

## Investigation of Pathological Findings Infected with *Aeromonas salmonicida* in Rainbow Trout (*Oncorhynchus mykiss* Walbaum, 1792) [1]

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[1] This paper is a summary of a MSc thesis and was supported by Adnan Menderes University Research Fund (Project no: VTF 04-003)

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Makale Kodu (Article Code): KVFD-2010-2490

### Summary

Experimental furunculosis caused by *Aeromonas salmonicida* was induced in rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792) using intraperitoneal injection and immersion challenge methods. A total of 65 (50 experimental and 15 control) rainbow trouts (weight 155±15 g and 20-25 cm in size) were used for this experimental work. *Aeromonas salmonicida* at a concentration of 3x10<sup>5</sup> cfu/ml (0.1 ml per fish) was intraperitoneally injected to twenty-five fish (first group). A second group of 25 fish was immersed in 30 l water inoculated to which 3 ml of bacterial inoculum (3x10<sup>5</sup> cfu/ml) had been added for one hour. Sterile saline solution (0.1 ml/fish) was intraperitoneally injected to control group. Necropsy of the fish was performed, macroscopic and microscopic findings were evaluated. The chronic form of disease was observed in both groups. Anorexia, irregular swimming, hemorrhages at the dorsal, ventral and pectoral base of fins were the main clinic and macroscopic findings. However, in some fish, diffused or focal scale eruption on the dermis from operculum to caudal fin, periocular hemorrhages and exophthalmos were observed. Similar microscopic findings were determined in the both experiment groups. Microscopically, foci of bacteria with no inflammatory response especially in the muscles, gills, heart, stomach, pyloric caeca, intestines, kidney, spleen and liquefactive necrosis in the muscles were the main microscopic findings.

**Keywords:** *Aeromonas salmonicida*, Rainbow trout, Experimental infection, Pathological findings

## *Aeromonas salmonicida* ile Enfekte Edilen Gökkuşığı Alabalıklarında (*Oncorhynchus mykiss* Walbaum, 1792) Patolojik Bulguların İncelenmesi

### Özet

Bu araştırma *Aeromonas salmonicida* ile deneysel Furunkülozis'in intraperitoneal yol ve immersiyon yöntemiyle oluşturulması amacıyla yapıldı. Bu amaç için, 50 adet deneme grubu, 15 adet kontrol grubu olmak üzere toplam 65 adet, 155±15 g ağırlığında, 20-25 cm büyüklüğünde gökkuşığı alabalığı (*Oncorhynchus mykiss* Walbaum, 1792) kullanıldı. İntraperitoneal yolla oluşturulan enfeksiyonda (birinci grup) 25 adet balığa 3x10<sup>5</sup> hücre/ml olacak şekilde 0.1 ml bakteri inokulatu verildi. İmmersiyon yöntemiyle uygulama yapılan diğer gruptaki (ikinci grup) 25 adet balık, 3x10<sup>5</sup> hücre/ml olacak şekilde 3 ml bakteri inokulatu verilen 30 l suda 1 saat bekletildi. Kontrol grubundaki 15 balığa ise 0.1 ml serum fizyolojik intraperitoneal olarak verildi. Bu balıkların sistemik nekropsileri yapıldı, makroskobik ve mikroskobik bulgular değerlendirildi. Her iki deneme grubunda da hastalığın kronik formu şekillendi. Balıklarda klinik ve makroskobik olarak, iştahsızlık, deri renginde koyulaşma, yüzme bozuklukları, dorsal, ventral ve pektoral yüzgeçlerin tabanında kanamalar görüldü. Ayrıca, bazı balıklarda, operkulumdan kuyruk yüzgecine kadar uzanan bölgede pullarda dökülmeler, perioküler kanamalar ve ekzoftalmus dikkati çekti. Her iki deneme grubunda da, mikroskobik olarak benzer bulgular görüldü. Balıklarda mikroskobik olarak kaslarda, solungaçlarda, kalpte, midede, pilorik keselerde, bağırsaklarda, böbreklerde ve dalakta yangısal bir yanıt görülmeksizin bakteri kümeleri ve kaslarda erime nekrozları saptandı.

