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Systemic Infections of *Aeromonas hydrophila* in Rainbow Trout (*Oncorhynchus mykiss* Walbaum): Gross Pathology, Bacteriology, Clinical Pathology, Histopathology and Chemotherapy

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Abstract: In the present study *Aeromonas hydrophila* outbreaks occurred for 3 months (July, September and October) in 1996 about 15-20 days after flooding in juvenile cultured rainbow trout (*Oncorhynchus mykiss* Walbaum) were investigated. Sensitivities of four isolates of *A. hydrophila* against 16 chemotherapeutants were determined. Minimum bactericidal concentrations (MBCs) of formalin to the isolates were between 6.4-8 μg/ml. 23 μg/ml dose of potassium per manganate ($KmnO_4$) and 15 μg/ml dose of Chloramine-T were effective to inhibit one of the isolates but not to the other isolates. Oral applications of ofloxacin or flumequine following bath disinfections with formalin controlled the natural infections. Pathogenicity of three isolates of *A. hydrophila* was tested by intramuscular injection into healthy 1-year-old rainbow trout. The lethal dosages of 50 % end point (LD_{50}) for the isolates were calculated as 4×10^4, 2×10^5 and 5×10^5, respectively. Natural and experimental infections caused dross clinical abnormalities such as abnormal movements, anorexia, darkening skin, pale and swollen gills, cherry-red coloured spleen, necrosis in liver, haemorrhage in kidney and serous exudates in intestine. Histopathological examination demonstrated pathological changes in gill, brain, heart, kidney, liver and intestinal tissues of naturally infected juvenile rainbow trout. Glutamate oxalacetate transaminase (GOT) enzyme and bilirubin (BIL) levels in blood serum of naturally infected rainbow trout were significantly higher than in healthy fish. No significant increases were observed in the serum L-lactate dehydrogenase (LDH), blood urea nitrogen (BUN) and creatinine (CRE) levels of diseased fish group. There was no significant a decrease in the mean concentration of glucose (GLC) of the naturally infected fish group.

Key words: Disease, rainbow trout, *Aeromonas hydrophila*, blood, histopathology, treatment

Introduction

Aeromonas hydrophila that predominantly exists in nature is being considered autochthonous inhabitants of aquatic environments (Nieto *et al.*, 1985; Toranzo *et al.*, 1986; Santos *et al.*, 1987, 1988, 1991; Knochel, 1989 and Anders and Anders, 1990; Loghothetis and Austin, 1996a; Austin and Austin, 1999). It comprises a portion of the normal microbial flora of different organs of healthy fish (Doukas *et al.*, 1998). Although some researchers claimed that *A. hydrophila* could be utilised as competitor bacteria to prevent *Flexibacter columnaris* infection (Chowdhury and Wakabayashi, 1989a, 1991b and Wakabayashi, 1991), it was demonstrated that this bacterium was an opportunistic pathogen for fish (Aoki and Egusa, 1971 and Loghothetis and Austin, 1996a). It could cause latent, chronic and acute infections under environmental and physiological stress conditions (Grizzle and Kiryu, 1993 and Harikrishnan *et al.*, 2003).

In this study the blood parameters of fish were used as indicators of their physiological state. The study of these parameters has become widespread in the identification of pathologies associated with infectious diseases (Studnicka and Siwicki, 1986; Nakano et al., 1995; Aydýn et al., 2000, 2001, 2002; Harikrishnan *et al.*, 2003), nutritional deficits deficits (Hrubec and Smith, 1999), toxicity (Everall *et al.*, 1992; Mughal *et al.*, 1993; Shakoori *et al.*, 1996; Hrubec and Smith, 1999), anoxic conditions and the other environmental stressors (Martinez *et al.*, 1994; Yang and Chen, 2003) encountered in fish farming.

The present study was designed to test the pathogenicity of *A. hydrophila* isolates in rainbow trout (*Oncorhynchus mykiss* Walbaum), and to investigate histopathological and clinical pathological effects of natural infection in diseased rainbow trout. A secondary goal was to develop adequate quarantine and therapy techniques for control of this disease.

