

Pathological Findings in Rainbow Trout (*Oncorhynchus mykiss* Walbaum, 1792) Experimentally Infected with *Yersinia ruckeri**

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Abstract: The aim of this study was to evaluate the pathological findings in rainbow trout experimentally infected with *Yersinia ruckeri*. For this purpose, 42 [1 control group (10 fish) and 1 experimental group (32 fish)] of rainbow trout were used. *Yersinia ruckeri* serotype I (R0072) at a concentration of 3×10^6 cfu/ml (0.1 ml per fish) was intraperitoneally injected into the experimental group, whereas the control group was subjected to the same volume of sterile saline. Following the intraperitoneal injection, between 4 and 26 days, fish that died due to natural infection and those killed were necropsied and macroscopic and microscopic findings were determined.

Macroscopically, in the acute form, hyperemia and hemorrhages were observed, especially at the base of the fins, around the anus and eyes, on the swim bladder and in the gills. In the chronic form, dark pigmentation or depigmentation of the skin and yellow-grayish discoloration in the liver were noted. In addition, the kidney and spleen were dark in color and smaller than those observed in the control fish. Microscopically, in the acute form, edema, hyperemia, hemorrhage and desquamation of the epithelial cells in the secondary lamellae and a significant decrease in the numbers of lymphoid cells in the kidney were seen. In the spleen, focal necrosis was the most important microscopic finding. In the chronic form, hyperplasia of the interlamellar cells of the primary lamellae, fatty changes in the liver and many large mononuclear cell proliferations with slightly incised nucleus in the hemopoietic tissue of the kidney were observed.

This is the first pathological study on rainbow trout experimentally infected with *Yersinia ruckeri* in Turkey.

Key Words: *Yersinia ruckeri*, rainbow trout, pathological findings

Deneysel Olarak *Yersinia ruckeri* ile Enfekte Edilmiş Gökkuşuğu Alabalıklarında (*Oncorhynchus mykiss* Walbaum, 1792) Patolojik Bulgular

Özet: Bu çalışmada deneysel olarak *Yersinia ruckeri* ile enfekte edilen gökkuşuğu alabalıklarında patolojik bulguların incelenmesi amaçlandı. Bu amaçla toplam 42 adet [1 kontrol grubu (10 balık) ve 1 deneme grubu (32 balık)] gökkuşuğu alabalığı kullanıldı. Daha sonra deneme grubundaki balıklara 3×10^6 cfu/ml bakteri içeren 0,1 ml'lik *Yersinia ruckeri* serotip I (R0072) suşu intraperitoneal olarak, kontrol grubuna ise aynı dozda serum fizyolojik intraperitoneal olarak verildi. Uygulamayı takiben 4. ve 26. günler arasında doğal infeksiyon sonucu ölen ya da öldürülen balıkların sistematik nekropsileri yapılarak makroskobik ve mikroskobik bulguları incelendi.

Makroskobik olarak akut formda, özellikle yüzgeç tabanlarında, gözler ve anüs çevresinde, hava kesesinde ve solungaçlarda hiperemi ve kanamalar gözlemlendi. Kronik formda, deri renginde açılma veya koyulaşma ile karaciğerde sarımsı-grı renk değişiklikleri belirlendi. Ayrıca böbrek ve dalağın kontrol gruplarına göre daha koyu renkli ve küçük oldukları görüldü. Mikroskobik olarak, akut formda, sekonder lamellerde ödem, hiperemi, kanama, epitellerde dökülmeler ile böbreklerdeki lenfoid hücrelerde belirgin azalmalar saptandı. Dalakta görülen en önemli bulgu ise fokal nekrozlar idi. Kronik formda ise solungaçlarda interlameller hücrelerde hiperplazi, karaciğerde yağlanma ile böbrekte çok sayıda iri yapılı ve çentikli çekirdekli lenfoid hücre proliferasyonları dikkati çekti.

Bu araştırma, Türkiye'de *Yersinia ruckeri* ile gökkuşuğu alabalıklarında deneysel olarak yapılan ilk patolojik çalışmadır.

