



Prevalence of diegenetic encysted metacercariae and their histopathological alterations in the Nile tilapia (*Oreochromis niloticus*), and the African catfish (*Clarias gariepinus*)

Doaa A. Yassen¹, Eman A. Abd El-Gawad^{1*}, Karima F. Mahrous², Khaled A. Abd El-Razik³, Ahmed A. Tantawy⁴, Amany A. Abbass^{1*}

- 1- Aquatic Animal Medicine department, Faculty of Veterinary Medicine, Benha University, Egypt
- 2- Genetics and Cell biology department, National Research Center (NRC), Egypt
- 3- Veterinary Infectious Diseases department, Veterinary Research Institute National Research Center (NRC), Egypt
- 4- Pathology department, Faculty of Veterinary Medicine, Benha University, Egypt

*Corresponding Authors: eman.mahmoud@fvtm.bu.edu; amanyabbass3@fvtm.bu.edu

ARTICLE INFO

Article History:

Received: Aug.17, 2023

Accepted: Sept. 3, 2023

Online: Sept. 12, 2023

Keywords:

African catfish;
Clinostomum spp;
Endoparasites;
Histopathology;
Nile tilapia

ABSTRACT

Parasitic infestation has a serious impact on fish health causing severe economic losses in fish farming as well as it has public health importance. This investigation was conducted on the prevalence of diagenetic encysted metacercariae (EMC) and its histopathological alterations in some freshwater fishes sampled from El – Riah El- Tawfiki, Qaliobia Governorate, Egypt. In this regard, a total of 600 randomly collected fish comprising 400 Nile tilapia (*Oreochromis niloticus*), and 200 African catfish (*Clarias gariepinus*) were examined macroscopically and microscopically from December 2020 to September 2021. The infested fishes showed white to yellowish cysts/nodules of variable size in different organs. The recorded EMC was identified as *Clinostomum* sp., *Euclinostomum* sp., *Haplorichiaed* sp., *Pygidiopsisigeneta* spp. *Cyanodiplostomum* spp, and *Prohemistomulum* spp. in the Nile tilapia and *Prohestomatide* spp. in African catfish. The overall prevalence of EMC was 29.25% in Nile tilapia and 22% in African catfish. The highest seasonal prevalence of EMC was 43% in summer for Nile tilapia and 34% in winter for African catfish. kidneys of Nile tilapia and musculature of African catfish recorded the highest tissue distribution of overall recorded EMC. Severe histopathological changes were observed in fish organs including gills, subcutaneous musculature, kidneys, liver, and ovaries. In conclusion, these results reflect the negative impacts of EMC on fish reproduction and general health status, therefore awareness about the control of fish parasites especially in natural resources should be conducted.

1.INTRODUCTION

The fish sector is considered one of the most important food manufacturing worldwide, because of the fast growth cycle of fish and their cheap, excellent source of superior protein and low saturated fat (FAO, 2016). Nile tilapia is one of the most widely cultivated freshwater fish worldwide due to its unique value for intensive culture (FAO, 2004). In Egypt, it constitutes over 65% of Egypt's total aquaculture production

(GAFRD, 2020), and it is the main fish species inhabiting Nile River. *Clarias gariepinus* is also one of the popular fish species that inhabit the Nile River and its tributaries and have been cultured with other fish species to expand the final output of fish farm productivity (Pouomogne, 2008). In the aquatic environment, fish are subject to different pollutants and environmental variations that favor stress on animals, making them vulnerable to disease outbreaks resulting in considerable economic losses (Abd El-Gawad *et al.*, 2012; El Asely *et al.*, 2015; El-Gohary *et al.*, 2020).

Fish parasitic diseases are common all over the world and one of the potential factors that restrict the development of total fish production (Bui *et al.*, 2019). In Egypt, parasitic diseases represent a large sector of fish diseases about 80% (GAFRD, 2020; Eldanasory *et al.*, 2022). Therefore, many previous studies have been conducted to explore the different parasites infecting various fish species (Eman *et al.*, 2014; Ghoneim *et al.*, 2015; Shaheen *et al.*, 2017; Mahmoud *et al.*, 2018; Younis *et al.*, 2022; Abd-ELrahman *et al.*, 2023). Endoparasites, particularly metacercaria of digenetic trematodes can affect the wild and cultured freshwater fish's health causing not only high economic losses but also can seriously affect human health, leading to sever digestive disorders (WHO, 2017). Fish-borne zoonotic trematodes such as *Clinostomum spp* are considered one of the most important fish trematodes of public health concern detected in several countries causing laryngo-pharyngitis and halzoun like disease in people who ingest raw or undercooked fish (Song *et al.*, 2018; Menconi *et al.*, 2020). All digenetic trematodes have similar life cycles that involve a definitive host (human, dog, or cat), snails (1st intermediate host) and fish act as 2nd intermediate host for larval stages of digenetic trematode. The longtime warm weather enables the outburst of parasites spread because of reproduction of the 1st intermediate host needed for parasitic life cycle (Younes *et al.*, 2016).

Histopathological inspection is one of the most key for diagnosis of parasitic diseases in aquatic animals (Noga, 2010). It considered as biomarker for aquatic environmental stressors from pollution or pathogens, and pointed to the relation between organ which infested, time of exposure, type of pathogen caused this alteration and the host immune response (Lehmann *et al.*, 2020; Tayel *et al.* 2020). The host tissues react to endoparasitic infestation by encapsulating the parasites with cell hyperplasia, resulting in nodules, which are easy to visually identify (Dezfuli *et al.*, 2007). Thus, the current study aimed to investigate the total and seasonal prevalence of encysted metacercaria of digenetic trematodes in Nile tilapia and African catfish with evaluation of the associated histopathological alterations in different body organs.

