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Araştırma Makalesi / Research Article

A Detailed Histopathological Research on *Myxobolus cerebralis* in Rainbow Trout Tissues

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Abstract

The causative agent of whirling disease, *Myxobolus cerebralis*, has a negative impact on salmonid fish and causes significant economic losses. Infected specimens of rainbow trout (*Oncorhynchus mykiss)* exhibiting clinical signs of whirling disease were sampled and subjected to a thorough histopathological examination in this study. The results showed *M. cerebralis* spores in brain, spleen, and gill tissues, with notable pathological changes such as inflammation, necrosis, and cyst formation. The study details the progression of *M.cerebralis* infections, highlighting significant tissue damage and immune responses in the host. The results demonstrate the importance of identifying the pathogen with histopathological methods to understand the impacts on fish health and provide insight into the host tissue response.

Keywords: Myxozoa, Whirling disease, Parasite, Histopathology.

Gökkuşağı Alabalığı Dokularında Myxobolus Cerebralis Üzerine Detaylı Bir Histopatolojik Araştırma

Öz

Dönme hastalığı etkeni olan *Myxobolus cerebralis*, salmonid balıklarını olumsuz etkileyerek önemli ekonomik kayıplara neden olmaktadır. Bu çalışmada, dönme hastalığının klinik belirtilerini sergileyen enfekte gökkuşağı alabalığı (*Oncorhynchus mykiss*) örneklenmiş ve kapsamlı bir histopatolojik incelemeye tabi tutulmuştur. Sonuçlar beyinde, dalakta ve solungaç dokularında M. *cerebralis* sporlarının iltihaplanma, nekroz ve kist oluşumu gibi dikkate değer patolojik değişikliklerle birlikte olduğunu göstermektedir. Çalışma, *M.cerebralis* enfeksiyonunun seyrini detaylandırarak, konakçıdaki önemli doku hasarını ve bağışıklık tepkilerini ortaya koymaktadır. Sonuçlar, patojenin balık sağlığı üzerindeki etkilerini anlamak ve konakçı doku tepkisine ilişkin veri sağlamak adına histopatolojik yöntemlerle tanımlamanın önemini göstermektedir.

Anahtar Kelimeler: Myxozoa, Dönme hastalığı, Parazit, Histopatoloji.

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1. Introduction

Myxospores are common parasites of fish all over the world, and many species have been documented to cause serious damage to important freshwater and commercial marine fish (Lom and Dykova, 1994; Dar et al., 2017). Especially salmonid species are highly susceptible to *Myxobolus cerebralis* (*M. cerebralis*) as the causative agent of whirling disease (O'Grodnick, 1979; Gilbert et al., 2001). Infected fish exhibit a variety of clinical symptoms, from severe skeletal abnormalities and blackened caudal areas to the whirling activity that gives rise to the disease's name. In juvenile fish, severe infections often lead to death (Gilbert and Granath Jr., 2001).

M. cerebralis was first documented in Germany by Hoffman, 1990 in salmonids, then several fish species were reported as infected with *Myxobolus sp*.; Indian major carp (*Labeo rohita*) (Gupta and Kaur, 2017), Iberian barbel (*Luciobarbus bocagei*) (Molnar et al., 2012), roach (*Rutilus rutilus*) (Molnar et al., 2010), gibel carp (*Carassius auratus gibelio*) (Zhang et al., 2010), wild mullet (*Mugil cephalus*) (Kim et al., 2013), tilapia (*Tilapia zillii*) and *Saratherodon melanotheron melanotheron* (Gbankoto et al., 2003), carp (*Cyprinus carpio*) (Liu et al., 2013) and rainbow trout (*Oncorhynchus mykiss)* (Hedrick et al., 1999; Arsan et al., 2007; Antonio et al., 2015). The life cycle of the Myxobolus species in rainbow trout is quite complex, using both fish and invertebrates hosts. The obligate host is defined as *Tubifex tubifex* which releases triactinomyxon spores, and after penetration, the sporoplasm generates plasmodia in tissues. The migration of the parasite through the cranium and spine causes lesions and inflammation that result in pressure on nerves and whirling behaviour (El-Matbouli et al., 1995, 1999; El-Matbouli and Hoffmann, 1998; Rose et al., 2000; Gilbert and Granath, 2001; Akram et al., 2023).

