NOTE

An iridovirus-like agent associated with systemic infection in cultured turbot *Scophthalmus maximus* fry in Denmark

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ABSTRACT: A disease in a Danish turbot *Scophthalmus maximus* rearing unit caused the death of approximately 50 000 fry corresponding to a mortality of 70 %. The initial signs of disease were reduced feed intake, lethargy and darkening in pigmentation, especially of the tail and fins. Later there was atypical swimming, e.g. swimming on edge, and spasms in the terminal stages. An iridovirus-like agent, measuring ca 170 nm in diameter (160 to 185 nm), was detected by electron microscopy in samples of fin, gill, liver, kidney, spleen, heart, pancreas, intestinal collagen, and in 1 of 3 brain samples examined. After decontamination of the facilities, no new outbreaks have occurred.

The conditions usually found in fish farming are apt to aggravate the consequences of a disease outbreak. Viral diseases especially spread quickly and result in dramatically high mortalities.

The viral agents detected in farmed turbot *Scophthalmus maximus* include *Herpesvirus scophthalmi* (Buchanan & Madely 1978), infectious pancreatic necrosis virus (IPNV) (Castric et al. 1987), and a virus suggested to be a member of the aquareovirus group (Lupiani et al. 1989). More recently viral haemorrhagic septicaemia virus (VHSV), a lyssavirus belonging to the rhabdoviridae, was found in market-sized farmed turbot (Schlotfeldt et al. 1991) and a picornavirus-like agent causing encephalomyelitis among turbot fry (Bloch et al. 1991) has been described.

This report describes detection of an iridovirus-like agent in turbot fry from a turbot rearing unit in Denmark.

Materials and methods. Disease occurred in 1 batch of fry in a Danish rearing facility in 1991. Very high mortality occurred, reaching 70 % or approximately 50 000 individuals. The initial symptoms were reduced feed intake, lethargy, darkening, especially of tail and

fins. Swimming was abnormal (on edge) and spasms occurred in the terminal stage after which the dead or dying fish sank to the bottom of the tanks. The only gross external sign of disease was some small white or greyish spots or swellings on the edge or surface of the fins of some of the diseased fry. After 1 wk with a daily mortality of approximately 10 %, the remaining fish were killed and the area, tanks and all facilities were disinfected with sodium hypochlorite and Lyso® (Schülke & Mayr, Germany). The disease did not reappear after this single outbreak. The production unit has an annual period of approximately 3 mo without any production and during this time the fish farm was cleaned and disinfected as described.

Diseased fish were subjected to bacteriological and parasitological examinations as previously described (Bloch et al. 1991). For electron microscopy moribund fry were collected and fixed in 2.5 % glutaraldehyde in a phosphate buffer (0.13 M). After dissection the various organs and tissues were post-fixed separately in 1 % osmium tetroxide and embedded in Vestopal-W (Serva) as previously described (Bloch et al. 1991). In addition to a fin with macroscopic alterations initially examined, 5 turbot, measuring 15 to 25 mm in length, from 2 tanks were examined. Three months after the onset of the outbreak, 3 additional fry from a third tank suspected to be infected were examined.

Results. Neither the bacteriological nor the parasitological examination yielded any significant findings. A preliminary electron microscopical examination of a fin from 1 fish revealed the presence of icosahedral virions. Similar virions were discovered in various tissues of 3 fry from one of the tanks. When calibrated with a cross grating (Polaron), the hexagonal virions were ca 185 nm from corner to corner and ca 160 nm from facet to facet measured in the least compressed direction.

The size and the cytoplasmic position of the particles together with the apparently 5-layered capsid and the slightly protruding vertices of the virion all presumptively identified them as members of the Iridoviridae (Fig. 1). Virions found in detached but still adhering epithelial cells were, however, smaller with a diameter of ca 135 nm. They had a more rounded outline and lacked the semi-translucent zone between the capsid and the dense part of the nucleoid.