2. MATERIALS AND METHODS

2.1. Fish sampling and ethical considerations

A total of 600 fish samples, representing 400 Nile tilapia and 200 African catfish was collected randomly from El – Riah El- Tawfiki and its tributaries during the period from December 2020 to September 2021 (100 Nile tilapia and 50 African catfish per season). The average body weight and length of Nile tilapia was 60 ± 30 gm and 13 ± 2 cm respectively, and 185 ± 30 gm and 20 ± 2 cm for African catfish. A live and freshly dead fishes were quickly transported in separate clean plastic bags to the diagnostic Lab of Aquatic Animal Medicine department, Faculty of Veterinary Medicine, Benha University, Egypt for clinical, parasitological, and histopathological examinations. The research was conducted according to the guidelines of the committee of animal's welfare and research Ethics of Benha University, Faculty of Veterinary Medicine (BUFVTM: 13-10-22), Egypt.

2.2. Clinical and Postmortem examination

All fishes were carefully examined by the naked eye for any external lesions, macroscopic cysts and or nodules in subcutaneous musculature, gills, branchial cavity, gill chamber, fins, and eye. After that, fish were dissected, and each organ was placed in a petri dish containing physiological saline and inspected for presence of any nodules or cysts according to **Noga (2010)**. Total and seasonal prevalence were estimated according to **Ezenwaji et al. (2005)**.

2.3 Parasitological examination

From each examined fish, a small part from different organs were taken and squashed by compression between two clean glass slides to examine and photograph the encysted metacercaria by using a light microscope. The cysts collected from the kidneys and branchial cavity of Nile tilapia were excised with a fine needle and washed in a petri dish containing 0.09% physiological saline and the larvae sandwiched between two clean slides and preserved in 10% neutral buffered formalin. Then, they were stained in acetic acid alum carmine, cleared, and mounted in Canada balsam according to the procedures described by **Shaheen et al. (2014)**. The identifications of recovered EMC were limited to genus level as the fish harbor mostly larval stages of digenetic trematodes and could not be distinguished to species level morphologically (**Hoffman, 2019**). All the observed EMC were identified according to **Shareef and Abidi (2012)**; **Caffara et al. (2014)**; **Shaheen et al. (2014)**; **Abd rabo et al. (2017)**; **Hamouda and Younis (2021)**; **Younis et al. (2022)**; and **Abd-ELrahman et al. (2023)**.

2.4 Histopathological examination

Specimens from the infested organs (liver, kidneys, gills/branchiostegal musculature) of Nile tilapia and (kidney, liver, subcutaneous musculature, and ovary) of African catfish were fixed in 10% neutral buffered formalin for 48 hours followed by dehydrating in a series of graded ethyl alcohol and cleared in xylol then embedded in

paraffin wax. Using a microtome, tissue paraffin sections of 5µm thickness were prepared. These sections were stained with Meyer's hematoxylin and eosin according to **Bancroft and Gamble (2007)**. Histopathological lesions were photographed using Nikon Eclipse E800 microscope.

3. RESULTS

3.1. Clinical picture

The examined Nile tilapia and African catfish appeared without any pathognomonic external lesions. Yellowish cyst with varying size in branchial cavity and branchiostegal musculature (**Fig.1A&B**) and faint grayish white cyst embedded in the kidney's tissues were recorded in Nile tilapia (**Fig.1C**) were recorded. The examined African catfish revealed macroscopic and microscopic whitish nodules in kidney with congestion and enlargement (**Fig. 2A**), liver (**Fig. 2B**), and musculature (**Fig. 2C**).



Figure 1: (A&B) Nile tilapia showed yellowish cyst in the branchial cavity and branchiostegal musculature (arrow); (C) grayish white cyst embedded in the kidney's tissues (arrow).

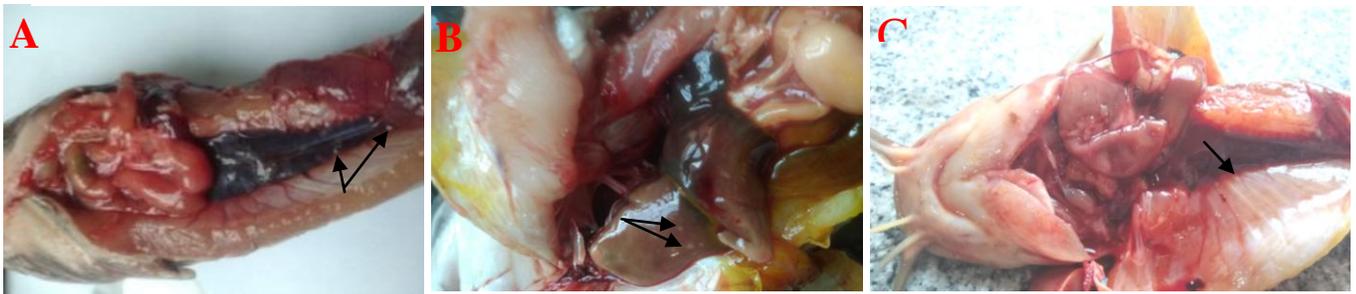


Figure 2: African catfish showed (A) whitish nodules in kidneys (arrow); (B) liver (arrow); (C) musculature (arrow).

3.2. Parasitological examination:

The recovered cysts from branchiostegal musculature and kidneys of Nile tilapia were identified as *Clinostomum* spp and *Euclinostomum* spp respectively. The excysted metacercaria of *Clinostomum* spp appeared flat elongated in shape, whitish yellow in color, has two long intestinal caeca, and two large oral and spherical ventral suckers (**Fig**

3A). The excysted metacercariae of *Euclinostomum* spp look like large leaf with laterally branched intestinal ceca and the ventral sucker was larger than oral sucker (**Fig. 3B**).

