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Original Research Article

Histological Effects of Nematode Infection in the Intestines of Freshwater Fish from the Khasa River in Kirkuk Province, Iraq

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Abstract: The presence of parasites, especially nematodes, in the intestines of *Cyprinus carpio* fish causes structural deformities of the intestine (Necrosis), accompanied by the occurrence of cases of rupture in the villi, as well as infiltration of inflammatory cells in the villi and degeneration of cells in the mucosal layer. The results of the current study, through the histological section of the intestines of infected and uninfected *Carasobarbus luteus*, showed the pathological effects of nematodes on the intestines of Leuciscus vorax and *Planiliza abu*, accompanied by necrosis of the villi, as well as necrosis and damage to the muscular layer and epithelial tissue, fibrin deposition in the mucosal layer, peeling of the submucosal layer, necrosis and rupture of the intestines of *Carasobarbus luteus*. In addition, the infiltration of inflammatory cells into the villi causes a clear decrease in the number of villi, the tearing of the superficial epithelial cells in the mucosal layer, as well as necrosis and rupture in the muscular layer, which causes a change in the function of the intestine and a decrease in its efficiency, represented by poor nutrition, weight loss, and cases of lethargy and inactivity. The attachment of the threadworm to the epithelial layer leads to the withdrawal of part of the epithelial cells and the dissolution, erosion and death of the cells.

Keywords: Nematode Infection, Carasobarbus luteus, Planiliza abu, epithelial cells.

INTRODUCTION

Fish living in several environments are exposed to different types of nematodes. The aquatic environment is the most stable and stable, as it provides most living organisms with suitable conditions for life, growth and reproduction [1-3]. The study is limited to nematodes that infect the intestines of fish. These worms often cause pathological effects ranging from mild to severe infection that leads to the death of fish. Symptoms of infection appear in internal inflammation, loss of appetite and weight loss due to the parasite sharing its food with the fish, which results in delayed growth and weak resistance of fish to diseases [4]. Nematodes are the most common parasites that infect fish. Different types parasitize the digestive tract and cause many serious diseases for fish [5, 6]. It also causes many diseases for fish because it lives inside the intestines and works to destroy the cells of the epithelial layer of the intestines. The reason for this is its ease of movement in the lining of the intestines, which destroys the mucous membranes and connective tissues of the intestines, causing peritonitis [7, 8].

Nematodes that infect fish cause various pathological histological changes in tissues and organs, including separation of muscles, necrosis of epithelial tissues as a result of worms adhering to these layers or due to mechanical action, or tissue hyperplasia as a result of the host's reaction, in addition to physiological and functional damage such as cell proliferation, immune mutation or strange behavioural response, or change in growth and reproductive damage [9, 10]. The current study describes the histological effects of nematode infection in the intestines of freshwater Fish from the Khasa River in Kirkuk Province, Iraq.

MATERIALS AND METHOD

Fish Samples Collected

Fish samples (*Cyprinus carpio*, *Carasobarbus luteus*, *Planiliza abu*, *Arabibarbus grypus*) were taken

from Khasa Dam, which is located 15 km from Kirkuk Governorate, during the study period from the beginning of January 2024 until September 2024. The number of fish examined during the study period was 541 fish. Fishermen used fishing nets and hook fishing in those areas. Live fish were transferred directly to the laboratory after being caught using a cork container containing a quantity of river or basin water. Dead fish were frozen until examined.

The fish were dissected according to the method (8) and (9) by making a longitudinal incision starting from the anus and heading forward to the mouth opening to search for nematodes. The internal cavity of the body was examined first, and then the intestines were isolated and placed in a Petri dish. Then, the intestines were examined under a dissecting microscope to search for nematodes.

Tissue Section of Preparation

To find out how these worms affected the infected fish, tissue sections were made from the fish's intestines once the dissection procedure was finished. A neutral formalin solution at a 10% concentration was used to fix the tissue research materials until tissue slices were ready. The intestinal tissue was removed and put in glass vials with a 10% formalin fixative solution. After that, the tissue slices were made using the procedures listed below:

Washing: The tissue sections were washed with tap water from the fixative solution for 15 minutes.

Dehydration

The sample is passed through intermittent ascending concentrations of ethyl alcohol 50%, 70%, 90% and 100% for 2-4 hours in concentration.

Clearing: The samples were passed through xylene for half an hour at each stage for Clearing.