The process of identifying the causative parasite, *M. cerebralis*, involves first confirming the developmental stages or spores in cartilage of tissue sections stained with hematoxylin and eosin (H&E) and then providing a definitive description of the spore consistent with its characteristic size and shape (Lom and Hoffman, 1970; Thoesen 1994);, however the direct fluorescent antibody test, the latter digestion concentration procedure and DNA-based diagnoses have been increasingly used for detection of the pathogen (Antonio et al., 1998).

In this study, *M. cerebralis* infection was determined in rainbow trout individuals with clinical signs from a rainbow trout farm and the histopathological characteristics were described. The formation and invasion in different organs were identified in detail.

2. Materials and Methods

Infected samples were determined in a rainbow trout farm that showed the clinical signs of "whirling disease". The moribund and recently dead samples were randomly collected and transferred to Izmir Katip Celebi University Faculty of Fisheries Fish Disease and Biotechnology Laboratory for clinical and pathological analyses.

Gill, liver, spleen, brain, and gut tissue of infected specimens were fixed in 10% neutral buffer formalin solution and the standard ethanol dehydration protocol was applied. The tissue samples were processed for embedding in paraffin and then sectioned at 4-5 µm thickness. The samples were deparaffinized with xylene and stained with hematoxtylin and eosin (H&E) (Humason 1979; Hedrick et al. 1999). The slides were examined under a light microscope (Olympus, CX22RFS1).

Additionally, for bacteriological analysis, kidney and spleen tissues were inoculated on tryptic soy agar (TSA, Merck, Germany) and BHIA (Roberts and Shepherd, 1997; Karagouni et al., 2005).

3. Findings and Discussion

The present study provides a comprehensive histological surveillance of *M. cerebralis* infection that occurred in rainbow trout tissues. Our results reveal significant insights into histopathological changes across various organs associated with Myxobolus sp infestations, including brain, spleen, and gill tissues, and elucidated the progression within the host tissues. This comprehensive analysis highlights the intricate relationship between *M. cerebralis* and rainbow trout, providing information on pathogenic outcomes as well as possible host adaptations.

The infected fish showed whirling swimming behaviour in a rainbow trout farm in Aegean Region turkey (Figure 1A) and showed obvious skeletal disfigurations such as skeletal deformities of the head (Figure 1B) and body (Figure 1C). The mortality rate was calculated as 70% during infection. Myxobolus species have been shown to cause serious economic losses and serious negative impacts on fish health (Lom & Dyková, 2006). The clinical signs of infection is reported as skeletal deformities, darkened skin and erratic swimming behaviour, also called 'whirling' (Halliday 1976; Rose et al., 2000; Kelley et al., 2004a). Before histopathological studies, these clinical signs were observed in rainbow trout and fish during the infestation. Blazer et al. (2004) released a comparative study to detect susceptibility to *M. cerebralis* and concluded that, between lake trout (*Salvelinus namaycush*), Atlantic salmon (*Salmo salar),* and rainbow trout, the highly susceptible specie is found to be rainbow trout with inflammatory responses. In this study, the behavioural disorders, clinical and pathological findings, and histopathological results detected in rainbow trout allowed us to identify the causative agent of the disease as *M. cerebralis*.