As shown in Figure 3, different types of microscopic EMC were observed in the liver of Nile tilapia. The EMC of *Haplorchid* spp appeared spherical, transparent with double layered cyst wall (**Fig. 3C**). EMC of *Pygidiopsisgeneta* spp also appeared as double wall layered cyst (**Fig. 4D**). The EMC of *prohemistomulum* spp was rounded in shape with double walled cyst and their color varied from grayish white to yellowish brown sheltered by a dense external wall and a tinny internal membrane and the EMC of *Cyanodiplostomum* spp was ovoid in shape surrounded by two transparent cyst walls (**Fig. 4E**). Other various forms of unidentified EMC in liver and muscles of Nile tilapia were observed (**Fig. 4F**).

Microscopic EMC in African catfish were looked as spherical or sub spherical in shape with double walled cyst and their color varied from grayish white to yellowish brown, which identified as *Prohestomatide* spp (**Fig.4A**). The EMC of *Cyanodiplostomum* spp was ovoid in shape surrounded by two transparent cyst walls (**Fig.4B**). Also, other unidentified types of EMC were observed in musculature (**Fig.5C**).

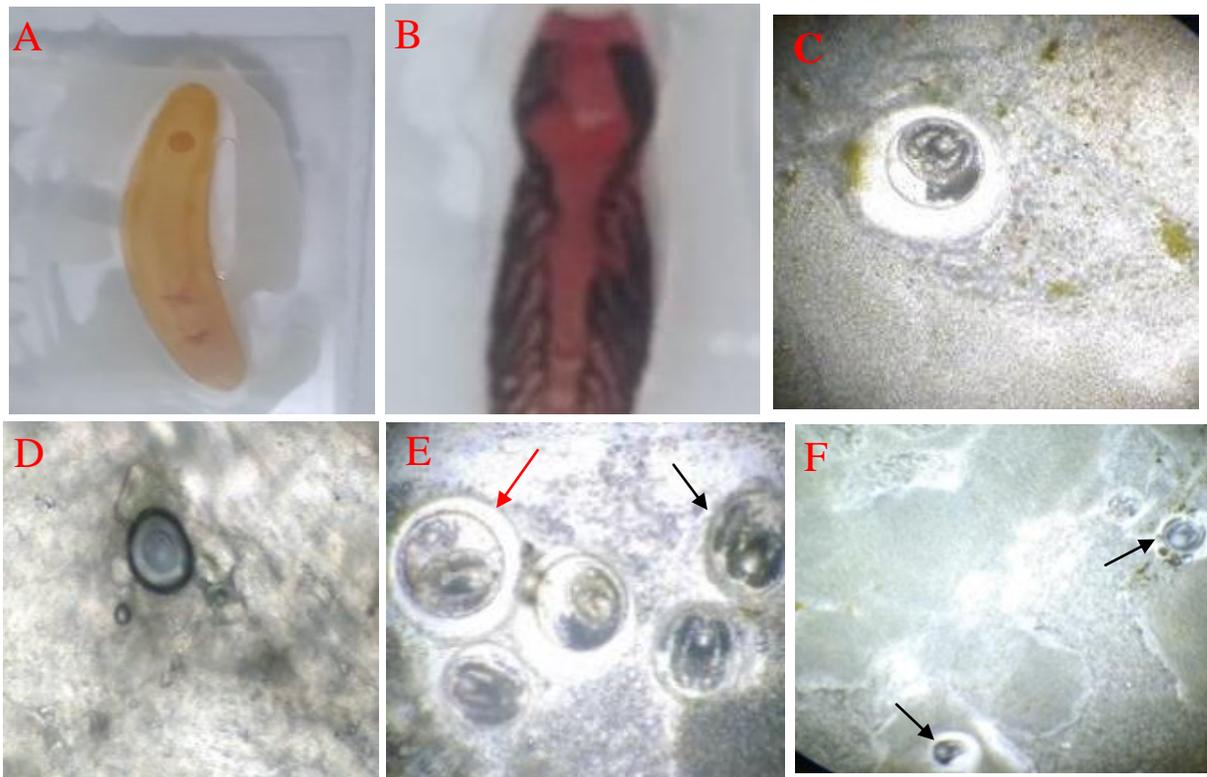


Figure 3: (A) *Clinstomum* spp from Nile tilapia, stained with acetic acid Alum carmine x10 (B) *Euclinostomum* spp. stained with acetic acid alum x10; (C-F) wet mount preparation from liver of Nile tilapia revealed EMC of *Haplorichiaed* spp x10 (C); EMC of *Pygidiopsisgeneta* spp x10 (D); EMC of *Prohemistomulum* spp (red arrow) and EMC of *Cyanodiplostomum* spp (black arrow) (E); and unidentified EMC (arrow) x10 (F).



Figure 4: Wet mount preparation from African catfish revealed microscopic EMC (x10); (A) EMC of *Prohemistomum* spp in liver; (B) EMC of *Cyanodiplostomum* spp in musculature; (C) unidentified EMC in musculature.

3.3. Seasonal Prevalence and tissue distribution of digenean EMC

The overall and seasonal prevalence of EMC recorded in Nile tilapia and African catfish were described in **Tables 1 & 2**. The total prevalence of all types of EMC in Nile tilapia was 29.25% with seasonal prevalence 20%, 26%, 43% and 28% in winter, spring, summer, and autumn respectively. The *Clinostomum* spp revealed prevalence (8%) with the highest seasonal prevalence 10% in spring and autumn followed by 9% in summer and 3% in winter. The total prevalence rate of *Euclinostomum* spp, was 5.75% with seasonal prevalence 5% in winter, 3% in spring, 11% in summer, and 4% in autumn. The other microscopic EMC recorded 15.5% total prevalence and the seasonal incidence was 12%, 13%, 23%, and 14% in winter, spring, summer, and autumn correspondingly. The recovered EMC from African catfish revealed overall prevalence 22% and seasonal was 34 %, 4%, 18% and 32% in autumn, winter, spring, and summer respectively.

