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ATYPICAL AEROMONAS INFECTION IN CULTURED SEA BASS *(DICENTRARCHUS LABRAX)* IN THE BLACK SEA

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Abstract

The Black Sea, an inland sea, has contributed about 70% to the total fish production of Turkey for many years. Because of its low salinity and appropriate water temperature, both freshwater and marine fish such as salmon, trout, and sea bass can be cultured in the Black Sea. The aim of this study was to identify the etiological agent of mortality that occurred in sea bass in the Black Sea during July 2002. Bacteria isolated from kidneys and livers of infected fish were identified as *Aeromonas salmonicida achromogenes* according to morphological and biochemical characteristics, API 20NE results, and an agglutination test for *A. salmonicida*. The diseased fish had petechial hemorrhages on the bottom of the fins and lesions on the lateral and ventral sides of the body. Cumulative mortality was approximately 20%. This is the first report of *A. salmonicida achromogenes* associated with sea bass in Turkey.

Introduction

The genus *Aeromonas* comprises gram-negative, cytochrome oxidase-positive, facultatively-anaerobic rods. All but one of the *Aeromonas* species are motile. *Aeromonas salmonicida* is nonmotile and divided into four subspecies according to the Bergey's Manual of Systematic Bacteriology: *salmonicida*, *achromonogenes*, *masoucida*, and *smithia*. *A. salmonicida* subsp. *salmonicida* is the typical strain, while the other three subspecies are atypical strains (Holt et al., 1994). However, a cytochrome oxidase-negative atypical *A. salmonicida* species, different from this species, has been described (Wiklund and Bylund, 1991; Wiklund et al., 1994). Atypical species have been identified as the cause of

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ulcer disease and atypical furunculosis (Hubbert and Williams, 1980; Wiklund and Dalsgaard, 1998).

Aeromonas salmonicida is pathogenic mainly for salmonid fish in fresh water but there is an increasing number of reports of infections in other fish species. Strains have been isolated from wild and cultivated fish in both marine and brackish water. Atypical species mainly infect temperate regions of the northern hemisphere: Canada, USA, Japan, and central and northern Europe, including the Nordic countries. However, atypical strains have also been isolated from fish in the Mediterranean and Australia (Wiklund, 1990; Wiklund and Bylund, 1991; Gudmundsdottir, 1998; Wiklund and Dalsgaard, 1998; Hiney and Olivier, 1999; Laidler et al., 1999).

Although there are several reports of survival of typical *A. salmonicida*, there are few reports dealing with the survival of atypical *A. salmonicida.* It was reported that typical *A. salmonicida* has a higher survival rate in the bottom sediment of brackish water than in fresh and salt water (Efendi and Austin, 1994; Wiklund, 1995a). Wiklund (1995b) reported that sediment can act as a reservoir for this pathogen and facilitate the spread of the disease. The occurrence of the pathogen in water bodies of high or low salinities may be limited, explaining why this disease usually occurs in brackish water areas in Norway, Sweden, Finland, and Iceland (Inglis et al., 1993; Gunnlaugsdottir and Gudmundsdottir, 1997; Wiklund and Dalsgaard, 1998; Hiney and Olivier, 1999).

The clinical and pathological features of infection by atypical *A. salmonicida* strains vary with many factors, e.g., environmental factors, virulence properties of bacterium, and host responses. Diagnosis is difficult as atypical strains belong to a heterogeneous group and infect various fish species in brackish, freshwater, and marine environments (Gudmundsdottir, 1998; Wiklund and Dalsgaard, 1998). Atypical *A. salmonicida* infections associated with disease outbreaks are similar to furunculosis, with loss of appetite, darkening in color, and mortality. External clinical signs often include hemorrhage at the fin bases and skin ulcers, or lesions on body sides, and pale gills. The course of the disease can be peracute, acute, subacute, or chronic as described for classical furunculosis. Pure cultures of bacterium can be isolated from internal organs such as the kidney, spleen, and heart (Paterson et al., 1980; Gudmundsdottir et al., 1997; Gudmundsdottir, 1998).

The Black Sea is an inland sea that has contributed about 70% to the total fish production of Turkey for many years. Because of its low salinity and appropriate water temperature, both freshwater and marine fish such as salmon, trout, and sea bass are cultured in the Black Sea. Disease outbreaks have been reported in trout cultured in the Black Sea (Karatas et al., 2004). In Turkey, typical furunculosis caused by *A. salmonicida* was first diagnosed in 1995, in cultured rainbow trout (*Oncorhynchus mykiss*; Timur et al., 1999), with no external or internal signs of disease except a darkening of color. Since then, it has caused serious problems in salmonid farms. However, until now, no atypical *Aeromonas* has been reported in any farmed or wild fish in Turkey.

