

NOTE

Herpesvirus-like particles in angelfish *Pterophyllum altum*S. Mellergaard^{1,*}, B. Bloch²¹ Danish Institute for Fisheries and Marine Research, Fish Disease Laboratory, c/o Royal Veterinary and Agricultural University, Bülowsvej 13, DK-1870 Frederiksberg C, Denmark² Department of Virology and Immunology, Royal Veterinary and Agricultural University, Bülowsvej 13, DK-1870 Frederiksberg C, Denmark

ABSTRACT: Herpesvirus-like particles were observed in adult angelfish *Pterophyllum altum* imported from the Amazon area. The viral particles were present in the nuclei of splenic macrophages and monocytes of dying fish. The case may represent a stress-mediated disease outbreak in which a latent herpesvirus infection was activated.

Herpesvirus infections have been recorded from a number of teleosts. The virus has been associated with hyperplastic skin lesions in walleye *Stizostedion vitreum* (Kelly et al. 1983), in carp *Cyprino carpio* (Schubert 1966), in masu salmon *Oncorhynchus masou* (Kimura et al. 1981), in Northern pike *Esox lucius* (Yamamoto et al. 1983), in golden ide *Leuciscus idus* (McAllister et al. 1985) and in smelt *Osmerus eperlatus* (Anders & Möller 1985). The virus has also been associated with more general disease manifestations in channel catfish *Ictalurus punctatus* (Fijan et al. 1970), in rainbow trout *Salmo gairdneri* (Wolf et al. 1975), in turbot *Scophthalmus maximus* (Buchanan & Madely 1978) and in masu salmon *Oncorhynchus masou* (Kimura et al. 1981, Tanaka et al. 1984).

The 3 affected adult angelfish *Pterophyllum altum* had been imported to Denmark from the Amazon and had been held by an experienced aquarist in an aquarium for 3 to 4 mo with 10 locally bred adult angelfish *Pterophyllum scalare* without any signs of disease. The problem showed up 2 d after the aquarist had made some minor changes in the aquarium decoration. During these changes the fish were present in the aquarium. The 3 *Pterophyllum altum* were found moribund while the 10 *Pterophyllum scalare* appeared normal. The moribund fish showed loss of equilibrium, swam occasionally in spiral movements, but mostly hung lifelessly at the water surface.

The moribund fish were killed by cutting the vertebral column behind the gills.

The fish had disseminated skin haemorrhages. In some of the haemorrhagic areas ulcerations were observed. The gills were pale. The liver and spleen were found to be distended.

Clinical signs were consistent with those of bacterial septicaemia in ornamental fish. However, to eliminate ectoparasites as causative agents a microscopical examination of smears of skin and gills was carried out. No parasites were detected. Therefore, this case was treated as a routine case and only cotton swabs were taken from the kidneys for bacteriological examination. However, no bacterial growth was detected on blood agar (Gibco) incubated at 20 °C for 1 wk. For this reason, only a limited pathological description of the condition is possible.

The spleens from the 3 fish were by chance removed to be used for histological demonstrations and were fixed in 4 % phosphate-buffered formaldehyde. Because the bacteriological samples were found sterile, the spleens were halved. One half were used for routine histological examination and the other half for electron microscopical examination. For routine histological examination, the specimens were embedded in paraffin wax and 4 µm thick sections were prepared and stained with Mayer's haematoxylin and eosin/phloxin (H&E).

The histopathological findings for the 3 spleens revealed different degrees of dilatation of the sinusoids. Fig. 1 illustrates the dilatation. The reticulo-endothelial cells of the sinusoids were hypertrophic and some were desquamated into the lumen (Fig. 2). The sinusoids contained a varying number of macrophages, many of which contained phagocytosed material. In the least affected spleen, erythrocytes were present; in the more severely affected spleens, the sinusoids were almost devoid of erythrocytes.

