

cent against second and third stage larvae; the same formulation was not effective against first stage larvae.

On the basis of these results it may be concluded that moxidectin 1 per cent injectable, at a single dose of 0.2 mg/kg body-weight subcutaneously, is efficacious for the control of *O. ovis* larvae, both in the early and advanced phases of infestation.

The same drug administered orally at a dose rate of 0.2 mg/kg cannot be recommended for use against bot fly infection in sheep.

These results are comparable with those obtained with ivermectin, which showed an efficacy of 98.5 per cent to 99 per cent (Puccini and others 1983, Schindler and others 1986) and are slightly better than those obtained with injectable rafoxanide (93.6 per cent) (Roncalli and others 1971, Arru and others 1982, Schindler and others 1986).

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Isolation of a parapoxvirus from pox-like lesions in grey seals

A. D. M. E. Osterhaus, H. W. J. Broeders, I. K. G. Visser,
J. S. Teppema, T. Kuiken

Veterinary Record (1994) 135, 601-602

IN Pinnipeds, parapox-like virus particles have been demonstrated on electron microscopical examination of pox-like lesions of grey seals (*Halichoerus grypus*) in Canada (Hicks and Worthy 1987) and the Netherlands (Osterhaus and others 1990) and of harbour seals at Sable Island (Wilson and others 1972). The lesions consisted mainly of firm elevated skin nodules which upon histological and ultrastructural examination showed typical features of pox lesions. Numerous attempts to isolate a parapoxvirus in cell culture from these lesions have been unsuccessful (Osterhaus and

others 1990). From a pox-like lesion of one grey seal, in which both orthopox- and parapox-like virus particles were visualised by negative contrast electron microscopy, only an orthopoxvirus was isolated in seal kidney cells (Osterhaus and others 1990).

The present study describes the successful isolation in seal kidney cells of a parapoxvirus from pox-like lesions of four grey seals, during the spread of this affection among a group of 10 one-year-old captive animals kept in a seal sanctuary in the United Kingdom.

Biopsies from pox-like lesions of six of these seals were collected. Part of these biopsies were used for histological examination to confirm their pox-like morphology and the remainder frozen at -70°C until use in virus isolation procedures.

Paired serum samples were collected from five grey seals: the first samples were taken at the beginning of the outbreak and the second samples about two months later. Serum samples were also collected from 164 free-ranging seals of different species on different continents as previously described (Osterhaus and others 1981) (Table 1). All the serum samples were stored at -20°C until use in an immunofluorescence assay (IFA) screening (see below).

Primary harbour seal kidney cells (SeKC) were prepared and cultured as monolayers in 25 cm² tissue culture flasks as previously described (Osterhaus and others 1985). Biopsy materials were homogenised and 5 per cent suspensions were prepared in culture medium without calf serum. Isolation procedures were carried out with these homogenates on the monolayer SeKC cultures as previously described (Osterhaus and others 1985). When cytopathic changes were observed, four passages of the cytopathic agent in the culture medium were made as described (Osterhaus and others 1985). Affected SeKC cultures were subjected to negative contrast electron microscopical examination for the demonstration of virus particles as previously described (Osterhaus and others 1990).

Serum samples were diluted 1:10 in phosphate buffered saline pH 7.4 and tested by indirect IFA on ethanol (-70°C) fixed slides with infected monolayer SeKC cultures, essentially as previously described (Osterhaus and others 1981).

Virus isolation procedures carried out with the biopsy materials from the lesions of six grey seals, resulted in the demonstration of focal cytopathic changes within three days in the SeKC cultures inoculated with materials from four of the animals (not shown). The samples from the other two animals proved to be unsuitable for virus isolation procedures, due to heavy bacterial contamination. The cytopathic agents present in the four cultures could be further passaged at least four times in the SeKC cultures. By negative contrast electron microscopy it was shown that in the cell cul-

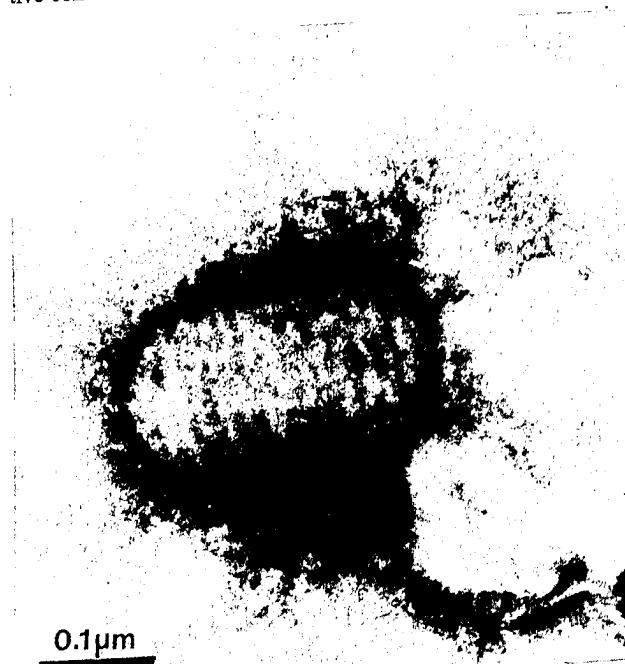


FIG 1: Negative contrast electron micrograph showing a typical parapoxvirus particle in SeKC cultures infected with culture medium materials after two passages

A. D. M. E. Osterhaus, Erasmus University Rotterdam, Institute of Virology, PO Box 1738, 3000 DR, Rotterdam, The Netherlands

H. W. J. Broeders, J. S. Teppema, National Institute of Public Health and Environmental Protection, PO Box 1, 3720 BA Bilthoven, The Netherlands

