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SHORT COMMUNICATION

ENTERIC RED MOUTH DISEASE IN CULTURED RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) ON THE BLACK SEA COAST OF TURKEY

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Abstract

Although *Yersinia ruckeri*, the etiological agent of the enteric red mouth disease (ERM), has been isolated from freshwater fish in several countries, there are few reports of its presence in cultured sea and brackish water fish. The aim of this study was to isolate and identify the etiological agent of disease outbreaks that occurred in rainbow trout cage farms on the Black Sea coast of Turkey in 2002. Clinical observation, biochemical, API 20 NE, and agglutination tests allowed the diagnosis of ERM caused by *Y. ruckeri*, which was successfully treated with medicated feed (oxytetracycline at 75 mg per kg body weight per day for 10 days).

Introduction

The genus *Yersinia*, of the Enterobacteriaceae family consists of Gram negative short rods. *Yersinia ruckeri* is an important primary pathogen of fish and the causative agent of enteric red mouth disease that primarily affects rainbow trout and other salmonids. Yersiniosis or enteric red mouth (ERM) is a serious infectious disease in the rainbow trout farming industry and causes economic problems in many countries. *Y. ruckeri* was reported in Atlantic salmon in Norway and Scotland, burbot in Canada, sturgeon in France, whitefish and Atlantic salmon in Finland, and rainbow trout in Greece, Turkey, Iran and Venezuela (Ross et al., 1966; Frerichs et al., 1985; Giorgetti et al., 1985; Bragg and Henton, 1986; Rintamaki et al., 1986; Sparboe et al., 1986; Dwilow et al., 1987; Vuillaume et al., 1987; Dear, 1988;

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Bullock and Cipriano, 1990; Savvidis, 1990; Timur and Timur, 1991; Alvarez et al., 1992; McCormic and McLoughlin, 1993; Petrie et al., 1996; Soltani et al., 1999). Although known to be a pathogen of freshwater fish, it was isolated from marine fish as well (Sparboe et al., 1986).

The disease was first reported in cultured rainbow trout in Turkey by Timur and Timur (1991) and later in cultured rainbow trout in freshwater (Cagırgan and Yüreklitürk, 1991; Karatas and Candan, 1997; Savaser and Diler, 1997).

The mode of transmission of ERM has been related to wild or farmed carrier fish and other putative vectors such as aquatic invertebrate and birds (Willumsen, 1989). The pathogen has been isolated from the feces of carrier fish even two months after an ERM outbreak (Rodgers, 1992). The survival of *Y. ruckeri* was examined by Romalde et al. (1994) who concluded that salinity may effect survival time of *Y. ruckeri* and that the strain can survive in 15‰ salinity for three months. Diler and Ekici (2003) found that the optimal growth for *Y. ruckeri* strains occurs at 5‰ and 15‰ salinity. Salinity of 9‰ did not prevent ERM, but mortality of rainbow trout was significantly reduced with each increase in salinity (Altınok and Grizzle, 2001).

For many years, the inland Black Sea has contributed about 70% of the total fish production of Turkey. Because of its low salinity (16‰) and appropriate water temperature, both fresh and marine fish are cultured in these waters. The Black Sea coast has appropriate salinity and temperature conditions for rainbow trout farming in net cages (Okumus et al., 1997; Sener, 2002). Rainbow trout production in net cages in the Black Sea is around 1700 tons yearly (Memis, 2003). The aim of this study was to isolate and identify the etiological agent of disease outbreaks among rainbow trout cage farms on the Black Sea coast of Turkey.

Materials and Methods

During May, 2002, unusual mortality outbreaks occurred among rainbow trout cage farms in Ordu Peninsula on the Black Sea coast of Turkey to which cultured rainbow trout were transferred during November 2001 at approximately 70-80 g.

During the outbreak, the seawater temperature was 16ºC and the fish averaged 250 g. Cumulative mortality reached approximately 3%. Diseased fish were transported to the Fish Diseases Laboratory and examined to diagnose the responsible agent. Samples from the kidney, liver, and spleen were inoculated on tryptic soy agar (TSA, Merck). The agar plates were incubated at 20ºC for 48 h. After purifying the cultures, biochemical and physiological tests were carried out on the isolates according to standard tube methods. Colonies were diluted in sterile pure water and API 20NE strips were inoculated according to the manufacturer's instructions at an incubation temperature of 20ºC for 48 h. The isolated bacteria were characterized using the API 20NE system and the identity was confirmed using a Bionor Mono Yr kit (BioNor Mono AS, BIONOR Aqua, Skien, Norway). Then the isolated bacteria were inoculated into Shotts-Waltman medium.

