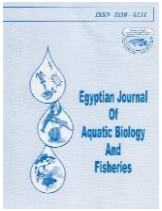
Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 26(4): 215 – 227 (2022)
www.ejabf.journals.ekb.eg



Hematological, biochemical, and histopathological alterations caused by the nematode parasite *Capillaria* sp. in the red tilapia (*Oreochromis* sp.) in Egypt

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ARTICLE INFO

Article History:
Received: April 16, 2022
Accepted: May 27, 2022
Online: July 7, 2022

Keywords:
Capillaria sp.,
Nematodes,
Red tilapia,
Hematology,
Biochemistry,
Histopathology.

ABSTRACT

Capillaria is a class of parasitic nematodes that are commonly found in fish as intermediate hosts to reach their final hosts, which include human. The present study was performed to analyze the prevalence of *Capillaria* sp. isolated from red tilapia fish (*Oreochromis* sp.) in Egypt. Fish were randomly collected alive or freshly dead, from May to November 2020 from a fish farm in Suez Governorate, Egypt. A total number of 190 freshwater fish with average weight and length as 26±0.35 g, and 11±0.25 cm, respectively were examined. The average body mass of infested males and females were 24±0.41 and 28±0.5 g, respectively, while the total length for both of them was 9.2±0.25 and 13±0.2 cm, respectively. Hematological and biochemical parameters were recorded in the infested fish. Also, histopathological examination of different organs of infested fish was done. Naturally infested fish revealed pathognomonic clinical signs such as excess mucus secretions, opening of the mouth and in some cases, appeared exophthalmia. A postmortem examination revealed an empty, shrinking stomach, as well as pale coloration of the internal organs. The total infestation prevalence was 41.1% with Capillariasis. Males showed more infestations (65.38 %) than females (34.62%). Regarding hematological and biochemical parameters, the study results showed a highly significant differences ($P \leq 0.05$) within infested and non-infested fish. Histopathological sections of the liver, spleen, and intestine of fish indicated that there were moderate abnormalities that occurred due to infestations**.**

INTRODUCTION

Fisheries play an important role in food security and as a source of income and social development in developing countries, while production of fish makes up 44% of total fish production in 2014, which was 74 million tons of fish worth 160 billion dollars, with all production directed for human consumption **(FAO, 2016)**. Fish production is not only important for gain money, but also for food security and social development in many countries, so the wide spread of a disease resulting from an interaction between pathogens, host and environment should be handled to overcome this problem **(Muktar *et al.*, 2016).**

Finfish considered as hosts to most ecto- and endoparasites, which are part of every ecosystem and affecting fish health **(Lieke *et al.*, 2020)**. In aquaculture, more than 50% of lost productivity caused by diseases; many causative agents such as high stocking densities and poor water quality are optimal conditions for the infestation and reproduction of parasites and other pathogens. The spread of infectious pathogens occurs due to unhygienic transportation of fish and equipment **(Assefa and Abunna, 2018).** Tilapia is the second most important cultured species after cyprinid fish, and farmed in over 100 countries worldwide, especially in China (1.8 MT), Indonesia (1.1 MT), and Egypt (875 thousand tones) **(Subasinghe, 2017)**.

The presence of fish diseases remains a major problem around the world, not only for losing productivity and decreasing marketability, but also for human diseases in many areas of the world due to the consumption of raw and uncooked fish meals. Several groups of helminthic parasites are known to infect fish and produce harmful effects on their hosts. Nematode worms are the largest group observed mostly in marine fish, and some of them infest fish at different stages of their life cycle as adults or larvae, causing damage **(Ţoţoiu *et al.*, 2013)**. *Capillaria philippinensis* is a tiny nematode parasite species of fish that consumes birds and is a member of the genus *Capillaria* that includes up to 250 members of parasites. Its importance arises from its ability to infect human and cause a serious illness that may end fatally if not diagnosed and treated in the suitable time **(Cross, 1992)**.

Endoparasitic infestation causes various problems in the organs and tissues of the host. The tissues react by encapsulating the parasites with cell hyperplasia, resulting in nodules, which are easy to visually identify **(Da Costa Eiras and Rego, 1989).** The parasite-host relationship may be important for environmental balance and depends on several factors, such as: parasite species, fish species attacked, the intensity of infection, and the organ affected.