Materials and Methods

Isolation and identification of bacteria: The bacteria were originally isolated from naturally infected juvenile rainbow trout (*Oncorhynchus mykiss* Walbaum) at different intervals during July (in Farm 1), September (in Farm 2 and 3) and October (in Farm 4) in 1996 20 days after fooling at four fish farms in the vicinity of Erzurum in the East Anatolian region in eastern Turkey. The mean individual weight of naturally infected fish was 15 ± 10 g in Farm 1, 30 ± 20 g in Farm 2 and 3, and 34 ± 20 g in Farm 4. The water temperatures in the fish farms were 17 ±

1 °C (in Farm 1) 15 ± 2 °C (in Farm 2 and 3) and 14 ± 3 °C (in Farm 4).

The infected fish were killed and gross clinical signs recorded during necropsy. In the isolation of bacteria from naturally infected fish, inocula were aseptically obtained from kidney, liver, spleen and muscle of each naturally infected fish and streaked on tryptone soya (TS) agar, GSP (*Aeromonas-Pseudomonas* selective) agar, MacConkey agar, Baird-Parker agar, *Yersinia* selective agar, *salmonella-Shigella* agar, thiosulfate citrate bile salt (TCBS) agar and Kligler (KG) agar. Cultures were incubated at 22 °C for 1-7 d. After incubation, the individual colonies grown on GSP agar, TS agar and MacConkey agar were enriched on GSP agar at 22 °C for 48 h, and then inocula were used in the identification tests (Plumb and Bowser, 1983; Anonymous, 1996). The bacteria isolated from fish were named isolate 1, 2, 3, 4, respectively to the Farm number. All of the bacteriological media used in this research was from Merck (Merck, Darmstadt, Germany).

In vitro efficacy of some disinfectants and antibiotics: Each pure culture of four bacterial isolates were added to sterile phosphate buffer solutions and their concentrations were adjusted with the use of a spectrophotometer as a 30 % transmittance (525 nm) with sterile phosphate buffer. Aliquots (0.1 ml) were used to test the sensitivity of the bacterial isolates to several chemotherapeutic and other antimicrobial substances. The agar disc diffusion method with Muller-Hinton agar (Bauer *et al.*, 1966) was employed to determine their sensitivity to chemotherapeutic agents (Table 2) according to National Committee for Clinical Laboratory Standards (NCCLS, 1992).

In vitro assays were conducted to determine the bactericidal concentrations of formalin, chloramine-T and potassium per manganate (due to their availability and cost). For the formalin and chloramine-T serial arithmetic dilutions of from 325 to 0.4 μg/ml in test tubes containing 5 ml sterile phosphate buffer solution were prepared. Aliquots (0.1 ml) of the standardized bacterial isolates were added to each tubes and left to stand at room temperature for 1 h, after which a loopful material from each tube was inoculated onto plates containing GSP agar. These were incubated for 7 days at 22°C and then examined for growth of *A. hydrophila*. In a similar manner the bactericidal effect of the $KMnO_4$ (serial arithmetic dilutions from 23.27 to 0.0125 μg/ml, being left to standard for 10 min after inoculation) were tested. Control tubes containing sterile phosphate buffer were each inoculated with aliquots of the standardized bacteria and cultirted onto GSP agar for 7 days at 22°C.

Chemotherapy: In order to treat naturally infected fish in rainbow trout farms, 20 mg/kg fish dosage of ofloxacin per day in Farm 1 and 2, and 50 mg/ kg fish dosage of flumequine per day in Farm 4 were orally used for 5 days after application of formalin bath for 3 days (50 mg/l of water for 1 h). 20 mg/kg fish dosage of ofloxacin per day was orally applied for 5 days after chloramine-T bath (20 mg/l of water for fish) for 3 days in Farm 3.