As in the initial fin sample, infected cells were observed in both the epithelium and in the underlying support tissue of the macroscopically opaque and slightly hyperplastic thickened parts of the fins of the 3 fry (Figs. 2 & 3). Some of the infected cells initially contained a lightly stained matrix which displaced and deformed the nucleus. Virions were usually present

Fig. 1. *Scophthalmus maximus*. Icosahedral virions in the cytoplasm of a hepatocyte of a turbot fry. Note the slightly protruding vertices and the apparently 5-layered capsid. Scale bar = 100 nm

within or around the matrix. In cells presumed to represent later stages of infection, the matrix had disappeared and dense masses resembling residual bodies or secondary lysosomes filled the cytoplasm. At this stage the cells contained few or no virions.

The most severely affected organs were the gills with infected cells both in the primary and the secondary lamellae. In the primary lamellae virions were observed in the interstitial cells/fibroblasts especially around the cartilaginous support structure (Fig. 4). Also, infected cells could be found free in the blood vessels. In the secondary lamellae the capillary could be completely occluded by infected cells (Fig. 5). The identity of these cells was difficult to determine due to their highly altered structure, but probably endothelial cells, pillar cells and leukocytes could be infected and occlude the capillaries. As in the fins, the epithelial cells of the lamellae could be infected.

The virus was detected in all 3 livers examined; however, infected hepatocytes were found in only 1 liver. In this specimen, virions were also found in non-hepatocytic cells adjacent to the sinusoids. In the other 2 livers, only a few fibroblasts lying together with collagen were infected. Virions were seen budding through the cell membrane (Fig. 6) with the envelope seeming to have an additional layer on the side adjacent to the virion. The diameters of the enveloped nucleocapsids were generally smaller than the diameters of nucleocapsids in the cytoplasm of intact cells and measured 140 to 165 nm.

Infected cells were also present in the haematopoietic tissue of the kidneys. The cells were often lysed and were therefore difficult to identify, but it appeared that both leukocytes and reticular cells could be infected.

Reticular cells also seemed to be a target for the virus in the only spleen examined, but as in the kidneys a few cells, apparently monocytes, were infected.

In the 2 hearts examined, the virus was observed in cellular debris along capillaries and in the cardiac myocytes.

Although the virus was generally not observed in the intestinal tissues, it was present in fibroblasts in the dense collagen surrounding a section of the intestine of 1 fish. The virus was similarly observed in a few fibroblasts adjacent to the pancreatic tissue and in the pancreas of 1 of 2 turbot.

Sections of the brain of all 3 fish were examined, but

Fig. 2. *Scophthalmus maximus*. Epithelial cells of a discoloured and slightly thickened fin. In some of the cells virions can be seen both in and outside the light matrix (m) that often displaces and deforms the nucleus (n). Some of the cells contain large dark inclusions (i) resembling residual bodies. Only few or no virions are present in such cells. Scale bar = 2 µm

Fig. 3. *Scophthalmus maximus*. Infected cells positioned between the basement membrane (b) of the fin epithelium and a fin ray (r). A few enveloped virions can be seen outside the cells. Scale bar = 1 µm

Fig. 6. *Scophthalmus maximus*. Virions budding through the cell membrane of a hepatocyte. Material seems to be layered on the inside of the envelope membrane. The diameter of the nucleocapsid is smaller (140 to 165 nm) than the diameter of virions found in cytoplasm. The clear zone between the core and the capsid is correspondingly smaller. Scale bar = 200 nm

the virus was seen in only one. As in other tissues with mixed cell types, the infected cells were usually difficult to identify because of the detrimental effects of the virus infection. The presence of the virus in cells covering a capillary could, however, indicate that astrocytes may be susceptible to infection.

The virus was never observed in fry from 2 of the 3 breeding tanks, although the fins of some turbot from these vessels were slightly swollen, similar to those seen in virus-infected animals. Examination by electron microscopy revealed the presence of bacteria both between the cells of the edematous epithelium and in vacuoles in these cells.

Discussion. Although the turbot virus examined in

this study should not yet be considered fully classified and therefore is referred to as an iridovirus-like agent, it most likely belongs to the family Iridoviridae.

