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Short communication

Systemic disease involving an iridovirus-like agent in cultured tilapia, *Oreochromis niloticus* L. – a case report

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Iridoviruses have been associated with systemic disease among an increasing assortment of wild and cultured fish populations (Langdon, Humphrey, Williams, Hyatt & Westbury 1986; Langdon, Humphrey & Williams 1988; Ahne, Schlotfeldt & Thomsen 1989; Pozet, Moussa, Torhy & de Kinkelin 1992; Bloch & Larsen 1993; Chua, Ng,Ng, Loo & Wee 1994; Matsuoka, Inouye & Nakajima 1996; Rodger, Kobs, Macartney & Frerichs 1997), including cichlid fishes (Armstrong & Ferguson 1989). Iridoviruses cause mortality among important food fish species (Langdon *et al.* 1988; Ahne, Ogawa & Schlotfeldt 1990; Bloch & Larsen 1993; Chua *et al.* 1994; Matsuoka *et al.* 1996), and the potential exists for the spread of iridoviral diseases between wild and cultured fish, as well as between imported and indigenous species (Langdon *et al.* 1988; Armstrong & Ferguson 1989; Langdon 1989; Hedrick, McDowell, Ahne, Torhy & de Kinkelin 1992; Hedrick & McDowell 1995; Rodger *et al.* 1997).The present report describes an outbreak of viral disease in cultured tilapia, *Oreochromis niloticus* L.

Twenty-five 3–6 cm tilapia were submitted alive by an aquaculturalist for diagnostic analysis because of elevated mortalities. The fish had recently been imported into Canada from a single source in Florida, and the submission included individuals from all four fibreglass tanks housing these recent additions. These four tanks, as well as two identical ones housing older 15–20 cm tilapia, shared a municipal water source in a recirculation system. The older tilapia were later submitted to this laboratory with clinical signs similar to those seen in the more recent imports.

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Affected fish were lethargic, swimming slowly or resting on the bottom of their tank. Grossly, these fish were bloated, they exhibited varying degrees of exophthalmia and gill pallor, and they were generally dark. Many fish also presented with erythema of the submandibular skin. Small numbers of monogenean trematodes were found in gill whole-mounts and in skin scrapings. The dominant internal gross features of this disease were severe abdominal ascites and marked pallor of the visceral organs, particularly the liver. Some individuals also exhibited petechial haemorrhages in the liver.

Swabs from eye lesions (two fish) and kidney tissue (one fish) were streaked onto trypticase soy agar (TSA, Difco) and incubated aerobically at 18 °C for 7 days. After incubation, bacterial isolates were subcultured onto TSA and identified using standard biochemical methods (MacFaddin 1980) as well as with commercially available identification strips (API20e, BioMerieux Vitek Inc., Hazelwood, MO). Large numbers of a mixed bacterial microflora were recovered from both fish with eye lesions. The microflora consisted of three colony morphologies, none of which appeared to be the predominant colony type. One isolate was identified as *Aeromonas hydrophila,* while the remaining two isolates were Gram-negative, oxidase-negative, non-fermentative, non-motile rods that grew at 37 °C. These isolates

did not react in API20e strips incubated at 18 or 37 °C. Low numbers of an axenic bacterial microflora were recovered from the kidney of the third fish. This organism was also identified as *Aeromonas hydrophila.*

Six fish were fixed in Bouin's fixative, sectioned sagittally and transversely, and then routinely processed for paraffin wax sections that were stained with haematoxylin and eosin. On light microscopy, the most prominent feature seen in the tissues examined was a diffuse distribution of hypertrophic cells seemingly within those tissues of mesodermal origin. Abnormal cells were found in cranial connective tissues, associated with endocardium, within hepatic parenchyma, throughout the spleen, and throughout the renal haematopoietic tissue. These cells were also present in the branchial vasculature and the choroid gland, as well as within intestinal lamina propria and serosa. Characterized by a heterogeneity in appearance, hypertrophic cells ranged from cells with copious foamy cytoplasm containing multiple pale zones and a displaced nucleus, to cells with a large foamy nucleus and taut or indistinct nuclear membrane capped by marginated chromatin, all surrounded by foamy basophilic cytoplasm, and finally to cells with greatly enlarged nuclei containing eosinophilic refractile nuclear inclusions and marginated chromatin surrounded by a thin ring of cytoplasm (Fig. 1). Round eosinophilic inclusion bodies were also intracytoplasmic in some instances. Affected tissues contained scattered necrotic cells and nuclear and cellular debris. In many cases, a mononuclear cell infiltrate appeared to be responding to the aberrant cells. In one exophthalmic fish, fungal hyphae had invaded retrobulbar tissue.