As presented in **Table 3**, The tissue distribution of overall recorded EMC among the examined Nile tilapia was 9.5% from kidneys, 8.25% from liver, 8% from branchiostegal muscle 3.25% from musculature, and only one case from intestine representing 0.25%. The tissue distribution of overall recorded EMC among the examined African catfish was 10.5% in musculature, 7% from liver, 4% from kidneys, and only one case represents 0.5% was reported in ovary.

Table 1: Total prevalence of different types of recovered EMC from examined Nile tilapia and African catfish.

Fish species	No. Examined	No. infested	% of infestation	Recovered identified EMC
Nile tilapia	400	117	29.25	<i>Clinostomum</i> spp. - <i>Euclinostomum</i> spp- <i>Haplorichiaed</i> spp. - <i>Pygidiopsisigeneta</i> spp - <i>Cyanodiplostomum</i> spp.- <i>prohemistomulum</i> spp.
African catfish	200	44	22	<i>Prohistantidae</i> spp- <i>Cyanodiplostomum</i> spp

Table 2: Seasonal prevalence of different types of recovered EMC among the examined the Nile tilapia and the African catfish.

Species	EMC in the Nile tilapia				EMC in African catfish	
	<i>Clinostomum</i> spp.	<i>Euclinostomum</i> spp.	Microscopic EMC	Total Infestation% with EMC	Microscopic EMC	Total Infestation% with EMC
Winter	3	5	12	20	17	34
Spring	10	3	13	26	2	4
Summer	9	11	23	43	9	18
Autumn	10	4	14	28	16	32

Table 3: Tissue distribution of overall recovered EMC among the examined Nile tilapia and African catfish.

Infested organs	Nile tilapia			African catfish		
	No. of examined	No. of infestation	% of infestation	No. of examined	No. of infestation	% of infestation
Liver	400	33	8.25	200	14	7
Musculature	400	13	3.25	200	21	10.5
Intestine	400	1	0.25	200	0	0
Kidneys	400	38	9.5	200	8	4
Gills/branchial cavity	400	32	8	200	0	0
Ovary	400	0	0	200	1	0.5

3.4 Histopathological examination:

The microscopic examination of different host tissues of fish infected with EMC revealed that the gills/ branchiostegal musculature had the highest number of metacercariae cysts, followed by subcutaneous muscles, liver, kidneys, and ovary. Many encysted metacercariae were found in the gill filaments and arches of diseased fish. Some of these metacercariae were alive and trapped in the lamellar cartilages, with chondrocyte growth forming a cartilaginous encapsulation. In the surrounding tissue of these EMC, there was severe congestion, hemorrhage, inflammatory cellular infiltration, and widespread loss of lamellar epithelium (**Fig. 5A**). Other metacercariae were dead cysts covered by thick multilayer capsules and bordered by a few inflammatory cells mixed with extravasated erythrocytes (**Fig.5B**). Furthermore, many metacercariae cysts enclosed by thin capsules were seen in the gill arches, together with widespread lymphocytic cellular aggregation (**Fig.5C**). Severe hyperemia was also found nearby some of these

cysts (**Fig.5D**). The livers of fish infected with encysted metacercariae were investigated, and many cysts were distributed throughout the hepatic parenchyma. These encysted metacercariae were surrounded by clear space and enclosed by a connective tissue capsule. The predominant microscopic hepatic microscopic alterations seen around these EMC were hyperemia and mononuclear inflammatory cell aggregation, as well as atrophy and necrosis of hepatocytes (**Fig.5E**). Moreover, the examined ovary displayed presence of few EMC surrounded by a fibrous tissue capsule with an increase in the number of atretic vitellogenic oocytes (**Fig.5H**).

Additionally, similar alterations, in addition to widespread hepatic steatosis and congestion of portal veins, were found only in the liver of tilapia infected with EMC. Similarly, the presence of EMC in the kidney was accompanied by a severe host reaction that included congestion, hemorrhages, and inflammatory cellular aggregation, activation of melanomacrophage centers, and extensive degeneration and necrosis of the renal tubular epithelium (**Fig.5F**). The examined subcutaneous skeletal muscles of fish infected with encysted metacercariae revealed presence of many parasitic cysts embedded in-between the muscle bundles, and the surrounding muscles showed hyaline degeneration with minimal mononuclear inflammatory cells infiltration (**Fig. 5G**).

