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VIRUS-LIKE PARTICLES IN THE RETINA OF THE SEA-BREAM, *SPARUS AURATA*

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Abstract

Cytoplasmic virus-like particles showing important similarities with the fish nodaviruses particles were found in nervous cells of the retina of hatchery-reared larval sea bream.

Introduction

In the past decade, the Nodaviridae have increased in importance as direct pathogens for various species of marine fish from aquaculture. Infections caused by viruses characterised and identified as members of Nodaviridae family have been reported in stripped jack (Mori *et al.*, 1990), in sea-bass and in barramundi (Comps et al., 1994).

Similar infections were also detected in several marine fish using histological methods, RT-PCR amplification technique, DNA probes and immunological tests (Bloch *et al.*, 1991; Nishizawa *et al.*, 1995; Comps *et al.*, *in press*).

Recently heavy mortalities (90%) associated with clinical signs of viral encephalitis were registered among hatchery-reared larvae of sea-bream. We report here the histopathological findings of the lesions found in the retina.

Material and methods

Larvae, 40 days old, which exhibited an abnormal swimming activity were collected in a French hatchery. Diseased larvae were fixed for histological examination, 20 in Bouin fixative and 12 in buffered glutaraldehyde. Ultrathin sections were stained by the method of Reynolds (1963). *In situ* hybridisation was performed using N12 probe made with cDNA of DIEV RNA2 (Comps *et al.*, in press).

Results

Light microscope examination of semi-thin sections revealed histopathological changes among some sampled animals treated for electron microscopy. Sections through the eyes an the brain showed an important necrosis in the inner nuclear layer of the retina (Fig. 1). The lesion appeared as a single vacuole, approximately 130 µm in size, surrounded by smaller vacuoles. Vacuoles contained cellular debris.

Ultrastructural examination showed that cellular debris found in the vacuoles and some damaged cells surrounding the vacuoles contained virus-like particles (VLPs) (Fig. 2). The size of VLPs was estimated to 22-25 nm. According their distribution, they exhibited paraspherical or polygonal profile. VLPs were often seen forming continuous lines (Fig. 2). Some of infected cells contained amorphous inclusions, 500-600 nm in size showing occluded particles (Fig. 3). In another cells, paracrystalline inclusions bodies characterised by a periodic structure were observed together with the VLPs (Fig. 4a and 4b).

In attempts of *in situ* hybridisation using N12 probe no reaction of hybridisation with cDNA of DIEV was observed.

Discussion

The size and the shape of the VLPs observed in the nervous tissue of the sea-bream are consistent with those of the nodaviruses