Infection experiments: A total of 130 rainbow trout, one-year-old with average 100 ± 22 g body weight, were used for the experiment. 13 different 700 l capacity fibre-glass tanks supplied with circulating freshwater (9 ± 1 °C) under continuous aeration were used in these studies. Forty fish were experimentally infected with each isolate of *A. hydrophila* from diseased rainbow trout obtained from Farm 1, 2 and 4, and divided into 4 subgroups each containing 10 fish. After an adaptation period of 15 days, the fish were injected with 10^4 live cells, 10^5 live cells, 5 x10^5 live cells and 10^6 live cells of each bacterial suspension into the muscle proximal to the dorsal fin. The remaining 10 fish were inoculated with sterile phosphate-buffered saline (PBS) and served as non-infected control fish. The lethal dosages of 50 % end point (LD_{50}) values were determined after 15 days of experiment using the method of Reed and Müench (1938) adopted by Plumb and Bowser (1983). The inoculated bacterium (*A. hydrophila*) was re-isolated from kidney of dead fish by using GSP agar and identified as *A. hydrophila* by characterization tests.

Clinical examination: During the experiments of the natural and experimental infection period, behaviours of the diseased fish as well as their gross external and internal signs were recorded.

Histopathological examination: Tissues of naturally infected fish were excised and placed in Bouin's fixative and processed for light microscopy by routine methods (Bullock, 1989), and then embedded in paraffin wax and 5μm sections were cut and the histological sections were prepared and stained with haematoxylin-eosin (H&E), eosin and Gram Brown&Brenn stains.

Sampling and analytical procedures: Blood analyses were conducted to compare blood parameters of fish using rainbow trout from each experimental group; 1) naturally infected 25 fish (showing clinical signs) and 2) non-infected healthy 25 fish. They were weighed, and 2.5 ml of blood was drawn from each by caudal vein puncture and immediately transferred into individual silicone-coated Vacutainer Tubes (Becton Dickinson). Blood was centrifuged promptly at 3, 100 x gravity for 10 min, and the serum was removed with a disposable transfer pipette. Concentrations of glutamate oxalacetate transaminase (GOT)and L-lactate dehydrogenase (LDH) enzymes, bilirubin

Table 1: Biological and biochemical characteristics of bacteria isolated from diseased rainbow trout; plus sign = yes, minus sign = no, O/F = oxidative/fermentative.

Characteristics	Result			
	Isolate 1	Isolate 2	Isolate 3	Isolate 4
Gram stain	-	-	-	-
Motility (room temperature)	+	+	+	+
Growth at 5 °C and 37 °C	+	+	+	+
Growth at 0 % NaCl	+	+	+	+
Growth at 5 % NaCl	-	-	-	-
Growth at KCN	+	+	+	+
Oxidase, catalase, lipase, DNA'ase, urease, arginine dihydrolase, lysine decarboxylase, ß-galactosidase	+	+	+	+
Ornithine decarboxylase, phenilalanine deaminase	-	-	-	-
Nitrate reduction and degredation of Tween 80	+	+	+	+
Degredation of pectine	-	-	-	-
Esculin, starch, casein, lecithin and gelatin hydrolysis	+	+	+	+
Haemolysis (human blood)	+	+	-	-
Haemolysis (sheep blood)	-	-	-	-
Production of H_2S	-	+	-	-
Metil-Red test	-	-	-	-
Voges-Proskauer test	+	+	+	+
Simmon's citrate	+	+	-	-
Sodium citrate	+	+	+	+
Indole production	+	+	+	+
Gas production from glucose	+	+	-	-
Acid production from carbonhydrates: Glucose, Mannitol, Fructose, Sucrose, Maltose, Arabinose, Salicin, Trehalose, Galactose, Glycerol Inositol, Sorbitol, Rhamnose, Melibiose, Amygdalin, Dulcitol, Raffinose, Xylose, Cellobiose, Erythritol,	+	+	+	+
Adonitol	-	-	-	-
Lactose	+	-	-	+
O/F	F	F	F	F

Table 2: Results of susceptibility test of *A. hydrophila* (4 isolates) to chemotherapeutants ([1] = NCCLS (1992), R = resistant, MD = moderate sensitive, S = sensitive, NA = not applied, NR = not reported, Sulp./Trim. = sulphamethoxazole/trimethoprim, Amp./Sulb. = amphicillin/sulbactam)

