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Listonella anguillarum Isolated from Hatchery-Cultured Red Porgy *Pagrus pagrus* in Turkey

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Abstract: A *Listonella anguillarum* infection in cultured red porgy (*Pagrus pagrus*) is documented for the first time in this report. The infection was identified in a commercial hatchery in the Aegean near Bodrum, Turkey. Diseased red porgy were characterized by lethargy, anorexia, fin erosion and abdominal swelling. Haemorrhagies on the operculum, fins and over the surface of the body were seen. Internally, the liver and the kidney were pale. The spleen was enlarged. Ascites in the abdominal cavity was also observed. The identification of bacterial strains isolated from the affected fish was based on morphological (Gram staining and wet-mouth preparation), physiological and biochemical tests (API 20E). Mono-Va agglutination kit was also included to confirm the identification of the isolated bacteria. The principal histological changes in this study were haemosiderosis in the spleen and kidney, a massive depletion of haemopoietic elements in the spleen, haemorrhagies in the liver, intestinal walls and under the muscle tissue.

Key words: *Listonella anguillarum*, red porgy, *Pagrus pagrus*, vibriosis

INTRODUCTION

The red porgy (*Pagrus pagrus*, L.) is a sparid fish species and is distributed in both sides of Atlantic Ocean and also in the Mediterranean Sea (Ruead *et al*., 1998; Hernández-Cruz *et al*., 1999). European aquaculture is limited to some marine fish including Atlantic salmon (*Salmo salar*), European eel (*Anguilla anguilla*), white sea bream (*Diplodus sargus*), gilth-head sea bream (*Sparus aurata*), European sea bass (*Dicentrarchus labrax*) and turbot (*Scopthalmus maximus*). However, because of overproduction in the aquaculture industry and saturation of the sea bream and sea bass markets, diversification is necessary (Golamazou *et al*., 2006). In Turkey, the majority of marine fish production is on gilthead sea bream (*S. aurata*) and sea bass (*D. labrax*). Apart from these fish species, cultures of sharpnosed sea bream (*Puntazzo puntazzo*) and white sea bream (*Diplodus sargus*) have started. In recent years, red porgy (*P. pagrus*) has been added to these types at levels of 50-100 tonnes and pilot production has been continuing in commercial hatcheries in a very small scale (Deniz, 2000; FISH, 2004).

The red porgy is an alternative species due to its high market value, good growth rates and adaptation for culture conditions (New, 1991; Kentouri *et al*., 1995; Hernández-Cruz *et al*., 1999; Papandroulakis *et al*., 2004; Katharios *et al*., 2006). However, there are several problems at culture studies about this fish species. Briefly, these are to obtain broodstocks, pigmentation on the skin colour, bacterial, viral and parasitic infections (Paulidis *et al*., 2002; Katharios *et al*., 2006).

Listonella (*Vibrio*) *anguillarum* is a Gram-negative, facultatively rod-shaped bacterium (Alsina *et al*., 1994; Austin and Austin, 1999). This species causes vibriosis and affects a range of freshwater and marine fish species including rainbow trout (*Oncorhynchus mykiss*), Atlantic salmon (*S.salar*), cod (*Gadus morhua*), saithe (*Pollachius virens*), sole (*Solea solea*), sea bass (*D. labrax*), red sea bream (*Pagrus major*), white sea bream (*Diplodus sargus*), common dentex (*Dentex dentex*), Japanese flounder (*Paralichthys olivaceus*), turbot (*S. maximus*) and Japanese and European eel (*Anguilla japonica* and *A. anguilla*) (Muroga, 1975; Muroga and Tatani, 1982; Yamanoi *et al*., 1988; Myhr *et al*., 1991; Company *et al*., 1999; Pedersen *et al*., 1999; Rodkhum *et al*., 2005; Golomazou *et al*., 2006) as well as crustaceans and bivalve molluscs species (Bowser *et al*., 1981; Bolinches *et al*., 1986). The present report presents the first *L. anguillarum* infection in farmed red porgy (*P. pagrus*) that was reported in sea bass, sea bream and also rainbow trout during the last years (Cagırgan, 1993; Tanrikul *et al*., 2004; Timur and Korun, 2004; Korun and Timur, 2005; Demircan and Candan, 2006) and it also describes the main pathological conditions observed in this fish species reared in one hatchery in Aegean region (Bodrum territory) in Turkey.

