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# *Arcobacter cryaerophilus* Infection in Cross Albino Rainbow Trout and Rainbow Trout (*Oncorhynchus mykiss* Walbaum): Bacteriology, Gross Pathology and Clinical Pathology

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**Abstract:** *Arcobacter cryaerophilus* was isolates from naturally infected rainbow trout, (*Oncorhynchus mykiss* Walbaum) and its pathogenicity was tested by intramuscular injection into healthy 1-year-old normally pigmented rainbow trout and albino crosses. Experimental infections caused deaths with gross clinical abnormalities such as exophtahlmia, liver damage, bloody hemorrhagic kidney and heart and swollen intestines. No significant differences in deaths were observed between the two infected fish groups. Significant increases were observed in the serum glutamate pyruvate transaminase (GPT) level of both the experimentally infected albino crosses and rainbow trout groups. The mean serum glutamate oxalacetate transminase (GOT) value in blood of infected rainbow trout was significantly higher than in healthy fish, but GOT activity in blood of experimentally infected albino crosses was significantly less than that of healthy fish. Glucose (GLC) levels in blood of both experimentally infected albino crosses and rainbow trout were significantly less than in healthy fish. Cholesterol (CHOL) concentrations in serum were not significantly different among the four treatments. Serum triglyceride (TG) concentration in blood of experimentally infected normally pigmented rainbow trout group was significantly higher than in healthy fish.

**Key words:** Disease, *Arcobacter cryaerophilus*, rainbow trout, albino crosses, GOT, GPT, glucose, cholesterol, triglyceride

## Introduction

Expansion of aquaculture worldwide has led to increased isolation of previously underfined bacteria in fish. Confinement of fish in culture conditions, movement of fish throughout the world, increase interest in pathogens of fish, advanced technology available to detect and characterize bacteria have led to new discoveries of fish pathogens.

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 *Comphylobacter cryaerophila* as a new species in the genus Campylobacter and it was included in the subsequent publication of Bergey's Manual of Systematic Bacteriology (Holt *et al*., 1994). The taxonomic status of this bacterium was later revised to the newly designated genus and species *Arcobacter cryaerophilus* (Vandamme *et al*., 1991). *A. cryaerophilus* exists in the normal microbial flora of freshwater fish and it is considered a pathogenic bacterium for rainbow trout (Aydin *et al*., 2000 and 2002).

In this study we used the blood parameters of fish as an indicator of their physiological state. The study of these parameters has become widespread in the identification of pathologies associated with infectious diseases (Grizzle and Kiryu, 1993; Nakano *et al*., 1995; Edsall, 1999 and Aydin *et al*., 2000, 2001 and 2002), nutritional deficits (Studnicka and Siwichi, 1986 and Nakano *et al*., 1995) physiological activity (Chen *et al*., 2002), toxicity (Everall *et al*., 1991 and 1992, Mughal *et al*., 1993; Ahmad *et al*., 1995; Shakoori *et al*., 1996; Adham *et al*., 2002; Pepeljnjak *et al*., 2002 and Young and Chen, 2003), anoxic conditions and the other environmental factors (Hrubec and Smith, 1999 and Atamanalp and Kele, 2003) in fish farming. We, therefore, investigated the effects of A. cryaerophilus on blood parameters of experimentally infected rainbow trout and on albino crosses of the same age. 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 they albino crosses are superior to normally pigmented rainbow trout with respect to resistance to bacterial infections.

## Materials and Methods

**Isolation and Identification of Bacteria:** The bacteria were originally isolated from naturally infected rainbow trout (*Oncorhynchus mykiss* Walbaum) at various time during the spring and summer in 1997, 1998 and 1999 at five fish farms in the vicinity of Bahkesir (Gone) and Canakkale in the Marmara region in western 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 blood fluid of naturally infected fish and streaked on enriched tryptic soya (TS) agar, Baird-Parker agar, Yersinia-selective agar, thiosulphate citrate bile salt (TCBS) agar, *Salmonella-Schigella* (SS) agar, *Pseudomonas*

*Aeromonas* selective (GSP) agar, Kligler (KG) agar, MacConkey agar and *Campylobacter* selective agar. Cultures were incubated at 25°C for 1-7 days.
Organ samples passaged on *Campylobacter* selective agar were incubated at 25°C and 30°C for 48h 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 *Camphlobacter* 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 and Austin and Austin, 1999). All of the bacteriological media used in this research was from Merck (Merck, Darmstadt, Germany).