Blood parameters studies are a valuable factor, because they provide relevant information about the physiological capacity of fish and serve as a useful tool for the evaluation of the immune system. Since such parameters can be considered as indicators of the capacity to transport oxygen (hemoglobin and hematocrit); hematological parameters may also provide additional information about the host's health and immune status, which are important indicators of changes to the host **(Ballarin *et al.*, 2004;Wells *et al.*, 2005;Tavares-Dias and Moraes, 2007)**.

The current study was carried out to evaluate nematode species (*Capillaria* sp.) infestations based on morphological characteristics in red tilapia fish (*Oreochromis* sp.), as well as their effect on their health status by measuring some hematological and biochemical parameters for infested and non-infested fish. Also histopathological pictures were introduced to detect the damage occurred by the parasitism.

2. MATERIALS AND METHODS

2.1. Fish samples collection

A total number of 190 *Oreochromis* sp. (red tilapia) were collected from May to November 2020 from fish farm in Suez city, Egypt by using nets of various sizes (50 and 70 mm). Fish were transferred alive to the fish diseases lab at the National

Institute of Oceanography and Fisheries (NIOF) branch in Suez City, Egypt, to complete parasitological examinations. Length was measured, weight and sex were recorded for each individual fish as well as clinical signs with postmortem findings were observed for fish samples to evaluate the degree of pathogenicity.

2.2. Clinical signs and postmortem examinations

Collected fish were examined for clinical and postmortem (P.M.) lesions according to the methods described by **Noga (2010)**.

2.3. Parasitological examinations

Fish were examined freshly under light and dissecting microscopes searching for any external parasites. After that, each fish was examined separately on a petri dish to complete internal examination by dissecting the abdominal line searching for parasites. Due to the smaller fish sizes, they opened vertically and the internal mucosa were extracted from the intestine, where they found to be heavy infested by nematode parasites with heavy egg distributions in the intestine and the abdominal cavity, which were later identified. For nematode morphological examination, samples are isolated freshly and put on clean slides with a drop of water or ethyl alcohol for fixation, then mounted with Canada balsam. Identification of parasites was done by following the steps according to **(Hoffman, 1967).** No need for staining because the samples were clear morphologically under the light microscope **(Bush *et al.*, 1997)**. The parasite prevalence (P %) was calculated according to the following equation **(Mgbemena *et al.*, 2020).**

$$\text{Parasite prevalence (P \%)} = \frac{\text{No. of infected fish}}{\text{Total no. of examined fish}} \times 100$$

2.4. Hematological and biochemical analyses

The blood samples were obtained from the caudal vein and separated into two aliquots **(Noga, 2010).** For hematological evaluations, the first aliquot was transferred into EDTA-containing labeled tubes of blood, for measuring the following: total leukocyte counts (TLC), differential leukocyte counts (DLC), total red blood cells (RBCs), hemoglobin content (Hb), hematocrit test (HCT), mean cell volume (MCV), mean corpuscular hemoglobin concentration (MCHC), mean cell hemoglobin (MCH), and platelet counts according to **Tran-Duy *et al.* (2008).** While the second aliquot was collected in a dry, clean tubes (without anticoagulants) and serum was collected for further biochemical tests to determine the liver enzymes, alanine amino-transferase (ALT) and aspartate amino-transferase (AST). Also, total protein (TP), albumen (ALB), globulin (GL), creatinine (CR), urea (U) and uric acid (UA) were measured according to **Blaxhall and Daisley (1973).**

2.5. Statistical analysis

SPSS biostatistics program 20 was used to compare the means with independent samples. T-test was applied to get the significant values ($P \leq 0.05$) which increased or decreased **(Dytham, 2011).**

2.6. Histopathological examination

The liver, spleen, and intestine were preserved in formalin 10% for 24 hours, then transferred to 70% ethyl alcohol for complete preservation. Methods for preparing complete pathological sections were done according to **Carleton *et al.* (1980)** and **Bancroft *et al.* (1996).**

3. RESULTS

3.1. Physical parameters and infestations

A total number of 190 *Oreochromis* sp. (red tilapia) were examined randomly and it was found that the average body mass recorded for all examined fish was 26±0.35 g with an average total length 11±0.25 cm. The average body mass of infested males and females was 24±0.41 and 28±0.5 g, respectively, while the total length for both infested males and females was 9.2±0.25 and 13±0.2 cm, respectively. It was found that 78 fish of 190 ones were infested with nematode species (41.1%). Most infestations were recorded in males (51 fish) with a prevalence of 65.38%, while the least percentage was recorded in females (27 fish) as 34.62%.