Anahtar Sözcükler: *Yersinia ruckeri*, gökkuşuğu alabalığı, patolojik bulgular

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Introduction

Yersiniosis or enteric redmouth (ERM) disease, caused by *Yersinia ruckeri*, is an acute to chronic bacteremic infection of trout and salmon, and is responsible for serious economic losses (1,2). The disease is characterized by the presence of hemorrhages around the mouth and anus, at the base of the fins and on the surface of internal organs (3-5).

ERM disease was first recognized as a serious bacterial disease resulting in mortalities in rainbow trout (*Salmo gairdneri* Richardson) in the USA in the early 1950s (1). Currently, ERM disease occurs all over the world (6-8). In Turkey, the first isolation of this bacterium was from a trout farm in Denizli in 1991 (9). The presence of the infection has also been reported in the Aegean and Marmara regions (10,11).

Yersinia ruckeri is a Gram-negative, rod-shaped, fermentative, peritrichously flagellated bacterium (2,3,12). ERM is most prevalent in rainbow trout (4,8); however, the disease has also been reported in other salmonids and other fish species (13-15). The disease is most contagious at water temperatures of 15-20 °C and the incubation period of infection is 5 to 10 days (4,10,16). Transmission of the disease is primarily of a direct horizontal nature from fish to fish through the water (6,7). The fish that were infected previously always remain carriers (1,6). The infection is generally seen in peracute or acute form, especially in young fish when there is a sudden increase in water temperature in spring, whereas chronic infections usually occur in the yearling fish in the early winter (3,4,7).

Most of the previous research on *Yersinia* infections in various fish species was generally based on bacteriological findings of *Yersinia ruckeri*. However, research investigating the pathological effects of this agent on fish is very limited. Therefore, the aim of this experiment was to examine the pathological findings of rainbow trout experimentally infected with *Yersinia ruckeri* in Turkey.

Materials and Methods

Forty-two [1control group (10 fish) and 1 experimental group (32 fish)] rainbow trout obtained from a commercial fish farm with no history of ERM disease (Bağcı Fish Company) and weighing about 85 ± 10 g were used. They were acclimatized for 28 days in 2

separate pools (1 for the control group and 1 for the experimental group) and were maintained at 19-20 ± 0.5 °C. Water flow in each pool was set at 7 l/min. During the investigation period, the pH and dissolved oxygen of the water in both pools were uniform (pH 7.5 and dissolved oxygen 7.5-7.9 mg/l) and the fish were fed daily with a commercial feed (No.2 Bağcı).

Prior to injection, the fish were lightly anesthetized with phenoxyethanol (0.25%) and *Yersinia ruckeri* serotype I (R0072) at a concentration of 3 x 10⁶ cfu/ml (0.1 ml per fish) was intraperitoneally applied to the experimental group, whereas the control group was subjected to the same volume of sterile saline. Four to 26 days after injection, dead or killed fish were necropsied and tissue samples were collected and fixed in 10% neutral-buffer formalin solution, embedded in paraffin, sectioned at 5 µm and stained routinely with hematoxylin and eosin (H&E). The selected kidney, liver, spleen and heart sections were stained by Brown and Brenn staining for bacteria. In addition, in order to evaluate the presence of lipid in the liver, modified McManus's Sudan Black B staining was also used (17).

Results

Following the intraperitoneal injection, initial mortalities in the parenterally infected fish were seen first on day 4 postinfection and reached a maximum on day 7 (Figure 1). In this study, fish that died by 13 day postinfection in the infected group were considered to have the acute form and fish that died between 13 and 26 days postinfection in the same group were considered to have the chronic form. In addition, *Yersinia ruckeri* was reisolated from the infected fish 4 days after injection. No *Yersinia ruckeri* or any other pathogenic bacteria were isolated from the surviving fish in the control group.

Macroscopic Findings

Acute form: Clinical signs were characterized by general lethargy and swimming difficulties and they were close to the surface of the water. Hemorrhages were observed around the anus and eyes, in the gills and at the base of the dorsal, ventral, and anal fins. The liver and spleen were enlarged (Figure 2). Hemorrhages were often noted in the swim bladder, liver and adipose tissue.