Imbibition and embedding

The samples were passed through three stages of paraffin wax with a melting point of 50-60 ℃ for two hours, each stage at 60 °C for imbibition. The samples were embedded using the same paraffin wax used in the imbibition process and placed in a wax melting furnace with a wax dispenser at 60 ℃. After that, they were poured into metal basin moulds and carried with plastic mould holders. Embedding cassettes were used to obtain wax moulds containing the models and ready for cutting the models.

Sectioning

The wax moulds containing the tissue sections are fixed on the microtome and cut into 4-micrometre-thick pieces. After making a light smear of Mayer's albumin, the cut sections are placed on glass slides with a small brush. Then, the sections are taken to a hot plate at 40-45°C to be brushed. The sections are left for 48 hours at room temperature to dry completely.

Staining

After dissolving the wax from the sections by xylene, the sections are passed through a series of decreasing concentrations of ethyl alcohol 100%, 90%, 70%, 50%, and 30% for two minutes for each stage and transferred to distilled water. Then, the sections are stained with hematoxylin for 5 minutes and washed with tap water for 5 minutes until their colour disappears. The blue colour appears in them (Bluing) and immersed in ethyl alcohol 70%, then placed in eosin solution for 5 minutes, washed with tap water for 5 minutes, and passed through a series of ascending concentrations of ethyl alcohol 30%, 50%, 90%, 100% for one to two minutes, then cooled with xylene and loaded with Distrene plasticizer xylene D.P.X medium and covered with a glass cover.

Photography

Photographing the tissue sections prepared to determine the histopathological effects on the intestines of fish infected with parasites using a digital camera attached to a compound light microscope.

RESULTS AND DISCUSSION

As a result of parasitic infections with nematodes in river fish, the effect of nematodes is more dangerous and harmful to fish, which was observed during the examination and dissection of the internal organs of infected and healthy fish, especially in the intestine area. Therefore, the study of the intestine area was chosen, and histological sections were made to know the effect of infection with nematodes and the occurrence of histological changes in the intestines of infected fish, including fish (*Cyprinus carpio, Carasobarbus luteus, Planiliza abu, Arabibarbus grypus*), which included: -

Cyprinus carpio

Through the current study, we notice the effect of the presence of parasites, especially nematodes, on the intestines of common carp fish, as it causes structural deformities of the intestines in the form of necrosis accompanied by the occurrence of cases of explosion in the villi, as well as infiltration of inflammatory cells in the villi and degeneration of the cells of the basement membrane layer, as shown in Figure (1) compared with the control group as in Figure (2), which shows the difference between the standard, uninfected form and the variable and different situation after the infection of the intestines.

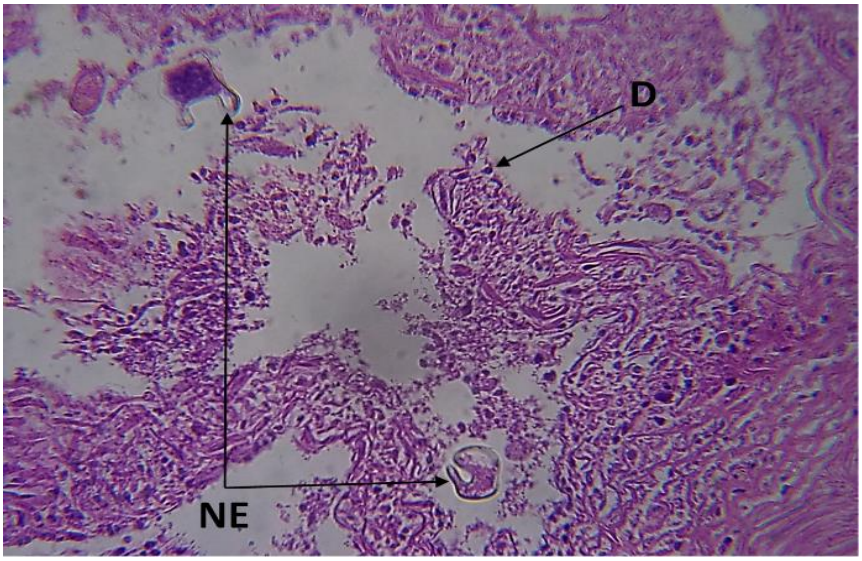

Figure (1): cross section of small intestine of infected *Cyprinus carpio* showed nematode (NE) and degeneration (D) of cells in mucosa layer