The aim of this study was to identify the etiological agent of mortality that occurred in sea bass cultured in the Black Sea during July 2002.

Materials and Methods

During June 2002, when the water temperature reached 16ºC, mortalities occurred in sea bass with a mean weight of 5-6 g cultured on the Black Sea coast of Turkey. Afterwards, in July 2002, mortality occurred in sea bass with a mean weight of 100 g as well. The cumulative mortality reached approximately 20% in both groups.

Autopsies were performed on diseased fish anesthetized with MS 222 (200 mg/l) in the Fish Diseases Laboratory of the Fisheries Faculty at Istanbul University. Samples from the kidney, liver, and spleen were inoculated onto tryptic soy agar (TSA; Jeney et al., 1992) and incubated at 20ºC for 7 days. Isolates were gram-negative, oxidase-positive, and similar to *A. salmonicida* in colonial morphology when subcultured before biochemical characterization. The pigment production ability of the isolates was investigated on TSA and brain heart infusion agar (BHIA). Biochemical and physiological tests were carried out on the isolates according to standard tube procedures. Colonies were diluted in pure sterile water and API 20NE strips (BioMerieux, France) were inoculated according to the manufacturer's instructions. Strips were developed and read after 48 h incubation at 20ºC.

Isolates were tested with a rapid agglutination reagent consisting of particles coated with a specific antibody (BioNor Mono AS, BIONOR Aqua, Skien, Norway). Antibiotic susceptibility tests and a test for sensitivity against O/129 (Vibriostat 150 µg) were performed with antibiotic tablets on Mueller-Hinton agar according to the specifications for the procedure and the zone of inhibition was measured.

Material for histopathological examination was obtained from one specimen. Samples were taken from the skin, muscle, gills, heart, intestine, liver, kidney, and spleen, and fixed in 10% formaldehyde for histopathological examination. After processing and wax embedding, sections were stained with hematoxylin and eosin (Roberts, 1978).

Results and Discussion

Petechial hemorrhages were observed on the bottom of the fins on fish of 5-6 g and on the sides of fish of 100 g (Fig. 1). Internally, creamy-white soft lesions in the kidney, liver, and spleen were present on fish of both weight groups. The spleen was swollen, the gills and liver were pale, and the bile spread (Fig. 2). Parasitological examination was negative. These clinical signs were not similar to atypical *Aeromonas* reports. But, the skin lesions corresponded to the description of infection with atypical *A. salmonicida* in marine species (Wiklund and Dalsgaard, 1995; Gudmundsdottir, 1998).

Plates inoculated with the kidney or liver of affected fish had growth of several kinds of bacteria but only one species was identified. Cream-colored colonies were subcultured at least once after isolation to obtain pure cul-

Fig. 1. Typical lesions in sea bass (*Dicentrarchus labrax*) infected with atypical *Aeromonas salmonicida*.

Fig. 2. Clinical pathology, including a pale liver and swollen spleen, in sea bass (*Dicentrarchus labrax*) infected with atypical *Aeromonas salmonicida*.

tures on TSA. The isolated bacteria were non-motile, fermentative, cytochrome oxidase-positive, gram-negative rods, and agglutinated in the agglutination test for *A. salmonicida.* Biochemical and morphological characteristics and API 20NE results are shown in Table 1. They did not produce brown, water-soluble pigment after three days of incubation on TSA and BHIA. The API 20NE profile of the isolated bacteria was 1050004.

The isolates were identified as *A. salmonicida* ssp. *masoucida*/*achromogenes* according to biochemical and morphological characteristics and API 20NE results. Isolates differed from *A. salmonicida mosoucida* on the basis of negative degradation of esculin, acid production from mannitol and glucose, Voges-Proskauer, and production of hydrogen sulfide according to Popoff (1984). Therefore, we determined that the isolated strains were *A. salmonicida achromogenes*. The atypical *A. salmonicida* isolated in the present study was similar to strains isolated by other researchers (Inglis et al., 1993; Wiklund et al., 1993; Austin et al., 1998; Hiney and Olivier, 1999; Laidler et al., 1999; Koppang et al., 2000).