3.2. Clinical picture

The fish infested with nematode parasites showed distended abdomens with pale coloration and poor swimming activity. They appeared to settle in the aquaria bottoms, which ultimately led to a high chance of not being taken for food or predation.

3.3. Parasitological Findings

The nematode parasites recorded in this study were identified morphologically as *Capillaria* sp. A lot of nematode eggs were observed freely inside the intestinal cavity (Fig. 1). Also, the body of female Capillaria showed its uterus carried a lot of peanut eggs (Fig. 1).

3.4. Hematological analyses

There were some changes between infested and non-infested samples in respect to Hemoglobin (Hb), red blood cells (RBCs), and hematocrit (HCT) levels where they showed significant differences ($P \leq 0.05$) between the two groups. The non-infested group recorded higher values compared to the infested one. Some parameters, such as mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and total leukocyte count (TLC), showed non-significant differences ($P > 0.05$) between the two groups, where the non-infected one recorded higher values. Differential leukocyte count showed variations between parameters, as the neutrophil counts and eosinophils showed non-significant differences ($P>0.05$) between infested and non-infested groups, where the values are nearly the same. Lymphocytes and monocytes observations showed a significant difference ($P\leq0.05$) between infested and non-infested groups and the higher values were recorded in the infested group. Basophils values weren't evaluated due to zero results, so they have no statistics. Platelets count (PL) showed a highly significant difference between infested and non-infested groups, with the higher values in the infested group (Table 1).

Fig. 1. Adult females of *Capillaria* sp.: (a) Sever concentrations of peanut eggs freely inside the intestine (10x), (b), (c), and (d) Adult females (fresh mount) with a clear uterus carried eggs (40x).

3.5. Biochemical parameters:

As shown in table 2, there were some changes between infested and non-infested samples as: Blood TP, ALB, and GL results showed significant differences between infested and non-infested groups with increasing values of non-infested ones. Blood CR and U results showed also significant difference between the two groups, but the values increased more in infested group than in the other one. Blood UA value showed non-significant difference between two groups of samples with increasing in infested group result. Blood ALT and AST activities showed a highly significant difference between the two groups, with the infested group showing greater increases in values than the other (Table 2).

Table (1). Hematological parameters between infested and non-infested fish.

Parameters	Infested	Non infested	*P value*
Hb (g/dl)	8.83±0.72	11.60±0.21	0.021*
RBCs (mm^3)	1.6±0.26	2.82±0.24	0.027*
HCT %	30.3±3.9	41.43±0.86	0.050*
MCV (μm^3)	140±19.35	193.97±8.35	0.063
MCH g/dl	45.43±1.12	50.9±2.17	0.089
MCHC g/dl	25.47±1.51	29.17±0.52	0.081
TLC (µl)	57±5.75	69±2.08	0.114
Neutrophils %	51.33±3.76	51±4.16	0.955
Lymphocytes %	53.33±2.96	40.33±0.88	0.014*
Monocytes %	10±0.58	7±0.58	0.021*
Eosinophils %	1.33±0.33	1±0	0.374
PL (µL)	79.33±3.84	52.67±1.45	0.003*

*Indicates significant difference between values of the two groups (*P≤0.05*). Hb: hemoglobin, RBCs: red blood cells, HCT: hematocrit, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration, TLC: total leukocyte count, PL: platelets.

Table (2): Biochemical parameter variations between infested and non-infested groups.

Parameters	Infested	Non infested	*P value*
TP (g/dl)	3.30±0.26	4.57±0.23	0.023*
ALB (g/dl)	2.53±0.34	4.23±0.47	0.043*
GL (g/dl)	1.75±0.15	3.83±0.34	0.005*
CR (g/dl)	0.43±0.03	0.25±0.02	0.005*
U (g/dl)	23.47±1.48	16.23±2.11	0.048*
UA (g/dl)	9.2±0.38	7.4±0.62	0.069
ALT (U/L)	30.83±1.68	16.13±0.69	0.001*
AST (U/L)	38.7±2.42	25.77±0.97	0.008*

*Indicates significant difference between values of the two groups (*P≤0.05*). TP: total protein, ALB: albumin, GL: globulin, CR: creatinine, U: urea, UA: uric acid, ALT: alanine amino-transferase, AST: aspartate amino-transferase.

