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RICKETTSIA-LIKE ORGANISM INFECTING JUVENILE SEA-BASS *DICENTRARCHUS LABRAX*

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Abstract

High levels of mortalities (20%) associated with abnormal swimming have been observed in farmed juveniles of sea-bass, *Dicentrarchus labrax*, in Mediterranean coast, southern France. Histopathology was obvious in the brain with nervous tissue necrosis and the presence of cells containing basophilic granules corresponding to small coccoid organisms. Ultrastructural examination revealed these organisms were rickettsia-like organisms morphologically close to *Piscirickettsia salmonis* .

Introduction

Abnormal swimming associated with viral nervous necrosis have been reported in larvae of several species of marine fishes (Bellance *et al.*, 1988; Bloch *et al.*, 1991; Breuil *et al.*, 1992; Glazebrook *et al.*, 1990; Yoshikoshi and Inoue, 1990). Similar clinical signs including spiral swimming, anorexia and mortality, were observed during the winter 1994 among juveniles of sea-bass ranging between 30 and 70 g. However microscope examination and immunological tests did not reveal the presence of DIEV causing encephalitis of the larval sea-bass (Comps *et al.*, 1994), in affected fishes. Further microscope study have shown that diseased fishes exhibited focal necrosis of mesencephalon tissues associated with inflammatory reaction. Rickettsia-like organisms were observed through sections of necrosed nervous tissues. We report in this paper the findings of histopathological investigations of the moribund sea-bass.

Material and methods

Fish

During the winter 1993-1994, pathological changes occurred in juvenile sea-bass, *Dicentrarchus labrax*, reared in floating cages on the Mediterranean French coast. The water temperature fluctuated during the epizootic between 12 °C and 15°C. Diseased sea-bass ranging between 30 and 70 g were sampled from three cages and processed for histopathological and ultrastructural examinations.

Light and electron microscopy

Five affected fishes were fixed *in toto* in 10 % buffered formalin. The different organs were separately embedded in paraffin and routinely sectioned (5 µm) and stained using haematoxylin and eosin. For electron microscopy, tissues collected from affected animals were fixed in 4 % glutaraldehyde in 0.4 M cacodylate buffer, post-fixed in 2% osmium tetroxide and embedded in Epon. Ultrathin sections were stained by the method of Reynolds (1963).

Results

Gross pathology

The most consistent sign of the disease was abnormal swimming and whirling behaviour. Affected fishes also displayed strong symptoms of meningitis and extensive haemorrhaging was observed on the surface of the brain.

During the first month after the outbreak of the disease, a mortality reaching 13 % occured in the most affected fish stocks. Additionally 10 % of diseased sea-bass exhibited signs of vibriosis (*Vibrio anguillarum*). Afterwards mortalities (monthly reported) decreased regularly: 3.5 % in January and February, 1.8 % in March and 0.5 % in April. The epizootic finished by the end of April.

Microscopic examinations

Squashes of brain from diseased sea-bass revealed the presence of cells which contained numerous basophilic inclusions, 0.5-1 µm in size (Fig. 2).

Histopathology

The significant histological changes were located into the brain. Focal necrosis associated with severe inflammatory reaction was detected in the anterior part of the medulla oblongata (Fig.1). Through these necrotic areas, some cells (macrophages) contained in cytoplasmic vacuoles, numerous coccoid elements which exhibited strong basophilia and were approximately 1 µm in size. Similar cells were also found isolated within the nervous tissue.

Electron microscopy

Thin sections through the brain lesions revealed that the basophilic granules belonged to a procaryotic organism. One or several procaryotic cells were enclosed within membrane-bound vacuoles (Fig. 3). They were paraspheric in shape and had a size ranging from 0.5 µm to 1.5 µm. The wall, 50 nm thick, consisted of two unit membrane-like elements separated by an electron lucent layer. Outer membrane appeared irregular and swollen. The peripheric zone of the cytoplasm was granular and electron-dense, while the central part was clearer and contained fibrils associated with finely granular material (Fig. 4). Some organisms were seen to be in course of binary fission. Other procaryotes contained polymorphous electron dense bodies (PEDB) surrounded by an unit membrane and containing numerous finely dense granules (ribosomes) (Fig.4). By TEM pathological some changes were observed in infected cells. Vacuoles containing the organisms were enlarged, sometimes occupying the whole cell. Remaining cytoplasm was finely granular and dense and contained pleomorphic electron dense inclusions (Fig. 3).

Discussion

The procaryotic organisation of the intracellular organism found in the brain of juvenile sea-bass allowed us to consider it as a rickettsia-like organism (RLO). Regarding its morphological characteristics and its multiplication which does not involve a chlamydia-like developmental cycle, it shares many common features with the members of the Rickettsiales. Moreover, because of its growth within membrane bound vacuoles in the cytoplasm of mononuclear cells and its rippled cell wall, it could be related to the tribe of Ehrlichieae (Ristic and Huxsoll, 1984).

This organism exhibits morphological similarities with *Piscirickettsia salmonis* , an important pathogen isolated from Coho salmon *Oncorhynchus kisutch* (Fryer *et al.*, 1990) and identified in other farmed salmonid fishes such as *Oncorhynchus mykiss, O. tshawystcha, and Salmo salar*) from southern Chile (Fryer *et al.*, 1992). However, we have to underline the presence of polymorphous electron dense bodies (PEDB) in the RLO of seabass . Such elements were not reported in *P. salmonis*. At the moment, their signification remains uncertain. In addition, we note that RLO-infection appeared to be located in the brain of sea-bass while in the salmonids, *P. salmonis* causes necrosis in different organs such as kidney, spleen, liver and intestines.