Light microscopy findings were consistent with a viral aetiology, and several more fish exhibiting clinical signs were prepared for ultrastructural examination. Samples (1 mm^3) of liver, spleen and kidney were trimmed from Bouin's-fixed tissues and placed into 2.5% glutaraldehyde in 0.1 M phosphate buffer for several days. Tissues were then rinsed in four changes of 0.1 M phosphate buffer and then post-fixed in 0.1 M phosphate buffer containing 2.0% osmium tetroxide. Standard processing for transmission electron microscopy then followed, beginning with dehydration of tissues in a graded ethanol series, followed by a propylene oxide rinse, epon resin infiltration using a graded series of epon–propylene oxide mixtures, and ending with two changes in 100% epon, and polymerization of epon-tissue samples at 60 °C for 2 days. Sections for electron microscopy (80–90 nm nominal thickness) were stained with lead citrate and uranyl acetate. Examination of hypertrophic liver, kidney and spleen cells revealed numerous internal polyhedral viral particles (Fig. 2).

Light and electron microscopy findings strongly suggested the involvement of a virus in this disease outbreak among cultured tilapia. The absence of bacteria and bacterial lesions in histological sections suggests that any bacteria recovered from these fish were secondary invaders. The size and symmetrical shape of the virion, as well as a replication process involving both nuclear and cytoplasmic phases (Murti, Goorha & Granoff 1985), suggest that this virion is a member of the family Iridoviridae. Attempts at isolation and further characterization are presently underway. Mao, Hedrick & Chinchar (1997) have recently characterized a number of iridoviruses isolated from various fish species.

Among published accounts of systemic iridoviral disease, the pathology observed in the tilapia, *Oreochromis niloticus* L., of the present report most closely resembles the findings of Armstrong & Ferguson (1989) for a systemic iridoviral disease in the chromide cichlid, *Etroplus maculatus.* This may be due to the fact that cichlids were affected in each case and/or because of a strain similarity for the respective iridoviruses involved. In support of the former, Leibovitz & Riis (1980) reported on a disease in ram cichlids caused by a virus other than an iridovirus which also was characterized by progressive cytomegaly. In each case there was a moderate hypertrophy to ballooning of cells, although the extreme cytomegaly of localized iridoviral diseases such as lymphocyctis did not occur. More recent reports of systemic iridovirus disease in redfin perch (Langdon & Humphrey 1987), sheatfish fry (Ogawa, Ahne, Fisher-Scherl, Hoffman & Schlotfeldt 1990), catfish (Pozet *et al.* 1992), turbot fry (Bloch & Larsen 1993), brown-spotted grouper (Chua *et al.* 1994) and angelfish (Rodger *et al.* 1997) share features of the pathological changes reported here for the tilapia, such as clinical signs (lethargy, petechiae, pigmentation darkening) and tissue tropisms (vascular system, reticuloendothelial elements of the spleen and kidney). However, each of these reports also describes a much more necrotizing disease than we saw in this disease in tilapia. Armstrong & Ferguson (1989) also suggested that the iridoviral disease of cichlid chromides produced less necrosis