**Anahtar sözcükler:** *Aeromonas salmonicida*, Gökkuşığı alabalığı, Deneysel enfeksiyon, Patolojik bulgular

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## INTRODUCTION

Furunculosis, caused by *Aeromonas salmonicida*, is a septicæmic and contagious disease characterized by the furuncle formation on the skin with necrotic and hemorrhagic muscle lesions<sup>1,2</sup>, and leads to outbreaks in many families of fish, especially Salmonidae and Cyprinidae<sup>3,4</sup>. In Turkey, *Aeromonas salmonicida* was isolated from the three fish hatcheries located in West-Aegean region in 2003<sup>5</sup>. The disease may be seen in peracute, acute, subacute and chronic forms. The peracute form of furunculosis frequently occurs in the fingerling fish. The acute form of furunculosis is common in growing fish and fish usually die within 2-3 days. Subacute and chronic forms are more common in older fish, and at the beginning of infection, deaths occur rarely, but the deaths rate gradually increases<sup>6</sup>.

*Aeromonas salmonicida* is a gram-negative, aerobic, non-motile, fermentative, non-spore forming, bacillus approximately 1.0 µm x 2.0 µm in size<sup>1,3,6</sup>. The cell wall of *Aeromonas salmonicida* possesses some virulent factors such as an additional cell envelope protein (A-Layer Protein) and lipopolysaccharid<sup>7-10</sup>. A-Layer Protein is important for virulence. Because *Aeromonas salmonicida*, possesses A-Layer Protein, has the ability to adhere, enter and to survive within macrophages<sup>7,11,12</sup>. In addition, the extracellular products (ECP) of *Aeromonas salmonicida* consisted of 70-kDa serine protease (extracellular protease, EP) and glycerophospholipid:cholesterol acyltransferase complexed with lipopolysaccharide (GCAT/LPS) play an important role in pathogenesis and virulence<sup>13-15</sup>.

The aim of this study was to investigate macroscopic and microscopic findings in rainbow trouts experimentally infected with *Aeromonas salmonicida* via the intraperitoneal route and immersion challenge methods.

## MATERIAL and METHODS

The ethics committee of Adnan Menderes University approved the design and protocol of the experiments (15.01.2004-2004/0013). In this research, a total of 65 (50 fish in experimental groups and 15 fish in control group) rainbow trouts (weight 155±15 g and 20-25 cm in size) obtained from a commercial fish farm with no history of furunculosis, were used. Fish were divided into three groups: First group (25 fish), second group (25 fish) and control group (15 fish). They were acclimatized for 14 days in three separate pools (one for control group and two for experimental groups). Water temperature was maintained at 15-18°C. During the experimental period, external filter (600 l/h) was used in all aquariums.

Dissolved oxygen of the water and pH in all pools were uniform (pH 7.25 and dissolved oxygen 9.1-9.4 mg/l). The fish were fed daily with a commercial feed (No.2 Bağcı). Before the intraperitoneal injections, fish were anaesthetized with 2-phenoxyethanol (0.25%)<sup>16</sup>. *Aeromonas salmonicida* (field strain) at a concentration of 3x10<sup>5</sup>cfu/ml (0.1 ml per fish) was intraperitoneally injected to twenty-five fish in the first group. The other twenty-five fish (the second group) were immersed into 30 l water inoculated with 3 ml of bacterial inoculum (3x10<sup>5</sup> cfu/ml) for one hour. The same procedure was repeated after three days. Sterile saline solution (0.1 ml per fish) was intraperitoneally injected to fifteen fish in the control group. Experiment was performed for 31 days. Necropsy of the fish was performed and tissue samples were collected and fixed in 10% formalin solution, embedded in paraffin, sectioned at 5 µm and stained routinely with Hematoxylin-Eosin (H&E). The selected kidney, liver, spleen, heart and gills were stained by Brown and Brenn staining method for the isolation of bacteria. In addition, to evaluate the presence of lipid in the liver, Oil Red O staining method was used<sup>17</sup>.