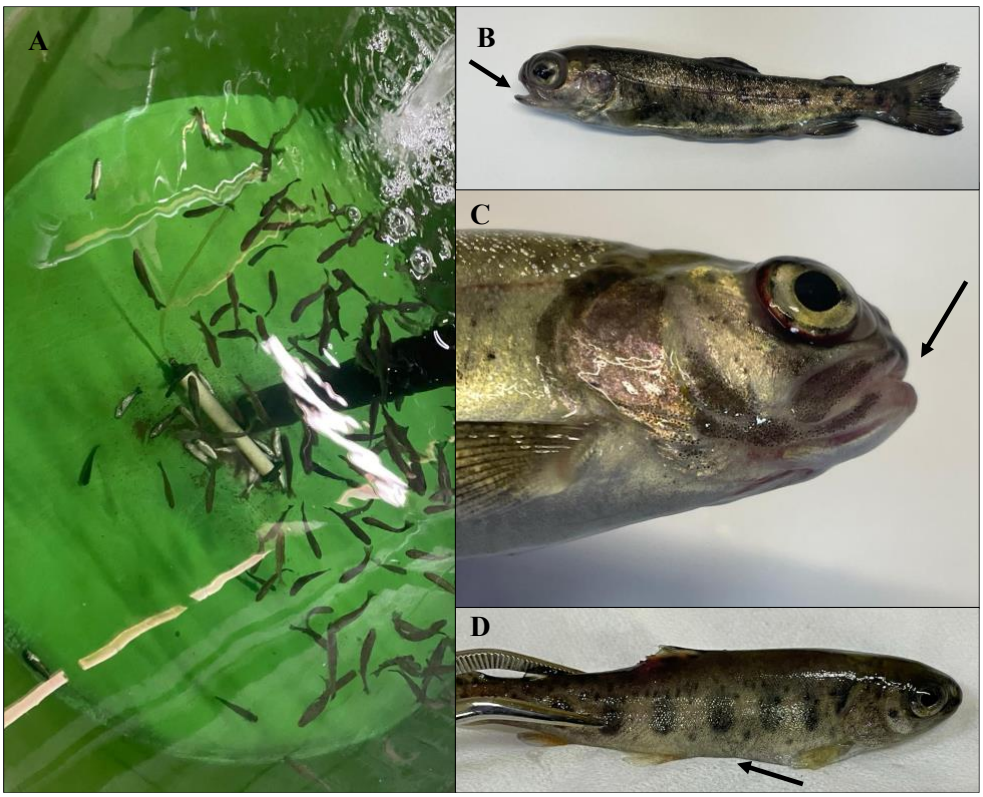

Figure 1.A: Suspected rainbow trout showed whirling behaviour, B-C: Skeletal deformities of the head, especially in the mouth area (arrow), D: Abdominal deformity due to anorexia (arrow)

There have been several diagnostic methods used, such as pepsin-trypsin digest (PTD), histopathology, polymerase chain reaction (PCR), nested PCR and quantitative real-time PCR for detection and quantification of *M. cerebralis* (Kelley et al., 2004a). However, the use of histopathological investigation has become essential for diagnosing Myxobolus infections. Using a microscopic examination of tissue sections, this approach can reveal the specific morphological changes caused by the parasite, including inflammation, cyst formation, size, shape, and the developmental stages of spores and necrosis of the tissue stained with hematoxylin and eosin (H&E) (Lom and Hoffman, 1970; Thoesen, 1994; Antonio et al. 1998; Feist & Longshaw, 2006). The detection of tissue damage, confirmation of infection, and Myxobolus spores are crucial for comprehending the dynamics of the disease and putting the right control measures in place (El-Matbouli and Hoffmann, 1989; Antonio et al., 1999). Since obvious clinical symptoms may not appear in the early stages of infection, histopathology is essential for a thorough assessment of the impacted fish populations (Antonio et al., 1999).

The results of the histological samples of this study were presented below. On examination of sections, the spores of *M. cerebralis* were found in the brain, spleen and gill tissues of the sampled individuals. The cysts were found to be oval or round, and the *M. cerebralis* were usually surrounded by a thin capsule and mature spores are in the majority (arrowheads). In the brain, plasmodium was clearly seen and the presence of mature myxospores associated with inflammotory cell infiltrations,

intracellular oedema, and necrosis was observed and presented in Figure 2. Significant pathological alterations are shown by histological examination of the brain tissue of rainbow trout (*Oncorhynchus mykiss*) infected with *M. cerebralis*. These changes are essential to understand the progression and impact of the disease. The Myxobolus species affects the cartilage and central nervous system of salmonid fish, severely affecting their neurological and anatomical systems. According to recent research, inflammation of the connecting cartilage after parasite removal causes restriction of the brain stem and spinal cord, which can lead to clinical symptoms and even death (Hedrick et al.,1998; Baldwin et al., 2000). The invasion of granulomatous inflammation of the vertebrate and skull, degeneration of the pathways between medulla and spinal cord and deformation in the lower brain stem appear to be the primary cause of the anomalies in the spinal cord and brain stem that are thought to be responsible for the abnormal behaviours in whirling disease (Rose et al., 2000).

