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A Comparative Investigation of *Arcobacter cryaerophilus* Infection among Albino Crosses and High- and Low-Body-Weight Rainbow Trout

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Abstract.—*Arcobacter cryaerophilus* was isolated from naturally infected rainbow trout *Oncorhynchus mykiss*, and its pathogenicity was tested by intramuscular injection into healthy 1-year-old high-body-weight (HBW) and low-body-weight (LBW) normally pigmented rainbow trout and albino crosses. Experimental infections caused deaths with gross clinical abnormalities such as exophthalmia, liver damage, bloody hemorrhagic kidney and heart, and swollen intestines. No significant differences in deaths were observed among the three infected fish groups. Hematocrit levels in blood of the experimentally infected HBW rainbow trout were significantly less than in healthy fish. No significant decreases were observed in the serum total protein of both the experimentally infected albino crosses and the high weight groups. Albumin and creatinine concentrations in serum were not significantly different among the three treatments.

Species of the genus *Campylobacter* can be found in the reproductive organs, intestinal tract, and oral cavity of humans and animals, with some being pathogenic (Holt et al. 1994). Neill et al. (1985) posed *C. cryaerophila* as a new species in the genus *Campylobacter*, and it was included in the subsequent publication of Bergey's Manual of Systematic Bacteriology (Smibert 1986). The taxonomic status of this bacterium was later revised to the newly designated genus and species *Arcobacter cryaerophilus* (Vandamme et al. 1991).

In this study we used the blood parameters of fish as indicators of their physiological state. The study of these parameters has become widespread in the identification of pathologies associated with infectious diseases (Eiras and Saraiva 1986; Studnicka and Siwicki 1986; Nakano et al. 1995; Yokoyama et al. 1996; Rodger and Richards 1998), nutritional deficits (Eiras and Saraiva 1986; Hrubec and Smith 1999), toxicity (Everall et al. 1991, 1992; Mughal et al. 1993; Shakoori et al. 1996; Hrubec and Smith 1999), anoxic conditions, and the other environmental stressors (Martinez et al. 1994; Val et al. 1998) encountered in fish farming. We, therefore, investigated the effects of *A. cryaerophilus* on blood parameters of experimentally infected high-body-weight (well-growing) rainbow trout *Oncorhynchus mykiss* and on albino crosses of the same age.

Aydin et al. (1997) suggested that poor-growing, normally pigmented rainbow trout are more resistant to infection than are the more robustly growing rainbow trout. Also, albino crosses, which grow better than normally pigmented rainbow trout, are more sensitive than those fish during the stress of transportation and oxygen depletion. Thus there is a great need to determine whether the albino crosses are superior to normally pigmented rainbow trout with respect to resistance to bacterial infections.

Methods

Isolation and identification of bacteria.—The bacteria were originally isolated from naturally infected rainbow trout at various times during the spring and summer in 1997 and 1998 at three fish farms in the vicinity of Balikesir (Gönen) and Çanakkale in the Marmara region in northwestern Turkey. 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, gills, and bloody fluid of naturally infected fish and streaked on enriched tryptic soy (TS) agar, Baird–Parker agar, *Yersinia*-selective agar, thiosulfate citrate bile salt (TCBS) agar, *Salmonella–Shigella* agar, *Pseudomonas–aeromonas*-selective agar, Kligler agar, Mac-

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Conkey agar, and *Campylobacter*-selective agar. Cultures were incubated at 25°C for 1–7 d.

Organ samples passaged on *Campylobacter*-selective agar were incubated at 25°C and 30°C for 48 h in an anaerobic jar with the aid of Anaerocult C mini (Anonymous 1996). After incubation, the individual colonies grown on *Campylobacter*-selective agar were enriched on *Campylobacter*-selective agar at 25°C for 48 h, and then inocula were used in the identification tests in both aerobic jars and anaerobic jars with the aid of Anaerocult C mini conditions (Plumb and Bowser 1983; Anonymous 1996; Austin and Austin 1999). All of the bacteriological media used in this research was from Merck (Merck, Darmstadt, Germany).

Infection experiments.—In all, forty-two 1-year-old fish, average body weights of 114.204 ± 6.07 g for F_1 albino crosses and 75.06 ± 10.22 g (high body weight; HBW) and 25.924 ± 2.1 g (low body weight; LBW) for normally pigmented rainbow trout, were obtained from a farm in northwestern Turkey. Four different 350-L concrete basins supplied with circulating freshwater (16 ± 1.5°C) under continuous aeration were used in these studies. Ten of the HBW and LBW rainbow trout and 12 of albino crosses were experimentally infected with an isolate of *A. cryaerophilus* from a diseased rainbow trout obtained from one of the farms mentioned above. A pure culture of the bacterial isolate was added to sterile phosphate buffer solutions and its concentration was adjusted to 30% spectrophotometric transmittance (at 525 nm) with sterile phosphate buffer. After an adaptation period of 15 d, the fish were injected with 5×10^5 live cells into the muscle proximal to the dorsal fin. The remaining 10 fish were inoculated with sterile phosphate-buffered saline (PBS) and served as noninfected control fish.