Although it is reported that chemotherapy may be difficult or ineffective for treatment of atypical *A. salmonicida* infections, the most used antimicrobial agents are oxolinic acid, oxytetracycline, and trimethoprim. In this work, all strains were sensitive to trimethoprim, flumequine, oxolinic acid, and oxytetracycline and resistant to O/129. Further, strains were resistant to amoxicillin, similar to results of Barnes et al. (1991). Antibiotic susceptibility test results are shown Table 2. Fish were treated with medicated feed at 75 mg/kg fish for 7 days. Mortality ceased during the medication period with oxytetracycline, but rose again after medication ceased.

Initially, it was thought that we might be dealing with *Piscirickettsia salmonis* because of the lesions on the body and the creamy-white soft lesions in the kidney, liver, and spleen. However, in the immunohistochemical tests, there were no positive reactions to application of antibodies against *P. salmonis* (P. salm-IHK) on sections from the liver, spleen, and kidney.

Histopathological findings included several large foci of necrosis surrounded by many

Table 1. Biochemical, morphological characteristics and API 20NE results of atypical *A. salmonicida* ssp. *achromogenes* isolated from the liver and kidney of sea bass.

Characteristic	
Gram stain	–
Cell morphology	Rod
Motility	–
Cytochrome oxidase	+
O/F test	+/+
O/129 (150 µg)	R
H_2S production	–
Indole	–
Brown pigment (TSA-3 days)	–
Growth in 0% NaCl	+
Growth in 3% NaCl	+
Growth in 7% NaCl	–
Growth in 22˚C	+
Growth in 37˚C	–
β-hemolysis	–
Tween 80	+
Catalase	+
Nitrat reduction	+
Triptophane	–
Arginine dihydrolase	–
Urea	–
Esculine	–
Gelatine	+
p-nitro-phenyl-β-D-galactopyranoxide	–
Glucose	+
Arabinose	–
Mannose	–
Mannitol	–
N-acetyl-glucosamine	–
Maltose	–
Gluconate	–
Caprate	–
Adipate	–
Malate	–
Utilization of Na-citrate	–
Sucrose	+
Lysine decarboxylase	–
Voges Proskauer	–
TCBS agar	–
Phenyl-acetate	–
Gas from glucose	–

+ = positive reaction; – = negative reaction; R = resistant

Table 2. Antibiotics susceptibility test results of atypical *A. salmonicida* ssp. *achromogenes*.

Antibiotic	
Trimethoprim	S
Flumequine	S
Oxolinic acid	S
Oxytetracycline	S
Sulfonamides	R
Cefepim	S
Cefoksitin	S
Cefuroxime	S
Imipenem	S
Ceftriaxone	S
Ofloxsacin	S
Cefoperazone-sulbactam	S
Meropenem	S
Gentamicin	S
Tobramycin	S
Netilmicin	S
Amikacin	S
Cotrimoxazol	S
Piperacillin-tazobactam	S
Ceftazidime	S
Aztreonam	S
Ciprofloxacin	S
Amoxicillin–calvulonic acid	R
Ampicillin	R
Ampicillin-sulbactam	R

S = sensitive; R = resistant

inflammatory cells (Fig. 3), bacterial colonization in the liver (Fig. 4), necrosis and a few small foci in the spleen (Fig. 5), and many large processes with a necrotic center and outer rim of inflammatory cells in the kidney (Fig. 6). In non-salmonids, there are several reports of signs of pathological disease in internal organs of fish infected with atypical *A. salmonicida*. In infection experiments with atypical *A. salmonicida* isolated from cod, degenerative changes and cyst formations were seen in the spleen and kidney of infect-

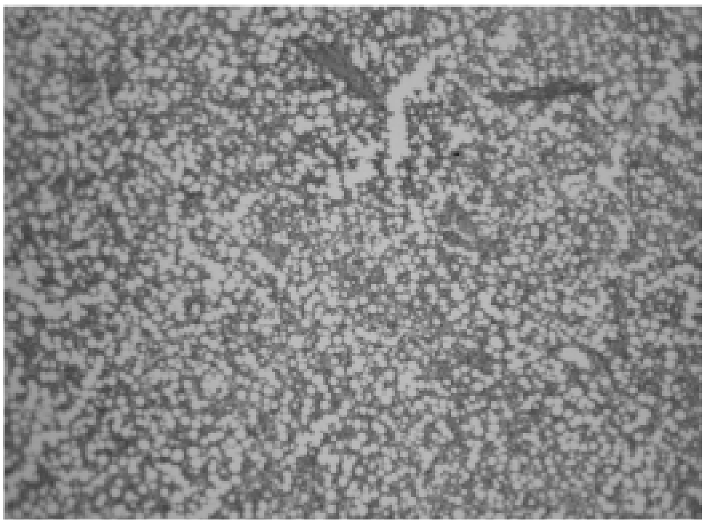

Fig. 3. Necrotic foci surrounded by inflammatory cells.