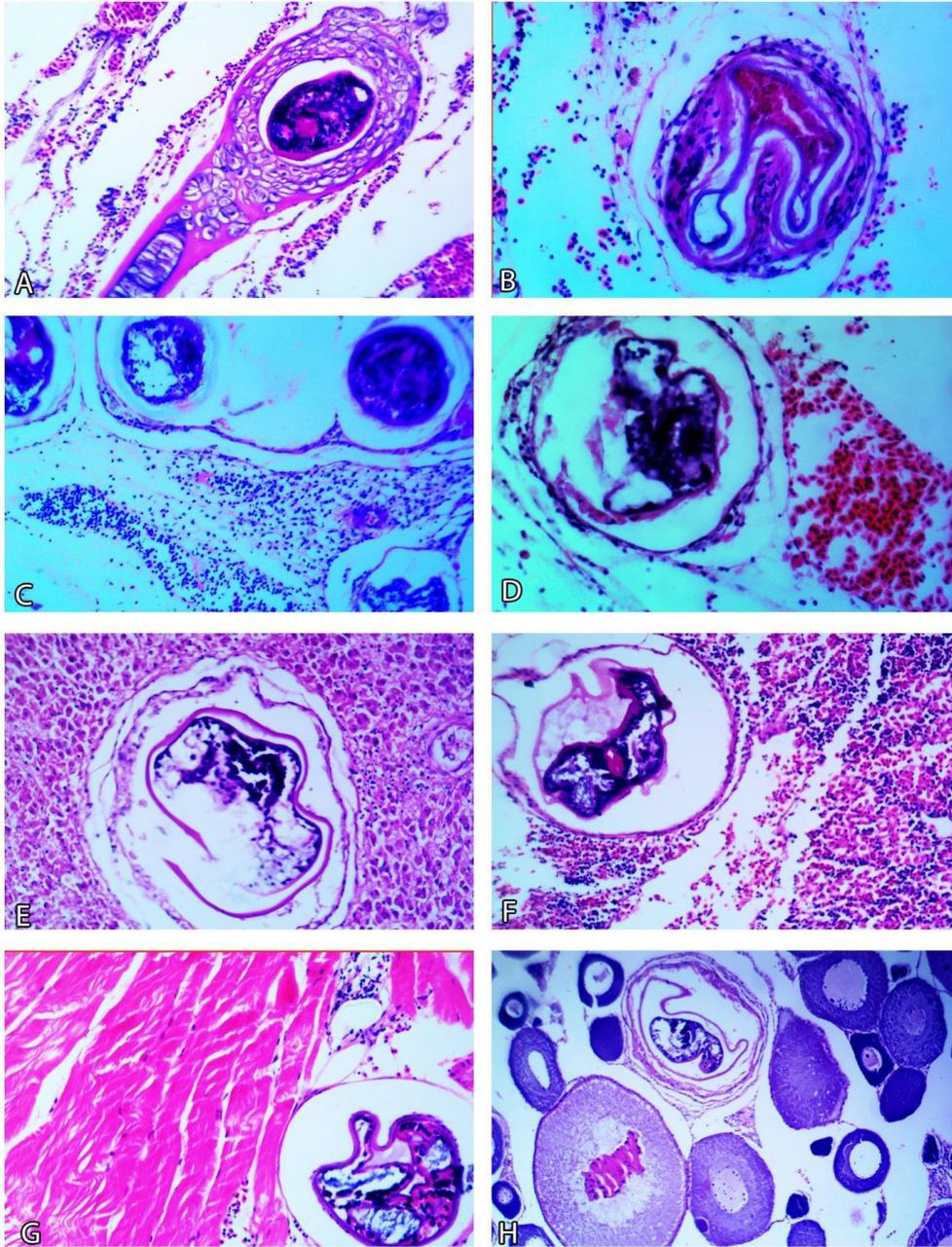


Figure 5: Histopathology of gills (A-D), liver (E), Kidneys (F), subcutaneous musculature (G) and ovary (H) of fish infected with EMC showing A, Cross section of the encysted metacercaria (EMC) within the gill lamellae with cartilaginous encapsulation, severe congestion, hemorrhage and extensive destruction of lamellar epithelium. B, Dead EMC surrounded with thick multilayer capsule and inflammatory cells mixed with extravasated erythrocytes in gill arch. C, several parasitic cysts surrounded with thin capsules and extensive lymphocytic cellular aggregation in gill arch. D, EMC with severe hyperemia and little inflammatory cells in gill arch. E, EMC surrounded with capsule compressed the adjacent parenchyma causing atrophy and necrosis of hepatocytes with aggregation of few mononuclear inflammatory cells. F, EMC within renal parenchymal with hemorrhage and extensive tubular necrosis. G, EMC with hyaline degeneration of skeletal muscles and little inflammatory cellular reaction. H, EMC surrounded by fibrous tissue capsule and embedded in ovarian tissue. H& E stain X200.

4. DISCUSSION

Parasitic diseases are among the common diseases affecting aquaculture production worldwide. Fish parasites affect the survival of fish and reducing host immunity making them vulnerable to other microbial infections, resulting in higher mortalities (El Asely *et al.*, 2015). Metacercaria of digenetic trematodes are one of the most important endoparasites responsible for great economic losses for both wild and cultured fishes (Abd-ELrahman *et al.*, 2023).

In the present study, the overall prevalence of recovered EMC was higher in Nile tilapia (29.25%) than African catfish (22%). Also, the highest infestation rate with EMC was in summer (43%) in Nile tilapia and in winter (34%) in African catfish. This result is comparable with previous findings by Soror *et al.* (2012); Abd Rabo *et al.* (2017); Attia *et al.* (2021); Salem *et al.* (2021) and Abd-ELrahman *et al.* (2023). However, Awadin *et al.* (2012) recorded that the total prevalence of EMC in African catfish was 93% and the peak reached in summer season. This variation in seasonal incidence of EMC is influenced by numerous reasons such as fish feeding habits, host immune response at different temperatures (EL-Shahawy *et al.*, 2017).

The recovered EMC of *Clinostomum* spp in this study, appeared as yellowish white to orange pea- like cyst in the branchial cavity of Nile tilapia. Similarly in several previous studies this parasite was detected in Nile tilapia (Shareef and Abidi, 2012; Hamouda and Younis, 2021; Abd-ELrahman *et al.*, 2023). Clinostomid parasites have low host specificity and can infect diversity of hosts, damaging and impairing them and, in severe cases, causing death (Shamsi *et al.*, 2021). The total prevalence for *Clinostomid* spp in this study was 8% with the highest seasonal prevalence 10% in spring and autumn and the lowest infestation was 3 % in winter. This result was nearly the same as that recorded in a previous study by Shaheen *et al.* (2014); EL-Shahawy *et al.* (2017); and Awosolu *et al.* (2018). Meanwhile, Taher (2009) and Salem *et al.* (2021) reported higher total prevalence of Clinostomid parasites in Nile tilapia reach to 62.25% and 74% respectively. These differences in prevalence may be associated with variances in habitat, environment water temperature, the abundantly of 1st intermediate hosts, and the profusion of aquatic piscivorous birds, which play an important role in completing the life cycles of digenetic trematodes (Vasemägi *et al.*, 2017; Mutengu & Mhlanga, 2018). The rainy season and snail availability might also play a key role in incidence variation during different seasons (Khan *et al.*, 2018). Taher (2009) reported also that the decreased prevalence of EMC during the cold seasons may be related to the death of the temperature dependent cercariae/metacercariae.