3.6. Histopathological results

No histological alterations were observed in the control liver and spleen of fish samples in all examined sections (Fig. 2a, c). The liver of infested fish revealed several pathological signs; the majority of the examined liver showed vacuolar degeneration and congestion of sinusoids, besides activation of melanomacrophage cells and mild hemosidrosis (Fig. 2b). The pancreatic tissues showed congestion of the portal vein and a few sections revealed atrophy of pancreatic cells. In addition, the splenic tissues of infested fish showed hemosidrosis and hyperplasia of melanomacrophages (Fig. 2d). No signs of alterations were detected in the control proximal intestine of red tilapia in the examined sections (Fig. 2e). Infected intestine with *Capillaria* sp. showed pathological signs include degeneration of mucosa epithelium resulting in lack normal architecture of intestinal villi, besides severe lymphocytic infiltrations (Fig. 2f).

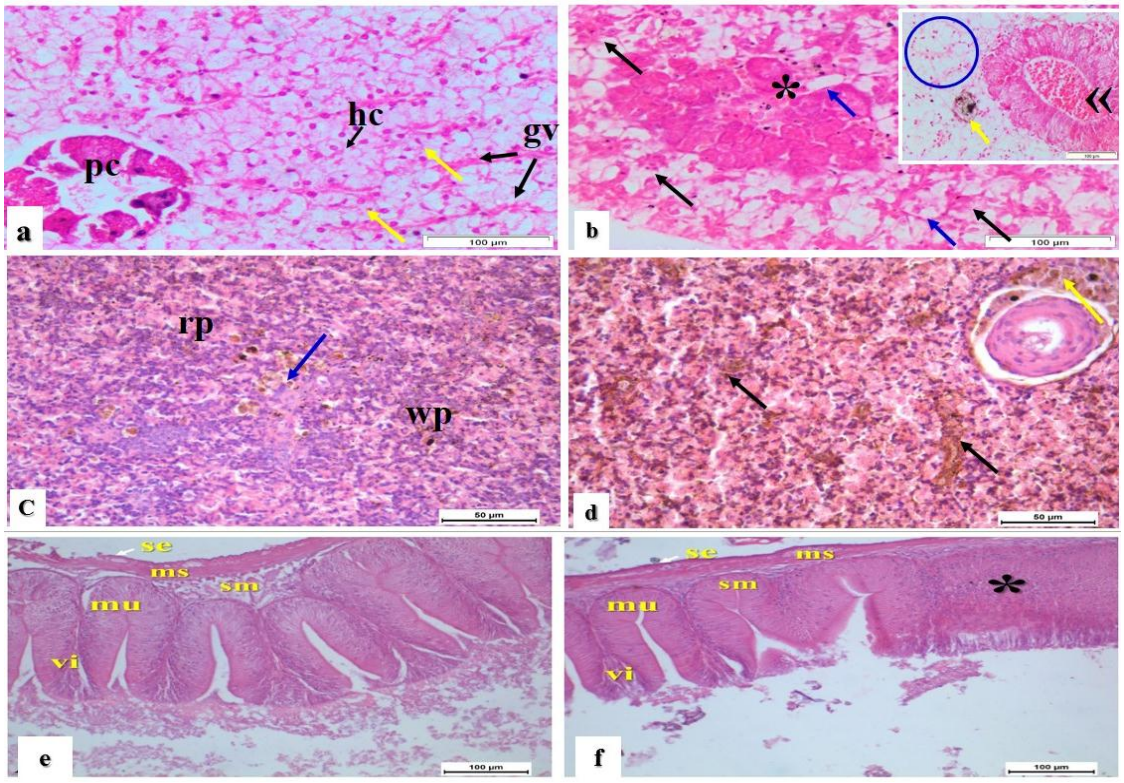

Fig. 2: **(a)** Section of control liver of red tilapia showed normal hepatocytes (hc), sinusoids (yellow arrows), pancreatic or acinar cells (pc) and glycogen granules (gv) (100x). **(b)** Section of infected liver revealed atrophy of acinar cells (asterisk), congestion of sinusoids (black arrows) and mild hemosidrosis (blue arrows) (100x). Window of **(b)** showed congestion of portal vein, severe moderate hydropic degenerations (circle) and activation of melanomacrophages (yellow arrows). **(C)** Control splenic section of red tilapia showed normal white (WP) and red (rp) bulbs, besides melanomacrophages (yellow arrow) (40x). **(d)** Section of infected spleen showed hemosidrosis (black arrows) and hyperplasia of melanomacrophage cells (yellow arrow) (40x). **(e)** Section of control proximal intestine of red tilapia showed normal layers of serosa (se), muscularis (ms), sub-mucosa (sm) and mucosa (mu) with folded villi (vi) (100x). **(f)** Section of infected intestine of red tilapia with *Capillaria* sp. showed degeneration of mucosa epithelium villi associated with severe lymphocytic infiltrations leading to lack villi structure (100x). H&E stain.

4. DISCUSSION

Helminthes are a large group of parasites in fish that are responsible for significant damage and pathogenic effects to their hosts, including emaciation and hindering growth of fish through absorbing a considerable amount of nutritive substances from the host's body, causing illness or even death **(Taha, 2018).** Nematodes are parasites affecting fishes and aquatic invertebrates, that acting as intermediate or final hosts during their life cycles. Infective L3 larvae may be accidently taken by human during eating raw or undercooked fish, causing anisakidosis **(Younis *et al.*, 2017).**

