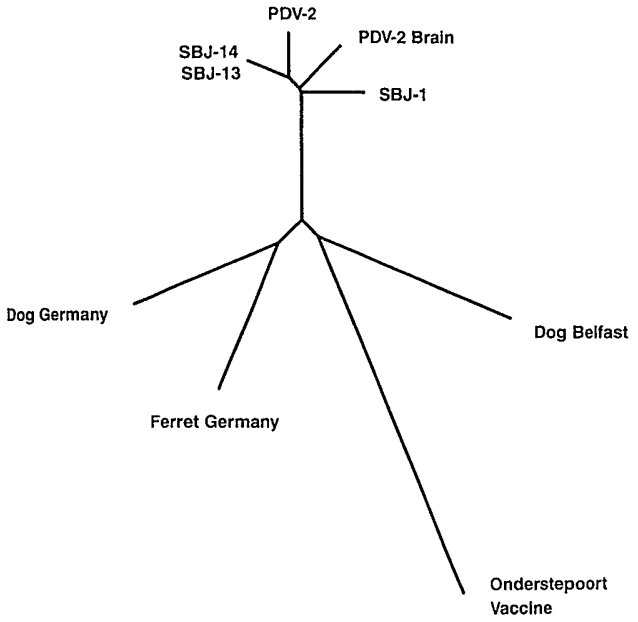

FIG 2: Phylogenetic trees showing the genetic relationships between the different viruses. The trees were derived by using the University of Wisconsin PHYLIP programs DNADIST and FITCH (Felsenstein 1989). The branch lengths are proportional to the estimated mutational distances between the sequences and the hypothetical common ancestors that existed at the nodes in the tree

(Chamberlain and others 1993). In the Lake Baikal epizootic the most likely source of infection was from land animals infected with the virus, because outbreaks of distemper are common in the large number of feral and domestic dogs around the lake. Canine distemper virus also infects a wide variety of other carnivore species and its effect on wildlife populations can be devastating (Appel 1987). Although the vaccine is known to be virulent in some carnivores (Carpenter and others 1976), it is highly unlikely that a vaccine strain was responsible for the disease in Baikal seals. The possibility that a Russian vaccine strain was responsible for the epizootic can be discounted because all the Russian vaccines originated from either the Rockborn or Onderstepoort strains and no other vaccine strains were developed independently. Recently, for reasons of reliability and high efficacy, commercial mink farmers in the Baikal region have been purchasing vaccines from abroad.

The observation that only a small proportion of the animals were positive in both the PCR and the ELISA is evidence that the virus is not circulating in the population as a very infectious agent, and may indicate that these viruses are transmitted by contact between terrestrial and aquatic animals. There is evidence that the European seal virus (PDV-1) caused an infection at a mink farm near the sea in Denmark (Blixenkrone-Møller and others 1989, 1990) and canine distemper virus has been isolated from a captive seal in Canada (Lyons and others 1993). It is therefore necessary to study the epizootiology of these virus diseases further, so that questions about the continued circulation of this virus in the seals of Lake Baikal or other wild animal populations, and the risks of interspecies infection, can be carefully assessed. The ability to amplify and sequence the genes of morbilliviruses which cause animal infections, without having to isolate the virus, will greatly facilitate this task.

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Abstract

Canine DNA microsatellites

MICROSATELLITES, which consist of repeated sequences of one to four base pairs in DNA, have been identified as part of a project to generate a genetic linkage map of the dog. They have been used to assign paternity in a number of doubtful cases. For example, all the pups in the litter of a bitch which had been mated on different days to two stud dogs were shown by DNA analysis to have been fathered by one of the dogs, and in another case it was possible to exclude a dog as the father of a litter. Such DNA analysis should also make it possible to register a pedigree dog with the appropriate kennel club even if its paternity had previously been unknown.

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