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RESEARCH ARTICLE

Pathological and Microbiological Investigations in Rainbow Trout (*Oncorhynchus mykiss* Walbaum, 1792) Naturally Infected with *Lactococcus garvieae*

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Summary

During the summer of 2006, an outbreak of lactococcosis took place in a rainbow trout farm in the southern Aegean region of Turkey. Clinically lethargie, erratic swimming, darkness in colour and exophthalmia were observed. Histopathological examination revealed epicarditis, peritonitis, enteritis and panophthalmitis. The gills had lamellar edema, hemorrhage and telangiectasia. Histopathological findings were also found in the liver, kidney, pyloric caeca and swim-bladder. In conclusion, the bacterium isolated from the rainbow trout farm was identified as *Lactococcus garvieae* by biochemically, and its pathological affects on the rainbow trout tissues were widely evaluated firstly in Turkey.

Keywords: *Lactococcus garvieae, Pathological and microbiological findings, Rainbow trout (Oncorhynchus mykiss Walbaum, 1792)*

Lactococcus garvieae ile Doğal Enfekte Gökkuşağı Alabalıklarında (*Oncorhynchus mykiss* Walbaum, 1792) Patolojik ve Mikrobiyolojik İncelemeler

Özet

Çalışmada, 2006 yılı yaz mevsiminde Ege Bölge'sinin güneyinde bulunan bir alabalık işletmesinde görülen laktokokkozis enfeksiyonu tanımlandı. Letarji, düzensiz yüzme, deride renk değişiklikleri ve ekzoftalmus gözlenen klinik bulgulardı. Histopatolojik incelemede, epikarditis, peritonitis, enteritis ve panoftalmitis görüldü. Solungaçlarda ödem, kanama ve telangiektazi belirgindi. Karaciğer, böbrek, pilorik kese ve hava kesesinde de histopatolojik bulgular mevcuttu. Mikrobiyolojik incelemeler sonucunda etkenin *Lactococcus garvieae* olduğu saptandı. Sunulan bu çalışma ile Türkiye'de ilk kez gökkuşağı alabalıklarında *Lactococcus garvieae* enfeksiyonunun patolojik bulguları kapsamlı olarak değerlendirilmiştir.

Anahtar sözcükler: *Lactococcus garvieae, Patolojik ve mikrobiyolojik bulgular, Gökkuşağı alabalığı (Oncorhynchus mykiss Walbaum, 1792)*

INTRODUCTION

The Lactococcosis genus was formerly known as the lactic group of streptococci and consisted of *Streptococcus lactis, Streptococcus cremoris* and *Streptococcus diacetylactis*. After the genus was established *Lactococcus garvieae (L. garvieae)* was also to this genus added [1]. *L. garvieae*, previously described as *Streptococcus garvieae*, was originally isolated from cow with mastitis in the United Kingdom [2]. Since that time, Gram-positive bacterium, *L. garvieae* (junior synonym of *Enterococcus serilicida*) has been isolated from various species of fish in

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different parts of the world including Japan [3], Australia [4], South Africa [5] and Europe [6]. The first microbiological description of *L. garvieae* in Turkey was in 1995 [7]. Since then, the bacterium has caused serious losses of rainbow trout in hatcheries of Turkey [8-10].

Bacteriologic and pathologic studies confirmed that *L. garvieae* was the etiological agent of a hemorrhagic septicemia in farmed trout, and the disease characterized with exophtalmos, darkness of the skin and haemorrhages in the internal organs [10-15]. The main histopathological findings in infected fish are epicarditis, peritonitis, enteritis, meningitis and panophthalmitis [12,16,17].

The purpose of this study is to elucidate the pathological and microbiological results of the lactoccosis of naturally infected with *L. garviea* in rainbow trout.

MATERIAL and METHODS

During the summer of 2006, an outbreak that occured in a fish farm in the southern Aegean region of Turkey. When the outbreak was determined in the fish farm, the mortality rates in affected fish ranged from 50% to 60%.

