

Pathological Investigations in Tench (*Tinca tinca* (L., 1758)) Naturally Infected with *Ligula intestinalis* Plerocercoids

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ABSTRACT

The aim of this study was to examine the pathological findings of 30 naturally infected tench (*Tinca tinca*) with *Ligula intestinalis* plerocercoids from Gölhisar Lake (Burdur) in Mediterranean Area of Turkey. Clinically and macroscopically, poor and irregular swimming, hemorrhages at the bases of fins and around the anus, the presence of the plerocercoids in the viscera together with perivisceral hemorrhages and distension of abdomen were generally observed in the diseased fish. Microscopically, hemorrhages, necrosis and mononuclear cell infiltrations were seen in the viscera invaded by plerocercoids. In conclusion, the cestode *Ligula intestinalis* plerocercoids' pathological effects on the tench tissues were widely evaluated for the first time in Turkey.

Keywords: *Ligula intestinalis*; Cestode; Plerocercoid; Tench; Pathology.

INTRODUCTION

Ligula intestinalis (*L. intestinalis*) is a pseudophyllidean cestode and common intestinal parasite of many fish species such as Tench (*Tinca tinca*) (1-3). It can be a major threat to natural and farmed fish species. *L. intestinalis* has been the subject of a number of studies because of the harmful effects on the body cavity of fish hosts. The plerocercoid stage infests a range of freshwater fish species, especially members of the *Cyprinidae* and *Catostomidae* family (2, 4). Although *L. intestinalis* has been the subject of a number of studies, there are differences in pathogenicity and parasite-host relationships (2). *L. intestinalis* is reported from tench in Mogan Lake, Beyşehir Lake, Kovada Lake, Abant Lake, Yeniçağa Lake and Terkos Lake in Turkey (2-7).

The tench is a Eurosiberian, warm-water, cyprinid fish species that has been cultured for centuries as a so-called secondary fish in polyculture with carp (*Cyprinus carpio* L.), in ponds in many European countries and the former USSR.

On the European market tench is bought as a food for stocking open waters (especially for recreational fishing) and as an ornamental fish (8). The management and/or creation of new freshwater bodies such as reservoirs is frequently accompanied by a programme of fish stocking and tench is one of the most widely distributed fish species in Turkey. There is relatively little information on the impacts of tench on aquatic systems (9-10).

The aim of this study is to elucidate the pathological findings of tench naturally infected with *L. intestinalis* plerocercoids.

MATERIALS AND METHODS

The data analyzed in this study describes the pathology of *L. intestinalis* in tench from Gölhisar Lake (Lake District, Burdur) in Mediterranean Area of Turkey. A total of 30 tench that were caught with nets of various mesh sizes (23 and 30 mm) between January 2016 and March 2016 were

examined. Specimens were placed in a aerated well 80-liters aquarium filled with lake water. The fish were maintained in the aquarium for 4-5 hours. Clinical signs (swimming performance and abdomen shape) were noted. Fish and plerocercoids were respectively measured and weighed to the nearest 1.0 mm and 0.01 g, respectively. The prevalence (percentage of hosts infected) and the intensity (number of parasites per infected host) were calculated.

After the transfer to the pathology laboratory, the fish were immediately necropsied. Plerocercoids larvae were found free in the abdominal cavity and entangled with viscera. Plerocercoids removed from the fish were counted, their length measured and weighed. The structure of the scolex and strobila were observed using a stereomicroscope. *L. intestinalis* plerocercoids show very limited structural differentiation. They were flat, unsegmented and had a tapering anterior end with two bothridia plerocercoids. The observed parasites were identified as *Ligula intestinalis* cestoda based on taxonomical features. Anterior end of strobila bluntly pointed with poorly developed scolex; bothria was represented only by two shallow tiny slits (11). Tissue samples taken from tench were fixed in 10% neutral-buffer formalin solution, embedded in paraffin, sectioned at 5 µm, stained routinely with haematoxylin and eosin (H&E) and examined microscopically.

RESULTS

Clinically, poor and irregular swimming, lethargy and abdominal swelling were seen in the diseased fish. Especially, infected fish were distinguished by their abdominal swelling and irregular swimming.

In total 30 individuals of tench fish were examined and 25 (prevalence 83.3%) specimens were infected by *L. intestinalis* plerocercoids (Figure 1). The number of detected individuals of *L. intestinalis* was 68 (Intensity: 2.72) and the numbers of detected plerocercoids in the fish varied from one and maximum five. Specimens of tench ranged from 19 to 38 cm in length, and 85 to 1040 g in weight. Plerocercoids of *L. intestinalis* ranged from 10 to 60 cm in length and 0.42 to 5.12 g in weight. The 30 tench individuals consisted of 19 (63.3%) males and 11 (36.7%) females.