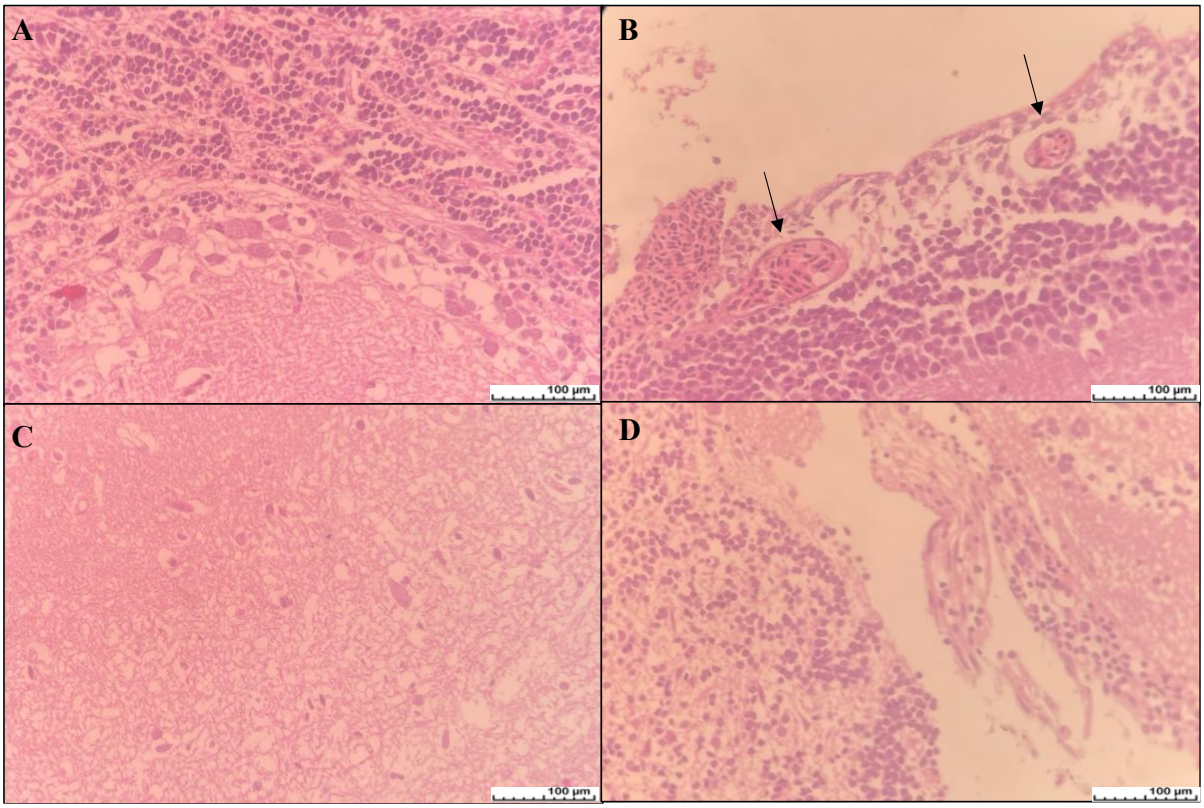

Figure 2. Brain tissues. A: Brain tissue with numerous intracellular aggregates of parastic stages, B: Plasmodium filled with spores in the interstitial tissue of the brain (arrow), C: Granulomatous response and inflammation in the brain tissue, D: Extensive presence of the parasite in the brain tissue (H&E x100)

Several concretional parasitic stages were determined in spleen tissue samples (Figure 3). Inflammatory cell infiltration was detected around the zone of infection in the spleen tissue associated with the dispersion (Figure 3). Kim et al. (2013) reported *Myxobolus episquamalis* infection in wild mullet (*Mugil cephalus)*, and histological analysis showed similar results to this present study.