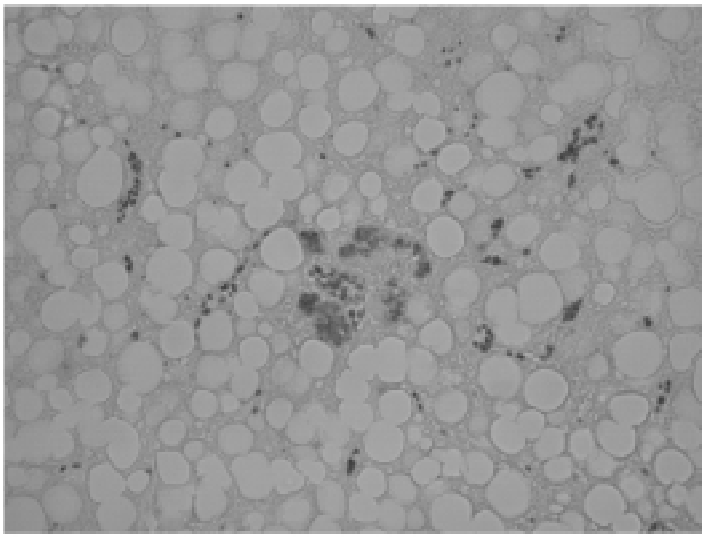

Fig. 4. Bacterial colonization in the liver.

ed cod (Morrison et al., 1984). Although atypical *A. salmonicida* ssp. *achromogenes* was reported in several fish species and countries, this bacterium had not previously been isolated from cultured sea bass (*Dicentrarchus labrax*) in the Black Sea.

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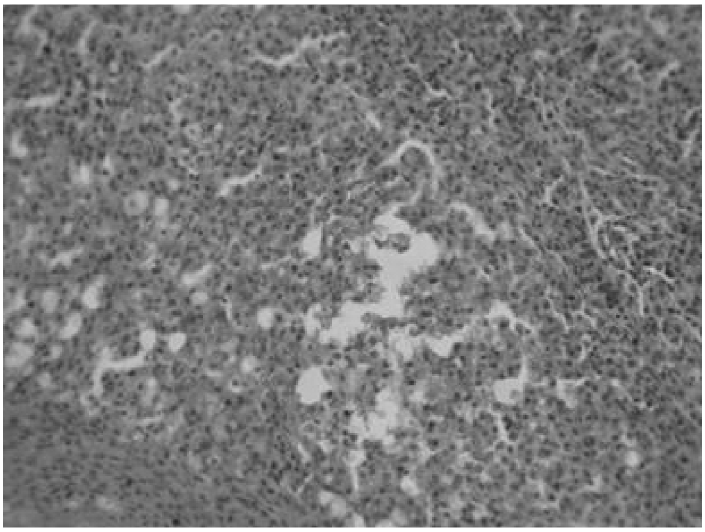

Fig. 5. Necrosis and a few small foci in the spleen.

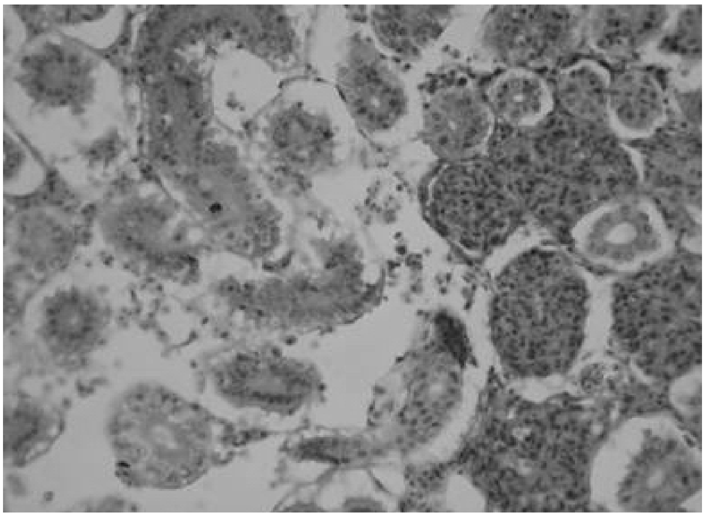

Fig. 6. Many large processes with a necrotic center and outer rim of inflammatory cells in the kidney.

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