Global Veterinaria 13 (2): 202-208, 2014
ISSN 1992-6197

DOI: 10.5829/idosi.gv.2014.13.02.84102

Parasitological and Histopathological Study of Digenetic Trematodes in Mullets from Lake Qarun, Egypt

[1]H.L. Kotb, [2]O.A. Mahdy and [3]I.B. Shaheed

[1]Directorate of Veterinary Medicine at Fayoum Province, Egypt
[2]Department of Parasitology, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt
[3]Department of Pathology, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt

Abstract: This study was carried out on 190 mullets collected from Lake Qarun, Egypt. All data concerning the season and species and weight of the fishes were recorded. The examined fishes were *Mugil capito* (N=85) and *Mugil cephalus* (N=105). Both parasitological and histopathological examinations of intestine, liver, spleen, pancreas, heart, kidney, eyes, muscles, swim bladder, gonads and brain were carried out. Out of 190 examined mullets, 124 fish (65.3%) had digenetic trematode; 74.2% in *Mugil capito* and 58.1% in *Mugil cephalus*. The recorded adult digenetic trematodes were; Haplosplanchnus Caudatus, Dicrogaster contractus, Haplosplanchnus Pachysomus, Lecithobotrys putrescens, Saccocoeluim tensum and Unicoelium fayoumensis. In addition, encysted metacercariae of Heterophyid sp., Prohemistomatid (Caythocotylidae) and Diplostomatidae were also recorded. The main pathological findings in mullets infested with adult digenetic trematoda were; desquamation of intestinal villi with hypertrophy the muscular layer, vacuolation of hepatocytes with thrombosis, degenerated renal epithelium, necrosis of pancreatic acini, gliosis and demyelination of the brain, cardiac edema with hemosiderin deposition in between myofiberlis, retinal vacuolation and edema, decreased number of testicular sperms with degenerated testicular germinal epithelium and degenerated epithelium of oocytes in ovaries. Mullets infested with encysted metacercariae showed Zenker's necrosis of the muscles and metacercariae was surrounded with fibrous tissue. Swim bladder showed vacuolation of gland epithelium with congestion. Generally there were hyperactivity of melanomacrophges centers in liver, spleen, Kidney and gonads. It was concluded that the infestation with trematodes induces characteristic pathological lesions in various organs of mullets which are helpful in their diagnosis.

Key words: Digenetic trematodes · Encysted metacercaria · Lake Qarun · Mullets and Pathology

INTRODUCTION

Lake Qarun is the third largest lake in Egypt which is located in Fayoum province about 80 kilometers southwest of Cairo. The lake is one of Egypt's most important natural landmarks and a resource that has helped support human culture for 8000 years. Therefore it is rich in both natural and archaeological resources [1].

Beside its importance in tourism, it has an international importance as feeding and rest place for migratory birds. Therefore, Lake Qarun has been declared as a Natural Protectorate in 1989. Historically, the lake was a freshwater lake that had several fish species. By time, its regime was changed to a drainage reservoir and became a salty lake with high salinity of 35,000 ppm [2].

Nowadays, the most common fishes in Lake Qarun are *Tilapia zilli, Mugil cephalus, Mugil capito* and *Solea vulgaris* [3].

Although, parasitic diseases have been increasingly considered a sanitary and economic threat to Mediterranean aquaculture over recent decades, the studies dealt with the parasitic fish diseases in Lake Qarun are still scarce. Digenetic trematodes are common in mullets causing severe economical losses [4, 5].

Corresponding Author: Iman B. Shaheed, Department of Pathology, Faculty of Veterinary Medicine, Cairo University, Giza, PO 12211- Egypt.

In addition, some of these parasites constitute a zoonotic importance [6, 7]. The incidence of helminthes infestation in Lake Qarun fishes was 68.8% [8]. Several fish parasites were recorded in Lake Qarun such as *Haplosplanchnus caudatus, H. pachysomus, Lecithobotrys putrescens* and *Dicrogaster contractus* [5, 9].

Histopathological examination is one of the most important methods for diagnosis of parasitic diseases in fishes [10, 11].

Although several previous histopathological studies have been carried out on the various species of fishes with various parasitic infestations [12-14], As far we know no pathological studies were carried out on parasitic fish diseases in Lake Qarun.