Clinical examination.—During the experimental infection period, behaviors of the diseased fish as well as their gross external and internal signs were recorded.

Sampling and analytical procedures.—As the fish become moribund (except healthy fish), they were weighed, and 4 mL of blood was drawn from each by caudal vein puncture and immediately transferred into individual 2-mL silicone-coated Vacutainer Tubes (Becton Dickinson: not containing EDTA) and 2-mL tubes containing EDTA. Blood in the coated Vacutainer Tubes was centrifuged promptly at 3,100× gravity for 10 min, and the serum was removed with a disposable transfer pipette. Concentrations of creatinine (CRE), albumin (ALB), and total protein (TP) were determined colorimetrically by using Spinreact kits and measuring absorbance at 492 nm with an AWARNES-Stat Fax 1904 spectrophotometer. Hematocrit (Ht) values of blood samples in EDTA-containing tubes were determined by the microhematocrit method outlined by Bullock (1989).

Statistical analysis.—To compare deaths of the infected fish groups, we used the hypothesis test of difference between two cumulative deaths (Anonymous 1993). The data obtained from blood analyses were subjected to nonparametric analysis of variance (ANOVA) by using the Minitab User Guide program (Anonymous 1993). A value of $P < 0.05$ was considered significant.

Results and Discussion

Identification of the Bacteria

After analysis of the characteristics listed in Table 1, all the isolates obtained from affected farms were identified as *A. cryaerophilus*, based on the revised taxonomic status of this bacterium as a newly designated genus and species. The isolates were almost identical with those of isolates of *A. cryaerophilus* from humans or other animals (Vandamme et al. 1991). Holt et al. (1994) reported that acidic and neutral products of carbohydrates were not produced by *Campylobacter* species, just as was seen in the present study. Bacteria were additionally passaged on selective media and the isolates only grew on *Campylobacter*-selective agar and TS agar.

Pathogenicity of the Isolate

Eight of 10 HBW rainbow trout infected with *A. cryaerophilus* died 7–21 d after inoculation. In the LBW fish group, 9 of 10 deaths occurred between 3 and 17 d after the bacterial inoculation. Six fish of the albino crosses group died in 24 h, and the remaining six died between 6 and 17 d after the inoculation. The stress of challenge could cause death of 50% of the albino crosses within 24 h. In contrast, no clinical signs or deaths were observed in the healthy fish injected with PBS for 1 month after the inoculation. Moribund stages for albino crosses were shorter than for the other groups. Cumulative deaths were not significantly different ($P > 0.05$) among the three infection treatments, although HBW fish have been reported to be more sensitive than the other groups to pathogens (Aydin et al. 1997).

TABLE 1.—Biological and biochemical characteristics of putative *A. cryaerophilus* isolated from diseased rainbow trout; plus sign = yes, minus sign = no, and NR = not reported.

Characteristic	Response	
	Isolates	Reference[a]
Gram stain	−	−
Motility (at 25°C)	+	+
Sheathed flagella	−	−
Growth on *Yersinia*-selective agar	−	NR
Growth on GSP agar	−	NR
Growth on Baird–Parker agar	−	NR
Growth on TCBS agar	−	NR
Growth on KG agar	−	NR
Growth on MacConkey agar	−	NR
Growth on SS agar	−	NR
Aerobic growth on complex solid media (TS agar)	+	+
Morphology of colonies	Small, white	Small, white
Morphology of cell	Rod	Rod
Oxidase	+	+
Catalase	+	+
Growth at 25°C	+	+
Growth at 42°C	+	+
Growth at 15°C	+	+
Growth at 3% NaCl	−	−
Growth at 6% NaCl	−	−
Urease, alkaline phosphatase, and arginine dihydrolase	−	−
Gelatin, esculin, and starch hydrolysis	−	NR
Degredation of Tween 20 and Tween 80	−	NR
in Kligler iron agar and triple-sugar agar:		
Acid–gas from glucose	−/−	NR
Acid from lactose	−	NR
Production of H_2S	−	NR
Methyl red test	−	−
Voges–Proskauer test	−	−
Simmon's citrate	−	NR
Valin utilization	−	NR
NO_3 reduced to NO_2	+	+
NO_2 reduction	−	−
Growth at KCN	+	NR
Indole production	−	−
Acid–gas production from carbonhydrates (Glucose, Melibiose, Maltose, Arabinose, Inositol, Rhamnose, Fructose, Sucrose, Mannitol, Sorbitol, Xylose, Sorbose, Mannose, Galactose, Dulcitol, Trehalose, Salicin, Dextrin, Inulin, Glycogen, Erythritol, Raffinose, and Adonitol)	−/−	NR

[a] = Holt et al. (1994).

