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MYCOBACTERIOSIS IN EUROPEAN SEA BASS, *DICENTRARCHUS LABRAX* L., CULTURED IN TURKEY

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Abstract

A case of mycobacteriosis in European sea bass *(Dicentrarchus labrax* L.) cultured in Turkey is described. Diseased fish were characterized by lethargy, lingering passively at the water surface, pale gills, and exophthalmia. Internally, grayish-white nodules were observed in the liver, spleen, and kidney. In one individual, a granulomatous mass of renal origin protruded into the abdominal cavity. The principal histological feature was the occurrence of characteristic granulomas with associated acid-fast, rod-shaped bacteria. This is the first confirmed case of fish mycobacteriosis in Turkey.

Introduction

Commercial culture of European sea bass (*Dicentrarchus labrax* L.) is widespread in Mediterranean countries, particularly France, Greece, Italy, Spain, and Turkey (Barnabé, 1990; Hossucu et al., 1990). Production has increased significantly in recent years from 46,202 tons in 1998 to 80,000 tons in 2002 (FISH, 2004). In Turkey, earlier bacteriological

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studies of moribund sea bass documented episodes of pasteurellosis (Cagirgan, 1993; Candan et al., 1996; Timur et al., 1999; Korun and Timur, 2005), vibriosis (Cagirgan and Yurekliturk, 1996; Korun, 2004), and *Aeromonas hydrophila* (Sahrikoglu and Candan, 2002). However, until the present study, there has been no evidence of mycobacterial infections. In this report, a case of mycobacteriosis in European sea bass from a closed-system fish farm in the Aegean region of Turkey is described.

Materials and Methods

In January 2003, sea bass *(Dicentrarchus labrax)* mortalities were noted at a daily rate of 0.3% (mean 17,000 fish per tank) in a closed-system production facility located between Canakkale and Lapseki on the Aegean coast of Turkey (Fig. 1). Fish were held in a mixture of pumped seawater and spring water at 21.1ºC, with 7.3-9.3 mg/l dissolved oxygen, pH 7.1, and 2.4% salinity. The capacity of the farm was 400 tons/y in 26 octagonal tanks. The feed conversation ratio (FCR) was 1.5-1.7.

Five moribund fish, weighing 200-250 g each, were selected from one of the tanks and autopsied under aseptic conditions. Samples taken from visceral organs, i.e., the spleen, liver, and kidney, were inoculated for bacterial isolation on Brain Heart Infusion Agar (BHIA; Merck, Germany) and Trypticase Soy Agar (TSA; Merck) supplemented with 1.5 % NaCl and Thiosulphate Citrate Bile Sucrose Agar (TCBS; Merck). Plates were incubated at 22ºC for two weeks.

To determine the presence of protozoa and metazoan parasites, gills, muscle, spleen, kidney, liver, gastrointestinal tract, and swim bladder were examined under a light microscope. Samples of muscle, gill tissues, spleen, kidney, liver, stomach, and intestine were fixed in 10% formalin for histological examination. Paraffin blocks were cut into 5 µm sections and stained with hematoxylin-eosin, PAS (Periodic-Acid-Schiff), Masson's trichrome, and Ziehl-Neelsen stains using routine procedures (Culling, 1963; Bullock, 1989).

Results

Affected sea bass displayed a loss of appetite, lethargy, and passive lingering at the water surface. Gross symptoms also included exophthalmia, pale gills, loss of scales, and ocular, opercular, and fin hemorrhages.

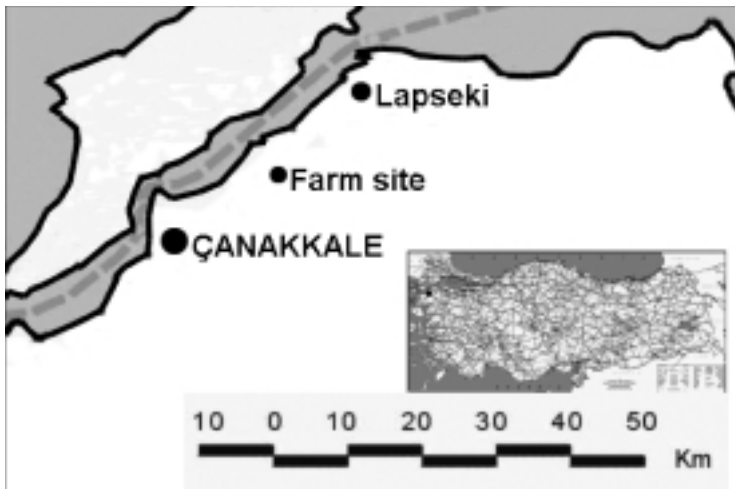

Fig. 1. Location of farm site between Canakkale and Lapseki, Turkey.

