

**Bilateral Exophthalmos: The Most Important Sign of Lactococcosis in Rainbow Trout (*Oncorhynchus mykiss*)**

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**Abstract:** In this study, we aimed to investigate rapidly isolation and identification of *Lactococcus garvieae* from rainbow trout (*Oncorhynchus mykiss*), reared Black Sea Region, Turkey. The fish with bilateral exophthalmos were collected from a commercial trout farm. The bacteria were identified as *L. garvieae* based on some biochemical tests and Polymerase Chain Reaction (PCR) assay. Following identification, the antibacterial susceptibility of bacteria was also determined for five different antibiotics by the disk diffusion method. All isolates were sensitive to florfenicol and amoxicillin-clavulanate and resistant to trimethoprim+sulfamethoxazole and enrofloxacin. Overall this study revealed that the bilateral exophthalmos may be considered as a pathognomonic symptom for lactococcosis in rainbow trout.

**Keywords:** Exophthalmos, Fish, Lactococcosis

**Bilateral Eksoftalmi: Gökkuşığı Alabalıklarında (*Oncorhynchus mykiss*) Lactococcosis'in En Önemli Göstergesi**

**Öz:** Bu çalışmada, Karadeniz Bölgesi'nde yetiştirilen Gökkuşığı alabalığından (*Oncorhynchus mykiss*), *Lactococcus garvieae* bakterisinin hızlıca izole ve tanımlanması amaçlanmıştır. Çift taraflı eksoftalmusa sahip alabalıklar ticari bir alabalık işletmesinden toplandı. Bakteriler bazı biyokimyasal testler ve moleküler (PZR) metodlar temelinde *L. garvieae* olarak izole edildi. İsimlendirilmelerini takiben bakterilerin ayrıca beş farklı antibakteriyeye karşı duyarlılıkları disk difüzyon metodu ile belirlendi. Bakteriler florfenikol ve amoksisilin-klavulünik asite karşı duyarlı, trimetoprim sülfametoksazole karşı ise dirençli bulunmuşlardır. Genel olarak bu ve benzeri çalışmalar çift taraflı eksoftalmusun gökkuşığı alabalıklarında Laktokokkozisin patognomik (ayırıcı) semptomu olarak düşünülebileceğini ortaya koymuştur.

**Anahtar sözcükler:** Eksoftalmi, Balık, Laktokokkozis.

**INTRODUCTION**

In recent years, the aquaculture activities have developed in Turkey, in the suitable sea and fresh water environments. Aquaculture industry especially focused on aquatic species such as rainbow trout and sea bass (*Dicentrarchus labrax*). According to the recent data, Turkey produced approximately 107000 tons of rainbow trout in a year (TSI, 2016). The development of fish production and overstocking bring about fish diseases and, as a result, unexpected economic losses. The effective fish health management is the most important issue in aquaculture activities (Kusuda & Kawai, 1998).

*Lactococcus garvieae*, the bacterial pathogen causing lactococcosis was first isolated from yellowtail in Japan (Kusuda, Kawai, Salati, Banner & Fryer, 1991) and

has afterward been found in many areas where salmonids are cultured such as Turkey (Ture, Haliloglu, Altuntas, Boran & Kutlu, 2014). The bacteria causes large economic losses in the aquaculture environment. It was also isolated from many animal species including cows, buffalos, poultry, cat, and dogs (Vendrell, Balcázar, Ruiz-Zarzuola, Gironés & Múzquiz, 2006). It was isolated from humans in some clinical cases. For this reason, it has been considered a potentially zoonotic pathogen (Chan et al., 2011).

*L. garvieae* is extremely virulent for various fish species. Rainbow trout is the most sensitive fish species for lactococcosis (Vendrell et al., 2006). In recent years, *L. garvieae* was one of the most reported bacteria species from farmed trout in Turkey. The bilateral exophthalmia was

typical external signs of affected fish in rainbow trout (Ture & Altinok, 2016).

In this study, we aimed to investigate rapidly isolation and identification of *Lactococcus garvieae* isolated from fish. This study is first to draw attention to exophthalmia in rainbow trout with lactococci.

### CASE REPORT

The study was carried out in late Summer 2017 in a rainbow trout farm located in Black Sea Region of Turkey. The 20 rainbow trout with bilateral exophthalmos were collected. The temperature of the water was 16.5°C during sampling. The weight of fish varied between 12.15-16.24g. Liver and head-kidney samples of fish were aseptically streaked on Tryptic Soy Agar (TSA, Merck) and incubated at 30°C for 24 h under aerobic conditions. The isolates were subcultured on the same media to check the purity of the isolate. The pure colonies were typically characterized by Gram staining and the following biochemical tests: cytochrome oxidase, catalase and motility (Austin & Austin, 2012).

Genomic DNA of bacteria was extracted with the QIAamp DNA mini kit (Qiagen). The precise identification of the presumptive *Lactococcus* spp. colonies were performed by PCR assay. Briefly, a forward primer pLG-1 (5'-CATAACAATGAGAATCGC-3') and a reverse primer pLG-2 (5'-GCACCCTCGCGGGTTG-3'), for PCR amplification of a less conserved region of the small subunit 16S rRNA gene sequence of *L. garvieae* were synthesized. DNA amplification was performed with PCR master mix (Qiagen) in a thermocycler (Applied Biosystems) as described by Zlotkin, Eldar, Ghittino & Bercovier, (1998). The PCR products were subjected to electrophoresis and run at 100 V for 30 min. The stained DNA bands were viewed by UV transillumination. The sizes of the PCR products were determined with the migration of 100-bp DNA ladder (Bio Basic).

Following identification, the antibacterial susceptibility of bacteria was determined for five different antibiotics by the disk diffusion method. The test was performed on Mueller Hinton Agar (MHA, Oxoid) plates. The commercial antibiotic disks (Oxoid) including florfenicol (FFC, 30 µg), enrofloxacin (ENR, 5 µg), erythromycin (E, 15 µg), trimethoprim-sulfamethoxazole (SXT; 25 µg) and amoxicillin-clavulanate (AMC; 30 µg) were used in this study. The test was done and described according to the Clinical and Laboratory Standards Institute guidelines (CLSI, 2014).

A total of 16 *L. garvieae* strains were isolated from the 20 fish samples. Affected fish had clinical signs indicating the characteristic of lactococcus infection such as bilateral exophthalmos and haemorrhages around the ocular

area (Figure 1). Identification of the Gram (+), oxidase, catalase, and motility (-) *L. garvieae* isolates were confirmed by PCR assay. Consequently, 16 isolates gave the expected 1100-bp PCR amplification product (data not shown).

All isolates were also sensitive to florfenicol and amoxicillin-clavulanate and resistant to trimethoprim+sulfamethoxazole and enrofloxacin by disc diffusion method.



**Figure 1.** Bilateral exophthalmos and haemorrhages around the ocular area in rainbow trout.

### DISCUSSION and CONCLUSION

*L. garvieae* has an increasing clinical significance in the field of the fishery, as well as human and veterinary medicine (Altun, Diler & Adiloglu, 2004; Vendrell et al., 2006). Studies on the detection and characterization of *L. garvieae* associated with cultured rainbow trout have been recently increased (Ture et al., 2016). In this current study, *L. garvieae* isolated from cultured rainbow trout with characteristic external signs. The exact identification was performed rapidly such as into the three days.

This study is the first report focused on exophthalmia in rainbow trout with lactococci. *L. garvieae* was isolated from 16 out of 20 fish samples (80%). Consequently, the bilateral exophthalmos may be considered as a pathognomonic symptom for lactococcosis in rainbow trout.

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