Therefore the aims of the present study were to investigate the types of digenetic trematoda infest *Mugil* fish in Lake Qarun and to clarify the main histopathological lesions induced by these digenetic trematodes.

MATERIALS AND METHODS

Collection of Fish Samples: During the period extending from October 2010 to November 2013, a total number of (190) Mugil samples was collected from Lake Qarun.

All data concerning the season and species and weight of fish were recorded. The collected fishes were identified according to Whitehead *et al.* [15]. The examined fishes included; *Mugil capito* (85) and *Mugil cephalus* (105).

The collected live and freshly died samples were transferred quickly to the laboratory for parasitological examination according to Whitehead *et al*. [15].

Parasitological Examination: Muscles specimens were collected from right and left head, trunk and tail regions at different depths. The specimens were dissected, mixed with few drops of saline solution, compressed between two glass slides and examined under microscope. The observed encysted metacercariae were fixed in phosphate buffered formalin according to Gamal *et al.* [8].

The body cavity of each fish was opened and examined by naked eyes for the presence of macroscopic parasites or abnormal lesions of the different organs. The viscera were removed and each organ was placed in a Petri dish with saline solution and examined for cysts and parasites using a dissecting microscope. Liver, spleen, pancreas, kidney, heart, swim bladder, eyes, gonads and brain were examined. The intestinal tract of each fish was opened by scissors, left in normal saline solution for few minutes, shacked and examined for visible parasites by using a hand lens. Binocular dissecting microscope was used for detection of microscopic helminthes parasites. The collected digenean worms were washed several times with normal saline solution and fixed in 10% formalin solution. These specimens were stained using Simichon's acetocarimine and then observed under compound microscope.

The eye balls were removed and kept in saline solution. Eye lens and surrounding tissues were fragmented with needle and pressed between two slides and examined with dissecting microscope.

Histopathological Examination: Small specimens of muscles, intestine, liver, spleen, pancreas, kidney, heart, swim bladder, eye, gonads and brain were taken from infested fishes and preserved in 10% neutral buffered formalin solution. The tissue was processed by conventional methods, sectioned at 4-6 microns and stained with hematoxylin and eosin according to Bancroft and Gamble [16].

RESULTS

Out of 190 examined mullets, 124 fish (65.3%) had digenetic trematodes. The incidence of digenetic trematodes was 74.2 % and 58.1% in *Mugil capito and Mugil cephalus.*

As regards the fish weight, 70% of digenetic trematodes infections were recorded in small fishes that weighted 50-100 gram.

Seasonally, winter showed the highest incidence of digenetic trematodes infection in mullets of Lake Quran while summer showed the lowest incidence as shown in (Table 1). The incidence of digenean trematodes infection was higher in females of mullets (62.9%) than males (37.1%). The incidence of different types of digenetic trematodes among the examined mullets were collected in (Table 2). Mullets had either adult digenean trematodes (58.9%), EMC (10.5%) or mixed infestations of both (30.6%).

Parasitological Findings

Encysted Metacercariae of Digenean Trematodes

Prohemistomatid Metacercaria: It was found in the muscles, liver, spleen and pancreas of examined mullets. The cyst was double walled and spherical in shape. The outer wall was fragile and easily ruptured and the

Table 1: Seasonal incidence of infection with digenetic trematodes in the examined mullets

	Winter			Spring			Summer			Autumn		
Mullets	Ex.	Inf.	%	Ex.	Inf.	%	Ex.	Inf.	%	Ex.	Inf.	%
M. cephelus	40	33	82.5	20	12	60	30	5	16.7	15	11	73.3
M.capito	20	17	85	20	13	65	20	5	25	25	15	60

Ex. = number of examined mullets, Inf. = number of infected mullets

Table 2: Incidence of the different types of digenetic trematodes among the examined mullets

	M. cephalus		*M. capito*	
Digenetic Trematodes	No.	%	No.	%
Unicoelium fayoumensis	9	14.8	10	15.9
Haplosplanchnus pachysomus	9	14.8	5	7.9
Haplosplanchnus caudatus	6	9.8	7	11.1
Dicrogaster contractus	2	3.3	5	7.9
Saccocoeluim tensum	5	8.1	8	12.7
Lecithobotrys putrescens	4	6.6	3	4.8
Heterophyid sp. metacercaria	2	3.3	2	3.2
Prohemistomatid metacercaria	4	6.6	3	4.8
Diplostomatid metacercaria	1	1.6	1	1.6
Mixed infection of adult and EMC	19	31.1	19	30.1
Total	61	100	63	100