Edema of varying degrees was observed in the splenic stroma. Necrosis of connective tissue cells was

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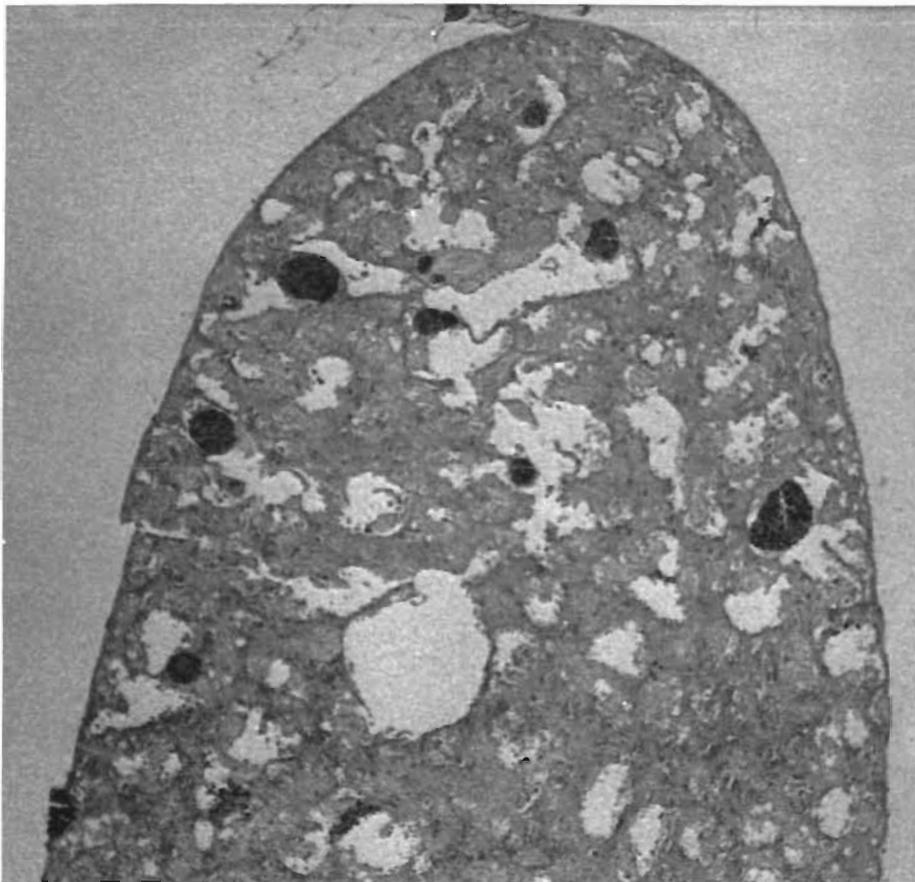


Fig. 1. *Pterophyllum altum*. Spleen from one of the severely affected angelfish showing dilatation of the sinusoids. 40 ×

present in some areas. Macrophages in the stroma contained a light brownish pigment in the H&E-stained specimens. A Perl's stain identified the pigment as haemosiderin. The macrophages of the least affected spleen contained almost no haemosiderin while the macrophages of the other 2 spleens contained large amounts of this pigment. This observation suggests that 2 of the fish had suffered from a severe haemolytic anemia.

For the electron microscopical examination the formaldehyde-fixed specimens were postfixed in 1% phosphate-buffered osmium tetroxide. After dehydration in acetone, the samples were embedded in Vestopal W (Serva).

Examination of the spleens revealed nucleocapsid-like particles of diameter ca 100 nm. The particles occurred in low numbers in the nuclei and occasionally in the cytoplasm of macrophages and monocytes in the splenic stroma and in the sinusoids. Some of the particles contained a dark, irregularly shaped core of average diameter ca 55 nm. Others contained only little core material or had a ring-shaped core (Fig. 3). In a few cases the nucleocapsid-like particles were seen budding through the nuclear membrane and regularly shaped enveloped particles were observed in the

perinuclear cisternae. More frequently, irregularly shaped, e.g. drop-shaped, dense particles containing one or sometimes more nucleocapsid-like particles were observed in vacuoles in the cytoplasm and in the intercellular space (Fig. 4). Nucleocapsid-like particles partly covered by narrow smooth cisternae could be observed in the cytoplasm (Fig. 5) indicating that the irregularly shaped dense particles had apparently been enveloped in the cytoplasm. The diameter of the smallest regularly shaped enveloped virions was ca 135 nm while the diameters of the more irregular particles were usually larger. All these features of the particles are typical of herpesvirus particles.