The total of 30 dead or euthanasied rainbow trout (weight 120±10 g) collected from the rearing pools were necropsied. Tissue samples (heart, liver, kidney, gills, stomach, pyloric caeca, intestines, spleen, swim-bladder, eyes, brain, skin and skeletal muscles) taken from rainbow trout were fixed in 10% neutral-buffer formalin solution, embedded in paraffin, sectioned in 5 µm and stained routinely with haematoxylin and eosin (H&E). The selected kidney, liver, spleen, heart, eye and gill sections were also stained by Brown and Brenn staining method for bacteria [18].

Tissue samples from heart, liver, kidney and spleen were taken for isolation of bacteria, and streaked directly onto tryptic soy agar (TSA, Merck) supplemented with 5% sheep blood. Biochemical tests on samples were carried out in API 20 Strep systems (Biomerieux, France), and the incubation was 72 h at 25°C [19].

RESULTS

Bacteriological Findings

After incubation for 48 h at 25°C, pure cultures of whitish colonies were observed. All isolates were non-motile, oxidase and catalase negative, and Gram-positive cocci short chain. Based on phenotypic characteristics, the bacterium was identified as *L. garvieae*. The results are presented in *Table 1*.

Pathological Findings

Lethargie, anorexia and darkness in colour on the skin were observed on the diseased fish. The infected fish were generally found to be swimming weakly and irregularly near the surface of the water.

Macroscopical findings: Redness of fins were determined, hemorrhages at the base of the pelvic and pectoral fins and around the anus were identified. Erosive- ulcerative changes in the skin were only seen in two fish. Uni- or bilateral exophthalmos occured frequently accompanied by hemorrhages in the ocular

Table 1. *The API 20 Strep profile for L. garvieae isolated from rainbow trout farm*
Tablo 1. *Alabalık işletmesinden izole edilen L. garvieae'nin API 20 Strep testine göre biyokimyasal özellikleri*

Properties							
Catalase	-	α-Galactosidase	-	ADH	+	Trehalose	+
Oxidase	-	β-Glukoronidase	-	Ribose	+	Inulin	-
VP	+	β-Galactosidase	-	Arabinose	+	Raffinose	-
Hippurate	-	Alkaline phosphatase	-	Mannitol	+	Amygdalin	-
Esculin	+	Leucine arylamidase	+	Sorbitol	-	Glycogen	-
Pyrrolidonylarylamidase	+	Motility	-	Lactose	-	β-Hemolysis	-

(+; Positive, -; Negative)

Fig 1. Petechial hemorrhages on the serosal surface of the pyloric caeca (arrows)

Şekil 1. Pilorik kese serozal yüzeyinde peteşiyel kanamalar (oklar)

cavity with the opacity of the cornea. The gill flaments were swollen, hemorrhagic and covered by mucus. The abdomen was distended, and a hemorrhagic serous fluid was found in the abdominal cavity. The petechial hemorrhages were also observed on the mesenterium and serosa of the some organs including stomach, pyloric caeca *(Fig. 1)* and swim-bladder. The liver was swollen and yellowish-white in colour. The spleen and kidney were generally enlarged and dark in colour. The pyloric caeca and intestines were swollen and they filled with reddish-mucus content.

Microscopical findings: The distribution of the microscopic findings were presented in *Table 2*. The most common microscopical findings were observed in the heart and peritoneum. The epicardial lesions consisted mainly of inflammatory cells and varying amounts of fibrin and cellular debris *(Fig. 2A)*. Within the myocardium, infiltrations of lymphoid cell *(Fig. 2B)* and activation of the ventricular endothelial macrophages were also present. Cellular or fibrinocellular peritonitis was generally observed *(Fig. 3A)*. Severe infiltrations of inflammatory cell and fibrin were generally detected in

Table 2. *Assesment of the microscopic findings in fish tissues infected with L. garvieae*
Tablo 2. *L. garvieae ile enfekte balık dokularında mikroskobik bulguların dağılımı*