**Infection Experiments:** In all 30 1-year-old fish average body weights of 120.14 ± 21.04g for $F_1$ albino crosses and 105.10 ± 14.34g for normally pigmented rainbow trout, were obtained from a farm in western Turkey. Four different 350 L concrete basins supplied with circulating freshwater (16 ± 1°C) under continuous aeration were used in these studies. The of the rainbow trout and 10 or 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 pre 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 $5x10^5$ live cells into the muscle proximal to the dorsal fin. The remaining 10 fish in both albino crosses and normally pigmented rainbow trout groups were inoculated with sterile phosphate buffered saline (PBS) and served as non-infected control fish.

**Clinical Examination:** During both the natural and experimental infection periods, behaviours of the diseased fish as well as their gross external and internal signs were recorded.

**Sampling and Analytical Procedures of Blood:** Blood analyses were conducted to compare blood parameters of fish from each experimental group 1) non-infected healthy 10 normally pigmented rainbow trout, 2) non-infected healthy 10 albino cross rainbow trout, 3) experimentally infected 10 albino crosses and 4) experimentally infected 8 rainbow trout.
As the fish become moribund (except healthy fish), they were weighed, and 3 mL of blood was drawn from each by caudal vein puncture and immediately transferred into individual silicone-coated Vacutainer Tubes (Becton Dickinson). Blood in tubes was centrifuged promptly at 3,100-x gravity for 10 min and serum was removed with a disposable transfer pipette. Concentrations of glucose (GLC), cholesterol (CHOL) and triglyceride (TG) were dtermined calorimetrical by using Spinreat kits and measuring absorbance at 492 nm with an AWARNES-State Fax 1904 spectrophotometer. Glutamate oxalacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) enzyme were determined by using Spinreact kits at 540 nm wavelengthy.

**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 for the Bacteria:** After analysis of the characteristics listed in Table 1, all the isolates obtained from affected farms were identified as *Arcobacter cryaerophilus*, based the revised taxonomic status of this bacterium 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 normally pigmented rainbow trout infected with *A. cryaerophilus* died 5-23 d after inoculation (80% mortality). All of the albino crosses died between 3-17 d after the inoculation (100% mortality). In contrast, no clinical signs or deaths were observed in the healthy fish injected with sterile PBS for

Table 1: Biological and biochemical characteristics of bacteria (Arcobacter cryaerophilus) isolated from diseased rainbow trout (plus sign = positive, minus sign = negative, NR = not reported)

| Characteristic | Response | |
|---|---|---|
| | Isolates | References* |
| 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 6% NaCl | - | - |
| Growth at 3% NaCl | - | - |
| Ureaase, alkaline phosphatase, arginine dihydrolase | - | - |
| Gelatin, esculin and starch hydrolysis | - | - |
| Degradation 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 |
| Iondole production | - | - |
| Acid/gas production from (glucose, arabinose, inositol, maltose, erythritol, fructose, rhamnose, sucrose, sorbitol, sorbose, mannose, adonitol, galactose, dulcitol, salicin, trehalose, xylose, dextrin, inulin, glycogen, raffinose) | - | NR |

* = Holt *et al.*, (1994)

1 month after the inoculation. Moribund stages for albino crosses were shorter than for the other group. Cumulative deaths were not significantly different ($P<0.05$) between the two infection treatments and this was similar to in previous report (Aydin *et al.*, 2002).