Plasmodia of the parasite was detected on the scales, skin, heart, kidney, intestine, pancreas, gills, stomach, liver, and spleen tissue of infected individuals, and mature spores were observed to exist freely in spleen tissue.

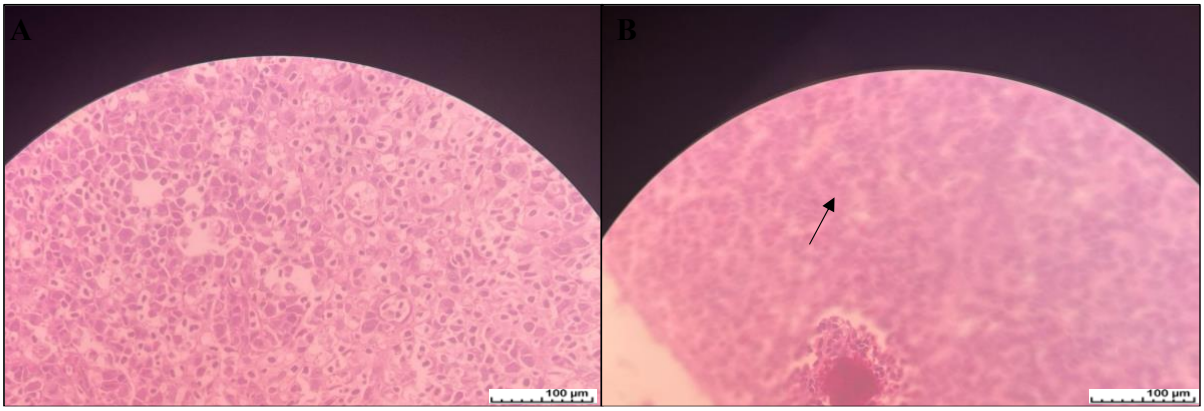

Figure 3. A: Aggregate of parasitic stages in spleen tissue, B: The presence of inflammatory cellular infiltration caused by *M. cerebralis* infection (arrow) (H&E x100)

Most cysts in the gill tissue were located in gill filaments that occupied the intervals between the respiratory lamellae. The presence of infection was associated with necrosis of cartilaginous tissues and inflammatory cell infiltrations in gill tissues that effected by mature *M. cerebralis.* Parasitic cysts were determined in the branchial cartilage and congestion was observed in the branchial blood vessels (Figure 4). Significant information on the pathogenic effects of these parasites on fish respiratory function is provided by histopathological examination of gill tissue from rainbow trout infected with species of Myxobolus. In some cases, the plasmodia leads to deformation of gill lamellae that causes loss of respiratory surface and damage 90% of the gill filament, observed as hyperplasia, vacuolization of the epithellium, and hypertrophy in the histological sections (Gupta and Kaur, 2017). Similar histological results have been reported from several researches (Antonio et al., 1998; Baldwin et al., 2000; Kelley et al., 2004b) from infected rainbow trout gill tissues, and especially, inflammatory cell infiltrations in the gill tissues and parasitic cyst formation were observed in the infected individuals in this present study.