Organs	Microscopic Findings	Number of Fish (n)			
		0	1	2	3
Heart	epicarditis	4	6	13	7
	infiltration of lymphoid cells in the myocard	15	8	5	2
Periton	peritonitis	12	4	7	7
	adhesion	18	6	3	3
	hemorrhage	10	4	10	6
	clump of bacteria	16	5	7	2
Gill	hemorrhage	8	11	5	6
	lamellar edema	5	10	7	8
	telangiectasia	10	7	9	4
	clump of bacteria	19	4	7	0
	increas numbers of EGCs	16	3	6	5
	hyperplasia of the interlamellar cell	16	7	5	2
	lamellar fusion	20	4	5	1
Eye	hemorrhage	6	7	8	9
	infiltration of inflammatory cell	10	8	7	5
	clump of bacteria	14	8	5	3
Liver	degeneration	12	8	10	0
	infiltration of lymphoid cell	17	2	7	4
	fatty droplets in the hepatocytes	13	6	8	3
Kidney	degeneration	13	7	5	5
	hyalin droplets in the epithel cells of the proximal tubuli	15	8	4	3
	increase in the melanomacrophage centres	14	3	5	8
Pyloric caeca	desquamation of mucosal epithelium	12	6	9	3
	increses of level of mucin	9	7	9	5
	increase number of the EGCs	12	3	10	5
Stomach	increase number of the EGCs	10	4	7	9
Intestine	hyperemia	5	5	8	12
	hemorrhage	6	3	7	14
	infiltration of inflammatory cell	16	7	5	2
Swim-bladder	hemorrhage	11	9	5	5
	infiltration of inflammatory cell	19	3	6	2
Spleen	clump of bacteria	20	4	6	0
Brain	meningitis	29	0	1	0
Skin	erosion - ulcer	28	1	1	0

(0; none, 1; mild, 2; moderate; 3; severe. n; number of fish)

the serosa of the pyloric caeca and mesenterium.

In the eyes, there were hemorrhages and infiltrations of inflammatory cell in the sclera and to a lesser extent in the choroid and retina. Clumps of bacteria were found in the lumens of the blood vessels *(Fig. 3B)* and hemorrhagic areas, and macrophages also contained similar bacteria. The gills showed vascular changes including edema, hemorrhage and telangiectasia *(Fig. 3C)*. Swelling of epithelial cells of the gills were common present, and some of epithelial cells were separated from the secondary lamellae. Small clumps of gram-positive bacteria were also determined in the blood vessels. Some fish had lamellar hyperplasia and fusion coincided with an increased of eosinophilic granular cells (EGCs) within primary lamellae.

Lesions of the liver included degeneration of the hepatoctys and perivascular and periductular lymphoid cell infiltrations *(Fig. 3D)*. The sinusoids were dilated, the hepatocytes were contained varying size lipid droplets. In the kidney, degeneration and hyaline droplets in epithelium of the proximal tubuli were generally found. In addition, an increases in melanomacrophage centres were also determined in some fish. In the spleen, the blood vessels were filled up erythrocytes, and some vessels were contained small clumps of gram-positive bacteria.

Histopathological findings were marked in the pyloric caeca to anterior intestines. Sections from the pyloric caeca showed that desquamation and necrosis of mucosal epithelium with increased level of the mucin in the lumen. Large numbers of the EGCs within submucosa of the pyloric caeca were also seen. Severe hyperemia and

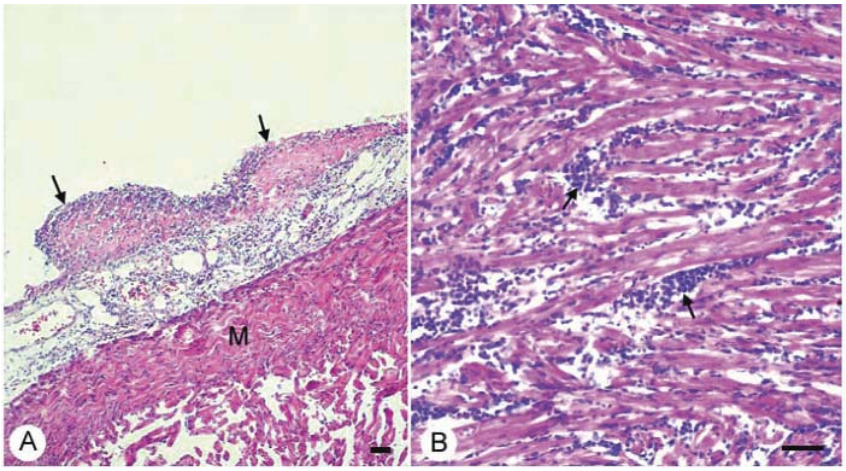

Fig 2. Heart. A. Epicarditis consisted of inflammatory cells and fibrin (arrows), (M: Myocard), HE., Bar= 50µm. B. Lymphoid cell infiltrations in the myocardium (arrows). HE., Bar= 50µm