Except for one individual with a distinctive lump in the abdominal region (Fig. 2), no other signs or lesions were externally visible. Internally, the spleen, kidney, and liver were pale and contained grayish-white nodules. Splenomegaly, hepatomegaly, ascitic fluid, and an empty gastrointestinal tract were observed in all fish. The distinctive abdominal swelling observed in one individual was found to be a granulomatous mass originating in the kidney and protruding into the abdominal cavity, with whitish nodules measuring 2-6 mm on the surface (Fig. 3). No fungi, ectoparasitic, or endoparasitic organisms were found in the affected fish, nor was any bacterial growth observed on the BHIA, TSA, or TCBS after two weeks.

In the histological sections of the abdominal mass, the granulomas (Fig. 4) had a necrotic core, surrounded by epithelioid macrophages, with acid-fast rods in the core, demonstrated by the Ziehl-Neelsen stain (Fig. 5). Acid-fast rods were also clearly visible in hepatic and renal granulomas, as well as in the surrounding tissues. In the kidney, lymphocyte and granulocyte infiltration was observed. Caseation-like necrosis at the center of the granulomas and the outer layers of epithelioid macrophages surrounded by fine fibrous connective tissue indicated that the disease had reached a chronic phase.

The liver contained sparse granulomas and foci of fatty degeneration (Figs. 6,7). In the spleen, in addition to granulomas, there was evidence of necrosis and reduction of parenchymatic tissue. Granulomas were also observed in the visceral fat and in the gill filaments. No obvious histopathology was observed in the heart muscle. In the histological sections of renal tissue, mycobacterial granulomas, lymphocyte infiltration, and amyloid deposits were observed (Figs. 8,9).

Discussion

Piscine mycobacteriosis has been reported in numerous marine and freshwater fishes. Clinical findings vary with the host species and environmental conditions. Nevertheless, inappetence, lethargy, and exophthalmia are typical, though general symptoms. Characteristic granulomatous lesions may occur in any organ, but the spleen and kidney are particularly commonly affected (Colorni, 1992; Frerichs, 1993; Toranzo et al., 2005). Affected marine fish in Mediterranean countries include European sea bass (*D. labrax*) cultured in

Fig. 2. Sea bass, the lower has a swollen abdomen (arrow).

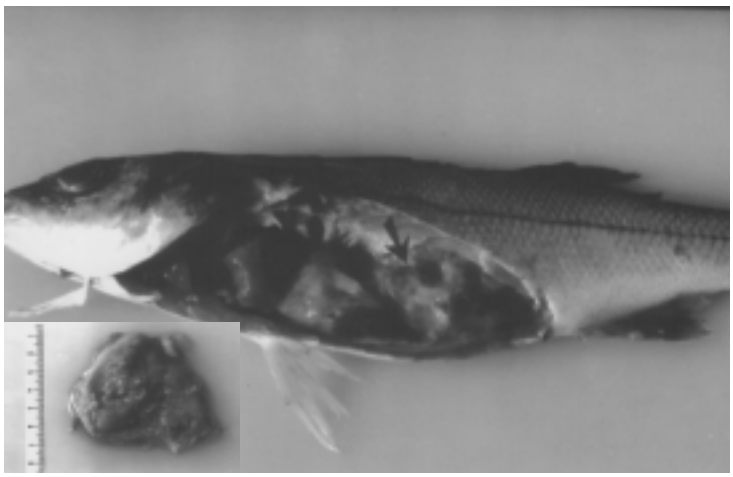

Fig. 3. Position of granulomatous mass in the abdominal cavity (arrow) and granulomas on the spleen and liver. Inset: Characteristic 2-6 mm granulomatous lesions on the mass.