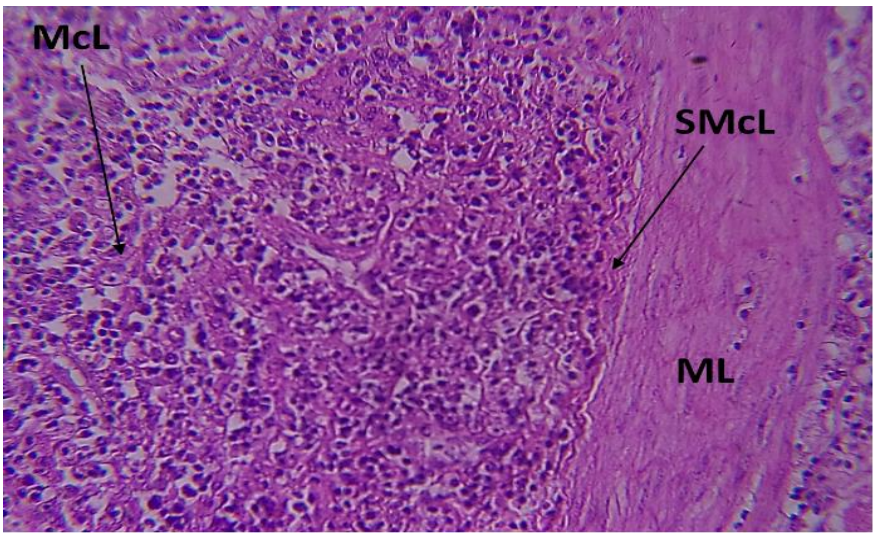

Figure (2): cross section of small intestine of normal *Cyprinus carpio* showed normal structure of mucosa layer (McL), submucosa layer (SMcL)and muscular layer (ML) H&E X400.

The reason for this difference or changes in the intestinal tissues is due to the attachment of the threadworm to the wall of the epithelial layer of the intestine through the mucous enzymes it contains, which leads to the withdrawal of part of the epithelial cells and their erosion and dissolution of its epithelial cells with their infiltration. This phenomenon may affect other layers of the intestinal wall, including the appearance of inflammatory cells (lymphocytes, plasma cells, macrophages). The villi become inflamed due to the presence of the worm, which causes friction with the villi, which reduces the absorption efficiency. That is, the presence of worms in large numbers in the intestines is a cause of obstruction of absorption and digestion, which causes severe damage to the intestines of fish, weight loss, and the death of fish [11]. This is consistent with the study [12], where he observed changes in the intestinal tissues of carp fish infected with the nematode worm, represented by the exposure of the intestinal mucosa, the formation of large, low villi, and the complete removal of the endothelial layer from the surface in areas that were penetrated by white blood cells.

The nematode causes the disintegration of the intestinal villi, the appearance of gaps and peeling of cells in the mucous membrane layer. This layered disintegration of the villi cells may be a cause of tissue poisoning in the fish intestines as a result of a malfunction in the fish's function, and mechanical damage in the area where the nematode is present causes inflammation or internal bleeding in the wall of the fish intestines [13].

In general, inflammatory cells were present at the beginning of the worm's presence when it tried to adhere to the intestinal walls using bothria, depending on the worm's size, especially in large worms. This may generate pressure on the intestinal wall and loss of the outer endothelial layer [12].

Leuciscus vorax

The results of the current study of the histological sections of the intestines of *Leuciscus vorax* infected with nematodes and those not infected are shown in Figures (3 & 4).

