Journal of Animal and Veterinary Advances 10 (8): 1019-1023, 2011
ISSN: 1680-5593

Mix Infections in Rainbow Trout (*Oncorhynchus mykiss Walbaum*): *Lactococcus garvieae* and *Vibrio anguillarum* O1

[1]T. Tansel Tanrikul and [2]Necdet Gultepe
[1]Faculty of Fisheries, Ege University, 35440 Urla, Izmir, Turkey
[2]Faculty of Fisheries, Canakkale Onsekiz Mart University, Canakkale, Turkey

Abstract: *Vibrio anguillarum* and *Lactococcus garvieae* which causes mixed infections were isolated for the first time from rainbow trout farms in the Southwest of Turkey. The outbreak occurred at 15°C water temperature in June 2009. The diseased fish showed erratic swimming, dark discoloration, bilateral exopthalmia, haemorhages on the fins, operculum, the liver, spleen and lower intestines with mortality ranging from 30-50% in the pools. The identities of the isolates were confirmed as *V. anguillarum* O1 and *L. garvieae* by biochemical and serological tests using API 20 Strep, API 20 E and API Strept system test strips and also *V. anguillarum* (ATTC 43305) used as antigens for agglutination. The epizootic disease was effectively controlled by oral administration of enrofloxacin.

Key words: *Lactococcus garvieae*, *Vibrio anguillarum*, mix infection, rainbow trout, strept system, antigens

INTRODUCTION

Lactococcus garvieae (*L. garvieae*) is the species of clinical significance in humans and animals. The original description of *L. garvieae* dates back to 1984 when it was first isolated from a mastitic udder of a cow in the United Kingdom (Vela *et al*., 2000). On the other hand, regardless of its limited importance as a mastitogenic agent, *L. garvieae* turned out to be the most important risk factor in the Mediterranean sea because it causes specific losses approximately 50% in the total production of in European trout industry (Ghittino and Prearo, 1992).

Prieta *et al*. (1993) reported *L. garvieae* as a fish pathogen in Europe. Bacteriologic and molecular studies confirmed *L. garvieae* as the etiological agent of a hemorrhagic septicemia in cultured trout characterized by bilateral exophthalmia, darkening of the skin, congestion of the intestine, liver, kidney, spleen and brain and by characteristic hemorrhagic enteritis (Domenech *et al*., 1993). In 1991, a new enterococcal species *Enterococcus seriolicida* was described as a new pathogen responsible for an infection of eels and yellowtail with symptoms identical to those caused by *L. garvieae* in trout (Rodkhum *et al*., 2005).

In Japan, fish diseases due to gram positive cocci were described as *Enterococcus seriolicida* during the 1970. Indeed, 16S rRNA sequencing and DNA hybridization studies confirmed that *L. garvieae* and *E. seriolicida* are the same species (Domenech *et al*., 1993). *Vibrio anguillarum* is one of the most important pathogens in fish of several countries and in many of fish species. Primary identification of *V. anguillarum* can be performed by using biochemical criteria such as those described by Baumann and Schubert (1994). The *V. anguillarum* usually attaches to the segments of intestine in rainbow trout and turbot (Olsson *et al*., 1996; Spanggaard *et al*., 2000).

The gut has also been reported to be the initial site of the infection in turbot larvae (Grisez *et al*., 1996). Vibriosis affects some fish species such as turbot (*Scophthalmus maximus*), pacific salmon (*Oncorhynchus kisutch*), atlantic salmon (*Salmo salar*), rainbow trout (*Oncorhynchus mykiss*), sea bass (*Dicentrarchus labrax*), striped bass (*Morone saxatilis*), cod (*Gadus morhua*), Japanese and European eel (*Anguilla japonica* and *Anguilla anguilla*), yellowtail (*Seriola quinqueradiata*) and ayu (*Plecoglosus altivelis*) (Rodkhum *et al*., 2005). The aim of the present study was to examine the mixed infections accounted for by *Lactococcus garvieae* and *Vibrio anguillarum* O1 in rainbow trout.

MATERIALS AND METHODS

Sampling: The outbreak was first observed in a rainbow trout fish farm in the Southwest of Turkey in June 2009. During the infection period, behaviors of the sick fish and mortalities were recorded.