Clinical observations for the two upper jaw; pale and hyperemic foci in the gill filaments; pale and yellow colour with hyperemic, hemorrhagic and necrotic areas in liver; bloody and watery inflammation and swelling of kidney; hemorrhages in muscle; hemorrhages and bloody fluid in intestine. Watery spleens were observed in infected fish as in previous report (Aydin *et al.*, 2002) Elongated spleens, observed in pervious report (Adydin *et al.*, 2002), were not present in these experimentally infected fish. The clinical signs of experimental infections such as exophthalmia, damage of liver, bloody kidney and hemorrhagic heart and swollen intestine in rainbow trout were similar to signs

Table 2: Nonparametric analysis of variance of blood characteristics of healthy and experimentally infected fish groups (minimum and maximum values in parenthesis)

| Test | Infected cross albino rainbow trout[1] (n = 10) | Healthy cross albino rainbow trout (n = 10) | Infected rainbow trout[1] (n = 8) | Healthy rainbow trout (n = 10) |
|---|---|---|---|---|
| GPT (u/1) | 83.7* (11-587) | 28.86 (13-51) | 120.7* (20-207) | 28.5 (5-107) |
| GOT (u/l) | 60.5* (10-199) | 357.55 (207-570) | 23.6* (8-48) | 7.2 (3-9) |
| GLC (mg/dl) | 2.9*** (0-7) | 42.54 (25-73) | 0.94*** (0-3) | 58.60 (46-102) |
| CHOL (mg/dl) | 91 (39-113) | 116.11 (82-295) | 92 (28-134) | 73 (18-126) |
| TG (mg/dl) | 94.5 (45-188) | 89.33 (59-233) | 131 (51-770) | 42.5* (14-180) |

1 = in the moribund stage n = number of samples examined
* = $p<0.05$ ** = $p<0.01$ *** = $p<0.001$

found natural and experimental A. cryaerophilus infections (Aydin *et al.*, 2000 and 2002). Degenerations of jaw and fins, observed in these experimentally infected fish and in previous reports (Aydin *et al.*, 2000 and 2002), were not observed in natural infections (Aydin *et al.*, 2000)

**Blood Analysis:** Blood analysis was conducted on 10 healthy (non-infected) normally pigmented rainbow trout, 10 healthy albino crosses, 10 infected albino crosses and 8 infected normally pigmented rainbow trout were significantly greater ($P>0.05$) than in healthy rainbow trout (Table 2). Significant differences ($p<0.05$) in the serum GOT levels were observed among four treatments (Table 2). Mean GPT concentration in infected albino crosses and infected rainbow trout were significantly higher than in healthy albino croses and healthy rainbow trout ($p<0.05$), However, the differences between the GPT levels observed the two experimentally infected fish groups and two healthy fish groups were not significant (Table 2). Significantly increases and decreases and relatively large statistical variations in the serum enzymes levels of infected albino crosses and rainbow trout groups may originate from effect of infection. Although the serum GOT and GPT values may decrease in chronic stress (Nakano *et al.*, 1995; Jeney *et al.*, 1996), *A. cryaerophilus* infection (Aydin *et al.*, 2000) and toxic effects of pollution (Mughal *et al.*, 1993); Ahmad *et al.*, 1995; Shakoori *et al.*, 1996 and Adham *et al.*, 2002), it can also dramatically increase with acute infectiosn (Studnicka and Siwicki, 1986), mycotoxin (Pepeljnjak *et al.*, 2002), hematotoxic effects (Everall *et al.*, 1991 and 1992) motile *Aeromonas* septicaemia (Grizzle and Kiryu, 1993) and *Serratia liquefaciens* infection (Aydin *et al.*, 2001). The enzyme-abundant tissues contribute to the aspect of the circulating enzyme pattern in the serum. When damage occurs in enzyme - abundant tissue, some enzymes leak from injured cells and the activities of serum enzymes will change. In this study, the increases in serum GOT and GPT activities in the three experimental infection groups might be an indication of considerable clinical damage caused by infections in the liver. This is because GOT, GPT and LDH activities in fish serum are known to be very useful as an index for diagnosis of liver function and metabolic defects of muscle (Everall *et al.*, 1991, 1992; Nakano *et al.*, 1995; Hrubec and Smith, 1999 and Adham *et al.*, 2002).

