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AN OUTBREAK OF A HERPESVIRUS INFECTION IN HARBOR SEALS (*PHOCA VITULINA*)

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ABSTRACT: During an outbreak of a herpesvirus infection in juvenile harbor seals, 11 out of 23 seals died. The duration of the disease in these 11 animals varied from 1–6 days. Nasal discharge, inflammation of the oral mucosa, vomiting, diarrhea and fever up to 40 C were observed in the first days of the disease. In later stages coughing, anorexia and lethargy occurred. Severe necrosis of the liver and interstitial pneumonia were the most striking histopathological findings.

INTRODUCTION

Recently, reviews on viral diseases in marine mammals have been published (Smith and Skilling, 1979; Britt and Howard, 1983). Virus isolation or identification has been related to disease in the case of San Miguel sea lion virus (Smith et al., 1973, 1978, 1981; Sawyer, 1976), seal pox virus (Wilson et al., 1969, 1972a, b; Wilson and Sweeney, 1970; Wilson and Poglayen-Neuwall, 1971), dolphin pox virus (Flom and Houk, 1979; Geraci et al., 1979), sea lion adenovirus (Britt et al., 1979; Dierauf et al., 1981), influenza A virus (Geraci et al., 1982, 1984) and rabies virus (Odegaard and Krogsrud, 1981). These reports concerned diseases of both free-ranging (Wilson et al., 1969, 1972a, b; Wilson and Sweeney, 1970; Wilson and Poglayen-Neuwall, 1971; Smith et al., 1973, 1978, 1981; Sawyer, 1976; Britt et al., 1979; Flom and Houk, 1979; Geraci et al., 1979, 1982; Smith and Skilling, 1979; Dierauf et al., 1981; Odegaard and Krogsrud, 1981) and captive animals (Geraci et al., 1984).

The present report describes the clinical and pathological features of an outbreak of a herpesvirus infection in harbor seals. Virological investigation has shown that a hitherto unidentified herpesvirus (seal herpesvirus = SeHV) was involved in this outbreak (Osterhaus et al., 1985).

MATERIALS AND METHODS

Eleven animals out of a group of 23 harbor seals, which had been found on the tidal sandbanks of the Dutch Wadden Sea, died during the outbreak. They were juvenile motherless animals, varying in age from some days up to 2 wk when they were found. They had been hospitalized in the Seal Nursery Station in Pieterburen, The Netherlands where the outbreak occurred in July and August 1984.

Gross pathology and histopathology of the 11 seals were carried out according to routine techniques. Formalin fixed sections of lung, liver, spleen, mesenteric lymph nodes, small intestine and oral mucosa were processed for histopathology using the following staining techniques: H&E, PAS, Gomori's reticulin, Weigert van Gieson (Luna, 1968).

Virological techniques used have been described elsewhere (Osterhaus et al., 1985).

Bacteriological examination was performed according to methods described by Cowan (1975). Samples were collected from liver, lung, spleen, small intestine, kidney and mesenteric lymph nodes as soon as possible, but in all cases within 24 hr after death. Incubation was performed at 37 C up to 72 hr under aerobic and anaerobic conditions (Gas Generating Kit, Anaerobic System, Oxoid, Code no. BR 38, Pharmachemie, Haarlem, The Netherlands) and in a carbon dioxide atmosphere (Gas Generating Kit, Carbon Dioxide System, Oxoid, Code no. BR 39, Pharmachemie, Haarlem, The Netherlands). Identification of the isolated bacteria was carried out according to general procedures (Buchanan and Gibbons, 1974; Cowan, 1975).

Liver tissues were examined for total mer-

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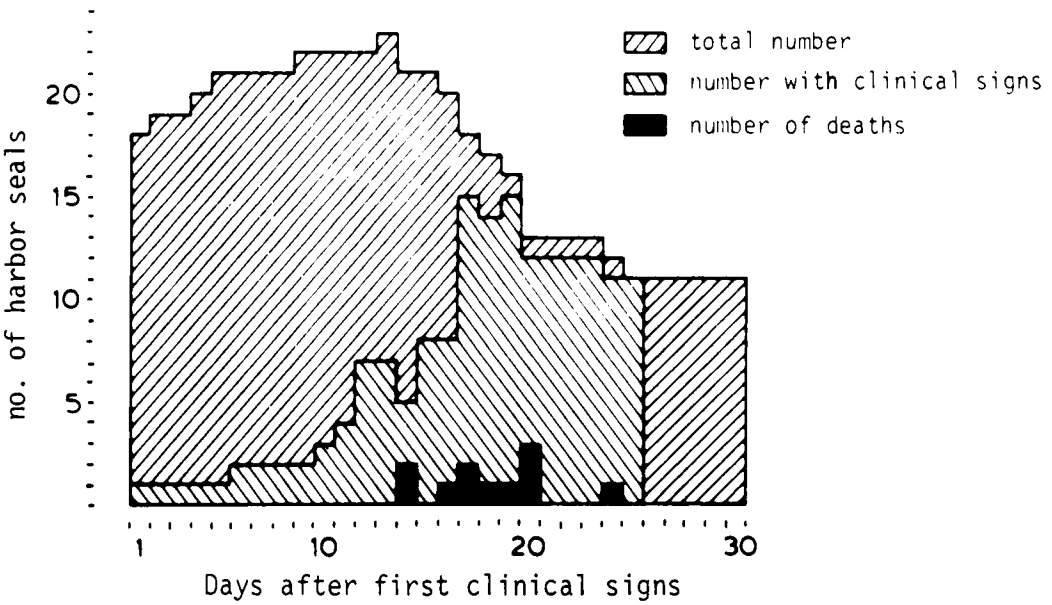