A total of 20 moribund fish of 80-200 g were collected from the farm and transported to the laboratory of fish diseases in Faculty of Fisheries, Ege University. Infected

Corresponding Author: T. Tansel Tanrikul, Faculty of Fisheries, Ege University, 35440 Urla, Izmir, Turkey

fish samples were described by their gross external and internal symptoms and examined by bacteriological investigations of some organs.

Isolation and identification of bacteria: Samples of spleen, kidney and liver were subjected to microbiological examination by inoculation on trypticase soy agar (Difco) supplemented with 5% defibrinated sheep blood (BTSA) and incubated at 21°C for 48 h. Pure cultures of the different colony types were obtained on BTSA.

A presumptive identification of the strains was performed by Gram staining, catalase, oxidase activity, motility and resistance to O/129 vibriostatic agent. API 20 Strep test and API 50 CH tests (BioMerieux S.A., France) were used to determine biochemical characteristics of *L. garvieae* according to the manufacturer's instruction at incubation temperature of 24±0.5°C. For the biochemical tests of *V. anguillarum*, API 20E system was carried out at 21°C for 48 h. (BioMerieux S.A., France). Slide agglutination test was performed on all isolated strains using raised rabbit serum against *V. anguillarum* O1 (ATTC 43305) as in that of Toranzo *et al.* (1987).

Antimicrobial sensitivity test: All isolates were tested for their sensitivity to 9 antibiotics on Mueller-Hilton agar using the Kirkby-Bauer disc diffusion technique (Stokes *et al.*, 1993). A loopful of a 24 h culture was placed in the center of the petri plate with media and spread with a dry swab.

Antibiotic sensitivity-impregnated discs were then placed on the plates and following incubation at 21°C for 24 h the zones of inhibition were measured.

RESULTS AND DISCUSSION

During the infection period, water temperature was measured as 15±0.5°C. Clinical signs of the disease were erratic swimming, bilateral exophthalmia and hemorrhage in the eyes, skin discoloration, yellowish and reddish necrotic lesions and ulcers in the abdominal muscle, hemorrhage and erosion in pectoral, abdominal and anal fins and also hemorrhage in mouth of some fish. Mortality rates varied from 30-50% in different pools of the farm.

Necropsy showed typical findings such as enlarged spleen, pale gills, liver and kidney as well as a petechial hemorrhage in liver, pyloric caeca, peritoneal and subperitoneal membranes and perivisceral fat in lower intestines. As a result of bacteriological culture from the diseased fish, *L. garvieae* and *V. anguillarum* from the same fish were grown whereas from the some fishes in the farm either *L. garvieae* or *V. anguillarum* were also isolated. Isolated streptococci were described as non-motile Gram (+) cocci in short chains and oxidase and catalase reactions were negative. Growth occurred at 24°C. The API 20 Strept rapid identification system resulted in a profile 5143110. According to the API 20 Strept test and API 50 CH test results, the biochemical characteristics of isolated *L. garvieae* are shown in Table 1 and 2, respectively. API 20 E profile for *V. anguillarum* was 3207726. The morphologic and penotypic characteristics of *V. anguillarum* isolated from the farm are shown in Table 3.

Table 1: The API 50CH profile for *L. garvieae* isolated from farm

Characteristics	Values	Characteristics	Values
Properties	+/-	Properties	+/-
Glycerol	-	Salicin	+
Erythritol	-	D-cellobiose	+
D-arabinose	-	D-maltose	+
L-arabinose	-	D-lactose	+
D-ribose	+	D-melibiose	-
D-xylose	-	D-sacharose	-
L-xylose	-	D-trehalose	+
D-Adonitol	-	Inuline	-
Methy-βD-xylopyranoside	-	D-melezitose	-
D-galactose	+	D-raffinose	-
D-glucose	+	Amidon	-
D-fructose	+	Glycogene	-
D-mannose	+	Xylitol	-
L-sorbose	-	Gentiobiose	+
L-rhamnose	-	D-turanose	-
Dulcitol	-	D-lyxose	-
Inositol	-	D-tagatose	+
D-mannitol	+	D-fucose	-
D-sorbitol	-	L-fucose	-
Methyl-αD-mannopyranoside	-	D-arabitol	-
Methyl-αD-glucopyranoside	-	L-arabitol	-
N-acetyl glucosamine	+	Potasium gluconate	-
Amygdaline	+	Potasium 2-ketogluconate	-
Arbutin	+	Potasium 5-ketogluconate	-
Esculin	+		