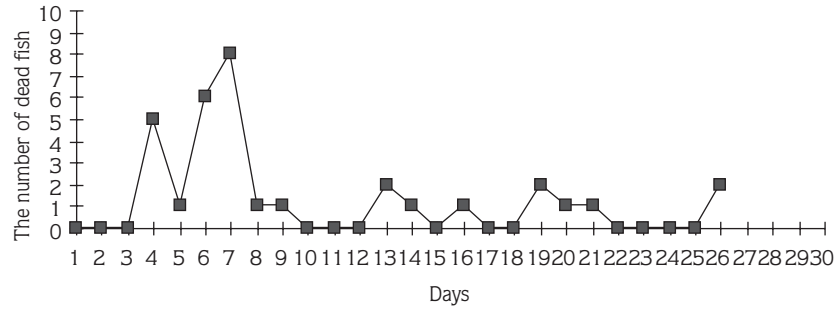


Figure 1. Distribution of the number of dead fish by day in rainbow trout following experimental infection with *Yersinia ruckeri*.

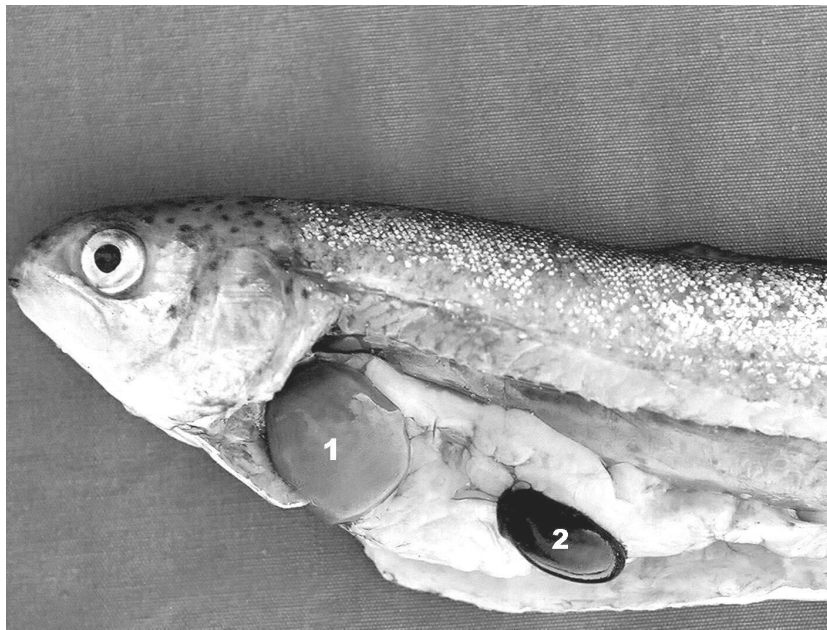


Figure 2. Enlargement in the liver (1) and spleen (2).

Some petechial hemorrhages were also seen on the surface of the stomach and in the lateral musculature. The stomach and pyloric caeca contained clear mucus. Furthermore, the intestines were occasionally flaccid and filled with a reddish fluid.

Chronic form: Clinical signs included loss of appetite and lethargy. Macroscopically, dark pigmentation or depigmentation of the skin, pale in the gills and exophthalmos were observed. Exophthalmos was sometimes accompanied by hemorrhages around the ocular cavity in some cases. The liver was slightly enlarged and yellow-grayish discoloration in the liver was obvious. The stomach, pyloric caeca and intestines

contained a thick and yellowish fluid. The kidney and spleen were dark in color and smaller than those observed in the control fish.

Microscopic Findings

Acute form: Hyperemia in the primary lamellae and edema in the secondary lamellae (Figure 3a) were often present. In the secondary lamellae, desquamation of the lamellar epithelial cells was also observed. These findings were most pronounced in all fish with the acute form. Furthermore, capillary dilatation of the secondary lamellae and diffuse hemorrhages in both the primary and secondary lamellae (Figure 3b) were also seen.