Chemotherapeutant (µg/disc)	Sensitivity (zone diameter -mm)				Reference (zone diameter = mm)		
	Izolat 1	Izolat 2	Izolat 3	Izolat 4	R	MS	S
Cephoperazon/Sulb. (75/30)	NA	S (22)	NA	NA	<15	16-20	>21
Flumequine (30)	NA	S (20)	S (27)	S (27)	NR	NR	NR
Nitrofurantoin (300)	NA	MS (16)	MS (15)	MS (15)	<14	15-16	>17
Erythromycin (15)	NA	R (8)	R (11)	R (11)	<13	14-22	>23
Enrofloxacin (5)	NA	S (22)	S (25)	S (25)	<14	15-17	>18
Sulp./Trim. (23.75/1.25)	R (0)	R (10)	MS (14)	MS (14)	<10	11-15	>16
Ofloxacin (5)	S (26)	S (25)	S (24)	S (24)	<12	13-15	>16
Tetracycline (30)	R (0)	R (0)	R (8)	R (8)	<14	15-18	>19
Netilmycine (30)	S (19)	S (20)	S (20)	S (20)	<12	13-14	>15
Kanamycin (30)	MS (17)	R (10)	R (12)	R (12)	<13	14-17	>18
Vancomycin (30)	R (0)	R (0)	MS (10)	MS (10)	<9	10-11	>12
Chloramphenicol (30)	R (10)	S (20)	MS (14)	MS (14)	<12	13-17	>18
Gentamycine (10)	S (16)	S (15)	S (17)	S (17)	<12	13-14	>15
Rifampycin (5)	R (0)	R (0)	R (0)	R (0)	<16	17-19	>20
Oxytetracycline (30)	R (0)	R (10)	R (14)	R (14)	<14	15-18	>19
Imipenem (10)	R (10)	NA	NA	NA	<13	14-15	>16
Cephoperazon (75)	R (0)	NA	NA	NA	<15	16-20	>21
Cefixime (5)	R (10)	NA	R (8)	R (8)	<15	16-18	>19
Ampicillin (10)	R (0)	NA	NA	NA	<13	14-16	>17
Chlarithromycine (15)	R (0)	NA	NA	NA	<13	14-17	>18
Amp./Sulb. (10/10)	NA	R (0)	R (0)	R (0)	<11	12-14	>15
Penicilin G (10 unit.)	R (0)	NA	NA	NA	<11	12-21	>22

Table 3: Pathogenicity of the isolates (*A. hydrophila*) isolated from diseased rainbow trout

Live cells inoculated to each fish	Dead/tested fish (% Mortalite)		
	Isolate 1	Isolate 2	Isolate 4
10^4	2/10 (20)	0/10 (10)	1/10 (10)
10^5	7/10 (70)	4/10 (40)	3/10 (30)
5 x 105	9/10 (90)	6/10 (60)	5/10 (50)
10^6	10/10 (100)	10/10 (100)	10/10 (100)
LD_{50}	4×10^4	2×10^5	5×10^5

Table 4: Non-parametric (ANOVA) analysis of blood parameters of healthy and naturally infected rainbow trout groups. [n = number the evaluated samples. Values along a row with asterisks were significantly different from other values in row ($P<0.05$)].

Test[1]	Naturally infected fish (n = 25)	Healthy fish (n = 25)
GOT (u/l)	1372 (857-1769)*	735 (443-844)
LDH (u/l)	1252 (457-1434)	1402 (735-2642)
GIC (mg/dl)	72 (35-84)	81 (78-98)
BIL (mg/dl)	0.7 (0.1-1.19)*	0.2 (0.1-0.4)
CRE (mg/dl)	0.9 (0.4-3.30)	0.5 (0.2-0.7)
BUN (mg/dl)	9 (0-12)*	1 (0-3)

[1]GOT = glutamate oxalacetate transaminase, LDH = lactate dehydrogenase, GLC = glucose, BIL = bilirubin, CRE = creatinine, BUN = blood urea nitrogen