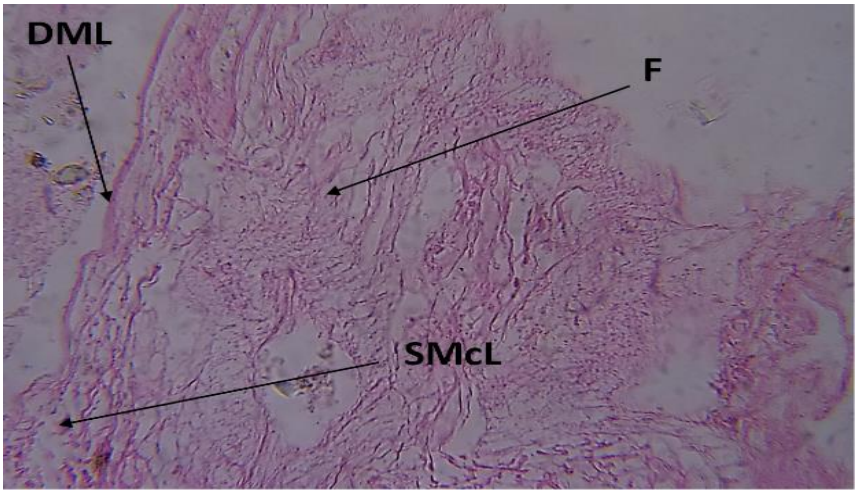

Figure (3): cross section of small intestine of infected *Leuciscus vorax* showed damage of muscular layer (DML), fibrin (F) deposition in mucosa layer and desquamation of submucosa layer (SMcL) H&E X400.

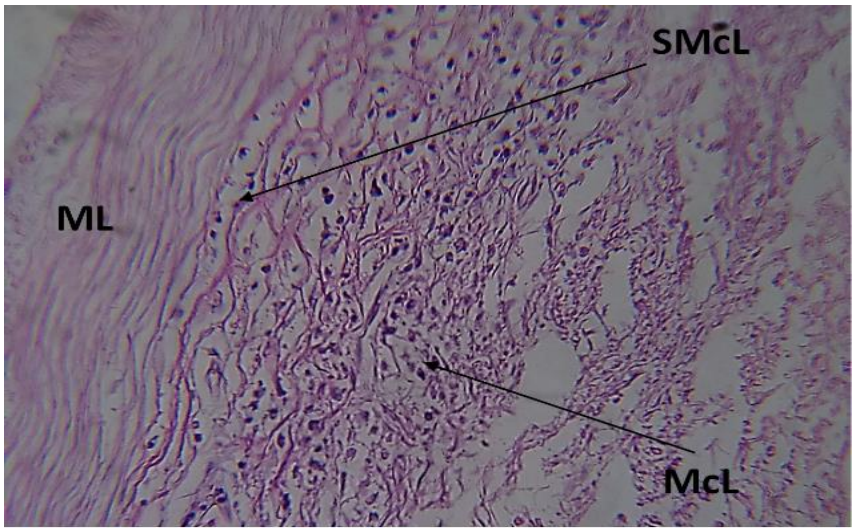

Figure (4): cross section of small intestine of normal *Leuciscus vorax* normal structure of mucosa layer (McL), submucosa layer (SMcL) and muscular layer (ML) H&E X400.

The results of the study showed the pathological effects of nematodes on the intestine of Leuciscus vorax, which are accompanied by villus necrosis, damage to the muscular layer, fibrin deposition in the mucosal layer, peeling of the submucosal layer and epithelial tissues, necrosis and rupture of the intestine of Leuciscus vorax [13]. Among the studies that indicated the histological changes in the intestine are the study of Nahal and his group [14], which indicated the histological changes of the intestine infected with nematodes, which include villus necrosis, necrosis and rupture of the muscular layer.

The current study shows that the worm causes damage differently in the intestines of infected fish, as the villi core is largely decomposed with lymphocytes and white blood cells, representing the host's reaction towards the nematode worm. It is known that this worm penetrates some layers of the walls of the infected intestine, especially when the snout or proboscis of the

parasite is relatively long, as this snout causes the closure of the intestinal cavity, especially when the worm is large in size and numerous. This is consistent with the study [14, 15], where he studied the effect of nematodes on the intestines of infected fish, which causes the decomposition of the cells that form the villi.

Carasobarbus luteus

The presence of nematodes, particularly in substantial numbers, induces histological abnormalities in the gut, including necrosis and rupture of the villi. We observe the infiltration of inflammatory cells into the villi and the adherence of the nematode to the epithelial layer, resulting in the retraction of certain epithelial cells and the disintegration and erosion of these cells. The intestinal villi manifested with the disruption of epithelial layers and cellular debris within the lumen of the infected intestine, alongside the degradation and loss of the basement membrane from the epithelial layers of the villus core. Concurrently, lymphocytes and white blood cells began to emerge during the collection due to the infection, accompanied by damage to the muscular layer [12]. Figures (5 & 6) show the histological section of the intestine of infected and uninfected *Carasobarbus luteus*.