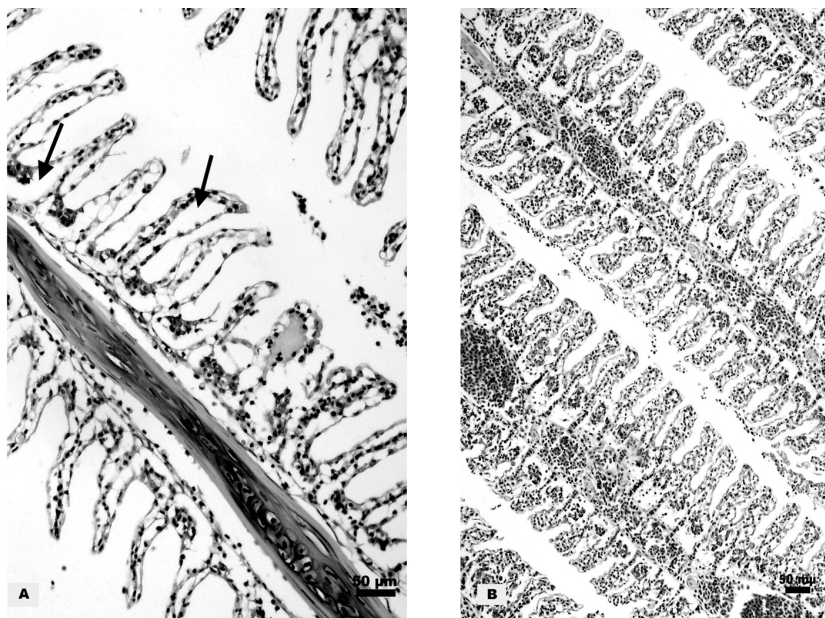


Figure 3. A: Edema in the secondary lamellae (arrows), H&E. B: Hyperemia and hemorrhage in the primary and secondary lamellae, H&E.

In the heart, swelling and activation of the ventricular endothelial macrophages were detected. In the perimysium of 4 fish, edema was observed. Mononuclear cell infiltrations in the epicardium were also present in 3 fish. In livers of all fish, hyperemia and hemorrhages were present. In addition, swelling of the hepatocytes was determined in some livers.

No noticeable histopathological changes were seen in the tubules of the kidney. However, cellularity of the glomerular tuft in 4 fish was characterized by proliferation of mesangial cells. In the kidneys, there were significant decreases in the numbers of lymphoid cells, and mononuclear cell proliferations, whose appearance was similar to that of macrophages with large size and round shape, were observed. In half of the fish with the acute form, the most important microscopic finding in the spleen was focal necrosis (Figure 4).

Chronic form: In the gills, interlamellar hyperplasia of the primary lamellae was detected. In the liver, discrete vacuoles were present in the cytoplasm of the hepatocytes (Figure 5). These vacuoles were recognized as fat droplets in paraffin sections stained with modified McManus's Sudan Black B. In some fish, severe fatty changes were also observed in the liver in which the

hepatocytes became small and the sinusoids disappeared. However, there were mononuclear cell infiltrations around the vena centralis and bile ducts in 4 fish.

No lesions in the glomerulus and tubules of the kidney were observed. However, large numbers of large mononuclear cell proliferations with slightly incised nucleus were observed in the hemopoietic tissue of the kidney in all fish with the chronic form (Figure 6).

Discussion

Mortality due to ERM disease in rainbow trout usually takes place in 5 to 10 days, depending on the size of the fish, and the general health and stress condition of the population (1,3). However, if the population has had prior exposure to and latent infection with *Yersinia ruckeri*, a stressful environment can result in mortality within 3 to 5 days (6). An intraperitoneal study by Busch and Lingg (16) indicated that mortalities first appeared at 6 days postinfection and reached a maximum rate at 9 days postinfection. Furthermore, Kubilay and Timur (18) reported in an experimental study in rainbow trout that initial mortalities in the parenterally infected population started on day 3 and ended 9 days postinfection. In this