This study revealed the prevalence of *Capillaria* sp. parasite, isolated from red tilapia fish as 41.1% (65.38% in males and 34.62% in females from total infestations), which nearby the results mentioned by **Aly (2013),** who stated that the prevalence of infection with *Capillaria* larvae was 50% in grass carp (*Ctenopharyngodon idella*) during 2003. Infections with zoonotic Anisakis nematodes larvae *Contracaecum* spp. were detected in five fish species from Nasser Lake, Egypt, one of them was *Oreochromis niloticus* with prevalence of 30%, and general body emaciation with a distended abdomen **(Hamouda and Younis, 2022)**. Also *Tilapia zilli* in Chanchaga River located in southern part of Niger State, have similar results as the infestation with nematodes like; *Camallanus* sp. was 36.1%; *Cucullanus* sp. was 25%; *Capillaria* sp. was 21.7%; *Eustrongylides* sp. was 9%; and *Alvinocaris markensis* was 5.3% **(Mgbemena *et al.*, 2020)**. The closely related percentages of nematode species infestations in the previous studies with this study, may be due to a similar conditions affecting the parasite distribution in Egypt and Africa (Niger), especially in *Oreochromis* fish species. Due to *Capillaria* sp. direct life cycle, the eggs embryonate in the soil or water, then ingested by the fish host and hatched inside the intestine into an infective larvae followed by the adult worm. Water pollution, bad farm management could be a main factor affecting parasite distribution, also migratory birds that could transmit infestation by consuming infested fish along their migratory pathways and then excrete eggs to infect another fish in other areas, helped in spread the parasite **(El-Dib and Doss, 2002).**

Abiyu *et al.* (2020), found that nematode species isolated from *Oreochromis niloticus* recorded prevalence 57.3%, which divided as *Contracaecum* was 54.4%, *Eustrongylides* was 1.8% and *Camallanus* was 1%, with slightly higher prevalence in females (58.2%) than males (56.4%). This disagree with what detected in this study where males infestation (65.38 %) was higher than females (34.62%), this variations may be regarding to several conditions such as fish age, size or the immune response toward the parasite attacking. The predatory behavior described by **Fessehaye *et al.* (2006)**, about active males and lazy females during spawning periods and their behavior of mouth holding eggs made them easily attacked by the parasites.

Hematological parameters are important for diagnoses the health status of the fish affecting with helminthes parasites, also to evaluate the nutritional and physiological status of the fish **(Chagas and Val, 2003)**. Many parasites, especially internal ones, can acclimate inside the host body with no damages, in a cohabitation

behavior, but this balance may be broken due to changes in the environmental conditions, parasite behavior, and host immune system **(Tavares-Dias *et al.*, 1999b)**. Hematological changes may occur to the host when increase the parasitic load, causing severe illness for the fish body as a result of parasite infestations, which act as a stressor lead to cellular immune response toward nematodes infestation as one of the defense mechanisms against parasites.