A great number of iridoviruses have already been isolated from fish or observed by electron microscopy in samples of fish tissue. Apart from 1 or 2 iridoviruses that do not seem to cause any overt disease, the piscine iridoviruses can be grouped according to the type of disease with which they are associated. Thus a group of 4 viruses are associated with infection of the integument, while another group of iridoviruses causes erythrocytic necrosis.

The present virus was observed in a variety of tissues and it therefore seems relevant to compare it with a group of iridovirus-like agents associated with severe systemic infection. Three of these viruses have already been isolated.

One of these, causing epizootic haematopoietic necrosis (EHN) in redfin perch *Perca fluviatilis* has been isolated in Australia (Langdon et al. 1986), but a number of other species of teleosts have been shown to be susceptible to this virus (Langdon 1989). The pathological findings in the affected species are not consistent, but renal haematopoietic necrosis seems to be the most common feature while hepatic, splenic and pancreatic necrosis and shedding of necrotic haematopoietic cells into circulation may occur. Another systemic iridovirus-like agent has been isolated from fry of sheat fish *Silurus glanis* in Germany (Ahne et al. 1989). The signs of disease are spiralic swimming and petechial haemorrhages in the skin. Other pathological characteristics are destruction of the renal haematopoietic tissue and the spleen. Necrotic foci in liver and pancreas, hyperplasia and oedema of the gill and diffuse myocarditis were other characteristic findings (Ogawa et al. 1990).

The third of the viruses, isolated from catfish *Ictalurus melas* in France (Pozet et al. 1992), produces similar histopathological alterations with destruction of the haematopoietic tissue of the kidney and the spleen as the principal findings. Oedematous alteration of the liver and extensive lamellar fusion and hyperplasia were also observed.

A comparison among these 3 iridovirus-like agents was published recently (Hedrick et al. 1992). In this report it is shown that the viruses are of the same size and morphology with a main diameter of 153 to 154 nm. Also the number and weight of the structural

Fig. 4. *Scophthalmus maximus*. Infected cells in the collagen positioned between a blood vessel (v) and the cartilaginous support rib (c) of a primary lamella. Scale bar = 1 µm

Fig. 5. *Scophthalmus maximus*. Part of a secondary lamella, the capillary of which is almost completely occluded by infected cells. Only the epithelial cells (e) can be identified with certainty. Scale bar = 2 µm

polypeptides are similar and an antigenic relationship is shown by cross immunofluorescence test. The 3 viruses furthermore exhibit a pronounced similarity to the slightly smaller frog virus 3 (130 to 140 nm) and the authors suggest that the 4 viruses should be grouped into the same genus.

Apart from the 3 piscine iridovirus-like agents mentioned above, 2 cichlide iridovirus-like agents also causing systemic disease have been found. The virus discovered in Ram cichlid *Apistogramma ramirezi* measured between 110 to 120 nm in diameter (Leibovitz & Riis 1980). The histopathological findings are similar to those observed in connection with the 3 viruses mentioned above, but degenerative changes in the eye seem to be more pronounced. The iridovirus-like agent found in the chromide cichlid *Etroplus maculatus* measured 180 to 200 nm in diameter (Armstrong & Ferguson 1989). The most striking pathological findings associated with the presence of the virus were altered cells in the renal haematopoietic tissue and the presence of abnormal cells throughout the circulatory system of the fish.

The pathological findings associated with these 5 viruses are not identical, but generally the same organs are involved. The present turbot virus has been observed in cells of the same tissues except the eye. The difference in size between the turbot virus and the other viruses mentioned should not be given too much weight as the diameter of the turbot virus seems to vary according to the state and position of the virus. It will therefore be of the greatest interest to compare the turbot virus with the members of the proposed group of piscine iridoviruses in order to clarify whether the turbot virus is identical with other members of the group and the turbot thus being just another host, or whether it is a different virus of its own. Such examinations, however, require that the virus can be cultivated. It is our hope that we will be able to do so in the near future, as we also intend to perform a transmission experiment in order to elucidate the pathogenicity of the virus.

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