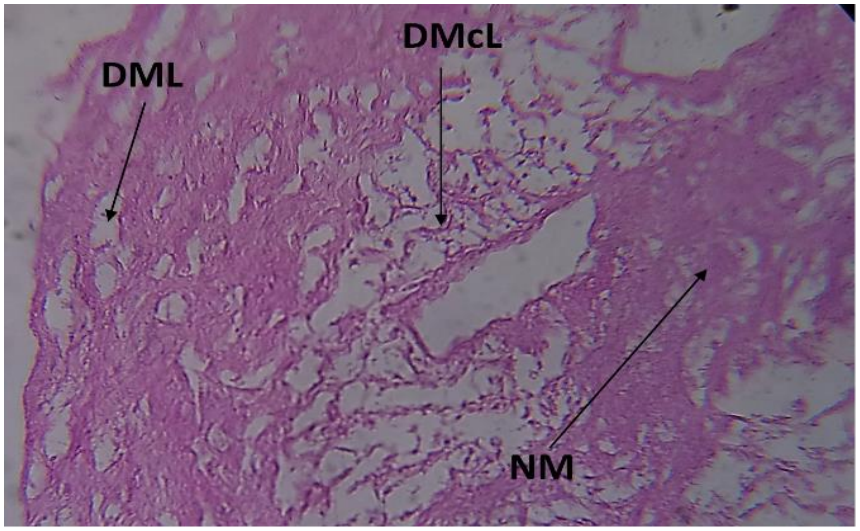

Figure (5): cross section of small intestine of infected Carasobarbus luteus showed damage of muscular layer (DML), damage of mucosa layer (DMcL) and necrotic materials (NM) H&E X400.

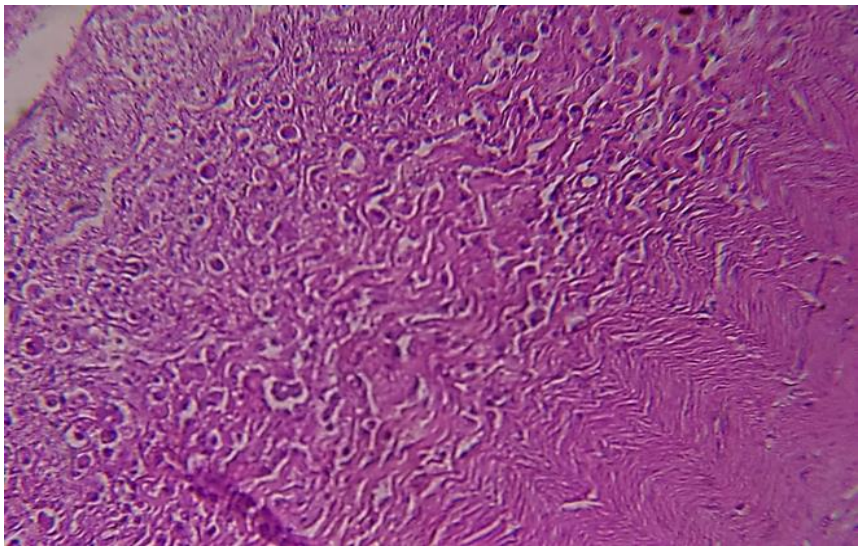

Figure 6: Cross section of small intestine of normal Carasobarbus luteus showed normal structure of mucosa layer (McL), submucosa layer (SMcL)and muscular layer (ML) H&E X400

Nematodes can cause physiological damage, such as cell proliferation, immune modification, proximal behavioural responses, growth and change, and necrosis and explosion. This is consistent with the study [16], which found that nematodes cause physiological damage, including cell proliferation and necrosis of some layers of the intestine.

Planiliza abu

The results of the current study showed Figures (7 & 8) the occurrence of necrosis of the villi accompanied by rupture as well as extensive erosion, causing an apparent decrease in the number of villi, as well as necrosis and rupture in the muscular layer, which causes a change in the function of the intestine and a decrease in its efficiency represented by poor nutrition, weight loss, and cases of lethargy and inactivity [12]. This results from the presence of nematodes in the intestine and their attachment to the intestinal wall and crowding, especially when they are present in large numbers, which causes damage to the layers of the intestine and villi [14].