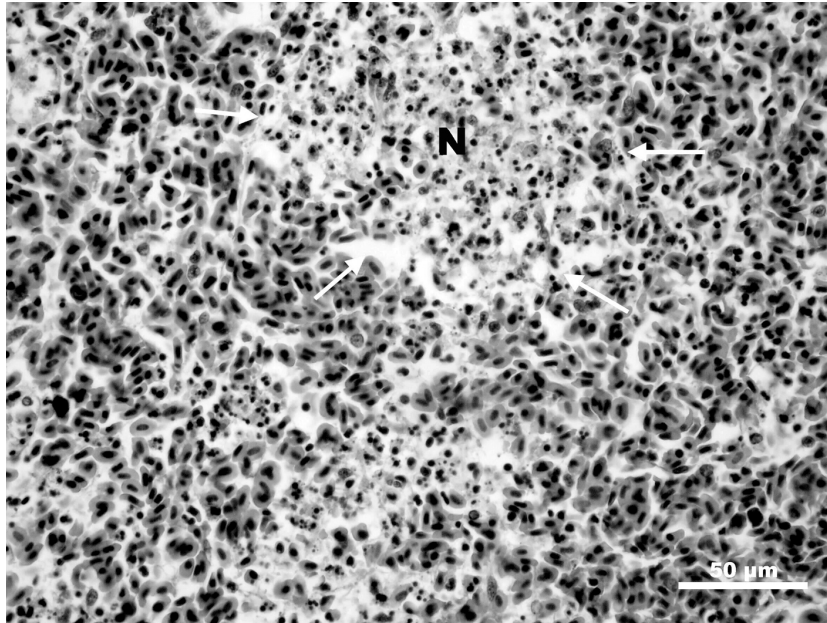


Figure 4. Focal necrosis (N) in the spleen (arrows), H&E.

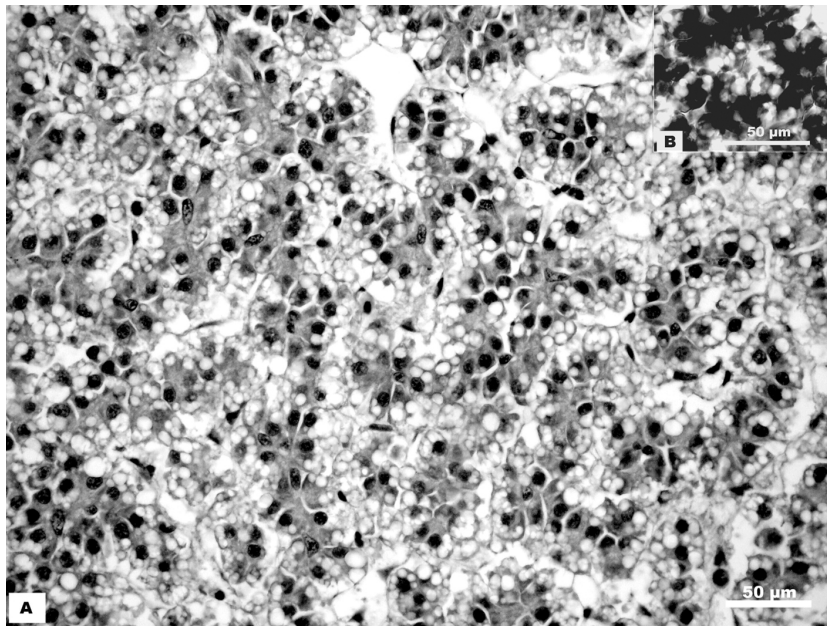


Figure 5. Fatty changes with discrete vacuoles in the hepatocytes A: H&E, B: Modified McManus's Sudan Black B.

study, following the intraperitoneal injection, mortalities first appeared at 4 days postinfection and reached a maximum rate 6-8 days after postinfection, which is in agreement with the literature (16,18).

Hemorrhages around the mouth, eyes, anus and vent, and at the base of the pectoral, pelvic and anal fins described in ERM disease might be considered the most obvious macroscopic finding of the disease in rainbow