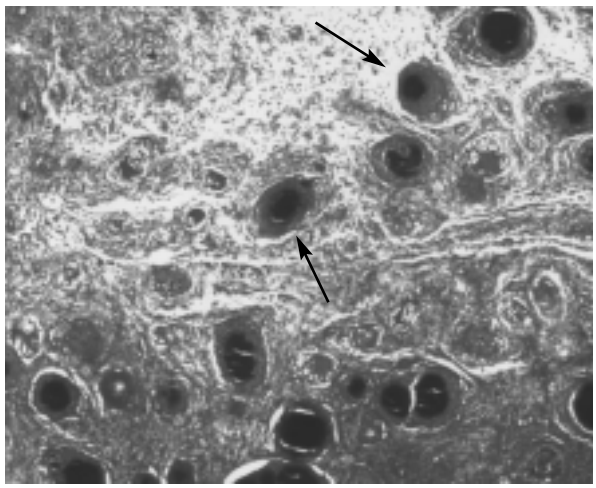

Fig. 4. Histological section through abdominal mass, showing numerous mycobacterial granulomas (arrow; Ziehl-Neelsen stain, x 50).

Greece, Israel, Italy (Colorni, 1992; Sechi et al., 2002; Ucko et al., 2002) and Turkey (present study). Skeletal deformities, chronic ulcers, and lordosis are at times observed in some affected species (Noga, 1996; Chinabut, 1999), however, these symptoms were not encountered in the present study.

The stock of diseased fish was destroyed before isolation of the mycobacterium responsible for the lesions could be carried out. However, the histopathological findings (presence of acid-fast rods in granulomatous lesions) are pathognomonic and conclusive for fish mycobacteriosis.

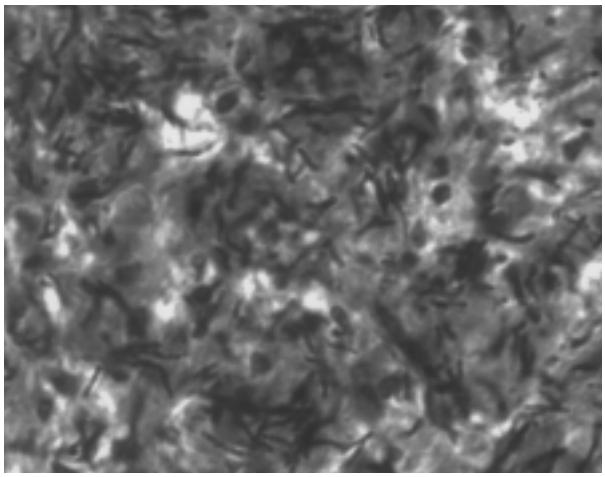

Fig. 5. Acid-fast, rod-shaped bacteria in high-power magnification of the granuloma core of an abdominal mass (Ziehl-Neelsen stain, x 2000).

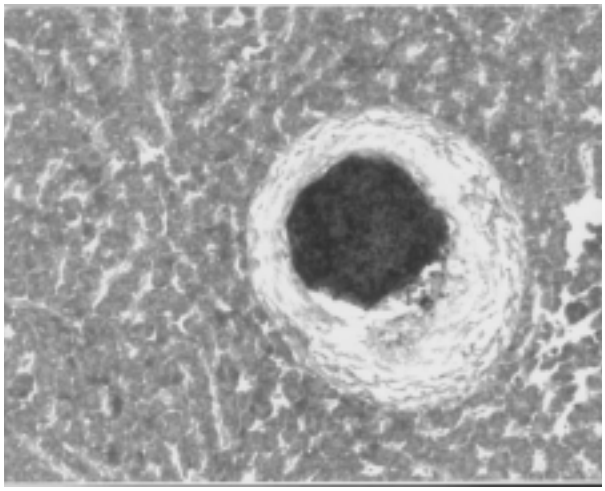

Fig. 6. Section of sea bass liver with a mycobacterial granuloma (Ziehl-Neelsen stain, x 300).