## RESULTS

In this experiment, chronic form of furunculosis was developed in the both groups. In the first group, the deaths began on 6<sup>th</sup> day and reached to maximum rate on 27-28<sup>th</sup> day; in the second group, the deaths began on 7<sup>th</sup> day and reached maximum rate on 23-25<sup>th</sup> day. The surviving fish were euthanased on the 31<sup>st</sup> day of the experiment [total 7 fish (3 fish from first group, 4 fish from second group)]. Fish in the control group were euthanased and changes were observed comparatively. *Aeromonas salmonicida* was reisolated from the infected fish and no *Aeromonas salminicida* or any other pathogenic bacteria were isolated from fish in the control group.

Clinical signs consisted of anorexia, darkening in the color or depigmentation of the skin and irregular swimming in the both groups.

### Macroscopic Findings

In both groups, macroscopically, hemorrhage at the dorsal, ventral and pectoral base of fins, diffused or focal scale eruption on the dermis from operculum to caudal fin were often noted. Furthermore, diffuse hemorrhages in the kidney, stomach and intestines; periorcular hemorrhages and exophtalmos; anemia; hyperemia or anemia in the gills; a great reduction in the size of kidney tissue; ascites and petechial hemorrhages on the perivisceral tissue (*Fig. 1*) were determined in some fish. Generally, due to anorexia, the stomach, pyloric caeca and intestines were empty.



**Fig 1.** Petechial hemorrhages on the perivisceral tissue (arrow)  
**Şekil 1.** Periviseral dokuda peteşiyal kanamalar (ok)

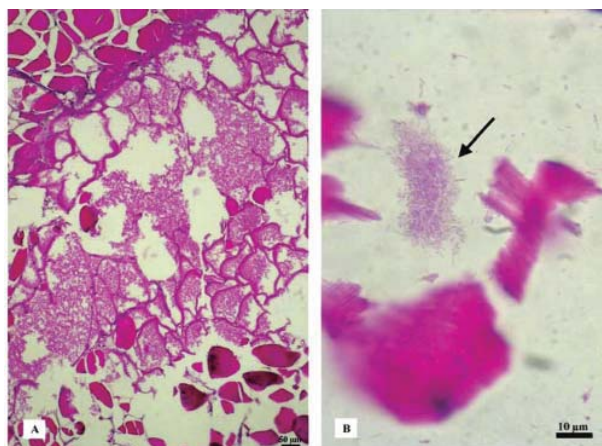
### Microscopic Findings

Similar microscopic findings were determined in the both experiment groups. The main microscopic findings were the foci of bacteria with no inflammatory response in the different tissue and organs, and liquefactive necrosis in the muscles.

The liquefactive necrosis was observed in the muscles (*Fig. 2A*). The sizes and the locations of the necrosis varied among the fish. Some of the necrotic muscle bundles were filled with the destruction products or they were completely empty.

Foci of bacteria with no inflammatory response were observed in the necrotic areas of muscle (*Fig. 2B*); around secondary lamellae in the gills; in the heart; serosal surface of the stomach, pyloric caeca and intestines; in the tubular lumen of the kidney; capsular surface of the spleen and kidney.

In the liver, discrete vacuoles were present in the cytoplasm of the hepatocytes. These vacuoles were



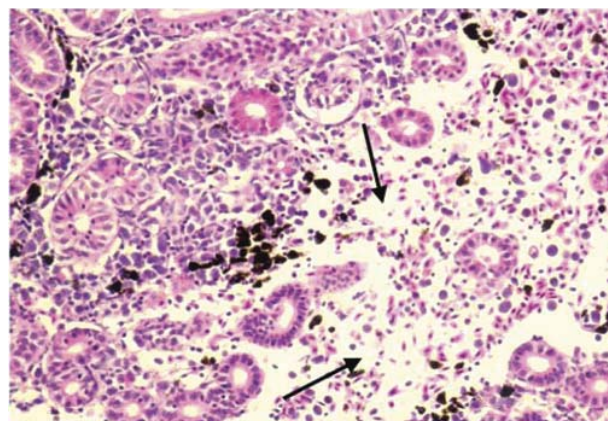
**Fig 2. A:** The liquefactive necrosis in the muscle tissue. HE. Bar, 50 µm, **B:** Foci of bacteria with no inflammatory response in the muscle tissue (arrow), HE. Bar, 10 µm

