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Treatment Methods of *Flavobacterium psychrophilum*: Cause of Rainbow Trout Fry Syndrome (RFTS) and Bacterial Cold-Water Disease (BCWD) in Turkey

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Abstract: Disease caused by the bacterium *Flavobacterium psychrophilum* in fry, fingerling and juvenile fish, were investigated in rainbow trout farm from 2000 to 2006 in Turkey. During the infection period, behaviors of the sick fish and mortalities were recorded. Infected fish samples were described using their gross external and internal symptoms and were examined by bacteriological investigations of some organs. In order to cure infected fish, seven different cures were applied in seven different periods. Best treatment cure was performed 6% mortalities with H_2O_2 (20 mL t^{-1} 0.5 h^{-1} bath) and florfenicol (15 mg kg^{-1} day^{-1} for 7 days orally).

Key words: *Flavobacterium psychrophilum*, rainbow trout fry syndrome, Bacterial cold-water disease, treatment

INTRODUCTION

During the last decade, disease caused by the bacterium *Flavobacterium psychrophilum* has been one of the most serious problems in fish culture and rainbow trout hatchery all over the world. *Flavobacterium psychrophilum* was first isolated in 1948 in the U.S.A.[1]. *F. psychrophilum*'s proclivity to induce epizootics at low water temperatures, the disease became known as Bacterial Cold-Water Disease (BCWD) or Low-Temperature Disease[2,3]. This disease is referred to as Rainbow Trout Fry Syndrome[4,5], Fry Mortality Syndrome[6] or visceral myxobacteriosis[7] but this disease caused by *F. psychrophilum* is known as Cold-Water Disease (CWD) in Turkey because of its occurrence in low water temperatures, often below 10°C. In feeding fry suffering from BCWD the mortality is lower, often around 20%. Fry and fingerlings with BCWD often have skin ulcerations on the peduncle, anterior to the dorsal fin, at the anus, or on the lower jaw[8]. Muscle lesions can also occur[8,9]. RTFS usually occurs during the first two months of feeding[4] and mortalities up to 70% have been reported[4,10]. Histopathological changes due to acute *F. psychrophilum* infections are similar in BCWD and RTFS. Infected fish suffer from an acute septicaemic infection with bacteria present in almost all organs[4,10,11].

This bacterium is capable of causing severe losses to rainbow trout hatchery. BCWD may cause losses of up to from 20[8] to 70%[4,10].

For the first time, *Flavobacterium psychrophilum* was isolated in 2000 in the Turkey but not reported. Nowadays, disease caused by the bacterium *Flavobacterium psychrophilum* is firstly problem happening in hatcheries. Some hatcheries has been controlling to this disease with basic sanitation and hygiene plans and also good stock management.

The aims of the present study were to determine what factors cause disease and to treatment and control of disease.

MATERIALS AND METHODS

Disease caused by the bacterium *Flavobacterium psychrophilum* in fry, fingerling and juvenile fish, were investigated in rainbow trout farm in 2000-2006. During the infection period, behaviors of the sick fish and mortalities were recorded. Infected fish samples were described using their gross external and internal symptoms and were examined by bacteriological investigations of some organs.

For the identification of samples of *F. psychrophilum* has been Cytophaga Agar (CA)[12] composed of tryptone, yeast extract, beef extract and sodium acetate. Identification of isolated *F. psychrophilum* is routinely made through morphological, biochemical and physiological characteristics[13].

In order to cure infected fish, chloramine-T (20 g t^{-1} 0.5 h^{-1} bath) and oxytetracycline (75 mg kg^{-1} day^{-1} for 10

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days orally)[14,15] for the first period infected group, chloramine-T (20 g t^{-1} 0.5 h^{-1} bath) and enrofloxacin (baytril) (50 mg kg^{-1} day^{-1} for 10 days orally) for the second period infected group, formaline (20 mL t^{-1} 0.5 h^{-1} bath) and oxytetracycline (75 mg kg^{-1} day^{-1} for 10 days orally) for the third period infected group, formaline (20 mL t^{-1} 0.5 h^{-1} bath) and enrofloxacin (50 mg kg^{-1} day^{-1} for 10 days orally) for the fourth period infected group and hydrogen peroxide (H_2O_2) (20 mL t^{-1} 0.5 h^{-1} bath) and oxytetracycline (75 mg kg^{-1} day^{-1} for 10 days orally) for the fifth period infected group, H_2O_2 (20 mL t^{-1} 0.5 h^{-1} bath) and enrofloxacin (50 mg kg^{-1} day^{-1} for 10 days orally) for the sixth period infected group, H_2O_2 (20 mL t^{-1} 0.5 h^{-1} bath) and florfenicol (15 mg kg^{-1} day^{-1} for 7 days orally) for the seventh period infected group were applied. Disinfection of eggs, hydrogen peroxide (75 mL^{-1} t^{-1} 20 m^{-1}) was applied to all periods for the prevention of disease.

RESULTS AND DISCUSSION

Clinical signs of disease were anorexia, lethargy and dark pigmentation of the skin, ascites and exophthalmia. At necropsy, an enlarged spleen, pale gills, liver and kidney, as well as a hemorrhagic protruding anus were typical findings. Diseased fingerlings and larger fish usually exhibit skin ulcerations, vertebral deformations have been described.