Şekil 2. Kalp. A. Yangısal hücrelerden ve fibrinden oluşan epikarditis (oklar), (M: Miyokard), HE., Bar= 50µm. B. Miyokardiyumda lenfoid hücre infiltrasyonları (oklar). HE., Bar= 50µm

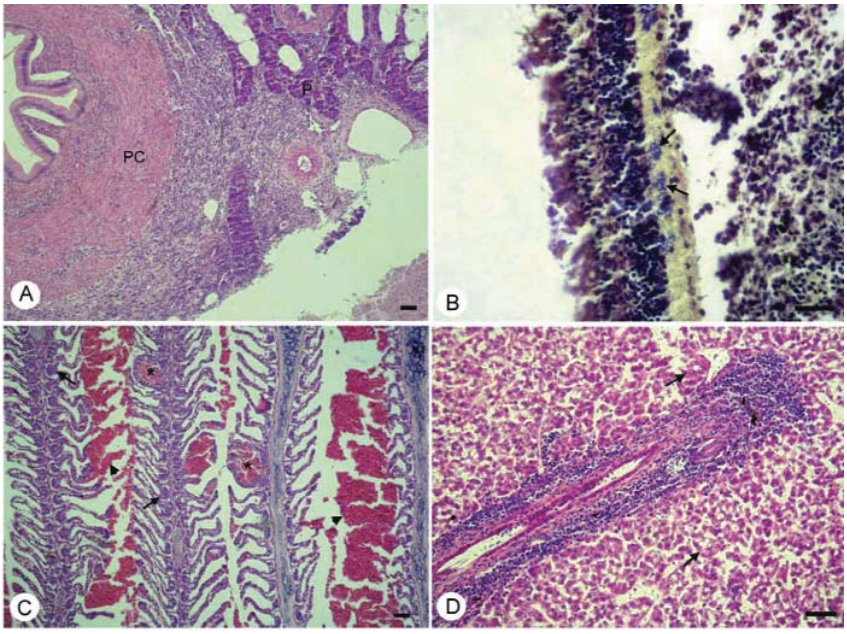

Fig 3. *A.* Peritonitis consisted of inflammatory cells. (PC: Pyloric caeca, P: Pancreas). HE., Bar= 50 µm. ***B.*** Gram-positive bacteria in the lumens of blood vessel of the eye (arrows), Brown-Brenn, Bar: 30 µm. ***C.*** Hemorrhages (arrow-heads), telangiectasia (*) and hyperplasia (arrows) in the gills. HE., Bar=50 µm. ***D.*** Degeneration in the hepatoctyes (arrows) and perivascular and periductular lymphoid cell infiltrations in the liver. HE., Bar=50 µm

Şekil 3. *A.* Yangısal hücrelerden oluşan peritonitis. (PC: Pilorik Kese, P: Pankreas). HE., Bar=50 µm. ***B.*** Gözde damar lümenlerinde Gram-pozitif bakteriler (oklar), Brown-Brenn, Bar=30 µm. ***C.*** Solungaçlarda kanama (okbaşları) telangiektazi (*) ve hiperplazi (oklar) HE., Bar=50 µm. ***D.*** Karaciğerde hepatositlerde dejenerasyon (oklar) ve perivasküler ve perikanaliküler lenfoid hücre infiltrasyonları. HE., Bar=50 µm

hemorrhages in the villus of the intestines were often determined. Infiltration of inflammatory cells in the lamina propria were also observed in some fish. The findings observed in the stomach were similar but less severe.