+ : Positive; - : Negative

In a slide agglutination tests with anti *V. anguillarum* O1 (ATCC 43305) serum showed that all strains were positive. Antibiyogram tests results for *L. garvieae* and *V. anguillarum* are shown in Table 4. There were parallelism in both *L. garvieae* and *V. anguillarum* in that they revealed identical antimicrobial susceptibility patterns, susceptibility to amoxicillin, ampicillin, enrofloxacin, flumequin, norfloxacin and to oxolonic acid and resistance to erythromycin, oxytetracycline and trimethoprim-sulphamethoxazole (1.25+23.75). Lactococcosis is a kind of disease caused by *L. garvieae* which has been particularly devastating freshwater culture of salmonid fish. The first outbreak of Lactococcosis was in the rainbow trout fish farm in Spain in 1988 (Ghittino and Prearo, 1992). Since then lactococcosis has caused heavy mortality in rainbow

Table 2: The API Strept profile for *L. garvieae* isolated from farm

Properties	Values
VP	+
Hippurate	-
Aesculin	+
Pyrrolidonylarylamidase	+
α-Galactosidase	-
β-Glukoronidase	-
β-Galactosidase	-
Alkaline phosphatase	-
Leucine arylamidase	+
ADH	+
Ribose	+
Arabinose	+
Mannitol	+
Sorbitol	-
Lactose	-
Trehalose	+
Inulin	-
Raffinose	-
Amygdalin	-
Glycogen	-
β-hemolysis	-

+ : Pozitive ; - : Negative

Table 3: The morphologic and biochemical properties for *V. anguillarum* isolated from farm

Properties	Values
Gram stain	-
Motility	+
Catalase	+
Oxidase	+
Growth in 0% NaCl	-
Growth in 7% NaCl	-
Sensitivity to o/129	+
Acid from: O/F	+
ONPG	+
ADH	+
LDH	-
ODC	-
Citrate utilization	+
H_2S	-
Urease	-
TDA	-
Indole	-
VP	+
Gelatin	+
Glucose	+
Mannitol	+
Inositol	+
Sorbitol	+
Rhamnose	-
Sucrose	+
Melibiose	-
Amygdalin	-
Arabinose	+

+ : Positive; - : Negative

trout in Italy, Australia, South Africa, England, France and Israel (Ghittino and Prearo, 1992; Prieta *et al*., 1993). The agent of this disease was first isolated as *Enterococcus seriolicida* from the trout farms in Turkey, 1997 (Cagirgan and Tanrikul, 1995) causing hemorrhagic septicaemia, ascites, bilateral exophthalmus and hemorrhage of the intestine, liver, kidney, spleen and brain. *L. garvieae* led to 80% of mortality in fish of 200±g

Table 4: Antibiotic susceptibility profile of the isolates of *V. anguillarum* and *L. garvieae*

	Isolates	
Antibiotic (μg $disc^{-1}$)	*V. anguillarum*	*L. garvieae*
Amoxycillin (25)	S	S
Ampicillin (10)	S	S
Enrofloxacin (5)	S	S
Flumequin (5)	S	S
Norfloxacin (5)	S	S
Erythromycin (5)	R	R
Oxolinic acid (30)	S	S
Oxytetracycline (30)	R	R
TMP-SDZ (25)*	R	R

*Trimethoprim-sulphadiazine (1/5); S : Sensitive; R : Resistant

at 18°C (Cagirgan and Tanrikul, 1997). Streptococcosis was also diagnosed from *Onchorhynchus mykiss* in Iran (Soltani *et al*., 2005). The affected fish in Iran have been showed to have the same symptoms such as erratic swimming, dark discoloration, haemorrhages on the base of fins and exophthalmia with cataract with fish mortality varying from 20-40%.

Internally, there were haemorrhagic livers, congestion of spleen and kidney and occasionally ascites in the peritoneal cavity. It was reported in Iran that they have the same symptoms as those found by us. However, the isolated pathogen was more similar to the *Streptococcus iniae* than *L. garvieae* (Cagirgan and Tanrikul, 1995; Soltani *et al*., 2005). There is no available information on isolation of *Streptococcus iniae* in Turkey but *L. garvieae* has been isolated rutinely (Domenech *et al*., 1993; Altun *et al*., 2004).