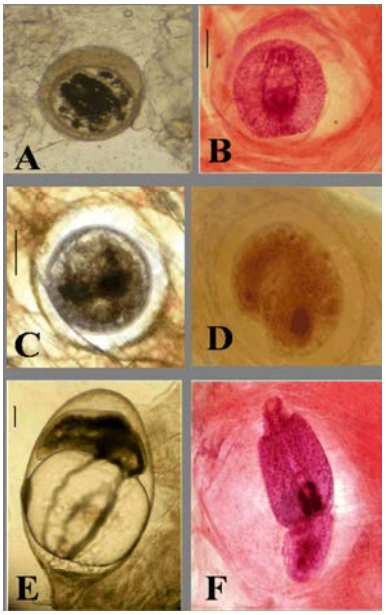

Fig. 1:
(A) *Prostomatidae* metacercaria (fresh specimens x400).
(B) *Prostomatidae* metacercaria (stained specimens x400).
(c) *Heterophyid* sp. metacercaria (fresh specimens x400)
(D) *Heterophyid* sp. Metacercaria (stained specimens x400).
(E) Diplostomatidae metacercaria (fresh specimens x400).
(F) Diplostomatidae metacercaria (stained specimens x400).

inner one was difficult to remove. The body of EMC was surrounded with a membranous wall containing fluid and the narrow part of the body had spines (Figure1 A, B).

***Heterophyid* Sp. Metacercaria:** It was found in muscles of the examined mullets. The cyst wall was globular to elliptical in shape and had two layers. The outer one was thin and transparent while the inner layer was homogenous with bright bluish color. Characteristic pigment granules in the body were also seen (Figure 1 C, D).

***Diplostomatid* Metacercaria:** It was found in the gonads and pancreas of the examined mullets. The cyst was double walled and elliptical to oval in shape. The outer wall was fragile and the inner one was difficult to remove. The EMC was clearly divided into coliform fore body and more or less cylindrical hind body (Figure 1 E, F).

Adult Digenean Trematodes

***Lecithobotrys Putrescens*:** It was recorded in the intestine of the examined Mugil capito. The worm was grayish white, elongated, tapering anteriorly and measuring 1-1.15 mm length. The whole body of the worm was covered by easily shed spines. The oral sucker is subterminal and larger than the ventral sucker (Figure 2A).

Unicoelium Fayoumensis: It was observed in the intestine of *Mugil capito*. The worm was fairly small, pointed and orange when fresh. The body was entirely covered by spines. The oral sucker was subterminal while the ventral one was rounded (Figure 2B).

Haplosplanchnus Pachysomus: It was seen in the intestine of mullets. The worms were fleshy, fairly large and orange when fresh. The body was somewhat fusiform, more pointed posteriorly than anteriorly. The body length measured 2.09-2.72 mm .The oral sucker was triangular, muscular and subterminal. The ventral sucker was long and club-shaped (Figure 2C).

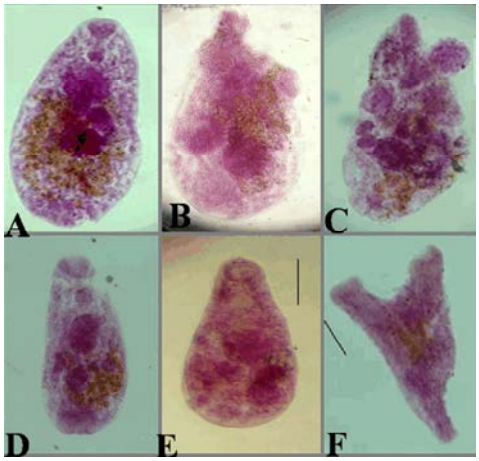

Fig. 2: Stained specimens of
(A) Lecithobotrys putrescens (x400).
(B) Unicoelium fayoumensis (x400).
(C) Haplosplanchnus pachysomus (x400).
(D) Dicrogaster contractus (x400).
(E) Sacoocoeluim tensum (x400).
(F) Haplosplanchnus caudatus (x400).