Susceptibility of the isolated strains to various antibiotics and a test for sensitivity against O/129 Vibriostat 150 µg were determined by the disc diffusion method on Müeller-Hinton agar (MHA) using commercially available discs.

Samples were taken from the skin, muscle, gills, heart, intestine, liver, kidney, and spleen, and fixed in 10% formaldehyde for histopathological examination. Sections were stained with hematoxylin and eosin (Roberts, 1978).

Results and Discussions

Exophthalmus, hemorrhages at the base of the ventral fin and mouth, swelling in the abdominal region, prolapsus, and hemorrhages in the anus were observed on postmortem examination of fish. The internal pathology involved hemorrhages in the muscle and swim bladder, and enlarged bright red spleen, kidney, and liver. The stomach and intestine were full of fluid. These results agree with earlier findings described by Bragg and Henton (1986), Sparboe et al. (1986), Vuillaume et al. (1987), Bruno (1990),

Savvidis (1990), Timur and Timur (1991), Alvarez et al. (1992), Inglis et al. (1993), and Petrie et al. (1996). Parasitological examination was negative.

Cream-colored, smooth, round colonies were isolated from the liver and kidney of four examined fish. Gram negative, motile, cytochrome oxidase-negative, fermentative, rod-shaped bacterial strains were identified in the isolated colonies. Green colonies with surrounding opaque haloes due to a zone of hydrolysis appeared on Shotts-Waltman medium. The biochemical, morphological, and physiological characteristics and API 20NE results of the isolates are shown in Table 1. The isolates tested positive in the agglutination tests for *Y. ruckeri*. Clinical observation, biochemical and API 20 NE test results, and the agglutination test led to the diagnosis of enteric red mouth disease (ERM) caused by *Y. ruckeri*. The result of the biochemical tests agree with Stevenson and Daly (1982), De La Cruz et al. (1986), Vuillaume et al. (1987), Savvidis (1990), Timur and Timur (1991), Inglis et al. (1993), McCormick and McLoughlin (1993), Savaser and Diler (1997), Soltani et al. (1999), and Oraic et al. (2002).

Histologically, there were several necrotic areas and a loss of hemapoietic tissue in the anterior and posterior parts of the kidney (Fig. 1). The liver was also necrotic and diffused vacuolation of hepatocytes were prominent (Fig. 2). Within the digestive tract, especially the intestine, necrosis of the mucosa and sloughing into the lumen occurred (Fig. 3). Our histological results were similar with the findings of Wobeser (1973), Bruno (1990), Timur and Timur (1991), Petrie et al. (1996), and Horne and Barnes (1999).

All strains were sensitive to trimethoprime (5 µg), flumequine (30 µg), and oxytetracycline (30 µg), and resistant to oxolinic acid (2 µg). The disease outbreak was treated with medicated feed (oxytetracycline, 75 mg/kg body weight/day for 10 days). Bacterial susceptibility to antimicrobial agents was similar to Giorgetti et al. (1985), Sparboe et al. (1986), Savvidis (1990), and Karatas and Candan (1997). In contrast, Dear's isolates were sensitive to oxolinic acid (Dear, 1988).

Table 1. Biochemical, morphological characteristics, and API 20NE results of *Yersinia ruckeri* isolated from the liver and kidney of rainbow trout cultured on the Black Sea coast of Turkey.

Characteristic	*Liver, kidney*
Gram	–
Motility	+
TSI	+
Cytochrome oxidase	–
O/F test	F
Nitrate	+
Triptophane	–
Urea	+
Esculine	–
Gelatin	+
Arabinose	–
Mannose	+
Gluconate	–
Caprate	+
Adipate	–
Citrate	+
Acid from glucose	+
Gas from glucose	–
Sucrose	–
Fructose	+
Mannitol	+
Hydrolysis of ornithine	+
Hydrolysis of lysine	+
Hydrolysis of arginine	–
H_2S production	–
Indole	–
Metil red	–
Sorbitol	–
Starch	–
Hydrolysis of gelatin	+
Methyl red	+
Voges Proskauer	–
Oxytetracycline	S
Flumequine	S
Oxolinic acid	R
Sulphonamides	R
Trimethoprine	S
Catalase	+
Growth on Shotts-Waltman	+

+ Positive reaction; – Negative reaction; F Fermentative, S Sensitive; R Resistant..