I. K. G. Visser, Seal Rehabilitation and Research Centre, Hoofdstraat 94a, 9968 AG Pieterburen, The Netherlands

T. Kuiken, Zoological Society of London, Marine Mammal Disease Research, Institute of Zoology, Regent's Park, London NW1 4RY
Dr Kuiken's present address is Department of Veterinary Pathology, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, Saskatchewan, Canada S7N 0W0



TABLE 1: Demonstration of parapoxvirus-specific antibodies in sera collected from different seal species from different countries

Species	Total numbers	Number positive	Number negative	Country
Harbour seal (<i>Phoca vitulina</i>)	37	12	25	United Kingdom
Grey seal (<i>Halichoerus grypus</i>)	11	3	8	United Kingdom
Harbour seal (<i>P. vitulina</i>) and grey seal (<i>H. grypus</i>)	53	11	42	The Netherlands
Ringed seal (<i>Phoca hispida</i>)	1	1	0	The Netherlands
Baikal seal (<i>Phoca sibirica</i>)	32	3	29	Russia (Lake Baikal)
Grey seal (<i>H. grypus</i>)	9	1	8	Canada
Harbour seal (<i>P. vitulina</i>)	21	2	19	Canada
Total numbers	164	33	131	

tures infected with culture medium materials after two passages, typical parapoxvirus particles were present (Fig 1).

In one of the five serum samples collected from the grey seals at the beginning of the outbreak and in all the five samples collected two months later, parapoxvirus-specific antibodies were demonstrated in the indirect IFA.

With the same assay it was shown that parapoxvirus-specific antibodies were present in 10 to 40 per cent of the sera collected from different seal species from northwest Europe, North America and Siberia (Table 1).

In the present communication the authors have demonstrated for the first time that a parapoxvirus associated with an outbreak of an apparently infectious skin disease in grey seals, can be isolated in vitro in primary SeKC from harbour seals. The presence of parapox-like particles has been shown by electron microscopy in the nodular lesions associated with this disease before and it is not clear why previous attempts to isolate the virus by the present authors and others have not been successful.

In the past, parapox-like lesions have predominantly been observed in limited numbers of animals in grey and harbour seal populations. As suggested before (Osterhaus and others 1990) immune suppression induced by phocine distemper virus (PDV) infections may have facilitated the massive infection in the group of 10 captive grey seals. Evidence for recent PDV infection in these one-year-old animals was obtained by showing that all these animals had PDV-specific serum antibodies, which at this age cannot be of maternal origin (Visser and others 1993). The isolation of the parapoxvirus in vitro has enabled the present authors to develop a serological assay (IFA), which allowed them to demonstrate that seroconversion took place in the grey seals during the outbreak, confirming that indeed the animals were infected with a parapoxvirus during the disease outbreak. Furthermore, it allowed them to screen serum samples from seals of different species at different continents. The results of this screening clearly indicate that parapoxvirus infections commonly occur in the seals of northwest Europe, North America, and in the isolated population of Lake Baikal seals in Siberia (three out of 32 tested).

It is interesting to note that parapoxvirus infections have also been demonstrated in different dolphin species of North and South America (Flom and Houk 1979, Geraci and others 1979, Britt and Howard 1983, Van Bressema and others 1993). Screening of 54 serum samples from eight different cetacean species stranded on the coasts of the UK, for the presence of antibodies to the parapoxvirus of the seals did not result in any positive samples (data not shown). Further serological and virological studies are needed to show whether this is due to lack of antigenic cross-reactivity between the parapoxvirus occurring in pinnipeds and cetaceans, or indeed to the relatively low frequency or absence of parapoxvirus infections in the cetacean species of northwest Europe.

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Abstracts

Rabbit haemorrhagic disease virus in Australia

THIS article reviews the origins and properties of rabbit haemorrhagic disease virus and considers its particular importance for Australia. It is suggested that it may provide a new and more acceptable means of controlling the rabbit population, in combination with myxomatosis. This is because the massive destruction of hepatocytes and the disseminated intravascular coagulopathy which are its chief pathological effects lead to the death of the rabbit relatively quickly and quietly, in a comatose state. It may be possible to identify the gene responsible for these effects and splice it into the genome of the myxomatosis virus, to enhance its effectiveness in the control of rabbits.

STUDDERT, M. J. (1994) *Australian Veterinary Journal* 71, 264

Absorption of colostral IgG by newborn rats

THE protein A-gold technique was used to demonstrate the process of absorption of gold-labelled IgG through the enterocytes of the small intestine of 12 newborn rats. In the cranial segment, labelled IgG molecules were observed to be attached to the wall of coated vesicles, suggesting that at this site the transport of colostral IgG was mediated by a receptor. However, in the transitional and caudal segments of the small intestine intracellular micropinocytotic transport predominated. Lysosomal structures in the enterocytes did not appear to impede the absorptive activity during the absorption period, which lasted 20 days.

JOCHIMS, K., KAUP, F.-J. & DROMMER, W. (1994) *Research in Veterinary Science* 57, 146

Ivermectin toxicosis in a kitten

A THREE-MONTH-OLD female kitten developed generalised ataxia, mild tremors, weakness, incoordination and miosis after being treated for an ear mite infestation with 0.3 mg ivermectin/kg bodyweight administered subcutaneously. The kitten became comatose within 12 hours, and despite supportive treatment, died seven days later. Although there are reports of large numbers of cats and kittens having been treated safely with ivermectin, it is suggested that caution should be exercised when ivermectin is administered parenterally to kittens.

LEWIS, D. T., MERCHANT, S. R. & NEER, M. (1994) *Journal of the American Veterinary Medical Association* 205, 584