Piscine mycobacteriosis is reportedly transmitted through infected feed, water, soil, and external parasites (Frerichs, 1993; Noga, 1996; Decostere et al., 2004). However, observations of mycobacteria in fish eggs and the presence of granulomas on the external wall of the egg suggest the possibility of vertical transmission (Chinabut, 1999). Although such a route has been reported for platy-fish, a viviparous species (Conroy, 1966), experimental egg-associated transfer was unsuccessful in oviparous species such as salmonids (Ross and Johnson, 1962). Since in this study the affected sea bass were offspring

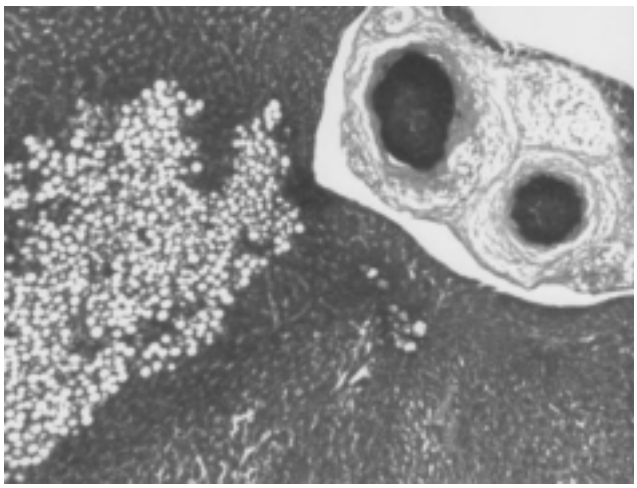

Fig. 7. Fatty degeneration (left) associated with mycobacterial granulomas in the liver (hematoxylin-eosin stain, x 100).

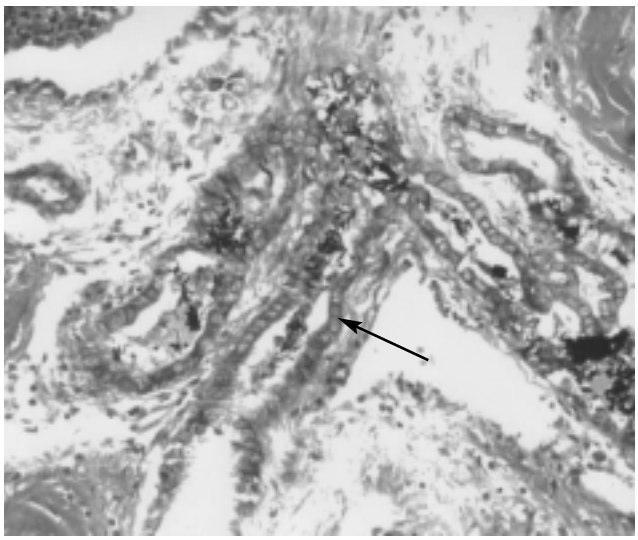

Fig. 8. Early granulomatous lesions in the kidney. Acid-fast rod-like cells can be seen in the renal tubuli (arrow; Zielh-Neelsen stain, x 400).

of breeders caught in the wild, we suspect the broodstock was the primary source of the infection. If the disease turns up again, future studies will focus on culturing, isolating, and characterizing this Turkish *Mycobacterium* strain, to evaluate its affinities to previously studied isolates of mycobacteria and of *Mycobacterium marinum* in particular.

Acknowledgements

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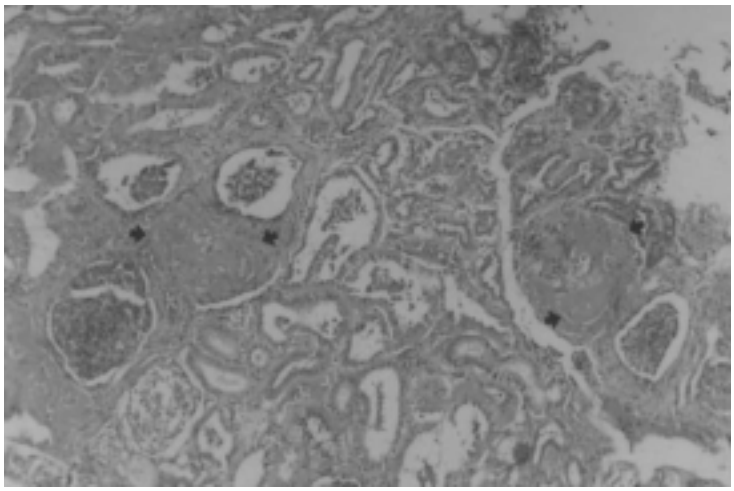

Fig. 9. Amyloid deposits (arrows) in the kidney (hematoxylin-eosin stain, x 100).

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