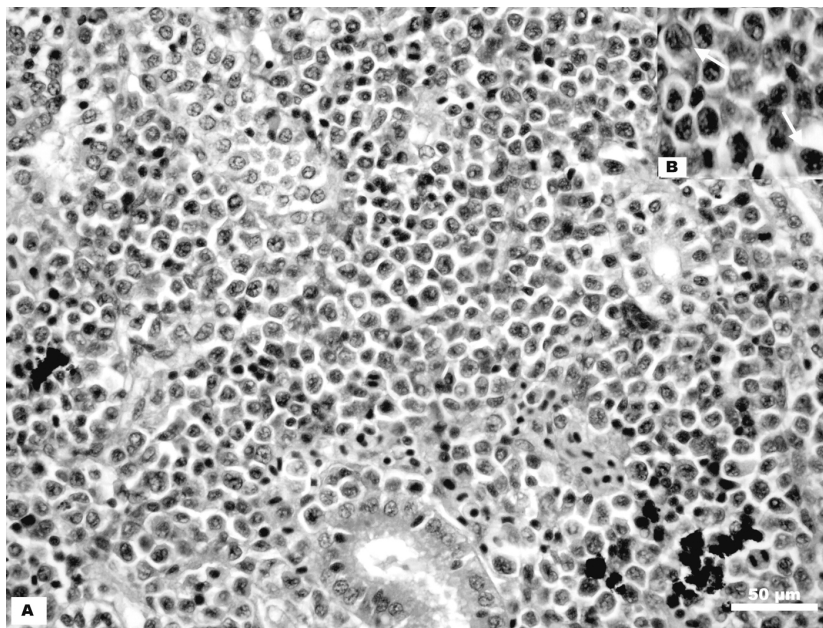


Figure 6. Large number of large mononuclear cell proliferation (A) with slightly incised nucleus (B, arrows) in the kidney, H&E.

trout (3,19,20). In this study, subcutaneous hemorrhages were seen especially at the base of fins and around the anus and eyes. Similar macroscopic findings were described by Frerichs et al. (19) in the atypical infection of rainbow trout with *Yersinia ruckeri*. However, hemorrhages around the mouth and oral cavity, giving the name ERM to the disease, were not observed in all fish. Rigos and Stevenson (5) also reported that hemorrhages were seen only at the base of the fins in all cases.

Vascular changes in the gills, including hyperemia and hemorrhages, have been described in rainbow trout naturally or experimentally infected with *Yersinia ruckeri* (5,6,21,22). In the present study, the histopathological changes in the gills of the fish were more severe in the acute form than in the chronic form. Furthermore, desquamation of the secondary lamellar epithelial cells was also observed in the acute form as this might be the result of edematous separation of the respiratory epithelium of the secondary lamellae. Interlamellar hyperplasia of the primary lamellae in all fish with the chronic form were observed in the gills. However, there have been no previous reports in the literature concerning hyperplasia of the interlamellar cells in the gills. Similar gill lesions were also seen in the control group.

Therefore, this might be related to the adverse water quality.

In the present study, petechial hemorrhages in fish were often observed on the surface of the swim bladder, liver, adipose tissue and also on the stomach and in the lateral musculature. Generally, similar macroscopic findings have been described in rainbow trout (12,19,23). Although there have been no reports on the cause of the petechial hemorrhages, a few authors (24,25) have suggested that they could be related to the extracellular products of *Yersinia ruckeri*.

Necrotic changes in natural or experimental ERM infections in rainbow trout were reported in the spleen, kidney and liver (3,4,8). In the present study, focal necrosis was observed only in the spleen in half of the fish with the acute form. In the chronic form, mononuclear cell proliferations in the kidney were observed. However, there have been no reports in the literature concerning proliferation of mononuclear cells in the kidney. Changes described in the spleen and kidney in this study may be considered the most prominent histopathological finding of ERM disease.

Some differences in the histopathological findings in the liver were recorded between the acute and chronic

forms in this study. In the acute form, vascular changes could be explained as a result of vascular damage. In the chronic form, there have been no reports in the literature concerning fatty changes in the liver in rainbow trout. Furthermore, infiltration of mononuclear cells in the liver was also reported by Busch (6). Therefore, we suggest that *Yersinia ruckeri* might be a causative agent of non-suppurative hepatitis in rainbow trout.

In conclusion, edema of the gills, focal necrosis of the spleen, mononuclear cell proliferations in the kidney and fatty changes in the liver in this study may be considered the most important pathological findings of ERM disease in rainbow trout. However, it was concluded that these pathological findings could be used to identify ERM disease, especially when microbial isolation is not possible.

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