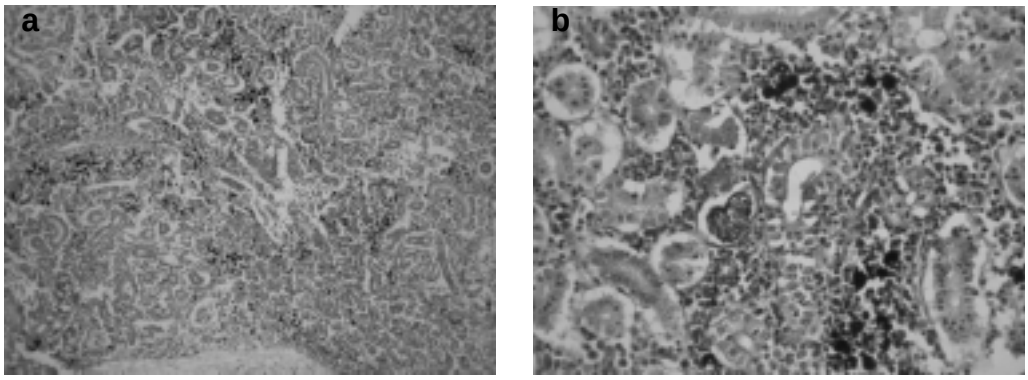

Fig. 1. Posterior kidney of diseased fish shows reduction of hemapoietic tissue and necrosis of the renal tubules: (a) hematoxylin and eosin stain x 10, (b) hematoxylin and eosin stain x 40.

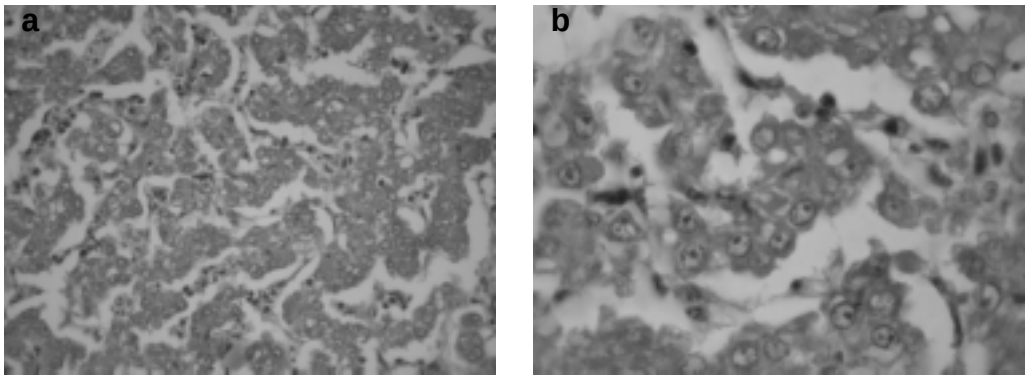

Fig. 2. Severe necrotic areas and extensive vacuolation of cytoplasm of hepatocytes: (a) hematoxylin and eosin stain x 10, (b) hematoxylin and eosin stain x 40.

This first report of the outbreak of enteric red mouth disease in rainbow trout raised in the Black Sea indicates that the long term survival of this bacterium in the salinity of the Black Sea enables it to cause outbreaks, confirming earlier reports that the salinity tolerance of *Y. ruckeri* causes mortality in rainbow trout (Thorsen at al., 1992; Altınok and Grizzle, 2001). It remains for future investigations to determine the source of the infection.

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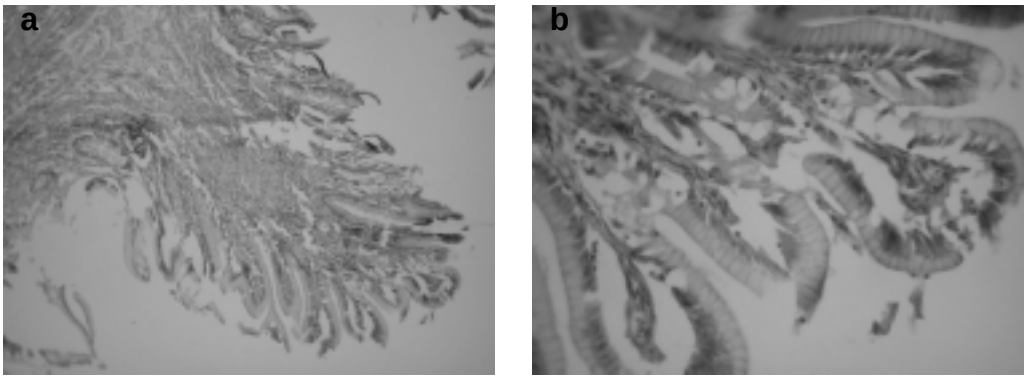

Fig. 3. Necrosis of the intestinal mucosa and sloughing into the lumen: (a) hematoxylin and eosin stain x 10, (b) hematoxylin and eosin stain x 40.

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