Dicrogaster Contractus: It was found in the intestines of mullets. It was an elongated worm measuring 0.73-0.83 mm in length. The body was mostly covered with spines. The oral sucker was oval and sub terminal (Figure 2D).

Saccocoeluim Tensum: It was found in the intestine of *Mugil capito*. The body was small, elongated and rounded at both ends. The body especially the anterior part was provided with minute spines. The body length was 0.56-0.82 mm. Oral sucker was subterminal and almost round in shape (Fig. 2E).

Haplosplanchnus Caudatus: It was observed in the intestines of the examined mullets. The body was Y-Shaped with unequal arms. The body measured 1.55-2.87 mm in length. The oral sucker was oval and subterminal (Figure 2F).

Histopathological Findings

Intestines: Adult digenean trematodes and encysted metacercariae were detected inside the intestinal lumen or embedded in the muscular layer causing its hypertrophy. Other sections showed the adult digenean trematodes embedded in between intestinal villi resulting in desquamation of intestinal villi or detected in the lumen. Appearance of rodlet cells and other inflammatory cells in submucosa were noticed (Figure 3A).

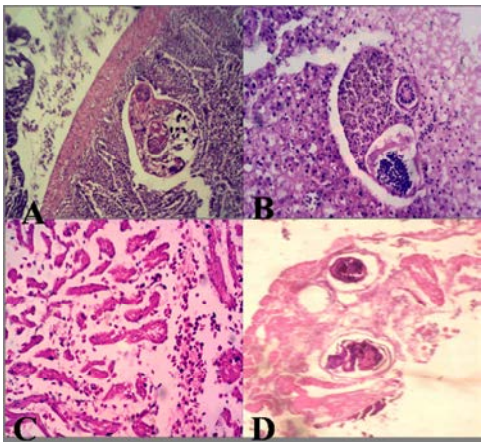

Fig. 3:
(A) Photomicrograph of Mugil capito, intestine, showing digenetic trematoda embedded in the submucosa with aggregations of excessive number of inflammatory cells (H&E X400).
(B) Photomicrograph of Mugil capito, liver, showing two encysted metacercariae were engulfed by melanomacrophges center cells and vacuolar hepatic degeneration (H&E X400).
(C) Photomicrograph of, Mugil cephalus, heart showing free RBCs, edema between muscles fibers and deposition of hemosiderin pigment (H&EX 400).
(D) Photomicrograph of, Mugil cephalus, muscles showing encysted metacercaria surrounded with a thick layer of fibrous tissue and surrounded with inflammatory cells (H&E X100).

Liver: Adult digenetic trematodes were observed inside the hepatic blood vessel of *Mugil capito* causing thrombosis and congestion of the blood vessels. Encysted metacercariae were detected in between the hepatocytes causing vacuolation of hepatocytes, hyperactivity of melanomacrophges centers and multiple inflammatory cells mainly eosinophilic granulocytes (Figure 3B). Some sections showed hepatic necrosis.

Heart: No adult or encysted metacercariae were seen in the heart but there were edema, hemorrhages and deposition of hemosiderin pigment between the cardiac muscle fibers of mullets infested with adult digenetic trematodes (Figure 3C).

Muscles: Encysted metacercariae were detected in muscles but in few cases it causing pressure atrophy and infiltration by inflammatory cells. EMC surrounded with fibrous tissue. Zenker's necrosis of the muscles was seen in some cases (Figure 3D).

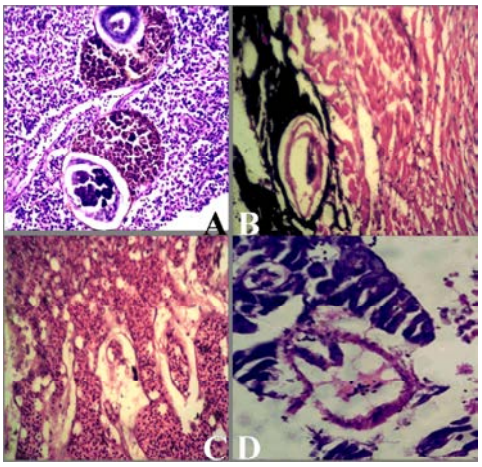

Fig. 4:
(A) Photomicrograph of Mugil capito, spleen showing hyperactivity of melanomacrophages centers (H &E X100).
(B) Photomicrograph of Mugil capito, swim bladder, showing encysted metacercariae surrounded with melanosis (H &E X100).
(C) Photomicrograph of, Mugil capito, eye, encysted metacercariae inside retina of the eye with severe congestion in arterioles and capillaries forming the rete mirabile (H &E X100).
(D) Photomicrograph of Mugil capito, pancreas, showing digenetic trematoda inside pancreatic tissue with severe necrosis in pancreatic acini (H&E X 400).