L. garvieae has been causing differents level of mortality in various countries but it has led to mortality of approximately 50% in total production accross Mediterranean of trout industry (Ghittino and Prearo, 1992). Eldar *et al*. (1999) reported the three biotypes of *L. garvieae*. Vela *et al*. (2000) also identified the thirteen biotypes. API profiles of Spanish strains were the similar to Turkish strains (Prieta *et al*., 1993). The present results show that the strains concerned were parallel with those found by Cagirgan (2004).

V. anguillarum is widespread across the world. *V. anguillarum* was isolated from salmon (Winton *et al*., 1983; Muroga, 1975) and from different marine species (Toranzo and Barja, 1990). Tanrikul *et al*. (2005) and Tanrikul (2007) reported that API 20 E strips were used for identification of *V. anguillarum* O1 which was isolated from both cultured sea bass (*Dicentrarchus labrax*) and rainbow trout (*Onchorynchus mykiss*).

According to the API 20 E test results; this isolate was identified as *V. anguillarum* O1 which were similar strains to those by Tanrikul *et al*. (2005) and Tanrikul (2007). So far, natural mixed infection has not been isolated in Turkey. Both *L. garvieae* and *V. anguillarum* O1 were isolated from infected fish.

Clinical signs are the same as those in other reported lactococcosis (Domenech *et al*., 1993; Cagirgan and Tanrikul, 1997, 1995; Salati *et al*., 1996) and vibriosis (Muroga, 1975; Toranzo and Barja, 1990). Antimicrobial susceptibility tests were conducted using from isolates of mix infection. *L. garvieae* and *V. anguillarum* were susceptible to amoxicillin, ampicillin, enrofloxacin, flumequin, norfloxacin and oxolonic acid. Endo *et al*. (1975) and Takahashi *et al*. (1976) proposed oxolonic acid and sulphonamids for vibriosis treatment, respectively. According to the Cagirgan and Tanrikul (1995) erythromycin and oxytetracycline were effective chemotherapeutic agents for streptococcosis.

Generally in Turkey, erythromycin is used against streptococcosis while oxytetracycline is also used against the rainbow trout fry syndrome. However, trimethoprim-sulphamethoxazole is employed against enteric red mouth. For these reasons, effects of some drugs are decreased for these bacteria. In this case, treatment with enrofloxacin was made against these two bacteria strains.

CONCLUSION

In this study, infected fish were treated with enrofloxacin (50 mg/kg/day for 7 days orally) and mortalities were completely controlled at the end of treatment period.

REFERENCES

Altun, S., O. Diler and A.K. Adiloglu, 2004. Genotyping of *Lactococcus garvieae* strains from rainbow trout (*Onchorhynchus mykiss*) by 16s rDNA sequencing. Bull. Eur. Assoc. Fish Pathol., 224: 119-125.

Baumann, P. and R.H.W. Schubert, 1994. Subgoup II Family *Vibrionaceae*. In: Bergey's Manual Systematic Bacteriology, Krieg, N.R. and J.G. Holt (Eds.). Williams and Wilkings, Baltimore, MD. USA., pp: 190-192.

Cagirgan, H. and T.T. Tanrikul, 1995. A new problem *Enterococcus*-like infection in rainbow trout (*Oncorhynchus mykiss*) farms in Turkey. J. Vet. Cont. Res. Inst., 19: 9-19.

Cagirgan, H. and T.T. Tanrikul, 1997. Lactococcosis in rainbow trout farm. Proceedings of the Mediterean Fish Congress, April 9-11, Izmir, Turkey, pp: 371-375.

Cagirgan, H., 2004. Biotyping of *Lactococcus garvieae* isolated from Turkey. EU J. Fish Aquat Sci., 21: 267-269.

Domenech, A., J. Prieta, J.F. Fernandez-Garayzabal, M.D. Collins, D. Jones and L. Dominguez, 1993. Phenotypic and phylogenetic evidence for a close relationship between *Lactococcus garvieae* and *Enterococcus seriolicida*. Microbiologia, 9: 63-68.

Eldar, A., M. Gloria, C. Ghittino, A. Zlotkin and H. Bercovier, 1999. Biodiversity of *Lactococcus garvieae* strains isolated from fish in Europe, Asia and Australia. Applied Environ. Microbiol., 65: 1005-1008.

Endo, T., K. Doi, H. Tanaka and Y. Watanabe, 1975. Effects of oxolinic acid on vibrio disease naturally contracted by rainbow trout. Fish Pathol., 10: 17-21.

