

Distemper virus in Baikal seals

SIR—An acute disease of Lake Baikal seals (*Phoca sibirica*) attributable to morbillivirus infection became evident in the autumn of 1987 when weakened seals crawled onto the lake's icy shores and died. Many of them had paralysed hind extremities and ophthalmitis. No similar event is known from records going back to the 1930s. By October 1988, several thousand of the 80,000–100,000 seals in Lake Baikal had died.

In December 1987, during an expedition to Ushkani Island on the border between middle and northern Baikal, one of us (V.S.K.) noticed that three of the five dogs that lived there and had close contact with seals had died with typical symptoms of canine distemper virus (CDV) infection.

In the same month, we observed signs of a similar disease in a 6-year-old female seal (code PB-6). Two days after capture the animal seemed to have recovered but after a further 3 days it again showed symptoms of acute disease — diarrhoea, ophthalmitis and convulsions of the hind flippers. It died 10 days after capture. Rectal temperature varied between 34 °C on days 1 and 10, and 24 °C on days 5–7. No severe pathology of internal organs was observed but cytological investigations of bladder epithelium showed intracytoplasmic eosinophilic inclusion bodies characteristic of canine distemper¹. Inclusions were also found in cells of liver, kidneys, spleen, lungs and in neurons of the brain and spinal cord.

When the sera of eight dead seals were studied, six gave weakly positive indirect haemagglutination reactions (titres of 1:10 to 1:80) with measles antigen², which is a close relative of CDV; all sera neutralized the cytopathic effect of CDV vaccine strain EPM³ with titres from 1:8 to 1:64.

To prove the presence of a CDV-like virus in seal tissues, we have recently used the technique of oligonucleotide probing. On the basis of the known sequences of CDV RNA^{4,5} we synthesized 14–40

deoxyoligonucleotide probes complementary to either virion RNA, or to the corresponding messenger RNA. The probes were designed to detect sequences that are either common to CDV and measles virus (probes 1–7, for gene *N* sequences, and probes 14–19 for gene *P* sequences), or sequences that differ between the two viruses (probes 8–13 for the gene *N* sequences that differ most). The precise sequences probed are shown in *a* in the figure. Probing revealed both virion and mRNA sequences in seal PB-6. These data suggest that CDV or a close relative was not only present but biologically active, inducing synthesis of its mRNA. These results and the preliminary immunological data were discussed at a conference held in Irkutsk in February 1988.

In June 1988, several hundred samples of blood and tissues of Lake Baikal seals were obtained during the spring cull. Of 83 spleen samples subjected to oligonucleotide probing, 8% gave a strong positive signal for CDV RNA and there was a total of 55% positive samples. The 45% negative samples confirmed that the assay is selective (*b* in the figure).

Along with CDV-neutralization tests, we also performed both radioimmuno-metric assays, using CDV vaccine absorbed on nitrocellulose and rabbit anti-seal IgG labelled with ¹²⁵I, and enzyme-linked immunosorbent assays (ELISA), using a monolayer of Vero cells infected with CDV in plastic wells and protein A conjugated with peroxidase⁶. A large proportion of animals tested contained antibodies against CDV in their blood.

In the neutralization assay, 55% of the sera had titres between 1:16 and 1:64; 50% were positive in the radioimmuno-metric assay; and 89% were positive by ELISA, with titres from 1:16 to 1:1,280. These data indicated that the whole population of Lake Baikal seals had been in contact with the virus, suggesting that the survivors should be immune to further infections. The lack of any unusual mor-

ality of seals in the autumn or winter of 1988 confirms that immunity is now established.

We mentioned⁷ CDV as the cause of the Baikal seal disease in September 1988 when, first from newspapers, and then from Dr A. Osterhaus, we learned that CDV or a closely related virus was also believed to have caused the disease of seals in Western Europe. This has been confirmed by several techniques^{8,9}. According to nucleic acid hybridization and immunological data^{10,11} the virus in European seals is not identical to CDV but a distinct seal virus (phocid distemper virus; PDV). Our data on Baikal seals prove only that they were infected by a morbillivirus closely related to CDV; its precise relationship to other morbilliviruses will only be established when the seal virus RNA has been sequenced. The fact that seals in Baikal and in Western Europe have been infected by very similar, or identical, viruses poses new questions concerning the origin and spread of the seal virus.

During a very recent visit, Dr A. Osterhaus brought us sera of European seals, some of which contained antibodies (with titres up to 1:1,080) to CDV in our ELISA. These data and those reported below by Osterhaus *et al.* strongly suggest that the Baikal and European seals have been infected by the same virus, or one that is closely related.

M. A. GRACHEV
V. P. KUMAREV
L. V. MAMAEV
V. L. ZORIN
L. V. BARANOVA
N. N. DENIKINA
S. I. BELIKOV
E. A. PETROV

Limnological Institute,
Siberian Division of the USSR
Academy of Sciences,
664033 Irkutsk,
Lermontova 281, USSR

V. S. KOLESNIK
R. S. KOLESNIK

Irkutsk Anti-Plague Institute

V. M. DOROFEEV

Moscow Bacterial Preparations Plant

A. M. BEIM
V. N. KUDELIN

Baikalsk Institute of Ecological
Toxicology

F. G. NAGIEVA

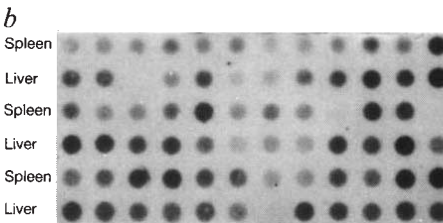
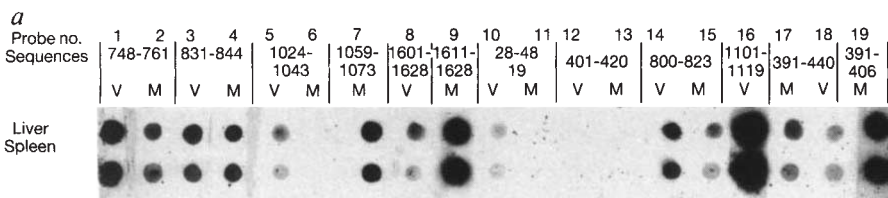
Moscow Research Institute of Viral
Preparations

V. N. SIDOROV

Institute of Nuclear Physics of the
Academy of Sciences of Uzbek, SSR

SIR—We have demonstrated the presence of an infectious morbillivirus in the organs of a Baikal seal (*Phoca sibirica*) collected on a recent visit to Lake Baikal.

The seal was one of three that had suffered from a canine distemper-like disease,



a, Oligonucleotide probing, using probes complementary to virion (V) or messenger (M) RNA, of liver and spleen of seal PB-6. Further details of the probes are given in the text. *b*, Oligonucleotide probing, using a mixture of probes 8, 10 and 12 in *a*, of spleens and livers from 36 seals without visible clinical symptoms culled in spring 1988.