Spleen: Encysted metacercariae were detected inside the spleen. It caused hyperactivity of splenic melanomacrophges (Figure 4A). Other sections showed severe congestion of the blood vessels.

Swim Bladder: Encysted metacercariae were seen inside the swim bladder. It showed severe congestion in blood vessels and vacuolation in glandular epithelium of gas gland. The encysted metacercariae were surrounded by melanin pigment (Figure 4B).

Eye: Encysted metacercariae were seen inside mullets' eyes causing severe congestion in the arterioles and capillaries forming the rete mirabile in the choroid body of the retina and edema of the lens causing cataract (Figure 4C).

Pancreas: Adult trematodes and encysted metacercariae were recorded inside the pancreas of *Mugil capito* causing severe necrosis of acini with the presence of multiple fat cells in between acini. The encysted

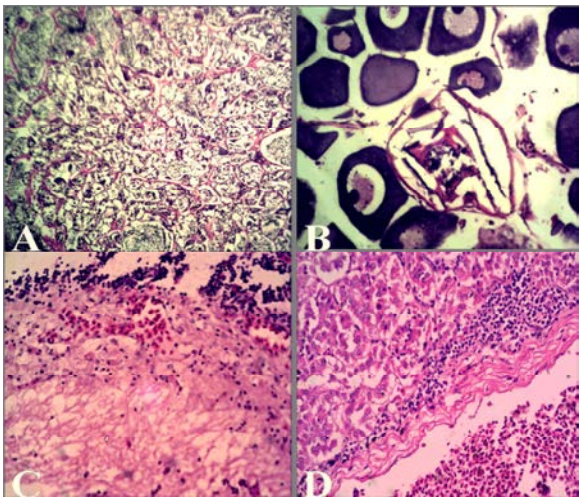

Figure 5:
(A) Photomicrograph of Mugil cephalus testis showing degenerative changes in germinal epithelium layer and decrease number of spermatocytes (H&EX 400).
(B) Photomicrograph of Mugil capito ovary, showing encysted metacercariae with aggregations of MMCs and congestion of blood vessels (H&E X400).
(C) Photomicrograph of Mugil cephalus brain showing congestion of blood capillaries, diffuse gliosis and demyelination (H&E X 400).
(D) Photomicrograph of Mugil cephalus kidney showing severe hemorrhage, hydropic degeneration of epithelial lining of renal tubules and inflammatory cells infiltration (H&EX 400).

metacercariae causing severe necrosis, pressure atrophy on pancreatic acini and severe congestion of pancreatic blood vessels (Figure 4D).

Gonads: Encysted metacercariae were recorded inside testes showed degenerative changes in germinal epithelium and decreased number of spermatocytes. Ovaries showed degeneration in follicular cell layer, edema in-between oocytes and congestion in blood vessels. The cysts were surrounded with melanomacrophges (Figure 5A&B).

Brain: No adult trematodes or encysted metacercariae were seen in the brain. There were severe congestion in blood capillaries, gliosis in the parenchyma of the brain, hemorrhages, deposition of hemosiderin pigment and infiltrations of inflammatory cells in the *Mugil capito* infected with digenetic trematodes (Figure 5C).

Kidneys: No adult dignean trematoda or encysted metacercariae were seen in the kidney. Various degenerative changes as hydropic degeneration in the epithelial lining of renal tubules were detected with congestion of renal blood vessels in *Mugil capito* infected with digenetic trematodes (Figure 5D).

DISCUSSION

Parasitic diseases are common in fishes and constitute a major economic and public health importance [9].

In the present study, the most commonly recorded digenetic trematodes in mullets from Lake Qarun were, *Haplosplanchnus caudatus, Haplosplanchnus pachysomus, Dicrogaster contractus*, *Lecithobotrys putrescens, Saccocoeluim tensum* and *Unicoelium fayoumensis.* Some of these parasites were previously reported in fishes from Lake Qarun [5, 8].