**Şekil 2. A:** Kaslarda erime nekrozu. HE. Bar, 50 µm, **B:** Kaslarda yangısal bir yanıt görülmeksizin bakteri kümeleri (ok), HE. Bar, 10 µm

recognized as fat droplets stained with Oil Red O method in frozen sections.

Hydropic degeneration in the proximal and distal tubules, reduction in the lymphoid cells of hematopoietic tissue (*Fig. 3*), and glomerular atrophy were determined in the kidney. The most important microscopic lesion in the spleen was necrosis in the lymphoid tissue.

The primary and secondary lamellar lesions consisted of edema, adhesion, hyperplasia, hyperemia and telangiectasia in the gills. Marked edema in the secondary lamellae was often present.



**Fig 3.** Reduction in the lymphoid cells of hematopoietic tissue in the kidney (arrows), HE. Bar, 50 µm

**Şekil 3.** Böbrekte hematopöietik dokunun lenfoid hücrelerinde azalma (oklar), HE. Bar, 50 µm

In addition, elevated numbers and degranulation of eosinophilic granular cells (EGC) in the submucosa of the stomach and pyloric caeca were determined. However, same finding was detected in only one fish's gill.

## DISCUSSION

In the chronic form of furunculosis anorexia, exophthalmos, darkening in the color of skin, irregular swimming, hemorrhages at the dorsal, ventral and pectoral bases of fins, and furuncle formation at the skin were reported as the main clinic and macroscopic findings<sup>6,12,14</sup>. In the present study, anorexia, irregular swimming, darkening in the color of skin, hemorrhages at the bases of fins, scale eruptions were observed following the inoculations. But furuncle formation was not seen.

It has been reported that the 70-kDa serine protease causes of liquefaction necrosis in the muscles<sup>13</sup>. In addition, on the other hand, GCAT/LPS combination with 70-kDa serine protease induces liquefaction necrosis in the muscles<sup>18</sup>. In the present study, the liquefaction

necroses in the muscles were located especially around foci of bacteria, and we suggested that liquefaction necrosis in the muscles could be related to the extra-cellular products of *Aeromonas salmonicida*.

One of the important microscopic findings of furunculosis is foci of bacteria with no inflammatory response in the dermis, muscle and internal organs<sup>1,19</sup>. Recent studies suggest that GCAT/LPS have haemolytic, leucocytolytic and cytotoxic activities<sup>6,12,20</sup>. That is because of these effects of GCAT/LPS, destruction of hematopoietic tissue occurs in kidney and spleen. Moreover, a few authors suggested that *Aeromonas salmonicida*, possesses A-Layer Protein, the ability to adhere, enter and, to survive within macrophages bring about phagocytosis inhibition<sup>7,11</sup>. Phagocytosis inhibition and hematopoietic tissue destruction constitute foci of bacteria with no inflammatory response in the dermis, muscles and internal organs. In this study, foci of bacteria with no inflammatory response in the muscles, gills, heart, stomach, pyloric caeca, intestines, kidney and spleen, and reduction in the lymphoid cells of hematopoietic tissue of the kidney were seen. As a matter of fact the selected tissue sections were stained by Brown and Brenn staining method for bacteria and positive results were obtained. We suggested that foci of bacteria with no inflammatory response in the muscles, gills and internal organs might be related to A Layer Protein and activity of GCAT/LPS.

In conclusion, in both groups, macroscopic and microscopic findings of this study might be considered important pathological findings of furunculosis in rainbow trout.

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