Euclinostomum spp appeared as round to oval grayish black cysts and give the area around it faint black color, as observed by Hassan *et al.* (2012); Mansour (2019). The total prevalence of *Euclinostomum* spp was 5.75%, with the highest rate of infection in autumn (11%) and the lowest rate recorded in summer (3%). This result nearly

resembles data obtained by **Mahmoud et al. (2018)** and **Areeda et al. (2019)** who recorded that the prevalence rate of *Euclinostomum* spp in Nile tilapia was 4.84% and 7.81%. respectively. On the other hand, **Shaheen et al. (2014)** recorded 18.3% total prevalence, with the highest seasonal prevalence in summer (27.3%), and the lowest infection rate was in winter (6.7 %). In addition, **Younis et al. (2022)** recorded infection rate with *Euclinostomum* spp. in Nile tilapia reach to 25.25%. **Ezzat et al. (2012)** reported that Nile tilapia collected from three different areas of Lake Nasser showed no infestation with *Euclinostomum* spp. These differences could be attributed to circumstances were occurred during these studies and differences in the species, sizes, weights, and number of examined fish (**Hamouda et al., 2018**). The other microscopic EMC in this study including *Haplorchid* spp, *Pygidiopsisgeneta* spp, *Prohemistomulum* spp, and *Cyanodiplostomum* spp were nearly isolated from Nile tilapia and African catfish as reported by **Shaheen, et al. (2014)**; **Abd Rabo et al. (2017)** and **Saad et al. (2019)**.

Regarding tissue distribution of the recovered EMC, kidneys tissue recorded the highest infestation rate (9.5%), followed by liver (8.25%), and gills/branchiostegal muscle (8%) in Nile tilapia. **Aly et al. (2005)** recorded that the most common sites of infestation with EMC in Nile tilapia were gills, followed by heart, liver, kidney, muscles. In addition, another study by **Eissa et al. (2011)** who reported that the susceptible organs for infestation with EMC were muscles, then gill filaments and kidney. This variation in EMC tissue distribution could be attributed to fish size difference, age of fish, fish feeding habits and/or immunological response to parasites as explained by **D'Silva et al. (2012)** and **Saad et al. [(2019)**. **Park et al. (2009)** and **Wang et al. (2017)** suggested that cercaria of *Clinostomum* spp pass in water current through the oral cavity, gill, and operculum and attach to these soft tissues easier than on the scaly surface. These parasites may feed on the mucous, blood, and tissue from the host, so they are commonly found in the branchial cavity and beneath the branchiostegal muscle. The highest prevalence of EMC in current study in African catfish was 10.5% in the subcutaneous musculature then liver (7%) after that kidney (4%) and only one case recorded in ovary. It could be attributed the highest infestation of musculature with EMC in African catfish to absence of scales which facilitates skin invasion by cercariae (**Mahboub et al. 2022**). **Sayed, et al. (2014)** recorded the most susceptible organ to EMC infestation in African catfish was liver then muscle and kidney. Meanwhile, **Jehan (1993)** showed that the highest susceptible organs were gills, liver, spleen, and kidneys.

In the present work, severe histopathological alteration induced by EMC in in different organs of Nile tilapia and African catfish contrasting to **Santos et al. (2017)** who, reported that there were no histological alterations connected to metacercaria infestation and indorsed this observation to the decreased load of infection. The recorded lesions in the present study were supported by previous reports showing that parasites can

cause extensive damage to the viscera, including the liver and musculature of many fish species (Adeyemo and Agbede 2008; Shareef and Abidi 2012).

The presence of live cysts in gills were enclosed by cartilaginous encapsulation and associated with severe congestion, hemorrhage and extensive destruction of lamellar epithelium. While the dead EMC surrounded with thick multilayer capsule and inflammatory cells mixed with extravasated erythrocytes and extensive lymphocytic cellular aggregation in gill arch. These lesions were nearly similar to those described by Younis *et al.* (2023) who found edema, hyperplasia, and fusion of the secondary gill lamellae in the gills of Nile tilapia and Hossain *et al.* (2007) and Fujimoto *et al.* (2014) who attributed to this effect of parasite on the gill epithelium. Moreover, Mood *et al.* (2010) explained the presence of metacercariae in the cartilage filaments, causing severe inflammatory response and hyperplasia of the cartilage of the primary lamellae. In the liver EMC compressed adjacent parenchyma causing atrophy and necrosis of hepatocytes with aggregation of few mononuclear inflammatory cells. This finding was in agreement with Elamei, (2001); Aly *et al.* (2005) and Khalil *et al.* (2014) who, observed the encysted metacercariae inside round capsules and were surrounded by clear space with mild hyaline degeneration and only a few inflammatory cell infiltrations. Also in the kidneys, presence of EMC was accompanied with hemorrhage and extensive tubular necrosis while, in muscle EMC was surrounded by fibrous tissue capsule and induced little inflammatory cellular reaction. Moreover, many EMC were embedded in ovarian tissue with increase in the number of atretic oocysts. These findings agreed with Abd Rabo *et al.* (2017) who, observed the changes on muscular as mononuclear leucocyte infiltration, pressure atrophy, intramuscular edema as well as focal necrosis.

5. CONCLUSION

This study concluded that EMC infestation in Nile tilapia and African catfish were persist all over the year and varied according to climatic changes, resulting in reducing the fish marketability. EMC infestation also induces severe histopathological lesions in affected organs that lower fish health status including growth, reproduction, and immunity. Therefore, snails must be effectively controlled, especially in natural water resources.

Conflict of Interests: The authors declare that there is no conflict of interests.

Funding: This research received no external funding.