Herpesvirus infections have mainly been associated with hyperplastic skin lesions. Only in channel catfish (Fijan et al. 1970, Wolf et al. 1972, Major et al. 1975), rainbow trout (Wolf et al. 1975) and masu salmon (Tanaka et al. 1984) have more generalized disease manifestations as observed in the angelfish *Pterophyllum altum* been described.

In channel catfish (Fijan et al. 1970), rainbow trout (Wolf et al. 1975), and masu salmon (Kimura et al. 1981) disease outbreaks were only observed in young fish. In contrast, adult fish were involved in the present case.

Common to both channel catfish and angelfish her-

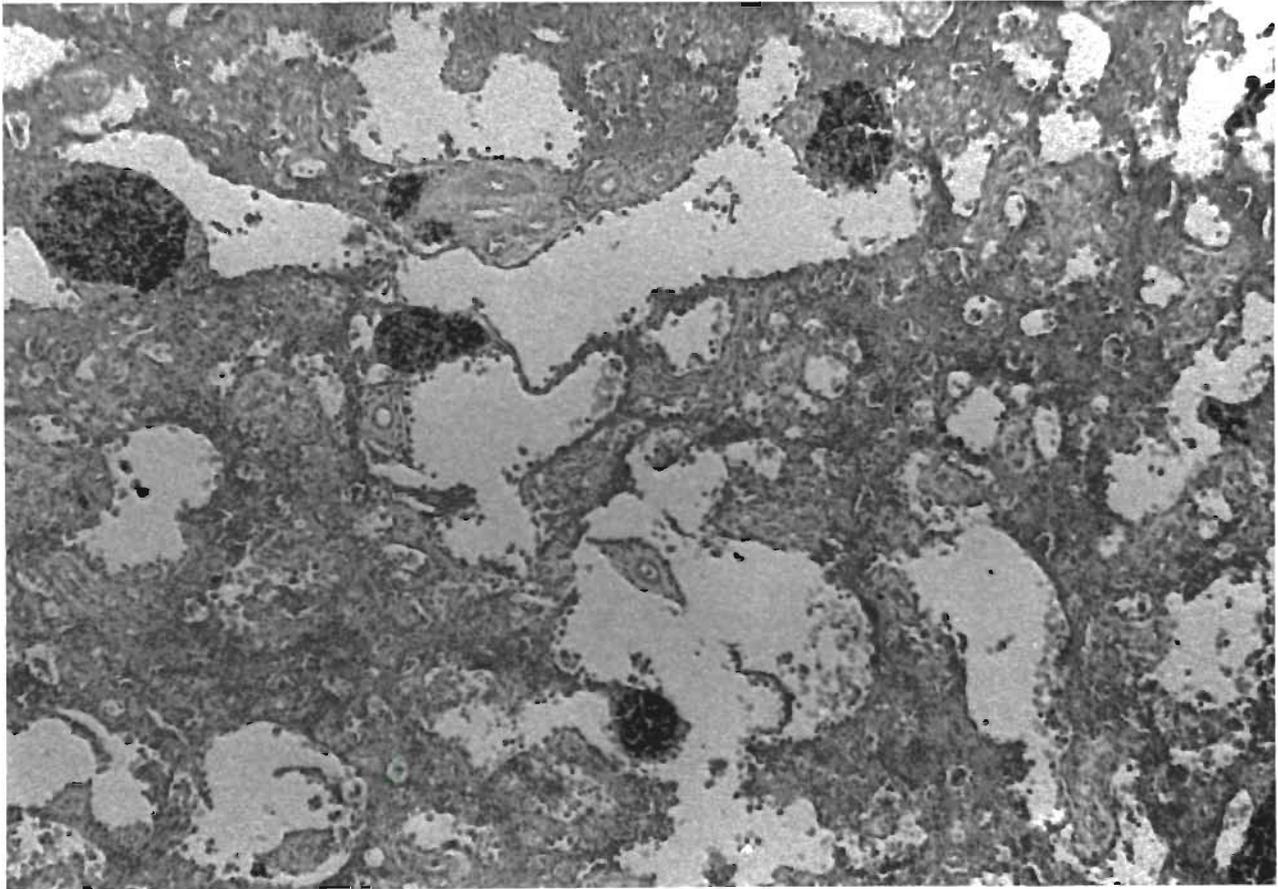


Fig. 2. *Pterophyllum altum*. Detail of Fig. 1. Note the desquamated reticulo-endothelial cells in the lumen of the dilated sinusoids. 100 ×