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MATERIALS AND METHODS

An epizootic was recorded in November 2003 in red porgy (*P. pagrus*) cultured one hatchery in Aegean Region (Bodrum territory) in Turkey. The salinity of the water was 20 $\%_0$ and the temperature was 22±1°C. The fish losses were found as 1.1 % per day. Ten fish (weighing from 26-39 g) were taken from octagonal concrete ponds supplied by a filtered, flow-through water supply and they were anaesthetized with 1.5 mL 2-phenoxyethanol (Fluka, Switzerland) per 1 L sea water. Samples were taken from internal organs such as spleen, liver, kidney and blood for bacterial isolations and seeded on Brain Hearth Infusion Agar (Merck, Germany) and Trypticase Soy Agar (Merck) supplemented with 2 % NaCl (BHIAS, TSAS). The inoculated plates were incubated at 24°C for 48 h and resulting colonies were subjected to standard bacteriology protocols (Holt *et al*., 1994; Austin and Austin, 1999) and API 20E system (BioMerieux, France) to determine morphological, physiological and biochemical properties. All the isolated bacterial strains were identified according to Baumann and Furniss (1994), Austin and Austin (1999). Mono-Va agglutination kit (*Vibrio anguillarum* from Bionor, Norway, Prod. Cod.: DE 020) was used to confirm the identification of the bacterial strains isolated from the diseased fish by following the manufacturer's instructions. The disk diffusion method was used to test the sensivity of the isolates to antibiotics on Mueller-Hinton Agar (Oxoid, England) by adding 1.5 % NaCl to one of seven antimicrobial substances including ampicillin (10 μg), compound sulphonamide (300 μg), erythromycin (15 μg), flumequine (30 μg), kanamycin (30 μg), novobiocin (15 μg) and tetracycline (30 μg) (Bauer *et al*., 1966; Alderman and Smith, 2001). For histological study, tissue samples were collected from the anterior and posterior kidney, liver, hearth, spleen, gastro-intestinal tract and skin and preserved in 10 % buffered formalin solution. After fixation process, tissues were processed by standard histological techniques and stained with hematoxyline and eosine stain and viewed under a light microscope (Bullock, 1989). For parasitological examinations, fresh smears from skin, gills, liver, kidney, stomach, intestine and kidney were examined microscopically at the laboratory of the hatchery (Collins, 1993; Christofilogiannis, 1993).

Table 1: Results of standard physiological, biochemical and API 20E tests of bacteria isolated from moribund red porgy (*Pagrus pagrus*)

Test	Standard method	API 20E
Motility	+	NA
Gram stain	-	NA
Oxidase	+	NA
O/F (Leifson)	F	NA
Indol	+	+
Voges-poskauer	+	+
Swarming	-	NA
Luminescence	-	NA
Metil-red	-	NA
ADH	+	+
LDC	-	-
ODC	-	-
H_2S production	-	-
Gelatinase	+	+
Urease	-	-
Amylase	+	NA
TDA	NA	-
Growth at		
4°C	-	NA
22°C	+	NA
37°C	+	NA
Growth in		
0% NaCl	-	NA
3% NaCl	+	NA
6% NaCl	+	NA
8% NaCl	-	NA
Acid production		
Amygdalin	NA	-
Arabinose	-	-
Glucose	+	+
Inositol	-	-
Lactose	-	NA
Mannitol	+	+
Mellibiose	NA	-
Rhamnose	NA	-
Sorbitol	NA	+
Sucrose	+	+
Citrate utilization	+	+
NO_2 production	+	+
ONPG	+	+
Haemolysis:		
Sheep	+	NA
Growth on		
TCBS	+,Y	
Resistance to		
0/129 (10 μg disk^{-1})	S	
0/129 (150 μg disk^{-1})	S	
		IZD (mm)
Ampicillin	R	(10)
C. sulphonamides	S	(25)
Erythromycin	S	(30)
Flumequine	S	(35)
Kanamycin	R	(10)
Novobiocin	S	(40)
Tetracycline	S	(30)

Symbols: NA; Not Available, F; Fermentative, Y; Yellow, S; Sensitive, R; Resistance, IZD; Inhibiton Zone Diameter, +; Positive, -; Negative

RESULTS AND DISCUSSION

The affected fish were characterized with lethargy, anorexia, exopthalmia, fin erosion and abdominal swelling. Haemorrhagies on the operculum, fins and over the surface of the body were seen. Internally, the liver and the kidney were pale. The spleen was enlarged. Ascites in the abdominal cavity was also observed. These clinical findings were similar to those previously reported for Vibrio infections in other sparid fish such as gilt-head sea bream and common dentex (Cagırgan and Yurekliturk, 1996; Balebona *et al*., 1998; Company *et al*., 1999).