6. REFERENCES

- Abd El-Gawad, E.A.; Abbass A.A. and Shaheen A. A. (2012).** Risks induced by pesticides on fish reproduction. *The Global Journal of Fisheries and Aquaculture Research.*, 4 (4): 329 – 338.
- Abd rabo, E. R.; Abou El Ezz, A. E.; Abbass, A. A.; Abdel-Gawad, E.A.; El Asely, A.M.; ElAbd, H. and Shaheen, A.A. (2017).** Isolation of some parasitic diseases from African catfish (*Clarias gariepinus*) in downstream El-Rahawy drain. *Benha Veterinary Medical Journal* 33 (1): 233-243
- Abd-ELrahman, S.M.; Gareh, A.; Mohamed, H.I.; Alrashdi, B.M.; Dyab, A.K.; El-Khadragy, M.F.; Elbarbary N K.; Fouad, A.M.; El-Gohary, F.A.; Elmahallawy, E.K. and Mohamed, S.A.A. (2023).** Prevalence and Morphological Investigation of Parasitic Infection in Freshwater Fish (Nile Tilapia) from Upper Egypt. *Animals.*, 13(6): 1088.
- Adeyemo, A.O. and Agbede S.A. (2008).** Histopathology of tilapia tissues harboring *Clinostomum tilapiae* parasites. *African Journal of Biomedical Research* 11:115–118.
- Aly, S.; Eissa, I.; Badran, A.; Elamie, M. and Hussain, B. (2005).** Pathological studies on encysted metacercariae infections among some freshwater fish in Egyptian Aquaculture. *Proceedings of Duetscher Tropentag, Hohenheim University, Stuttgart, Germany*, pp.11-13.
- Areda, T.A.; Mitiku, M.A.; Woldearegay, Y.H.; Teklu, A. and Getachew, S. (2019).** Prevalence of major parasites of Nile tilapia (*Oreochromis niloticus*) in southwest Showa zone selected fish farms, Oromia region, Ethiopia. *International Journal of Fisheries and Aquatic Studies.*, 7(3): 165-170.
- Attia, M.M.; Abdelsalam, M.; Korany, R.M.S. and Mahdy, O.A. (2021).** Characterization of digenetic trematodes infecting African catfish (*Clarias gariepinus*) based on integrated morphological, molecular, histopathological, and immunological examination. *Parasitology Research.*, 120: 3149–3162.
- Awadin, W.; Zahran, E. and Zaki, V.H. (2012).** Impact of some prevalent parasitic diseases on pathological alterations in African catfish (*Clarias garpienus*) in Dakahlyia Governorate, Egypt. *The Global. Journal of Fisheries and Aquaculture Research.*, 5(5): 16-32.
- Awosolu, O.B.; Simon-Oke, I.A.; and Oyelere, A.A. (2018).** Studies on the prevalence and distribution of parasites of tilapia fish (*Oreochromis niloticus*) from Igbokoda River, Ondo State, Nigeria. *Molecular Pathogens.*, 9 (1): 1-4.
- Bancroft, J.D. and Gamble, M. (2007).** *Theory and practice of histological techniques*, 5th edn. Churchill Livingstone, London, pp 125–138.
- Bui, S.; Oppedal, F.; Sievers, M. and Dempster, T. (2019).** Behavior in the toolbox to outsmart parasites and improve fish welfare in aquaculture. *Reviews in Aquaculture*, 11(1): 168-186.

- Caffara, M.; Davidovich, N.; Falk, R.; Smirnov, M.; Ofek, T.; Cummings, D.; Gustinelli, A. and Fioravanti, M.L. (2014).** Redescription of *Clinostomum phalacrocoracis* metacercariae (Digenea: *Clinostomidae*) in cichlids from Lake Kinneret, Israel. *Parasite* 21:32.
- Dezfuli, B. S.; Giari, L.; Simoni, E.; Menegatti, R.; Shinn, A. P. and Manera, M. (2007).** Gill histopathology of cultured European sea bass, *Dicentrarchus labrax* (L.), infected with *Diplectanum aequans* (Wagener 1857) Diesing 1958 (Diplectanidae:Monogenea). *Parasitology Research.*, 100: 707–713
- D'Silva, J.; Bhuiyan, A.I. and Bristow, G.A. (2012).** Distribution of helminth parasites in size groups and organs of hilsa shad, *Tenulosailisha*. *Dhaka University Journal of Biological Sciences.*, 21(1): 55-66.
- Eissa, I.A.M.; Gado, M.S.; Laila, A.M.; Mona, S.Z. and El-Deen, A.N. (2011).** Field studies encysted metacercariae infested natural male tilapias and Monosex tilapias in Kafr El -sheikh governorate fish farms. *Life Science Journal.*, 8: 7-12
- El Asely, A.M.; Abd El-Gawad, E.A.; Soror, E.I.; Amin, A.A. and Shaheen, A.A., 2015.** Studies on some parasitic diseases in *Oreochromis nilotica* fish hatchery with emphasis to life stages. *Journal of Advanced veterinary research.*, 5(3): 99-108.
- Elamei, M.M.M.T. (2001).** Studies on the diseases resulting from encysted metacercariosis in some fresh-water fishes. M.V.Sc. Thesis (Fish diseases and management). Fac. Vet. Med. Suez Canal Univ.
- Eldanasory, F.; Eladawy, R.S.; Elnoby, H. and Elkeblawy, R.M. (2022).** An analytical study of the marketing of freshwater fish in Kafr El-Sheikh Governorate. *Journal of Sustainable Agricultural Sciences.*, 48(1): 49-61.
- El-Gohary, F.A.; Zahran, E.; Abd El-Gawad, E.A.; El-Gohary, A.H.; Abdelhamid, F.M.; El-Mleeh, A.; Elmahallawy, E.K.; Elsayed, M.M. (2020).** Investigation of the Prevalence, Virulence Genes, and Antibiogram of Motile Aeromonads Isolated from Nile Tilapia Fish Farms in Egypt and Assessment of their Water Quality. *Animals.*, 10: 1432.
- Elkamel, A. A.; Sayed, G.M.; Ahmed, S.M.; Arafa, M.I. and Abdel Lateif, R.S. (2014).** Studies on some factors affecting metacercarial infections in African sharp-tooth catfish [*Claris gariepinus*] in Assiut Governorate. *Assiut University Bulletin for Environmental Research.*, 17: 25-36.
- El-Shahawy, I.S.; El-Seify, M.O.; Metwally, A.M. and Fwaz, M.M. (2017).** Survey on endoparasitic fauna of some commercially important fishes of the river Nile, southern of Egypt (Egypt). *Revue De Medecine Veterinaire.*, 168(4-6): 126-34.
- Eman, M.Y.; Nahla, H.S.; Eissa, I.A.M. and Mona, S.Z. (2014).** Parasitological studies on the isopoda (Cymothoidae) parasites infesting some marine fishes at Suez Canal area at Ismailia Province, Egypt with a key to the cymothoid genera. *Life Science Journal.*, 11(1): 227-231.