Ghittino, C. and M. Prearo, 1992. Report of streptococcosis in rainbow trout (*Oncorhynchus mykiss*) in Italy: Preliminary note. Bull. Italian Soc. Fish Pathol., 8: 4-11.

Grisez, L., M. Chair, P. Sorgeloos and F. Ollevier, 1996. Mode of infection and spread of *Vibrio anguillarum* in turbot *Scophthalmus maximus* larvae after oral challenge through live feed. Dis. Aquat. Org., 26: 181-187.

Muroga, K., 1975. Research on *Vibrio anguillarum* and its infections. J. Fac. Fish. Anim. Husbandry, 14: 101-215.

Olsson, J.C., A. Joborn, A. Westerdahl, L. Blomberg, S. Kjelleberg and P.L. Conway, 1996. Is the turbot, *Scophthalmus maximus* (L.), intestine a portal of entry for the fish pathogen *Vibrio anguillarum*. J. Fish Dis., 19: 225-234.

Prieta, J., A.M. Domenech, J.F. Fernandez-Grayzabal, M.D. Collins and U.M. Rodrigues *et al*., 1993. Lactococcicosis de la trucha arco iris (*Oncorhynchus mykiss*). Med. Vet., 10: 367-373.

Rodkhum, C., I. Hirono, J.H. Crosa and T. Aoki, 2005. Four novel hemolysin genes of *Vibrio* anguillarum and their virulence to rainbow trout. Mic. Pathogenesis, 39: 109-119.

Salati, F., P. Tassi and P. Bronzi, 1996. Isolation of an *Enterococcus* like bacterium from diseased Adriatic sturgeon *Acipenser naccarii*, farmed in Italy. Bull. Eur. Assoc. Fish Pathol., 16: 96-99.

Soltani, M., S.H. Jamshide and I. Sharifpour, 2005. Streptococosis caused by *Streptococcus iniae* in farmed rainbow trout (*Oncorhynchus mykiss*) in Iran: Biophysical characteristics and pathogenesis. Bull. Eur. Assoc. Fish Pathol., 25: 95-1006.

Spanggaard, B., I. Hube, J. Nielsen, T. Nielsen and L. Gram, 2000. Proliferation and location of *Vibrio anguillarum* during infection of rainbow trout, *Oncorhynchus mykiss* (Walbaum). J. Fish Dis., 23: 423-427.

Stokes, E.J., G.L. Ridgway and M.V.D. Wren, 1993. Laboratory Control of Antimicrobial Chemotherapy. In: Clinical Microbiology, Stokes, E.J. (Eds.). 7th Edn., Edward Arnoid, London, pp: 234-280.

Takahashi, T., S. Inoue, K. Hiramoto and K. Miyazawa, 1976. Amounts of indestion and concentrations in tissue resulting from oral administration in *Engraulis japonica* of sulfamonomethoxine. Fish Pathol., 11: 1-4.

Tanrikul, T.T., 2007. Vibriosis as an epizootic disease of rainbow trout (*Onchorynchus mykiss*) in Turkey. Pak. J. Biol. Sci., 10: 1733-1737.

Tanrikul, T.T., H. CagIrgan and E. Toksen, 2005. The use of API 20E for the identification of isolated *Vibrio* sp. from cultured sea bass (*Dicentrarchus labrax* L.). J. Fish. Aquat. Sci., 20: 483-490.

Toranzo, A.E. and J.L. Barja, 1990. A review of the taxonomy and seroepizootiology of *Vibrio anguillarum*, with special reference to aquaculture in the Nortwest of Spain. Dis. Aquat. Org., 9: 73-82.

Toranzo, A.E., A.M. Baya, B.S. Robertson, J.L. Barja, D.J. Grimes and F.M. Hetrick, 1987. Specificity of slide agglutination test for detecting bacterial fish pathogens. Aquaculture, 61: 81-97.

Vela, A.I., C. Vazquez, A. Gibello, M.M. Blanco and M.A. Moreno *et al*., 2000. Phenotypic and genetic characterization of *Lactococcus garvieae* isolated in Spain from Lactococcosis outbreaks and comparison with isolates of other countries and sources. J. Clin. Microbiol., 38: 3791-3795.

Winton, J., J. Rohovec and J. Fryer, 1983. Bacterial and Viral Diseases of Cultured Salmonids in the Pacific Northwest. In: Bacterial and Viral of Fish, Crosa, J.H. (Eds.). Academic Press, Washington Sea Grant, Seattle, USA., pp: 1-20.