Mortalities rates were 20% in first period group, 17% in second period group, 19% in third period group, 21% in fourth period group, 13% in fifth period group, 9% in sixth period group and 6% in seventh period group. Using chemotherapeutics and cure were given Table 1.

Clinical signs are the same other report to this infection. Typical clinical signs of bacterial coldwater disease include lethargy, dorsal skin erosion, ascites (accumulation of fluid in the peritoneal cavity), bilateral exophthalmia, pale gills and hemorrhagic vent with trailing mucoid casts. It often causes death if untreated[4,7,10,16-20]. Signs associated with neurological disruptions can also occur, including whirling behavior around the longitudinal axis, post-cephalic protrusion of the cranium, spinal deformities and loss of melanocyte control in the posterior body, which is also known as black-tail[16,21]. Concurrent problems, such as infestation with parasites, are common[22].

Several authors have advised that different chemotherapeutics and different doses for treatment to RFTS. Researchers reported that, in early stages of external infections, bath treatments with either water

Table 1: Treatment cures, using chemotherapeutics and mortality rate

Cures	I	II	III	IV	V	VI	VII
Chloramine T[A]	X	X					
Formaline[B]			X	X			
H_2O_2[C]					X	X	X
Oxytetracycline[D]	X		X		X		
Enrofloxacin[E]		X		X		X	
Florfenicol[F]							X
Mortality (%)	20	17	19	21	13	9	6

A = 20 g t^{-1} 0.5 h^{-1} bath; B = 20 mL t^{-1} 0.5 h^{-1} bath; C = 20 mL t^{-1} 0.5 h^{-1} bath; D = 75 mg kg^{-1} day^{-1} for 10 days orally; E = 50 mg kg^{-1} day^{-1} for 10 days orally; F = 15 mg kg^{-1} day^{-1} for 7 days orally

soluble oxytetracycline at 10-50 mg L^{-1} or quaternary ammonium compounds at 2 mg L^{-1} have been recommended[14, 23]. Sulfisoxazole was effective against *F. psychrophilum* among feeding fry when administered either as a therapeutic treatment at 220 mg kg^{-1} day^{-1} for 10 days or as a prophylactic therapy offered at 88 mg kg^{-1} day^{-1} for 26 days[24,25]. Sulfamethazine was also effective when administered at concentrations from 220-440 mg kg^{-1} day^{-1} in starter diets and at concentrations of 110 mg kg^{-1} day^{-1} in pelleted feeds[15].

In Europe, RTFS has been successfully controlled using oxytetracycline at 75-300 mg kg^{-1} day^{-1} for 10-14 days amoxicillin at 80-100 mg kg^{-1} day^{-1} for 7 days; and florfenicol at 10 mg kg^{-1} day^{-1} for 10 days[26-29].

Incorporation of the antibiotic oxytetracycline into fish food was traditionally the most common form of treatment for bacterial coldwater disease. The drug is usually effective in hatcheries experiencing the condition for the first time, but resistance towards this drug is developing[26]. Nevertheless, oxytetracycline remains the treatment of choice in North America. Amoxicillin is now commonly used in Europe, but resistance to it is also increasing[22]. Increasing incidence of resistant bacteria and recurrent outbreaks of disease shortly after a treatment continues to be problems and no alternative treatments are currently available[30].

Researchers reported that, oral antibiotics do not always provide satisfactory control against *F. psychrophilum* because the most severe epizootics often occur among fry before they have begun to feed or within a few weeks of when the fry were placed in ponds. Often when water temperatures are very low it is difficult to achieve a therapeutic dose in fish and treatments must be repeated. In order to be effective, fish culture personnel should diligently monitor their stocks for any behavioral or clinical signs of disease to ensure that treatments are initiated early in the disease process; - before fish become in appetent and refuse to feed[3].

In the present study, the treatment of RFTS, which is disease caused by the bacterium *Flavobacterium psychrophilum* in fry, fingerling and juvenile fish, was using seven different cures in seven

different periods. Oxytetracycline, enrofloxacin, formaline and chloramine-T have been using to fish disease control and treatment for a long time in Turkey. For this reason, *F. psychrophilum* may be improved resistant to these antibiotics and chemotherapeutics. *F. psychrophilum* isolates divided in susceptible and resistant clusters reflecting the reduced efficacy in practice when using oxtetracycline and amoxicillin[31]. The most recent isolates were less susceptible to amoxicillin and oxolonic acid, whereas resistance to oxtetracycline seemed stable over the last 5 years. Apparently, *F. psychrophilum* carries intrinsic resistance towards the potentiated sulfonamides and in correlation with this, they found very few susceptible isolates. All isolates were susceptible to florfenicol. In Turkey, hydrogen peroxide was became using one years ago, which is very effective for prevention *F. psychrophilum* in hatcheries. In this study, best treatment performed with H_2O_2 (20 mL t^{-1} 0.5 h^{-1} bath) and florfenicol (15 mg kg^{-1} day^{-1} for 7 days orally) in seventh period.

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