**Glucose Values:** Mean GLC levels in both experimentally infected albino crosses and infected normally pigmented rainbow trout were significantly less ($P<0.001$) than in noninfected albino crosses and rainbow trout (Table 2). However, the differences between the GLC concentrations observed the two infected fish groups and the two noninfected fish groups were not significant (Table 2). The low GLC levels in experimentally infected fish groups may come from hypoglycemia due to the increase or decrease in activities of liver enzymes under stress given by the infections. This theory may be supported by the results of serum enzyme analyses showing the significant increases in GOT level of serum of infected rainbow trout group and in GPT values of serum of both infected albino crosses and rainbow trout group and a significant decrease in GOT level of albino crosses group. Also, the decreases in blood GLC may account for degeneration of muscular tissue. The blood GLC levels of healthy fish groups show no disagreement with the literature values (Goss *et al.*, 1988; Pagnotta *et al.*, 1996; Van Raaij *et al.*, 1996; Wood *et al.*, 1996a h; Haman and Weber, 1996; Haman *et al.*, 1997; Aydin *et al.*, 2000 and 2001). 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; Aydin *et al.*, 2000 and 2001 and Yang and Che, 2003).

**Cholesterol Levels:** CHOL values in serum of the infected albino crosses were less than that of noninfected albino crosses but the difference was not statistically significant ($p>0.05$; Table 2). The CHOL of the experimentally infectd rainbow trout was higher than that of healthy fish but again the difference was not statistically significant (Table 2). Although the serum cholesterol values may significantly decrease with natural and experimental infections of A. cryaerophilus (Aydin *et al.*, 2000) and effects of toxic agents (Everall *et al.*, 1991, 1992; Mughal *et al.*, 1993), it can also increase with *Serratia liquefac* infection (Aydin *et al.*, 2001), nephrocalcinosis (Chen *et al.*, 2002) and toxicity of heavy metals (Yang and Chen, 2003). Researchers have stated that the blood CHOL of

healthy rainbow trout can also show considerable variation (Jeon *et al.*, 1995a, b; Hrubec and Smith, 1999; Aydin *et al.*, 2001 and Atamanalp and Kele, 2003).

**Triglyceride Values:** TG concentrations of both experimentally infected albino crosses and rainbow trout groups were significantly greater ($P<0.05$) than in healthy rainbow trout (Table 2). However, the differences between the TG levels observed the two infected fish groups and the noninfected albino crosses were not significant (Table 2) *A. cryaerophilus* infection could cause the increase in serum TG values of rainbow trout. The serum TG concentrations may significantly decrease in natural and experimental infections of *A. cryaerophilus* (Aydin et al., 2002). However, high levels of TG can occur with infections (Aydin *et al.*, 2001 ; and Yang and Chen, 2003) and toxicity of heavy metals (Yang and Chen, 2003). It is known that CHOL, TG and lipoprotein values are relatea with one another and these are connected with the metabolism of lipids and functions of the liver and kidney (Aydin *et al.*, 2000, 2001 and Yan and Che, 2003), but further studies are needed to explain these biochemical characteristics in fish.

In conclusion, these results demonstrated that *A. cryaerophilus* could be a significant pathogen for rainbow trout. Mortality rates did not differ significantly between the albino crosses and normally pigmented rainbow trout groups infected with *A. cryaerophilus*. The most discernible signs of experimental infections were exopthalmia, pale and hemorrhagic liver, bloody kidney, watery spleen, hemorrhagic heart and swollen intestine. Another point of this study is that *A. cryaerophilus* infections could cause the decrease in GOT live in serum of albino crosses and in GLC values in blood of both albino crosses and rainbow trout while GPT level in two infected groups and GPT value in infected rainbow trout showed significantly increase from that of healthy fish.

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