Macroscopically, darkening in the color of the skin, periocular hemorrhages, hemorrhages at the base of fins and around the anus, the presence of the plerocercoids in the viscera together with perivisceral hemorrhages and distension

of the abdomen were generally observed in the diseased fish. The liver was generally swollen and yellowish-white in color and the spleen was enlarged and dark in color. Unilateral exophthalmos and opacity of the cornea together with hemorrhage were seen in two fish. The gill filaments were swollen and covered by mucus.

Microscopically, hemorrhages (Figure 2), necrosis and mononuclear cell infiltrations were seen in the viscera invaded by plerocercoids. Sections from intestines showed dilatation of the villi, picnotic nuclei and nucleoli in the mucosal epithelial cells (Figure 3), increase of the Eosinophilic Granular Cells (EGCs) and mononuclear cell infiltrations in the submucosa and necrosis in the muscular layer. In the liver, severe and diffuse steatosis was observed. These hepatocytes with sharp cytoplasmic borders contained varying size lipid droplets. In addition, thrombosis and vasodilatation were noted in the liver. In the spleen, congestion was present with blood vessels filled with erythrocytes. The gills showed lamellar hyperplasia and marked edema in the primary lamellae and hyperemia and edema in the secondary lamellae (Figure 4). In the eyes of two fish, there were hemorrhages (Figure 5) and hyperemia of the sclera.

DISCUSSION

Cestode plerocercoids are some of the most harmful parasites of the body cavity of fish. The cestode *L. intestinalis* have a global distribution and are parasites of fish in lakes and reservoirs mainly in cyprinid fish (4). In fish, the larval stage (plerocercoids) of *L. intestinalis* is a most important tapeworm, which can be a significant threat to natural and farmed fish populations all over the world (12-13). In this study, tench from Gölhisar Lake naturally infected with *L.*



Figure 1: Macroscopic appearance of a tench (*Tinca tinca*) with two plerocercoids of *Ligula intestinalis*.

intestinalis were used as material and pathological findings were evaluated.

In a study, 310 freshwater fish were studied to determine the prevalence of *L. intestinalis* plerocercoids in Kars Plateau of North-Eastern Anatolia and plerocercoids were found in 2.6% (8/310) of the studied fish (14). In another study, more than 1,300 *Engraulicypris sardella* from Lake Malawi were examined and about 54% of the fish had infections of between one and eight *L. intestinalis* plerocercoids (15). In the present study a total of 30 tench were examined and 25 (prevalence 83.3%) of fish were seen naturally infected with *L. intestinalis* plerocercoids and the number of detected ple-

roceroids in the fish were between one and five. Ligulids are large parasites and can be as large as 20 cm length. The total weight of worm tissue may exceed the body weight of the fish host (4). In this study, specimens of tench were measured and ranged from 19 to 38 cm length and 85 to 1040 g in weight. Plerocercoids of *L. intestinalis* were measured and ranged from 10 to 60 cm in length and 0.42 to 5.12 g in weight.

When plerocercoids are present in the abdominal cavity of infected fish, a continuous compression and distortion occurs on the abdominal organs and viscera. Accordingly, disturbances can be seen in their normal functions and histological structures. In addition, infected fish often show

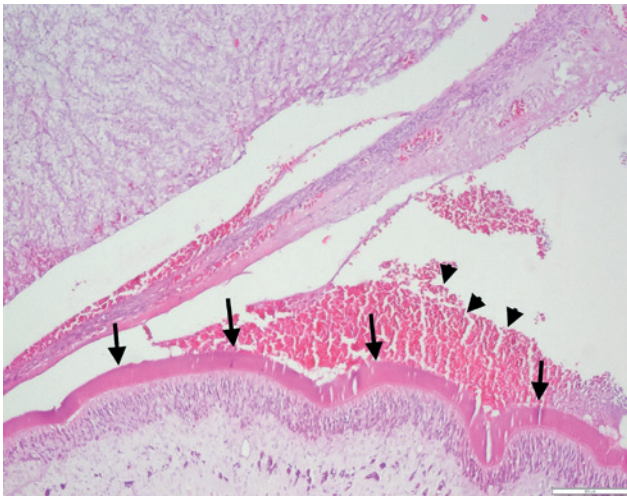


Figure 2: Hemorrhages (arrow heads) in the viscera invaded by plerocercoids (arrows), Hematoxylin-eosin stain; bar= 200 μ m.