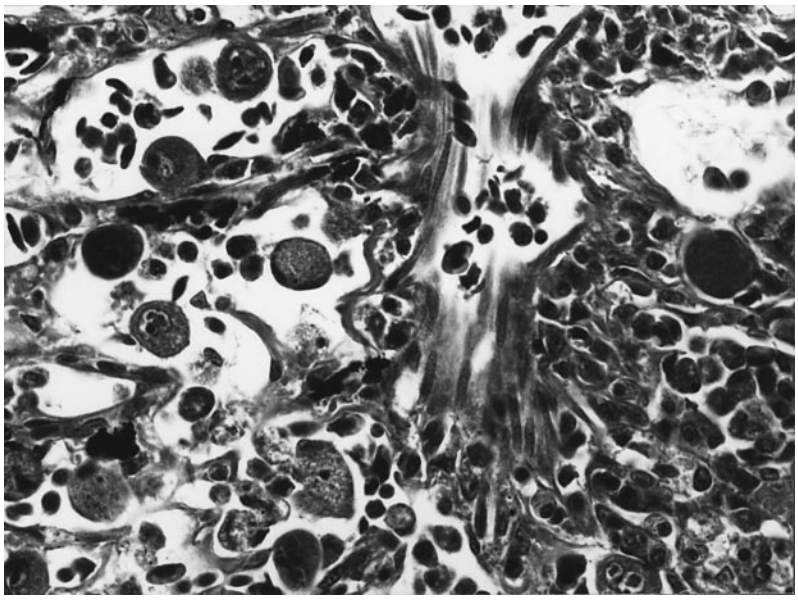

Figure 1 Section through choroid gland of eye of infected tilapia showing several hypertrophic cells, mostly within blood vessels (H&E, ×450).

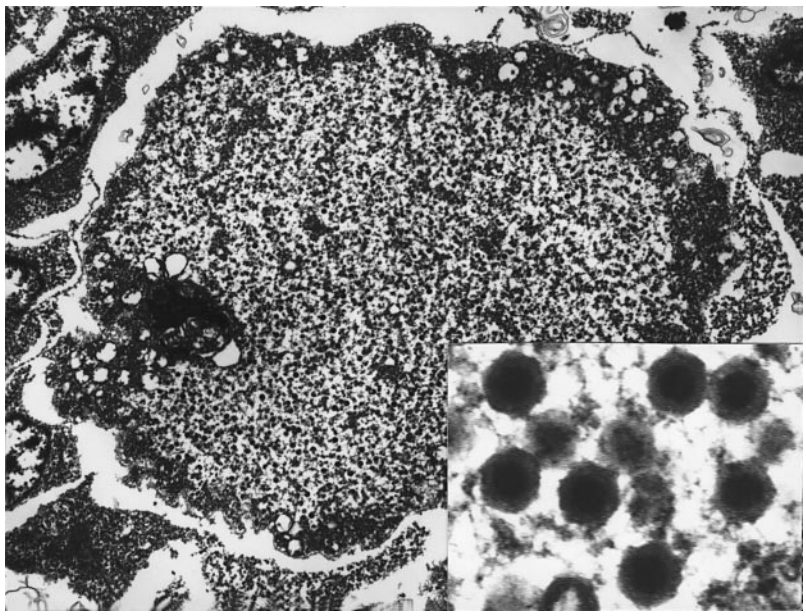

Figure 2 Electron micrographs of cytomegalic cell from anterior kidney showing enlarged nucleus surrounded by thin rim of cytoplasm (×6000). Insert shows enlargement of virus-like particles (×60 000).

than may be seen with other systemic iridoviral diseases, such as epizootic haematopoietic necrosis (EHN) in redfin perch, *Perca fluviatilis* L. (Langdon & Humphrey 1987).

Further studies to follow the sequential pathology of the iridovirus in tilapia and other species are ongoing in an attempt to provide detailed comparisons of this disease to other reported systemic iridoviral diseases of fish. Tilapia are some of the most important species of cultured fish in the world, and their culture in, and dissemination to, new geographic areas of the world such as Canada

continues to expand. Additional investigations are required to address the significance of this viral disease to tilapia aquaculture in general, as well as to the health of other indigenous fish species, both wild and cultured.

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