This study revealed significant difference ($p \leq 0.05$) with lower values of Hb and RBCs count in infested group than non-infested one, which may be due to possible passage of *Capillaria* sp. from the stomach to the mesentery leading to possible hemorrhage causing anemia. **Corrêa *et al.* (2013)** recorded low hematological parameters of *Hoplias malabaricus* affected by L3 larvae of *Contracaecum* sp. nematodes. There is no significant difference ($p > 0.05$) in MCV, MCH, MCHC, and TLC count values in this study, with lower values in infested group than non-infested one. This confirmed the normochromic normocytic anemia, which agreed with what mentioned by **Kundu *et al.* (2016)** who recorded decreasing in the same parameters after being infested by nematode parasite *Eustrongylides* sp. in the freshwater fish *Channa punctatus*.

This study recorded a significantly difference ($p \leq 0.05$) with higher values of platelets and differential count of infested group than non-infested one, this group of cells could participate in inflammatory responses and their phagocytic activity that has been considered to have an organic defense function, as in different species of animals **(Grecchi *et al.*, 1980; Nagasawa *et al.*, 2014)**. Neutrophils showed no significant difference between the two groups, that agreed with what mentioned by **Tavares-Dias *et al.* (1999a),** who did not recorded significant changes in the level of circulating neutrophils in the bloodstream of *P. mesopotamicus* when parasitized with *Argulus* sp. that might be due to the *Capillaria* sp. parasite had an extracellular effect on the host cells. Monocytes showed a significant difference ($p \leq 0.05$) with higher values in the infested group than the non-infested one, which was agreed with what was mentioned by **Furtado *et al.* (2019),** who recorded an increase in monocyte count in *Oreochromis niloticus,* parasitized by *Argulus* sp., *Lamproglena* sp. and *Epistylis* sp. The variations in platelets and differential counts regarding to immunological responses of the host cells toward the parasitism action **(Silva-Souza *et al.*, 2000; Nakayasu *et al.*, 2005).**

This study confirmed that the infestation with *Capillaria* sp. was responsible for a series of biochemical changes in the host fish. The liver acts as the main metabolic center of detoxification, biosynthesis, and excretion of cholesterol. The necrosis of hepatocytes by parasitic infestations, leads to a decreasing of total protein levels in the serum as a result of decreasing protein synthesis. The loss of protein from serum may leads to an increasing the levels of transaminase activity, indicating the rapid consumption of reserve foods like protein and carbohydrates under stress conditions **(Goel and Gupta, 1985)**. Total protein, albumin, and globulin levels in this study showed significant difference ($p \leq 0.05$) with lower values in infested group than non-

infested one. This is due to the parasitic infestation, which causes proteolysis of TP levels and freeing the amino acids and their profiteering in different metabolic processes. These findings are agreed with **Kundu *et al.* (2016)** who found that the TP levels was increased but without changes in globulin levels, during infestation of three species of helminthes parasites, *Eustrongylides* sp (Nematodes), *Euclinostomum heterostomum* (Trematodes) and *Pallisentis allahabadii* (Acanthocephalan), in *Channa punctatus* Bloch, 1793 of Nadia district in West Bengal, India. Also, there is a significant increase in ALT and AST activities in infested fish than non-infested ones, which was agreed with what stated by **Kundu *et al.* (2016)** and **Svoboda *et al.* (2001).** They found an elevation in blood ALT and AST liver activities of *Channa punctatus* due to *Eustrongylides* sp. infection that was due to the liver functions disturbance and to the hepatocytes injuries**.**

Histological sections of liver, spleen, and intestine of the infested fish showed mild to severe hemorrhage, necrosis, and hyperplasia indicating the mild effect of *Capillaria* sp. parasites in the intestine with a little effect on the visceral organs as liver and spleen. This is may be due to the freely movement inside the intestine and the toxic substances released by the parasite that spread to other tissues. **Furtado *et al.* (2019)** found that mild hyperplasia and hypertrophy of the goblet cells distributed below the epidermis, being indicative of an immunological reaction caused by the parasite. The severity of histopathological changes caused by nematodes parasites, is correlated with the depth of the penetration in the host tissues.

5. CONCLUSION

The aim of this study is to investigate the pathogenic effect of *Capillaria* sp. parasite on hematological, biochemical, and histopathological changes in red tilapia fish (*Oreochromis* sp.), providing a basis for future investigations to discover more detailed morphological and genetic identifications of this widely distributed parasite helping in understand the host-parasite relationships and zoonosis of this parasites, which may helpful on fisheries management and public human health in Egypt.

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