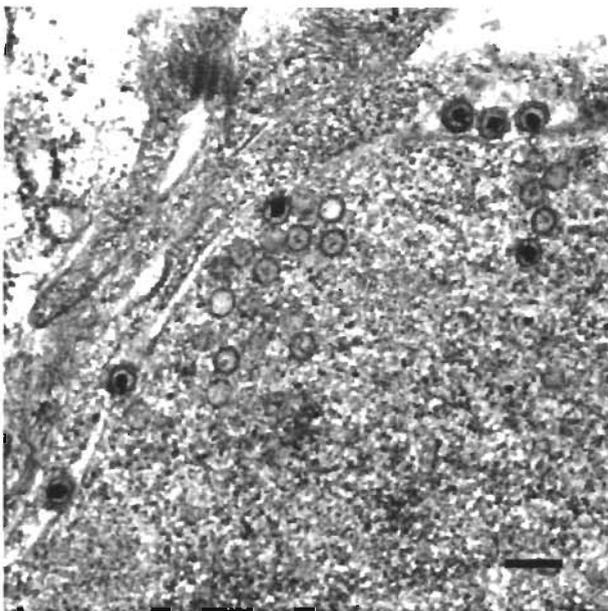


Fig. 3. *Pterophyllum altum*. Herpesvirus-like particles in a macrophage in the splenic stroma. Almost empty as well as complete nucleocapsid-like particles can be seen in the nucleus, while enveloped particles are present in the distended nuclear cisterna. Bar = 200 nm

pesvirus infections is the presence of skin haemorrhages. In angelfish the ulcerations might have aggravated the disease condition by causing osmoregulatory stresses. In channel catfish, Major et al. (1975) described phagocytosis of erythrocytes in both kidney and spleen and regarded it as a sign of erythrocytic degeneration. The observation of haemosiderosis in the spleens of 2 of the 3 angelfish indicates that erythrocytic degeneration may have been associated with the infection.

The disease outbreak may have been precipitated by the aquarist's manipulations within the aquarium. Stress caused by these manipulations activated a possible latent herpesvirus infection in either the imported or the 'home' bred angelfish. However, the very short interval between the stress and the appearance of the clinical signs indicates that the imported feral angelfish *Pterophyllum altum* were latent carriers of the herpesvirus.

The 'home' bred angelfish *Pterophyllum scalare* did not show any disease signs and it is not known whether they were also carrying the virus. Samples of these fish were not made available for examination and so this question remains unanswered.