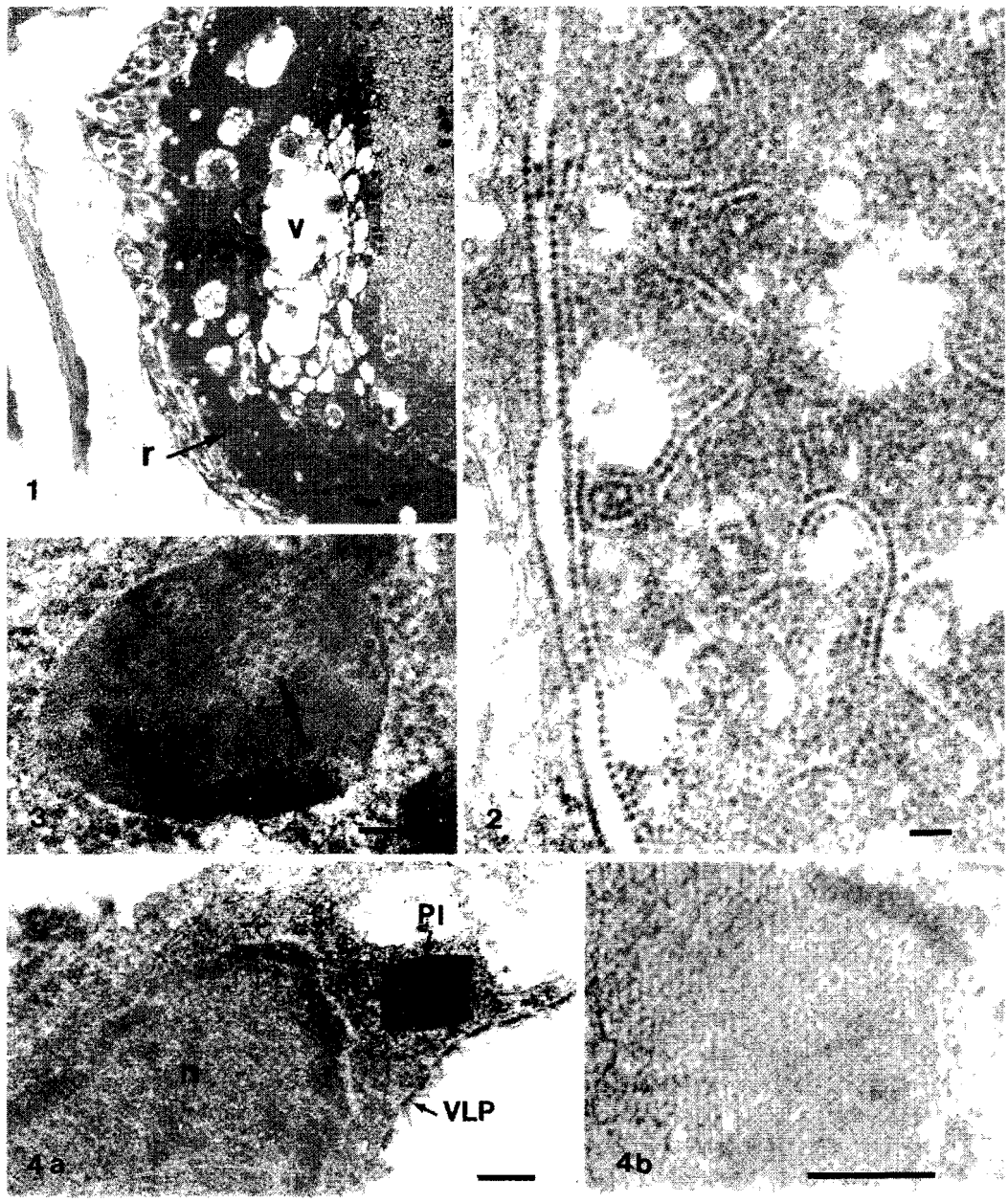

Fig. 1. Section through the retina .(r) showing vacuoles (V) in the layer of nervous cells. Toluidine blue. Bar = 10 µm. **Fig. 2.** Cross section of infected nervous cell. A large amount of virus-like particles are scattered throughout the cytoplasm. E.M. Bar = 100 nm. **Fig. 3.** Amorphous cytoplasmic inclusion body containing occluded VLPs (arrow). EM. Bar = 100 nm. **Fig4.** (a) Electron micrograph of infected cell containing a paracrystalline inclusion (PI). Virus-like particles (VLP). Nucleus (n). Bar = 200 nm. (b) Higher magnification of the Figure 4a showing periodic structure of the PI. Bar = 50 nm.

reported in stripped jack, sea-bass and barramundi and with those of virus particles described in several species of fish exhibiting nervous necrosis, such as turbot (Bloch *et al.*, 1991), Japanese parrotfish (Yoshikoshi and Inoue, 1990) or redspotted grouper (Mori *et al.*, 1991). However, the presence of paracrystalline inclusions through the cytoplasm of infected cells was not reported in other fish species than sea bream. Although no signal of hybridisation with the cDNA of DlEV was detected on sections of larvae processed for *in situ* hybridisation using N12 probe, we cannot conclude at present that this agent differs from DlEV, because characteristic lesions of nodavirus infection were not histologically apparent on the investigated sections (larvae fixed by Bouin medium).

Further studies involving collect of new infected animals for purification of VLPs would help identify this agent and clarify his relatedness with the fish nodaviruses.

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