FIGURE 1. Course of morbidity and mortality during an outbreak of seal herpesvirus (SeHV) infection in the Seal Nursery Station, Netherlands.

cury in four animals and for PCB's in two. Mercury was determined by flameless atomic absorption spectrophotometry (Thorpe, 1971). PCB analyses were performed by glass capillary gas chromatography (Kerkhoff et al., 1982).

RESULTS

The course of the disease is presented in Figure 1. The onset of disease was observed on 11 July 1984. At that time the nursery station housed 18 animals. New input of animals from the Dutch Wadden Sea resulted in a total number of 23 seals on 23 July 1984. Seven of these were affected on 23 July. The first two animals died on 24 July, the last one on 3 August. Signs observed were nasal discharge, inflammation of the oral mucosa, vomiting, diarrhea and fever with temperature up to 40 C (normal rectal temperature ranges from 36.5–37.8 C). In later stages coughing, anorexia and lethargy occurred. During the outbreak, diseased animals were kept indoors in pens with floor heating and antibiotic therapy was given (cefuroxim, Glaxo, and netylmicine, Schering, i.m.). For practical reasons the less severely diseased animals were kept in outside shelters with access to a basin. The duration of illness in the 11 seals varied from 1–6 days. The surviving seals (n = 12) have been placed back into the Wadden Sea according to the usual procedure after a successful nursery period.

Small erosions were observed in the oral mucosa. In most animals emphysema and pneumonia were seen. The livers were somewhat enlarged, their surfaces exhibiting a mottled appearance. Other organs showed no specific alterations.

Alterations of the liver were confined to the parenchyma and consisted of dystrophic degeneration varying in severity up to massive coagulation necrosis (Fig. 2). No specific zonal pattern was observed. Necrotic liver tissue was sharply delineated (Fig. 3). Sinuses were dilated in extensively altered areas. Small reactive foci consisting of mononuclear cells were present in the altered liver parenchyma. In some areas dissociation of liver cells was obvious. No reaction of Kupffer cells was present. Spaces of Disse were filled with