Clinical Signs of Infected Fish

The clinical observations for the three treatments were as follows: fin rot; darkened or faded surface pigment; ulcerated lesions on skin; deformation of the upper jaw; pale and hyperemic foci in the gill filaments; pale and yellow color with hyperemic, hemorrhagic, and necrotic areas in liver; bloody and watery inflammation and swelling of kidney; hemorrhages in muscle; and hemorrhages and bloody fluid in the intestine. Elongated spleens were present in the albino crosses and HBW rainbow trout, whereas watery spleens were observed in LBW rainbow trout and in some albino crosses; hemorrhages were observed in the spleens of LBW fish. The clinical signs of experimental infections such as exophthalmia, liver damage, bloody kidneys, hemorrhagic hearts, and swollen intestines were all similar to signs found in natural and experimental *C. cryaerophila* infections (Aydin et al. 2000). Degeneration of the jaw and fins, observed in these experimentally infected fish and in previous reports (Aydin et al. 2000), were not observed in natural infections.

Blood Analysis

Blood analysis was conducted on 10 healthy (noninfected) HBW rainbow trout, 10 healthy albino crosses, 10 infected albino crosses, and 8 in-

TABLE 2.—Nonparametric analysis of variance of blood characteristics of healthy and experimentally infected fish. Blood samples of low-body-weight rainbow trout were not analyzed because of hemolysis. Parenthetical values are ranges. Values along a row with asterisks were significantly different from other values in row ($P < 0.05$).

Test[a]	Infected albino crosses rainbow trout ($N = 10$)	Infected high-body-weight rainbow trout ($N = 8$)	Healthy rainbow trout ($N = 10$)	Healthy albino crosses rainbow trout ($N = 10$)
CRE (mg/dL)	0.44 (0.00–0.88)	0.62 (0.24–0.70)	0.20 (0.20–0.40)	0.28 (0.25–0.44)
TP (g/dL)	2.70 (1.80–4.40)	3.80 (0.00–5.20)	4.10 (3.20–4.40)	3.86 (1.80–4.10)
ALB (g/dL)	1.65 (1.00–3.10)	1.55 (0.00–2.30)	1.80 (0.20–2.10)	1.68 (0.90-1.80)
Ht (%)	23.00* (13.00–36.00)	33.50 (10.00–43.00)	42.00 (30.50–51.00)	24.50* (12.00–34.00)

[a] CRE = creatinine, TP = total protein, ALB = albumin, and Ht (%) = hematocrit.

fected HBW rainbow trout. The purpose of this examination was to determine the possible characteristic alterations of blood parameters in response to experimental infections with this pathogen.

Total protein levels.—Total protein values in serum of the infected albino crosses were lower than that of noninfected albino crosses but the difference was not statistically significant ($P > 0.05$; Table 2). Also, the TP of experimentally infected rainbow trout was less than that of healthy fish but, again, the difference was not statistically significant. Relatively large statistical variations were determined in both the infected rainbow trout and albino crosses groups. The mean serum TP value of healthy fish was within the normal limits given for rainbow trout (Shimma et al. 1981, 1982, 1984; Goss and Wood 1988; Schipper et al. 1994; Wang et al. 1994; Jeon et al. 1995; Hrubec and Smith 1999).

Albumin values.—Serum albumin concentrations of both the infected albino crosses and rainbow trout groups were less than in healthy rainbow trout (Table 2) but not significantly so. The mean serum ALB concentrations in fish groups were within the normal limits indicated for rainbow trout (Jeon et al. 1995; Hrubec and Smith 1999).

Creatinine values.—The creatinine concentrations in both the infected albino crosses and the rainbow trout groups were greater than that of healthy fish groups (Table 2) although not significantly so. These values were within the normal limits for blood CRE in rainbow trout (Shimma et al. 1981, 1982, 1984; Hrubec and Smith 1999). Nelson et al. (1999) reported that copper-induced gill proliferation and gentamycin-induced renal tubular injury did not affect serum CRE concentrations in goldfish. No information is currently available in the literature regarding the effect of bacterial infections on CRE values.

Hematocrit.—Mean Ht values in infected albino crosses ($P < 0.01$) and infected HBW rainbow trout ($P < 0.05$) were significantly less than in noninfected rainbow trout (Table 2). However, the differences between the Ht levels observed in the two infected fish groups and the noninfected albino crosses were not significant. Significant decreases in blood Ht of infected HBW rainbow trout may be a consequence of bacterially mediated hemolysis. The lower Ht might be expected in infected HBW rainbow trout, as has been reported with other bacterial infections (Eiras and Saraiva 1986; Martinez et al. 1994; Rodger and Richards 1998) and parasitic infections (Yokoyama et al. 1996). Wide ranges of variation in the blood Ht values of experimentally infected fish groups may also originate from the effect of infections. Hematological changes within fish species have been attributed to variations in age, season, genetic strain, physiological status (Rodger and Richards 1998), toxicants, nutritional factors (Lygren et al. 1999), water quality and temperature, stressors (Eiras and Saraiva 1986; Martinez et al. 1994), anoxic conditions, other environmental factors, and altitude (Val et al. 1998).

In conclusion, these results demonstrate that *A. cryaerophilus* could be a significant pathogen for rainbow trout. Mortality rates did not differ significantly between the albino crosses and the LBW and HBW rainbow trout groups infected with *A. cryaerophilus*. The most discernible symptoms of experimental infections were exophthalmia, pale and hemorrhagic liver, bloody kidney, elongated and watery spleen, hemorrhagic heart, swollen intestine, and decreased Ht.

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