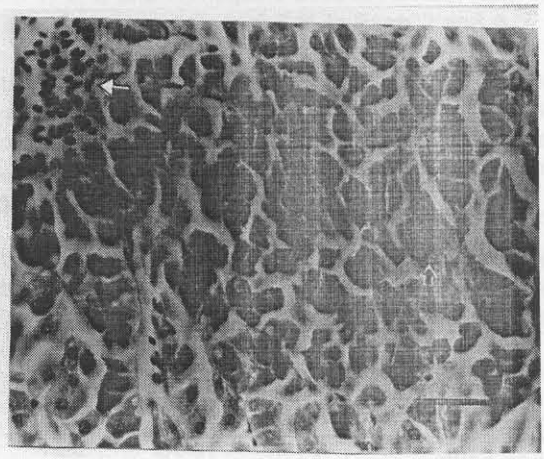

Fig. 1: Cytoplasmic vacuolation in hepatocytes (dark arrow), intravascular congestion (white arrow) in the liver tissue of rainbow trout naturally infected with *A. hydrophila* (H&E x400). Bar = 80 μm.

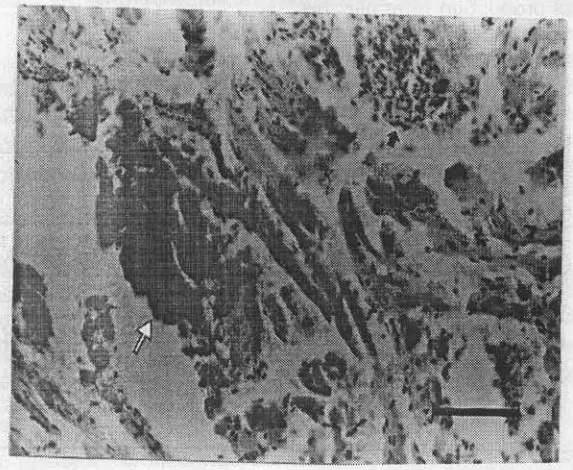

Fig. 2: Haemorrhage of cardiac muscle (white arrow) and thrombus (dark arrow) in the heart tissue of rainbow trout naturally infected with *A. hydrophila* (H&E x200). Bar = 160 μm.

CRE values: CRE level in the serum of naturally infected fish group was higher than that of healthy fish group (Table 4) although not significantly so ($P>0.05$).

BIL levels: Serum BIL concentrations of the naturally infected rainbow trout were significantly greater ($P<0.05$) than in healthy rainbow trout (Table 4), which may cause bilirunaemia.

BUN concentrations: BUN values in blood of the naturally infected fish were significantly higher than in healthy fish ($P<0.05$; Table 4).

Discussion

The identification test results of *Aeromonas hydrophila* were almost identical with those of isolates from fish (Nieto *et al.*, 1985; Toranzo *et al.*, 1986; Kuge *et al.*, 1992; Aydin *et al.*, 1997; Doukas *et al.*, 1998; Austin and Austin,

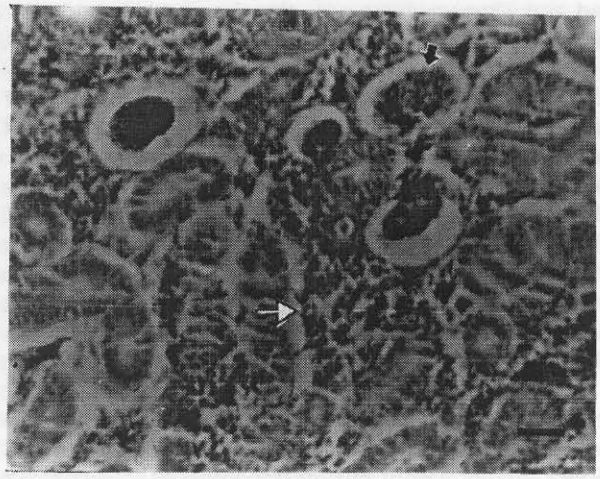

Fig. 3: Interstitial (white arrow) and glomerular (dark arrow) lymphocyte infiltration in the kidney of rainbow trout naturally infected with *A. hydrophila* (H&E x200). Bar = 80 μm.