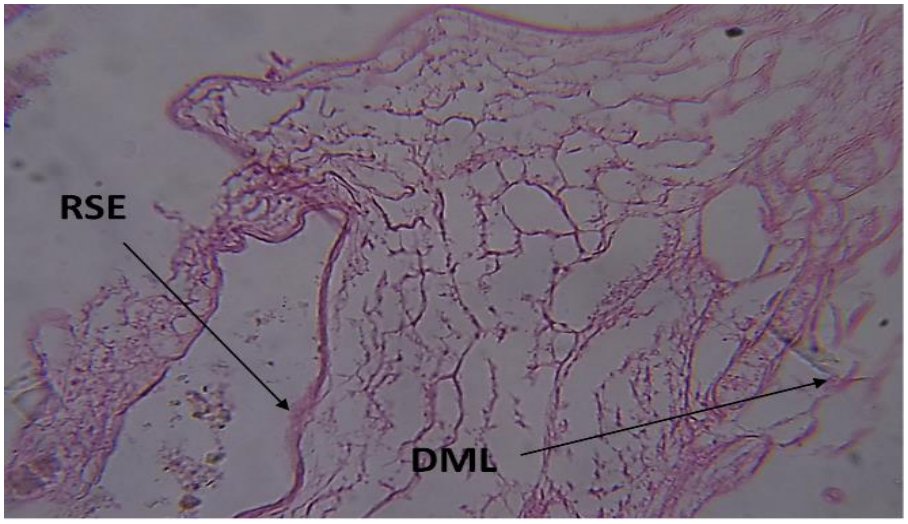

Figure (7): cross section of small intestine of infected *Planiliza abu* showed damage of muscular layer (DML), rupture of surface epithelium cells (RSE) in mucosa layer H&E X400.

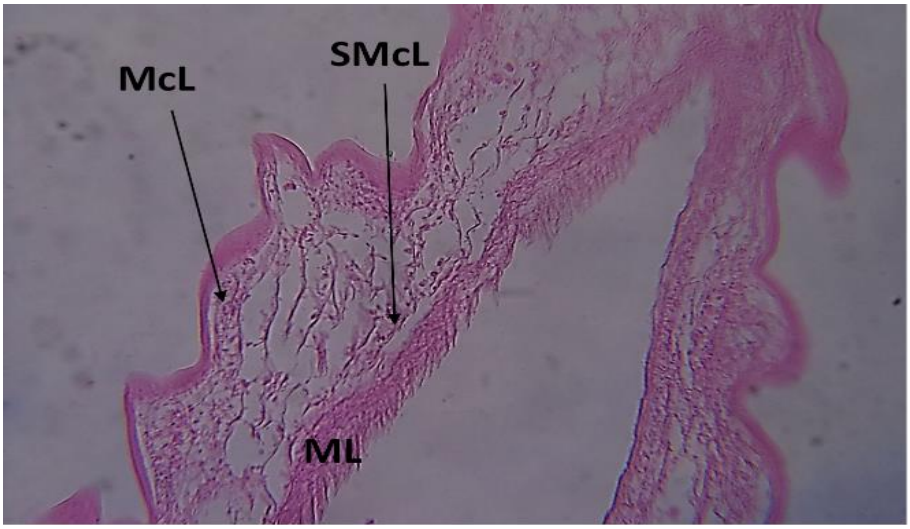

Figure (8): cross section of small intestine of normal *Planiliza abu* showed normal structure of mucosa layer (McL), submucosa layer (SMcL)and muscular layer (ML) H&E X400.

Histological changes of the intestine infected with the nematode worm are represented by the appearance of intestinal villi with rupture of the mucous membrane and cellular debris in the intestinal obstruction, as well as the loss of the basement membrane from the lumen of the villi and damage to the lumen of the muscular layer, which may lead to rupture and complete removal of the epithelial layer in the lumen of the intestine with the presence of white blood cells, especially lymphocytes [17-19].

CONCLUSIONS

This study aims to investigate the pathogenic effect of nematode histopathological changes in Freshwater Fish from the Khasa River in Kirkuk Province, Iraq; the histological changes observed with

nematode infestations were severe inflammatory reactions with myodegeneration and tissue damage, including marked cellular infiltration. Providing a basis for future investigations to discover more detailed morphological and histopathological identifications of this widely distributed nematode, helping in understanding the host-parasite relationships and zoonosis of these parasites, which may be helpful in fisheries management and public human health in Iraq.

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