NOTE

Intestinal microsporidiosis in European seabass (Dicentrarchus labrax L.) farmed in Italy

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Abstract
The occurrence of intestinal infections due to microsporidia in European seabass (Dicentrarchus labrax) farmed in Italy is described. The infected fish showed emaciation and low growth rate associated with a low level mortality. Morphological, histological and molecular analysis suggest that the microsporidian is a member of the genus Loma.

The increase of marine fish production in recent decades throughout the Mediterranean Basin has led to a raised awareness of those risk factors that may limit or even cause a decrease of the fish production. In addition to technical and management factors, health problems have become increasingly important not only to prevent substantial losses of aquaculture production, but also in relation to host welfare and wholesomeness of the final product. Microsporidians are obligate intracellular parasites that have been described from a wide range of vertebrate and invertebrate hosts. In recent years Microsporidia have aroused enormous interest in fish pathology mainly for their potential high pathogenicity, with possible negative effects on production (Awakura, 1974; Dyková and Lom, 1980; Dyková, 1995, Hirt et al., 1999), and for the supposed zoonotic potential of some fish microsporidia such as Pleistophora-like (Sli/g292o et al., 2000). Moreover the absence of effective therapeutic agents and the poor knowledge on the life cycle of several fish Microsporidia make the control and prevention of microsporidioses difficult to manage in aquaculture.

In this research note, the occurrence of intestinal microsporidiosis in European sea bass (Dicentrarchus labrax) farmed in Italy is described.

During an examination of European sea bass from three different Italian inland marine...
farms, a significant proportion of the fish were strongly emaciated with low growth rate associated with a low level mortality. The parasitological examination showed the presence, in the intestinal wall, of cyst-like structures ranging from 50 to 500 μm in diameters (Figure 1). They were full of ovoid spores measuring $3.5 \pm 0.31 \times 1.8 \pm 0.15 \mu m$ ($n = 35$) (Nis Elements D software, Nikon) with conspicuous posterior vacuole. No Microsporidia were found in other organs and tissues. Histologically, the cyst-like structures were xenomas, characterized by the persistence of the hypertrophic host cell nucleus and/

Figures 1-4. Intestinal microsporidiosis due to *Loma* spp. in European sea bass *Dicentrarchus labrax*.

**Figure 1.** Microsporidian xenomas in gut wall, particular of spores (inset): fresh mount.

**Figure 2.** Xenomas surrounded by a thin capsule containing spores and fragments of the host cell nucleus. HE, ×120.

**Figure 3.** Cross-section through the intestine of an infected *D. labrax*. A number of xenomas are scattered mainly in the lamina propria, rarely in the submucosa. HE, ×10.

**Figure 4.** Mature xenoma surrounded by granulomatous host reaction. HE, × 120.
or fragmented together with the scattered presence of mature spores among several developmental stages (Figure 2). Xenomas were present mainly in the lamina propria of the intestine, single or sometimes clustered, rarely in the submucosa (Figure 3). The highly infected areas showed the epithelium sloughing-off. Inflammatory reactions were not observed around the xenomas during their early developmental phases, while mature xenomas were surrounded by granulomatous reaction with necrosis (Figure 4). In advanced stages of infection a complete destruction of the xenomas wall was observed together with an increase amount of granulation tissue characterized by fibroblasts, macrophages, lymphocytes, plasma cells and newly formed capillaries. The cytoplasm of the macrophages appeared engulfed by phagocytized spores. The morphological characters of the spores and xenomas suggested that the microsporidian was a member of the genus *Loma*.

DNA extraction was performed from xenomas (QIAamp DNA mini Kit, Qiagen) and the majority of the SSU rRNA was amplified with the primers V1_f and 1492_r (Nilsen, 2000). The PCR product (1,189 bp) was sequenced (PRIMM, Milano Italy) in both direction and submitted for database similarities searching using BLAST (Altschul et al., 1990). The sequence obtained was 1189 bp long with a GC content of 55.59%. The BLAST search gave the highest identity (99.5%) with *Loma psittaca* (FJ843104, Casal et al., 2009) found in *Colomesus psittacus* (banded puffer) from estuarine region of the Amazon River.

In Europe the *Loma* species described to date in marine fish are *L. diplodae* in the efferent blood vessel of gill filaments of *Diplodus sargus* from the Mediterranean French coast (Bekhti and Bouix, 1985) and *L. dimorpha* from the connective tissue of digestive tract of *Gobius niger* and *Zosterisessor ephiocephalus* in France (Loubès et al., 1984), and *Lipophrys pholis* and *Gobius niger* both from the northwest coast of Spain (Leiro et al., 1994; Arias et al., 1999).

Up to now microsporidia have not been described in European seabass over the world except for an unknown microsporidia recorded by Alvarez-Pellitero et al. (1993) in the intestine, swim bladder and kidney of wild European seabass.

Further studies have to be carried out in order to clarify the origin/natural host and the correct affiliation of the intestinal microsporidia here described, which may represent a future threat of health and productivity in Mediterranean marine aquaculture.

**References**