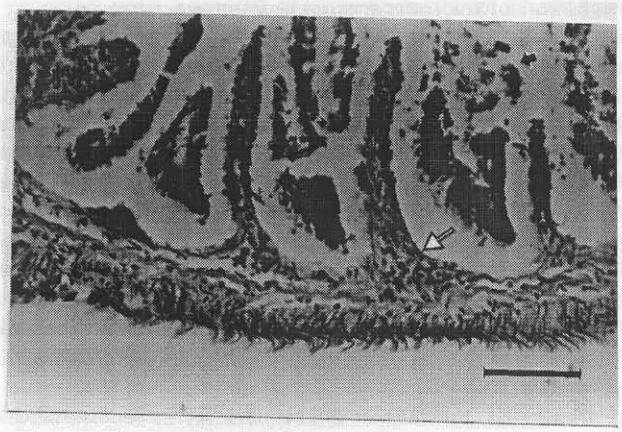

Fig. 4: Mononuclear inflammatory cell infiltration in lamina propria (white arrow) and of intestinal mucosa poured into lumen (dark arrow) in the intestine tissue of rainbow trout naturally infected with *A. hydrophila* (H&E x200). Bar = 160 μm.

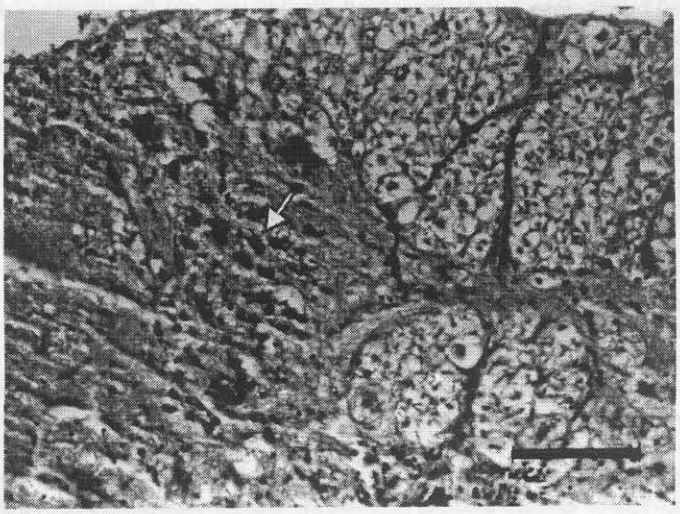

Fig. 5: Ischemia (arrow) in neurons of brain tissue (H&E x200). Bar = 160 μm.

1999; Popovic *et al.*, 2000), human or other animals (Chein *et al.*, 1993; Chien and Chieh, 1994; Holt *et al.*, 1994). *A. hydrophila* is one of the bacteria which may occur dominant or predominant in water of ponds, sediments and mud-guard (Santos *et al.*, 1987, 1988; Knochel, 1989; Anders and Anders, 1990; Loghotheites and Austin, 1996a; Popovic *et al.*, 2000). Also, this bacterium comprises a portion of the normal microbial flora of fish and other aquatic animals, and is an opportunistic pathogen for fish (Kuge *et al.*, 1992; Loghotheites and Austin, 1996a, b; Aydin *et al.*, 1997; Doukas *et al.*, 1998; Popovic *et al.*, 2000). Some researchers reported that *A. hydrophila* and its adhesin prevented infections by inhibiting fish pathogens such as *F. columnaris* (Chowdhury and Wakabayashi, 1989a, b, 1990; Wakabayashi, 1991) and *Vibrio* species (Fang *et al.*, 1998). However, several investigators stated that this bacterium was clearly a pathogenic for fish causing systemic infections (Kuge *et al.*, 1992; Grizzle and Kiryu, 1993; Loghotheites and Austin, 1996a, b; Aydin *et al.*, 1997; Doukas *et al.*, 1998; Austin and Austin, 1999; Popovic *et al.*, 2000). *A. hydrophila* infections have been reported in rainbow trout (Aydin *et al.*, 1997), aquarium fish and carp (Timur, 1984, 1986; ªim°ek *et al.*,1997) in Turkey. During the present study, *A. hydrophila* caused natural epizootic outbreaks in four populations of juvenile cultured rainbow trout. The form of *A. hydrophila* infection in our study was almost identical with acute MAS (Kuge *et al.*, 1992; Grizzle and Kiryu, 1993; Aydin *et al.*, 1997; Austin and Austin, 1999). Infection appeared about 20 days after flooding possibly due to the reduced resistance of the fish and the increases in microbial activity after physical water pollution and

incubation of microorganisms in 20 days.