Because of its location in the brain of sea-bass, we hypothesised that this RLO is the most likely cause of the abnormal swimming behaviour although its pathogenicity was not yet experimentally confirmed.

While this is the first report of Rickettsiales in sea-bass there are other recent examples in non-salmonid fish such as Nile tilapia (Chen *et al.*, 1994) and blue eyed plecostomus (Khoo *et al.*, 1995). Until recently,

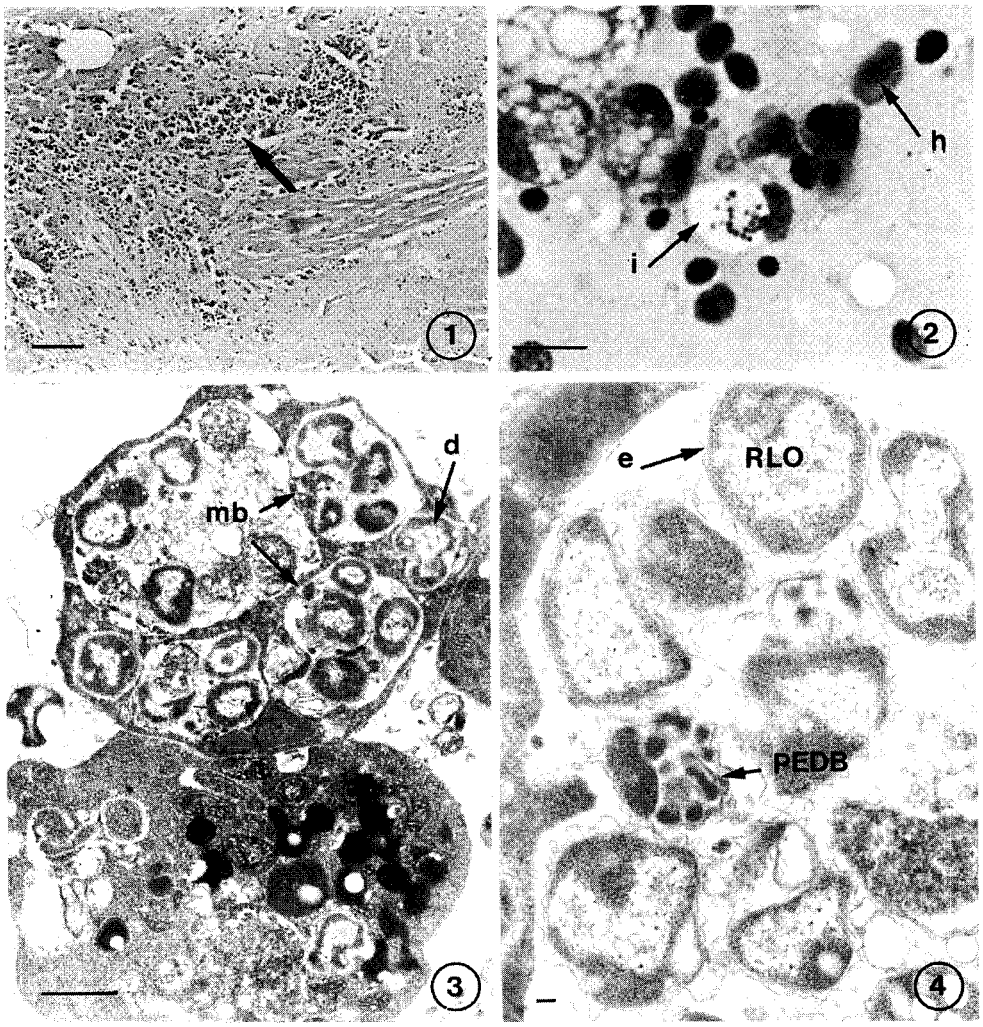

Fig. 1. Section through the brain showing necrosis of nervous tissue. Bar = 100 µm.
Fig. 2. Brain smear stained by Giemsa method: macrophage containing basophilic granules (i); haemocyte (h). Bar = 10 µm.
Fig. 3. Electron micrograph of macrophage containing rickettsia-like organisms within membrane-bound vacuoles (mb); organism undergoing binary fission. Bar = 1 µm.
Fig. 4. Electron micrograph showing the fine structure of rickettsia-like organisms: note the rippled outer membrane (e) ;one of them contains PEDB. Bar = 100 nm.

only a Chlamydia-like organism causing epitheliocystis infection, was reported in this fish species (Paperna and Baudin-Laurencin, 1979).

The origin and the mode of entry were not assessed here. However, susceptibility of sea-bass to this RLO might result of concomitant infection such as the vibriosis

which occured at the outbreak of the disease. As suggested by Rodger and Drinan (1993) and Cvitanich *et al.*, 1991) some other marine species could interfere as reservoirs for rickettsia-like organisms. Note that Rickettsiales have been previously reported in Crustacea (Bonami and Pappalardo, 1980) and in bivalve mollusc (Comps, 1980), from Mediterranean coast. Further studies are in course in order to determine the exact relationship of the RLO here described with *P. salmonis.*

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