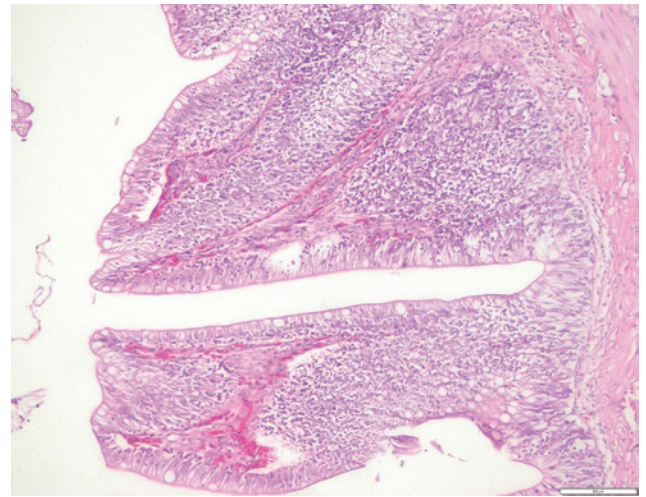


Figure 3: Dilatation of the villi, picnotic nuclei and nucleoli in the mucosal epithelium cells of intestines, Hematoxylin-eosin stain; bar= 100 μ m.

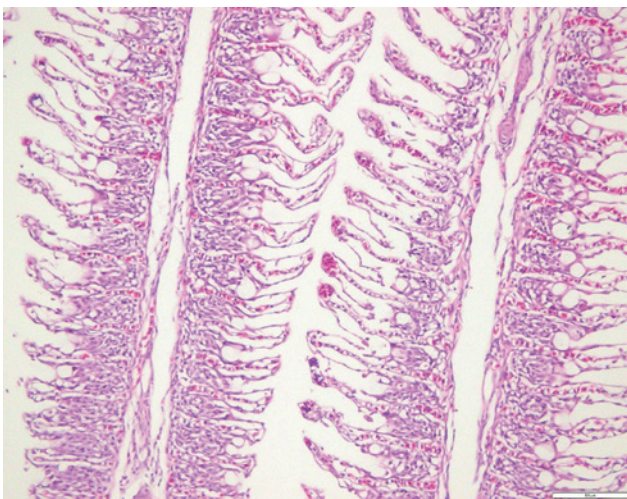


Figure 4: Marked edema in the primary lamellae; hyperemia and edema in the secondary lamellae of gills, Hematoxylin-eosin stain; bar= 100 μ m.

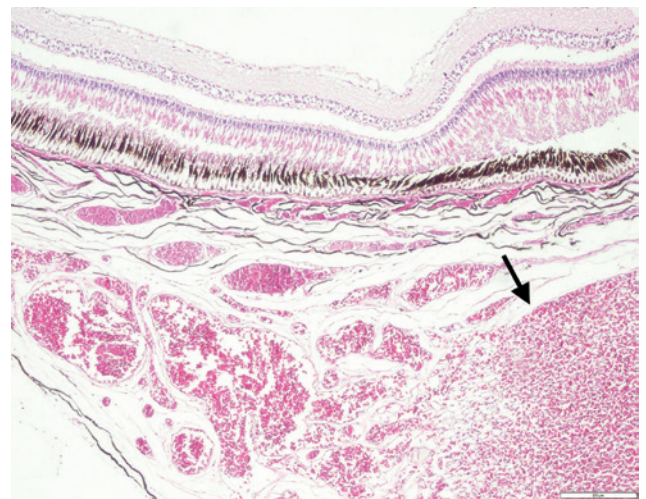


Figure 5: Hemorrhages (arrow) in the sclera, Hematoxylin-eosin stain; bar= 200 μ m.

swimming disorders and are more susceptible to predation. These fish can be recognized by their swollen abdomens (4, 16). In the present study, as a result of pressure of plerocercoids of *L. intestinalis* in the abdominal cavity of the fish, hemorrhages, necrosis and mononuclear cell infiltrations were seen in the viscera; dilatation of the villi, pyknotic nuclei and nucleoli in the mucosal epithelium cells and necrosis in the muscular layer were observed in the intestines. In addition, in agreement with the literature, poor and irregular swimming, abdominal swelling and susceptibility to hunting were noted in the infected fish. Infection also caused cholangiohepatitis, metaplastic hyperplasia and thrombosis that resulted from the compressive effect on the liver (16). In this study, severe and diffuse steatosis, thrombosis and vasodilatation were noted in the liver. It was considered that compressive effects of plerocercoids on liver may have caused diffuse steatosis in liver. This compressive effect may also cause circulatory disturbances and congestion in the spleen.

Due to their external placement the gills of the fish are affected by infectious agents such as parasites and non-infectious causes such as pollutants and genetic factors (17-18). In the present study, some pathological findings such as lamellar hyperplasia, adhesion and marked edema in the primary lamellae, hyperemia and edema in the secondary lamellae were observed. It was considered that these pathological findings in gills might have occurred in relation to the combined effects of the parasite causing induced circulatory disturbance and water pollution in the Gölhisar Lake.

In conclusion, to the best of our knowledge, this study constitutes the first description of findings of pathological effects of the food borne parasite *L. intestinalis* plerocercoids in the tench fish in Turkey.

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