Bacterial isolates were resistant to tetracycline, amphicillin, chloramphenicol (one isolate), erythromycine, potentiated sulphonamides, as previous researchers reported (Aoki and Egusa, 1980; Aoki, 1988; De Paola *et al.*, 1988; Aydin *et al.*, 1997). In agreement with the literature, isolates of *Aeromonas hydrophila* in this study were resistant to penicillin (Aoki and Egusa, 1971; Popovic *et al.*, 2000), oxytetracycline (Plumb *et al.*, 1995), and sensitive to chloramphenicol (two isolates; Aoki and Egusa, 1971, 1980; Liu and Wang, 1991; Popovic *et al.*, 2000), flumequine (Liu and Wang, 1991; Popovic *et al.*, 2000), enrofloxacin (Bragg and Todd, 1988; Plumb *et al.*, 1995), netilmycine, gentamycine, ofloxacin (Aydin *et al.*, 1997), and moderate sensitive to kanamycin (one isolate) and nitrofurantoin (Aoki and Egusa, 1971; Aydin *et al.*, 1997). Our isolates were resistant to penicillin G, which was probably R-plasmid-mediated because of the ability of *A. hydrophila* to produce ß-lactamase (Aoki, 1988; Popovic *et al.*, 2000). Also, drug- resistant strains of *A. hydrophila* can proliferate in direct response to enhanced use of antimicrobial compounds in fish culture. According to the results of this study, enrofloxacin, ofloxacin, gentamycine, netilmycine and flumequine antibiotics could be recommended to treat fish infected with *A. hydrophila*.

For treatment, formalin or chloramine-T was recommended to farmers, but to Austin and Austin (1999), the MBC of $KMnO_4$ was higher than could be tolerated by rainbow trout. The dosage of formalin used in the present study was higher than dosages recommended (Scott, 1993; Austin and Austin, 1999) but no toxicity was observed for fish in this application.

Mortality rates of these farms in this study were low, however, mortalities had reached between 56-70 % in these farms where fish was not treated in 1995 (Aydin *et al.*, 1997). The difference in mortality rates between treated and non-treated fish in farms showed that treatments could be effective.

According to the criteria of pathogenicity defined by Santos *et al.* (1987, 1991), two isolates were included in the highly virulent category, but Isolate 4 was classified weakly virulent by criteria of Nieto *et al.* (1985).

Clinical signs of natural and experimental infections were similar to previous findings (Timur, 1986; Chein *et al.*, 1993; Grizzle and Kiryu, 1993; Chien and Chieh, 1994; Aydin *et al.*, 1997; ªim°ek *et al.*, 1997; Doukas *et al.*, 1998; Popovic *et al.*, 2000; Harikrishnan *et al.*, 2003).

In the present study, absence of histopathological change in muscle could be due to acutely characteristic of disease and an early stage in development of infection. However, researchers have reported muscular degeneration and necrosis in the fish infected with *A. hydrophila* (Chein *et al.*, 1993; Grizzle and Kiryu, 1993; Chien and Chieh, 1994; Aydin *et al.*, 1997). Acute inflammation and thickness of lamellae were similar to those reported by Grizzle and Kiryu (1993) and Aydin *et al.* (1997). Inflammation and lipoid vacuoles in hepatocytes were present, as documented by researchers (Chein *et al.*, 1993; Grizzle and Kiryu, 1993; Chien and Chieh, 1994; Aydin *et al.*, 1997). Focal necrosis in cardiac muscle, observed in previous reports (Roberts, 1989; Aydin *et al.*, 1997), was not observed in natural *A. hydrophila* infection. The presence of inflammation in kidney was similar to the findings of previous studies. (Chein *et al.*, 1993; Chien and Chieh, 1994; Aydin *et al.*, 1997). Inflammation and mucosa poured into the lumen in intestine tissue were detected, as reported by Timur (1986). Ischemia in brain neurons might be a result of infection although a similar structural change with *A. hydrophila* infections has not been described in previous reports.