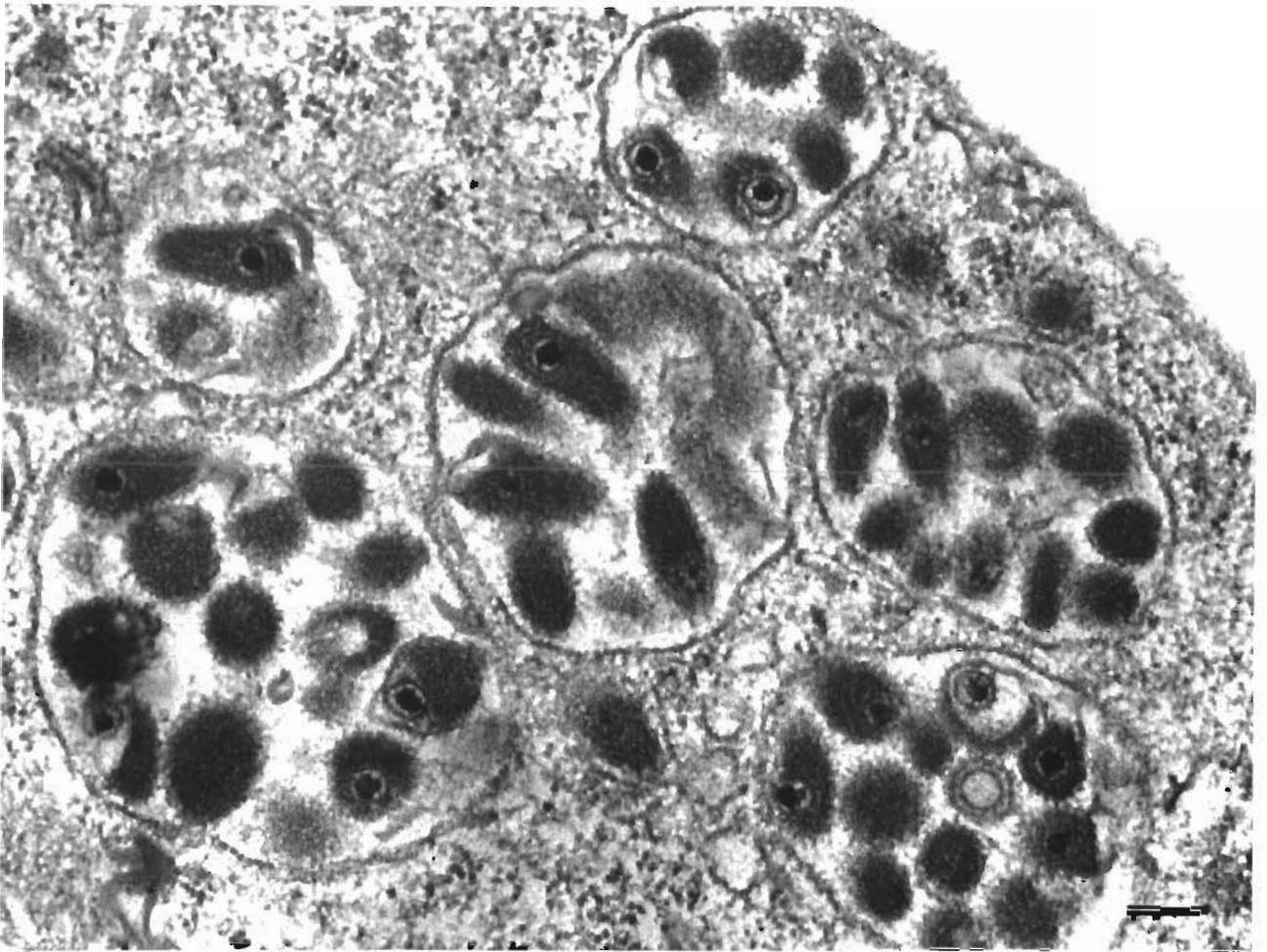


Fig 4 *Pterophyllum altum*. Drop-shaped herpesvirus-like particles in vacuoles in the cytoplasm of an infected cell. The space between the nucleocapsid-like inner particle and the surrounding unit membrane is most often filled with a strongly osmiophilic material. Bar = 200 nm

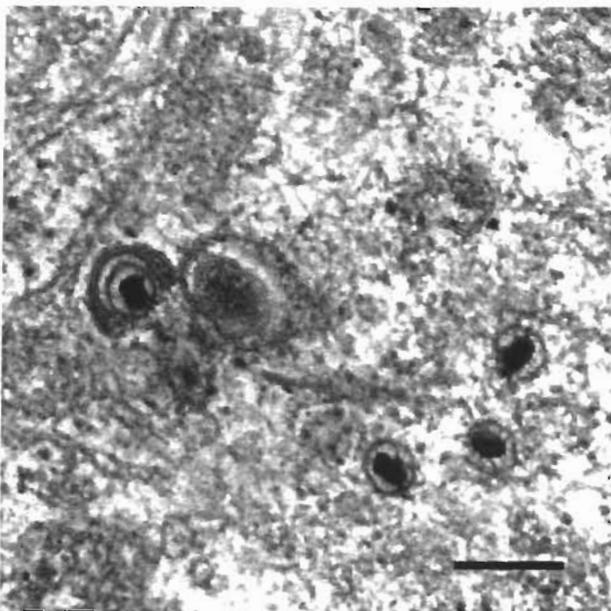


Fig 5. *Pterophyllum altum*. Nucleocapsid-like particles in the cytoplasm of an infected cell. One of the particles is partly surrounded by a narrow cisterna. Bar = 200 nm

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