The present study showed that *M. cephalus* and *M. capito* in Lake Qarun were highly infected with digenetic trematodes. This result was agreed with the finding of a previous study [5]. This could be attributed to the high population of these mullets inside the lake and to their feeding habits as an omnivorous species which feeds on plankton, thus it is more susceptible to trematodes' infection than other marine fishes. In this respect, there are indications that the infection with digenetic trematodes has increased in the last few years due to high production of fish and shellfish in unhygienic fish ponds and anthropogenic activity [17].

Regarding the season, the highest incidence of digenean trematodes was in winter. This might be due to the high population of migratory birds inside the lake at this time of the year. These birds act as a final host for most fish parasites. Similar findings were mentioned previously by Al-Bassel *et al.* [5]. In contrast, the highest infection rate (52.5%) was recorded in summer at Lake Manzala, Egypt [17]. This could be explained by the difference between lakes and species of the examined fishes. The incidence of digenetic trematodes infection is higher in *M. capito* than in *M. cephalus*. Similar finding was mentioned before by Saoud *et al.* [1].

In the present study, small fishes showed higher incidence of digenetic trematodes' infections than large ones. This could be explained by the lower immunity of young fishes than adults. Similar explanation was mentioned previously by Salem *et al.* [18].

The present study showed that infestation with digenean trematodes had characteristic histopathological findings. The most commonly affected organs in the infested mullets were; spleen, liver, intestine, pancreas, gonads, eye, muscles and swim bladder. Similar histopathological and ultrastructural investigations of these organs in fishes harboring an extensive infection of larvae of an unidentified digenean trematode were reported by Dezfuli *et al.* [14].

The tissue response against the digenetic trematodes varies according to the affected organ. In intestine, it showed only inflammatory cells infiltrations while in liver, spleen and gonads showed hyperactivity of melanomacrophages centers (MMCs). Macrophage aggregates (MAs), alternatively known as melanomacrophages centers (MMCs) were recorded as focal accumulations of macrophages which has immunological response and usually containing the pigments of hemosiderin, lipofuscin, ceroid and melanin [12, 19]. These aggregations were also seen in kidney and in other organs, especially in relation to inflammation. In addition, macrophage aggregates qualify as anatomical and cytological biomarkers since they are known to change in number, size and pigments content in relation to fish health and environmental degradation [19]. This explains the observed hyperactivity of melanomacrophes centers in the examined liver, spleen and ovaries of mullets infected with digenean trematodes. In this respect, metabolic difference was found in MMCs among different organs in their morphometric characteristics and biochemical and elemental composition [19]. They added that MMCs have several functions in teleost fish as capture and storage the cations, the phagocytosis of cellular debris and immunological reactions.

The present results showed numerous rodlet cells which have large granules and rod-like core in the infected intestines as a defense mechanism. Rodlet cells seemed to be recruited when helminthes affected epithelial tissues [13].

Both adult trematodes and EMC were observed in the intestine, liver and pancreas causing mechanical compression of the pancreas and liver and vacuolar degeneration of hepatocytes. Similar findings were recorded by Hauck and May [20].

The presence of fat cells in pancreas infected with digenetic trematodes could be attributed to the mechanical injuries and pancreatitis which resulted in escape of pancreatic lipase from the pancreatic ducts and splitting the abdominal fat at the vicinity of the pancreas.

Although neither adult nor metacercariae of digenentic trematodes were seen inside the brain, kidneys and heart of the examined mullets,

degenerative changes were observed in these organs due to the circulating toxins released from the adult digenean trematodes.

In conclusion, adult *Haplosplanchnus caudatus, Dicrogaster contractus*, *Haplosplanchnus pachysomus*, *Lecithobotrys putrescens, Saccocoeluim tensum* and *Unicoelium fayoumensis* and EMC of *Heterophyid* sp. *Prohemistomatid* and *Diplostomatidae* were the common digenean trematodes that causing various pathognomonic lesions in mullets from Lake Qarun.