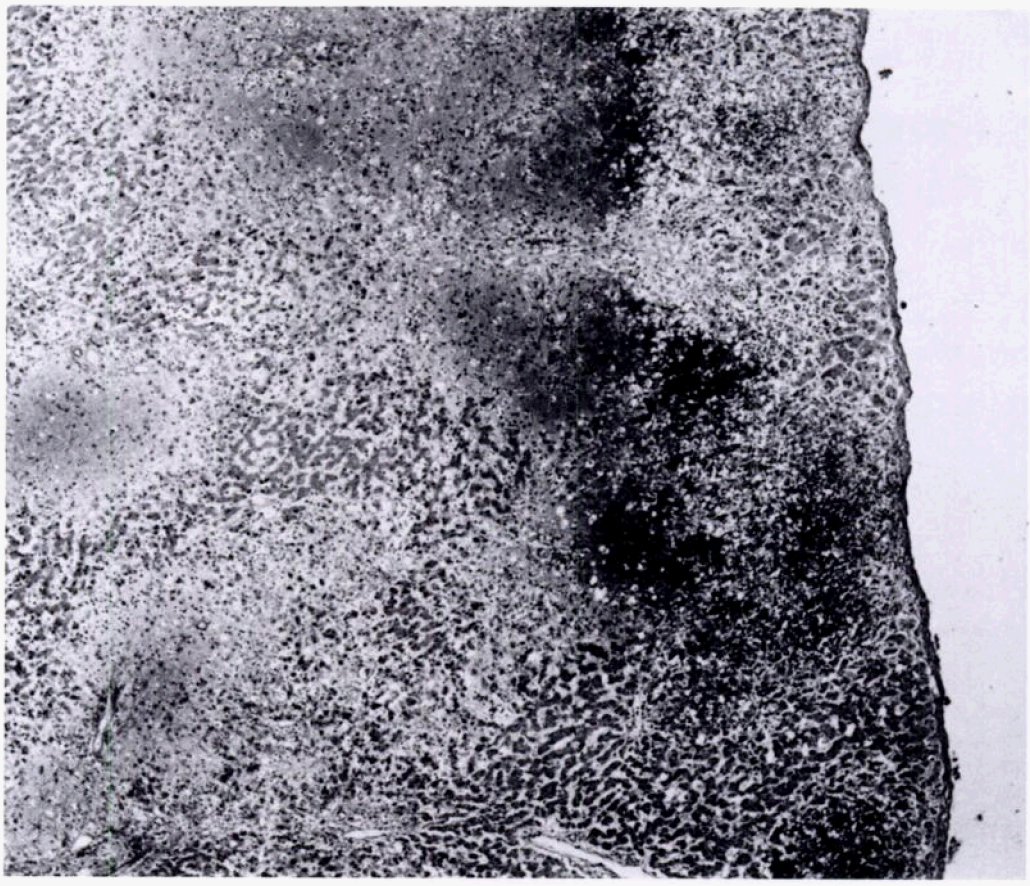

FIGURE 2. Massive coagulation necrosis in the liver of a harbor seal without zonal pattern. H&E, ×10.

proteinaceous fluid. The reticulin fiber pattern was unchanged, i.e., no collapse had taken place. Inclusion bodies were not observed.

Interstitial pneumonia with mononuclear infiltration was found. In addition small developing foci of exudative, fibrinous pneumonia were observed. Moderate emphysema was seen in the alveolar areas. The bronchial epithelium was unaltered.

The distal tubules of the kidneys showed degenerative changes of the epithelium. A nonspecific ulceration of oral mucosa was found in most cases. Distinct depletion of lymphocytes and erythrophagia by macrophages were observed in spleens and lymph nodes. No pathological lesions were found in brain tissue.

A hitherto unidentified herpesvirus (seal herpesvirus = SeHV) was isolated from all nine harbor seals examined (Osterhaus et al., 1985). Virological examination was not performed in the first two fatal cases of this outbreak. SeHV isolation procedures were performed in seal kidney cell cultures and confirmed by indirect immunofluorescence assays (Osterhaus et al., 1985). Intranuclear inclusion bodies could not be demonstrated in the monolayers exhibiting cytopathic changes (Osterhaus et al., 1985). The virus was isolated from the lungs in eight of nine seals, from the liver in three of nine seals, from the brain of both seals examined, from the kidneys of one seal examined and not from the oral lesions of two seals examined.

