NOTE

Cryptosporidium sp. (Apicomplexa) from cultured turbot Psetta maxima

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Abstract
During a survey conducted in a fish farm located in NW Portugal Cryptosporidium sp. sporogonial stages were detected in bile ducts epithelial cells of turbot (Psetta maxima). Fibrosis surrounding the infected bile ducts and necrosis of adjacent hepatic cells were observed.

The turbot, Psetta maxima (Linnaeus, 1758) is a very important marine fish farming species in Europe. In the last years it is estimated that the European production of turbot is about 6.000 tonnes per year (FAO Fishery Statistic, 2002) being the main producer countries located in Southwestern Europe. In Portugal production is about 350 to 400 tonnes per year. One of the major problems to the intensive culture of this fish species is the susceptibility to several pathogens. Fish coccidia have a considerable pathogenic potential, especially in intensive fish cultures where it can quickly spread and cause high losses (Lom & Diková, 1992). Cryptosporidium molnari Alvarez-Pellitero & Sitjà-Bobadilla, 2002, a parasite frequently found in young gilthead sea bream (Sparus aurata L.) and European sea bass (Dicentrarchus labrax L.), and Cryptosporidium scophthalmi Alvarez-Pellitero, Quiroga, Sitjà-Bobadilla, Morondo, Palenzuela, Pádrós, Vásquez & Neto, 2004 from cultured turbot are coccidian species that cause important histopathological damage in the stomach and intestinal epithelial cells and are associated with trickling mortalities and retarded growth (Alvarez-Pellitero & Sitjà-Bobadilla, 2002; Alvarez-Pellitero et al., 2004; Sitjà-Bobadilla et al., 2006). This paper reports the first occurrence of a bile duct Cryptosporidium species in a turbot population also infected with a bacterium and a parasite ciliate.

From March 2004 to February 2005 surveys were conducted in a turbot farm located in NW Portugal in order to determine the cause/s of mortality. Around 110 fishes were examined (total weight 20 to 1020g). Moribund fishes were killed by spinal cord severance and necropsy. For bacteriological studies swabs from several organs were taken.

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Figures 1 to 3. Developmental stages of Cryptosporidium sp in the bile ducts epithelial cells of turbot, Psetta maxima. Figure 1. Fibrosis (F) and necrotic hepatic tissue (NT) in turbot liver infected with Cryptosporidium sp. Figure 2. Mature oocysts with 4 sporozoites (arrows). Figure 3. Oocysts in different stages of sporogony and a free sporozoite (arrow). (H&E).

to different medium culture. Simultaneously squash smears and samples for histology were taken from different organs. Histological material were fixed in 10% buffered formalin, embedded in paraffin, routinely processed, sectioned at 2 μm and stained with haematoxylin and eosin (H&E).

Macroscopically fish were emaciated, with darkened and ulcerated skin, haemorrhages
at the base of the fins, exophthalmia, with distension of the abdominal cavity and ascites. Examined fishes were infected by several pathogens. Gram-positive cocci identified as *Streptococcus parauberis* were detected in several organs. The detailed identification and the pathology caused by this bacterium will be reported elsewhere. Scuticociliate belonging to the Philasterida were detected in skin, gills and ascitic fluid (see Ramos et al., 2007). Histological sections of the liver showed the occurrence of ovoid oocysts in different stages of sporulation inside a parasitophorous vacuole in bile ducts epithelial cells (Figure 1). Mature oocysts, 4 to 8 μm long, contained four naked elongate sporozoites (Figure 2). In some cases sporozoites were released from the oocysts (Figure 3). Other developing stages were not observed. Levels of infection were not determined as this parasite was observed accidentally. Fibrosis surrounding the infected bile ducts and necrosis of adjacent hepatic cells were observed (Figure 1). Epithelial bile ducts host cells seem not to be seriously damaged.

The absence of sporocysts and the presence of 4 sporozoites in the oocysts are features of the genus *Cryptosporidium* Tyzzer, 1907. According to Lom & Dyková (1992) the site of infection of piscine coccidia is very often extra-intestinal. As far as we know this is the first report of a piscine *Cryptosporidium* detected in bile ducts. Recently Alvarez-Pellitero et al. (2004) described *C. scophthalmi* from intestine and stomach of turbot from different farms on NW Spain coast. The oocysts of the Coccidia detected in this study were bigger than that ones described by these authors; however due to the scarcity of available material a specific identification of this parasite was not possible. As fishes were infected with several pathogens it is not known the effects and possible mortalities caused by this cryptosporidiosis.

**References**