REFERENCES

1. Saoud, M.F., A.A. Ashour, M.M. Ramadan and D.A. Lamloom, 1990. Helminth parasites of fishes from two inland lakes in Egypt. The Japanese Journal of Parasitology, 39(3): 258-264.
2. Gian, G. and A. Zidan, 2003. Water quality of lake Qarun, Egypt. International Journal of Environmental Studies, 60(6): 651-657.
3. Mohamed, F.A., 2009. Histopathological studies on *Tilapia zilli* and *Solea vulgaris* from Lake Qarun, Egypt. World Journal Fish and Marine Sciences, 1(1): 29-39.
4. Southgate, P., 1993. Diseases in Aquaculture, In Brown, L. (Ed), Aquaculture for veterinarians, fish husbandry and medicine, Pergamon Press, Oxford, England, pp: 91-131.
5. Al-Bassel, D., A. Al-Swaehly, A. Abdel-Baki, M. Atwa and R. Al-Shawsh, 1999. Parasites of mullets from two different waters. Fish Biology and Fisheries, 3(3): 259-278.
6. Elsheikha, H.M. and A.M. Elshazly, 2008. Host-dependent variations in the seasonal prevalence and intensity of heterophyid encysted metacercariae (Digenea: Heterophyidea) in brackish water fish in Egypt. Veterinary Parasitology, 153(1-2): 65-72.
7. Shareef, P.A. and S. Abidi, 2012. Incidence and histopathology of encysted progenetic metacercaria of *Clinostomum complanatum* (Digenea: Clinostomidae) in *Channa punctatus* and its development in experimental host. Asian Pacific Journal of Tropical Biomedicine, 2 (6): 421-426.
8. Gamal, A. Z. A. El-Shahawi and D. Al-Bassel, 1992. General Survey of the helminthes parasites infecting the common fishes in some inland water in Egypt. Proceeding of Zoology Science, A.R.E. 23 (2): 227-241.
9. Al-Bassel, D. A. and A.A. Hussein, 2012. Survey on parasites infecting mullets from Egypt and Libya. Egyptian Academic Journal of Biological Sciences, 4(1): 9-19.
10. Lom, J. and I. Dykova, 1992. Protozoan parasites of fishes. Development in Aquaculture and fishers science, 2: 315.
11. Edward, J.N., 2010. Fish disease: diagnosis and treatment. Second Edition, Wiley-Black Well, USA.
12. Wolke, R.E., 1992. Piscine macrophage aggregates: A review. Annual Review of Fish Diseases, 2: 91-108.
13. Reite, O.B., 2005. The rodlet cells of teleostean fish: their potential role in host defense in relation to the role of mastcells/eosinophilic granule cells. Fish and Shellfish Immunology Journal, 19(3): 253-267.
14. Dezfuli, B.S., A. Lui, F. Pironi, M. Manera, A.P. Shinn and M. Lorenzoni, 2013.Cell types and structures involved in tench, *Tinca tinca* (L.), defense mechanisms against a systemic digenean infection. Journal of Fish Diseases, 36(6): 577-585.
15. Whitehead, P.J., M.L. Bauchot, J.C. Hureau, J. Nielson and E. Tortonese, 1984. Fishes of the north-eastern Atlantic and Mediterranean (Vol. 1). UNESCO, Paris, pp: 510.
16. Bancroft, J.D. and M. Gamble 2013. Theory and practice of histological techniques. 7th. Edition, Churchill Livingstone, Edinburgh, London, Melbourne and New York, pp: 252.
17. Ghobashy, M., A.M. Soliman and E.A. Hassan, 2010. Responses of the Mullet, *Liza auratus* and the Cichlid, *Oreochromis niloticus* from Lake Manzala (Egypt) to Heterophyd Infection. Journal of International Zoological Research, 6: 13-23.
18. Salem, L.M., Y. Metawea and H. Elsheikha, 2010. Prevalence of heterophyiosis in *Tilapia* fish and humans in Northern Egypt. Parasitology Research, 107(4): 1029-1034.
19. Ribeiro, H.J., M.S. Procópio, J.M. Gomes, F.O. Vieira, R.C. Russo, K. Balzuweit, H. Chiarini-Garcia, A.C. Castro, E. Rizzo and J.D. Corrêa, 2011. Functional dissimilarity of melanomacrophage centers in the liver and spleen from females of the teleost fish *Prochilodus argenteus*. Cell Tissue Research, 346(3): 417-425
20. Hauck, A.K. and E.B. May, 1997. Histopathologic alterations associated with Anisakis larvae in Pacific herring from Oregon. Journal of Wildlife Diseases, 13 (3): 290-293.