The significant increases in serum LDH levels, which have been reported in fish with *A. hydrophila* infections (Brenden and Huizinga, 1986; Grizzle and Kiryu, 1993), were not observed in the naturally infected fish of our study but were unsignificantly less. The higher levels of serum GOT could be expected in fish infected with *A. hydrophila* as indicated by several workers (Brenden and Huizinga, 1986; Grizzle and Kiryu, 1993). The enzyme-abundant tissues contribute to the aspect of the circulating enzyme-pattern in the serum. When damage occurs in enzyme-abundant tissue, some enzyme leak from injured cells and the activities of serum enzymes will change. In this study, the increase in serum GOT activity may be an indication of considerable clinical damage and histopathological changes caused by the infection in the liver. Because activities of hepatic enzymes such as GOT, GPT and LDH in serum of fish have been known to be very useful as an index for diagnosis of liver function (Everall *et al.*, 1992; Mughal *et al.*, 1993; Shakoori *et al.*, 1994; Ahmad *et al.*, 1995; Nakano *et al.*, 1995; Aydin *et al.*, 2000, 2001 and Adham *et al.*, 2002).

The lack of significant differences observed between GLC concentrations of treatments may partially be due to the relatively 1arge statistical variation determined in the diseased group (Düzgüne° and Akman). Wide range of variation in the blood GLC values of naturally infected fish may also originate from hypoglycaemia due to the increase in activity of GOT under stress given by the infection. The serum GLC concentrations may significantly decrease with *A. hydrophila* infection (Harikrishnan *et al.*, 2003). Blood GLC level in fish is known to be very useful as a criteria for diagnosis of liver and muscle tissues function (Shakoori *et al.*, 1996; Wilkie *et al.*, 1996; Aydin *et al.*, 2000, 2001 and Yang and Chen, 2003).

The excessive BIL concentration in serum of the naturally infected fish might be a result of cessation of hepatobiliary functions, and reduce of tolerance and inhibition recovery from bilirunaemia following the *A.*

hydrophila infection insult. Hyperbilirunaemia has been reported with bacterial infections such as gill disease (Studnicka and Siwicki, 1986) and Jaundice disease (Sorimachi *et al.*, 1993). The mean serum BIL value of healthy fish was within the normal limits given for rainbow trout (Brenden and Huizinga,1986; Mattsoff and Nikinmaa, 1988; Grizzle and Kiryu, 1993 and Aydin *et al.*, 2001).

The lack of significant differences observed between CRE concentrations of treatments may partially be due to the relatively 1arge statistical variation determined in the diseased group (Düzgüne° and Akman). An unsignificant elevation might be expected in naturally infected fish, as has been reported with *Serratia liquefaciens* infection (Aydin *et al.*, 2001) and *Arcobacter cryaerophilus* infection (Aydin *et al.*, 2002). The changes in BUN, UA and CRE concentrations in blood have frequently been used in fish as an indicator of gill and kidney dysfunction (Nelson *et al.*, 1999; Adham *et al.*, 2002; Yang and Chen, 2003). In the present study, a wide range of variation and the significant increases in the BUN values of naturally infected fish group may also originate from the effect of infection, and this result may be an indication of considerable clinical damages and histopathological changes caused by the infection in the gill and kidney. Further studies are needed to explain effects of bacterial infections on BUN and CRE values.

In agreement with several researchers (Timur, 1984, 1986; Nieto *et al.*, 1985; Santos *et al.*, 1987, 1988, 1991; Grizzle and Kiryu, 1993; Aydin *et al.*, 1997; Doukas *et al.*, 1998 and Austin and Austin, 1999), *Aeromonas hydrophila* must be considered as a potential bacterial pathogen for salmonids and other fish.

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