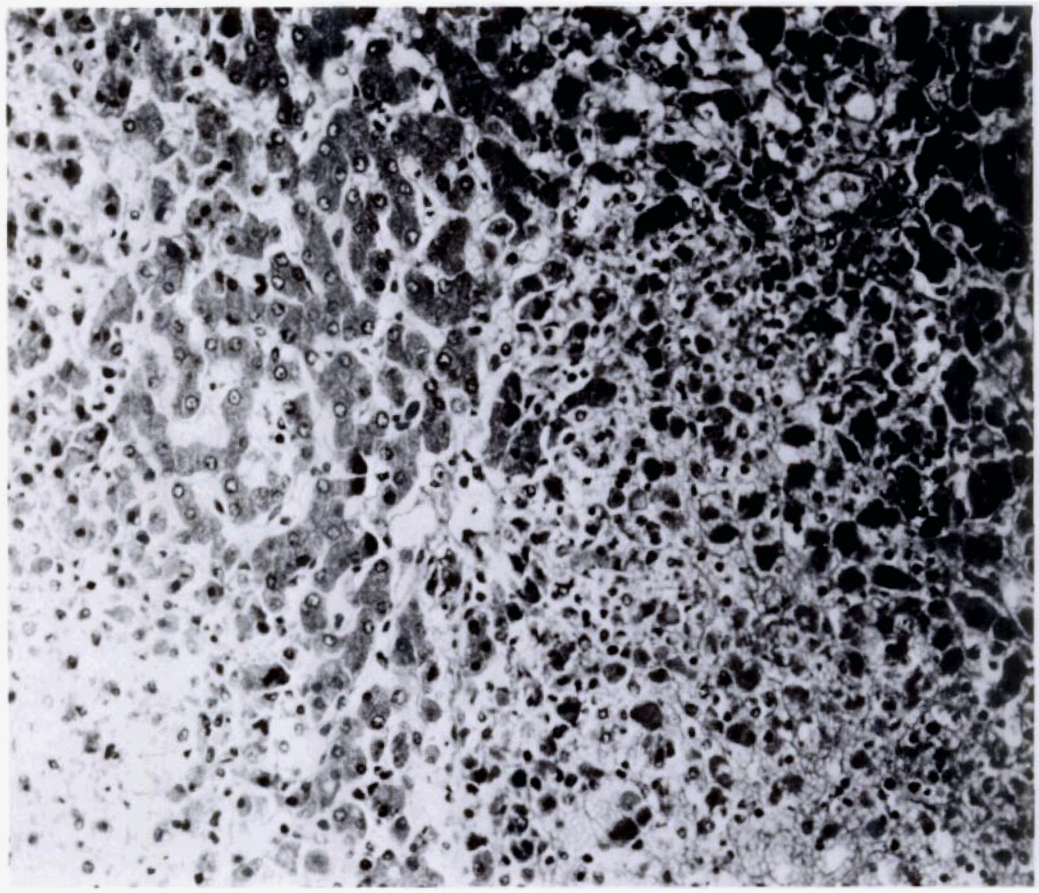

FIGURE 3. Necrotic liver tissue in a harbor seal. (Magnification of central part of Fig. 2.) H&E, ×40.

Various bacteria (*E. coli*, *Proteus vulgaris*, *Providencia stuartii* and *Streptococcus faecium*) were isolated from lung, liver and kidney in the first three cases. Subsequent bacteriological examinations revealed no pathogenic bacteria.

Toxicological analyses performed in four seals showed that levels of total mercury in the livers varied from 6.8–12.1 mg/kg dry weight. Levels of PCB's in the livers varied from 9.4–29 mg/kg (fat weight) or from 0.38–1.3 mg/kg (wet weight).

DISCUSSION

The cause of death in the seals was assumed to be a combination of extensive necrosis of the liver parenchyma and interstitial pneumonia. The lung lesions were quite distinct from those seen in influenza A virus infections. Bronchial epithelium was not altered in the SeHV infection. The absence of influenza A (H7N7) virus infection, which may cause severe pneumonia and high mortality in seals (Geraci et al., 1982), was demonstrated serologically in this outbreak (Osterhaus et al., 1985). Acute pneumonia may explain the more or less pronounced alveolar emphysema which was observed.

The only viral hepatitis described in aquatic mammals is the sea lion hepatitis which is caused by an adenovirus (Britt et al., 1979; Dierauf et al., 1981). Microscopically this viral hepatitis differs from SeHV hepatitis by the presence of numerous in-

tranuclear inclusions. A clear zonal pattern of the hepatic lesions based on the microcirculatory acinar concept of the hepatic structure was also absent in the seals. The absence of any induction of repair in the liver agrees with the acutely progressive course of the disease.

Necrosis of the liver can also be caused by mercury or PCB's (Cheville, 1983). Comparison of the residual total mercury and PCB's found in these seals with concentrations found in other studies indicated that these pollutants were not responsible for this disease outbreak (Drescher et al., 1977; Reijnders, 1980, 1984).

None of the bacteria isolated is likely to have caused the disease. Therefore the value of the antibiotic therapy remains doubtful.

The isolation of a hitherto unknown herpesvirus from nine seals suggested its etiologic role in this outbreak because SeHV was isolated from all animals which were examined virologically, but not from a seal which had obviously died from another cause outside the nursery station (Osterhaus et al., 1985). Furthermore seroconversion to SeHV was demonstrated in surviving seals (Osterhaus et al., 1985). An antigenic relationship of SeHV was demonstrated with canine herpesvirus and feline herpesvirus-1 although SeHV proved to be different from these and other mammalian herpesviruses (Osterhaus et al., 1985).

It is not unusual in herpesvirus infections that crowding of animals, as inevitably occurs in a facility like the seal nursery station, plays an important role in spreading the disease.

The family Herpesviridae is divided into three subfamilies: Alpha-, Beta- and Gammaherpesvirinae (Roizman et al., 1981). SeHV was shown to be a probable member of the Alphaherpesvirinae (Osterhaus et al., 1985). Lesions associated with a generalized infection with a member of the subfamily Alphaherpesvirinae are acute pneumonia, focal hepatitis, acute tubular degeneration in the kidneys, acute lesions in the oropharyngeal mucosa and encephalitis (Cheville, 1983; Jones and Hunt, 1983; Jubb et al., 1985), which have been described in a wide range of vertebrates (Roizman et al., 1981; Cheville, 1983). Severe clinical disease resulting in high mortalitv is encountered most frequently in young animals (Cheville, 1983) as was the case in this outbreak. The absence of intranuclear inclusions both in infected tissues and in infected cell cultures was somewhat unexpected in infections with members of the Alphaherpesvirinae.

Further observations are needed to determine why SeHV infection has not been recognized before and where it may have its natural, possibly inapparent origin.

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