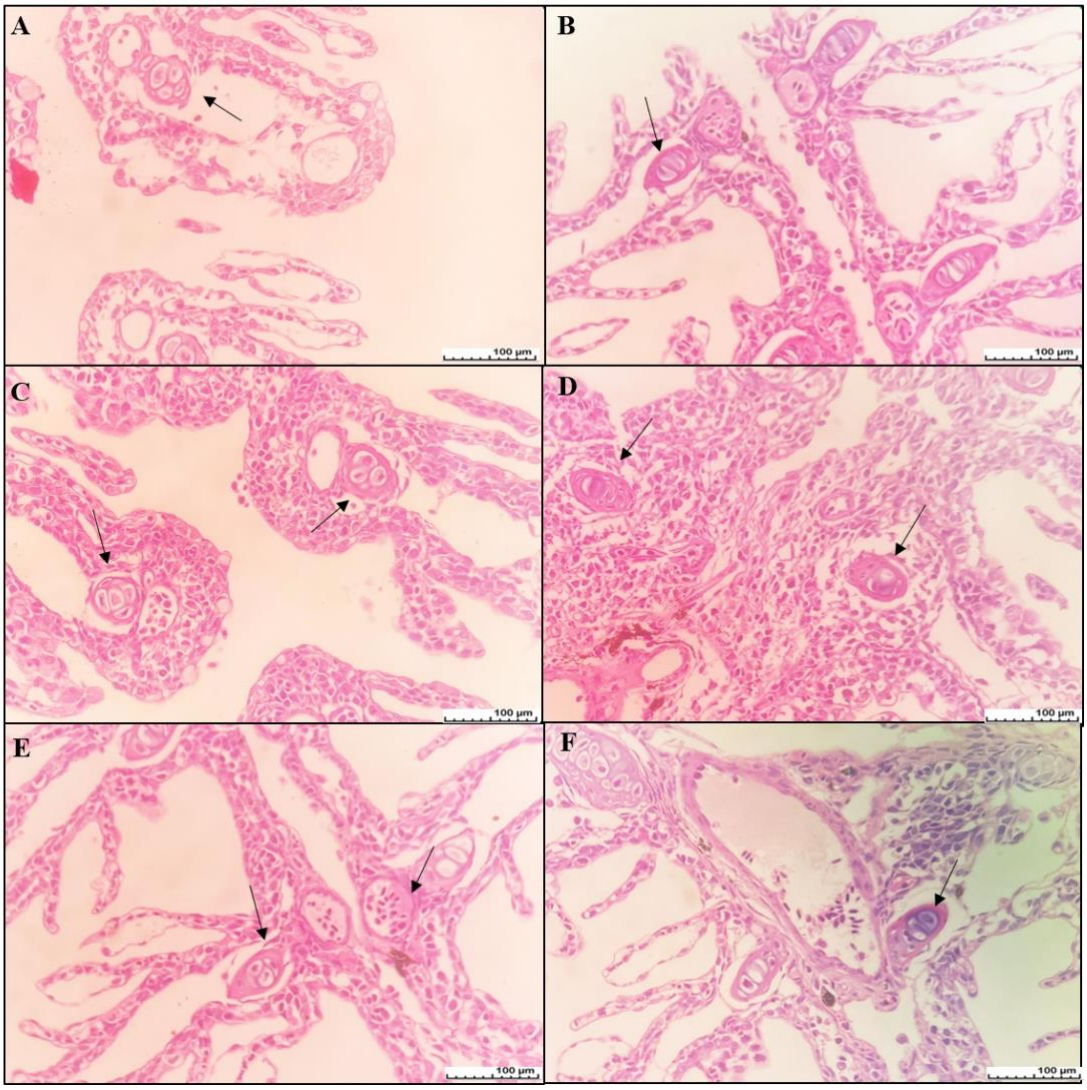

Figure 4. A, B, C, D, E, F: Different views of gill filaments with cysts of *M. cerebralis* in rainbow trout (arrows) (H&E x100)

4. Conclusions and Recommendations

In conclusion, the histopathological examination provides a comprehensive understanding of the impacts of *M. cerebralis* infections on the tissues of rainbow trout. The observed clinical and pathological changes caused by *M. cerebralis* in various organs are highlighted by the reported histological abnormalities, including hyperplasia, inflammatory responses, hypertrophy, and tissue degredation. These results show the importance of clarifying host-parasite interactions of myxozoan infections in fish. The immunological and molecular processes behind the tissue response that have been studied with further research need to be investigated in order to develop effective management strategies against Myxobolus infections in aquaculture.

Authors' Contributions

All authors contributed equally to the study.

Statement of Conflicts of Interest

There is no conflict of interest between the authors.

Statement of Research and Publication Ethics

The author declares that this study complies with Research and Publication Ethics.

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