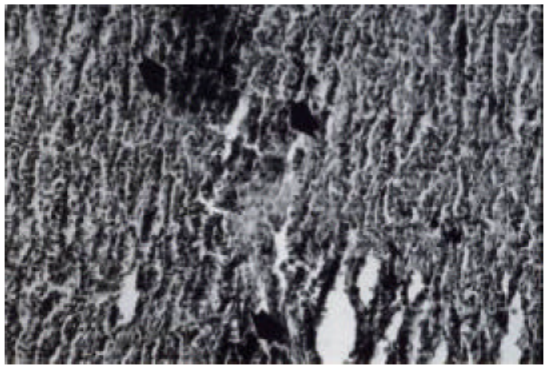

Fig. 1: Massive haemosiderin deposits (arrowed) and depletions of white and red pulpas in the spleen H+E×250

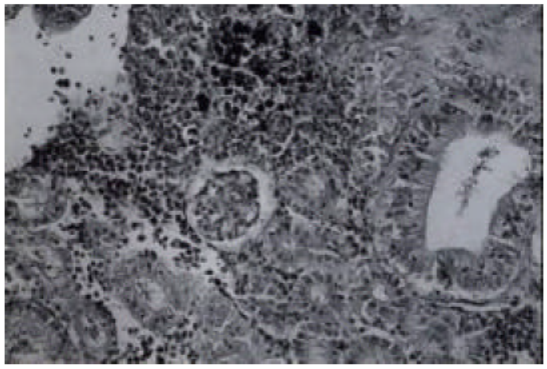

Fig. 2: Vacuoler degeneration and haemosiderin deposit (arrowed) H+E×250

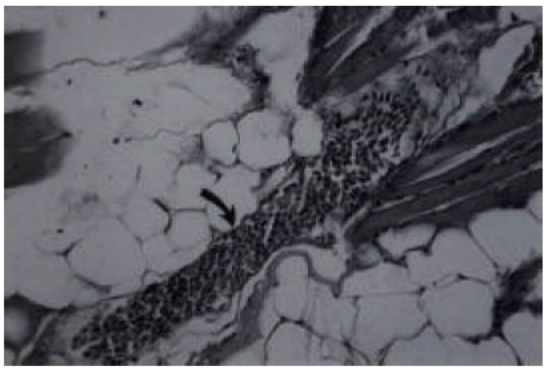

Fig. 3: Haemorrhagies (arrowed) under the muscle tissue H+E×500

After incubation, the bacterial strains (n = 12) isolated from moribund fish produced convex, yellowish-brown, bright colonies that were Gram-negative, motile, cytochrome oxidase and catalase positive, fermentative and sensivity to vibriostatic agents (10 and 150 μg $disk^{-1}$: Table 1). TCBS agar (Merck) showed yellow colonies. Isolated bacterial strains had similar phenotypic properties to other *Listonella anguillarum* strains published by Baumann and Furniss (1994), Balebona *et al.* (1998), Austin and Austin (1999) showing that our isolated strains belonged to this species. Identification was also confirmed by using the API 20E system (Table 1, identification profile number 3247524). The Mono-Va agglutination kit caused significant agglutination in the *L. anguillarum* strains while the control reactant did not. These results were in agreement with the findings of Romalde *et al.* (1995). The *L. anguillarum* strains were sensitive to chemical such as compounds sulphonamide, erythromycin, flumequine, novobiocin and tetracycline (Table 1). The treatment with flumequine in feed (50 mg kg^{-1} body weigth day^{-1} for 7 days) resulted to be effective to control fish mortalities. The principal histological changes in this study were haemosiderosis (an increase in the amount of haemosiderin caused by an increase in the rate of destruction of erythrocytes) in the spleen and kidney (Fig. 1 and 2), a massive depletion of haemopoietic elements in the spleen, vacuoler degeneration in the liver tissue, haemorrhagies in the liver, intestinal walls and under the muscle tissue (Fig. 3). These are similar to the findings reported by Ransom *et al.* (1984), Mellergaard and Bagge (1998), Agius and Roberts (2003).

According to the findings of this report, a *Listonella anguillarum* infection in cultured red porgy (*Pagrus pagrus*), including lesions and daily fish losses and treatment, is documented for the first time.

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