The swim-bladder wall was usually edematous. In some cases, there was mild hemorrhages and inflammatory cell infiltrations in the serosa. Meningitis in one fish and erosive-ulcerative dermatitis in two fish were also present.

DISCUSSION

Lactococcosis is one of the infectious diseases with significant economic and sanitary repercussions for trout farms in Mediterranean countries during the summer months [6,20-22]. The first description in Europa of *L. garvieae* as a fish pathogen was in 1993 [20]. Micro--biologically the bacterium was first isolated from a trout farm in 1995, in Turkey [7]. Since then, the bacterium has causes serious losses of rainbow trout in farms in Turkey as well as in most other European countries [8-10,13,22]. For this reason, *L. garvieae* has been considered as the new primary causative agent of rainbow trout in Turkey [8-10].

It is reported that outbreaks of lactococcosis in particular are mostly related to poor enviromental conditions and other stress factors [15,23]. The water temperature and quality are the most important factors influencing the onset of the disease outbreaks [15,22,23]. In the present study, the results obtained from the fish farm, strongly support the connection between environmental stress and outbreak of the lactococcosis. Because when the disease was occured, the water temperautre was over 18°C and the pools were also overcrowded.

Clinical signs and gross findings in *L. garvieae* infection in rainbow trout described here are similar to those reported in rainbow trout and other fish [10,12-14,16]. But, these lesions are similar to some fish diseases caused by different agents in rainbow trout [24]. Therefore, a presumptive diagnosis of the lactococcosis in rainbow trout can not be made on the basis of clinical signs and gross findings.

Lactococcosis is characterized by hemorrhagic septicemia in fish [11,12,25]. *L. garvieae*, agent of lactococcosis produces lesions in the vascular endothelium that causing blood extravasation, leading to hemorrhages and petechias at the surface internal organs. Over the external surface, it also intensely affects the most irrigated tissues such as perianal or buccal area and fins [25]. Diffuse hemorrhages related to haemorrhagic septicemia in the external surfaces, eyes and on the serosal surfaces of the fish in the present study are similar to those described by other investigators [11,12,16].

The lesions characterized by epicarditis, peritonitis and panophthalmitis in the present study are comparable those of lactococcosis in fish reported by the others [12,17]. Therefore, these lesions in the rainbow trout could be considered as the most characteristic histopathologic findings of the lactococcosis. Fibrinous meningitis associated with the lactococcosis have also been described in fish [16,17], but in the present study, the meningitis was determined in only one fish. In addition, the histopathological findings of the intestine, kidney and spleen manifested in this study are different from those described by some authors [12,16,17], especially with the lack of any extensive erosions with pseudomembrane-like formation of the intestines and any necrosis of the kidney and spleen [16,17]. Moreover, periductular and perivascular lymphoid cell infiltrations observed in the liver of some fish have not been described previously for lactococcosis.

The oral route is the main route of transmission of *L. garvieae* have been proposed [26,27], but the results from this study suggest that the gills and eyes are a major site of attachment and the proliferation of *L. garvieae* during infection period. After the bacteria penetrate through the gills and eyes, the spread of blood vessels and finally causing septicemia.

In this study, the isolated bacteria was identified as *L. garvieae* using the API 20 (25°C, 72h) system. The biochemical properties of bacteria isolated from rainbow trout in this study are very similar to those described in other studies [9,13,17,19,21].

It is widely accepted idea that *L. garvieae* is considered as one of the main pathogen of fish, but numerous cases of infections with *L. garvieae* in human were also reported [28-30]. However, in a recent report [31], sporadic human case related to consumption of raw fish foods infected with *L. garvieae* was also described. The relationship between outbreaks of *L. garvieae* and human infections has not been proved until now, and our results cannot support that *L. garvieae* might be considered potentially a zoonotic bacterium to humans.

In the present study, the bacterium isolated from the rainbow trout was identified by biochemically, and its pathological affects on the rainbow trout were firstly evaluated in Turkey. It was also reported that *L. garvieae*, agent of lactococcosis, might cause outbreaks with high mortality in rainbow trout in Turkey especially in hot seasons.

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