- Ezenwaji, N. E.; Aguiwo, J. N.; Ilozumba, P. C. O. and Ezenwaji, H. M. G. (2005).** Helminth endo- parasites of Mochokids in a tropical rainforest river system. *Animal Research International.*, 2(2): 346-352.
- Ezzat, S.M.; ElKorashey, R.M. and Sherif, M.M., 2012.** The economic value of Nile Tilapia Fish "*Oreochromis niloticus*" in relation to water quality of Lake Nasser, Egypt. *Journal of American Science.*, 8(9): 234– 247.
- FAO. (2004).** Fish Stat J Software for Fishery and Aquaculture Statistical Time Series; FAO Fisheries Division: Rome, Italy.
- FAO. (2016)** National aquaculture sector overview, Kenya. Retrieved from http://www.fao.org/fishery/count rysec tor/naso_kenya /en.
- Fujimoto, R.Y.; Neves, M. D. S, Santos, R.F.B.; Cruz, C.; Diniz, D.G. and Eiras, J.C. (2014).** Histopathological evaluation of seven Amazon species of freshwater ornamental armored catfish. *Acta Scientiarum. Biological Sciences.*, 36(3): 349-355
- GAFRD. (2020).** General Authority for Fish Resources Development. Statistical Report on Fish Production. Cairo, Egypt.
- Ghoneim, W. M.; Khalil, R. H., Saad, T. T., Tanekhy, M. and Abdel-Latif, H. M. (2015).** Ectoparasite fauna of cultured African catfish, *Clarias gariepinus* (Burchell, 1822), El-Behera Province, Egypt. *International Journal of Fisheries and Aquatic Studies* 3(1): 19-22.
- Hamouda, A.H. and Younis, A.E. (2021).** Characterization of *Clinostomum cutaneum* and *Clinostomum phalacrocoracis* in tilapia species of Aswan Governorate, Egypt: A morphological, molecular and histopathological study. *Aquaculture Research.*, 52(12): 6726-6740.
- Hamouda, A.H.; Sorour, S.S.; El-Habashi, N.M. and Adam, E.H.A. (2018).** Parasitic infection with emphasis on *Tylodelphys spp.* as new host and locality records in Nile perch; *Lates niloticus* from Lake Nasser, Egypt. *World's Veterinary Journal.*, 8(1): 19-33.
- Hassan, E.A; Soliman, M.F.M. and Ghobashy A. A. (2012).** Some factors affecting metacercarial infections in *Tilapia Zilli* from Lake Timsah, Ismailia, Egypt. *Egyptian Academic Journal of Biological Sciences.*, 4: 21-28.
- Hoffman, G.L. (2019).** Parasites of North American freshwater fishes. Cornell University Press
- Hossain, M.K.; Hossain, M.D. and Rahman, M.H. (2007).** Histopathology of some diseased fishes. *Journal of Life and Earth Science*, 2(2), 47-50.
- Jehan, F.A.K.A. (1993).** The role of fish in transmitting some parasites to man. M. V. Sc. Thesis (Zoonosis), Fac. Vet. Med., Alex. Univ., Egypt.
- Khalil, M.I.; El-Shahawy, I.S. and Abdelkader, H.S. (2014).** Studies on some fish parasites of public health importance in the southern area of Saudi Arabia. *Revista Brasileira de Parasitologia Veterinária.*, 23: 435-442.

- Khan, S., Ahmed, S.; Serajuddin, M. and Saifullah, M.K. (2018).** Variation in seasonal prevalence and intensity of progenetic metacercariae of *Clinostomum complanatum* infection in *Trichogaster fasciatus* fish. Beni-Suef University Journal of Basic and Applied Sciences., 7(3): 310-316.
- Lehmann, N.B.; Owatari, M.S.; Furtado, W.E.; Cardoso, L.; Tancredo, K.R.; Jesus, G.F.A.; Lopes, G.R. and Martins, M.L. (2020).** Parasitological and histopathological diagnosis of a non-native fish (*Oreochromis sp.*) with a noticeable presence in a natural Brazilian river environment. Journal of Parasitic Diseases, 44: 201-212.
- Mahboub, H.H.; Nada, H.S.; Abdel-Ghany, H.M.; Ghanem, R.; Ahmed Ismail, T. and Abdel Rahman, A.N. (2022).** Detection, diagnosis, Koch's postulate, hepatorenal and antioxidant indicators for some systemic pathogenic fungi invading the liver and kidneys of African catfish (*Clarias gariepinus*) in Egypt with a histopathological approach. Aquaculture Research., 53(7): 2670-2685.
- Mahmoud, W G.; Elsharawy, N. T. and Hashem, M. (2018).** Prevalence of Metacercariae in Nile Tilapia (*Oreochromis Niloticus*) at Assuit Province and the Effect of Freezing on Its Viability. Alexandria Journal of Veterinary Sciences., 59 (1): 49-56
- Mansour R. (2019).** First record of *Euclinostomum heterostomum* from the naturally infected heron "Ardeola ralloides" in Egypt: a light & scanning electron microscopy study. Egyptian Journal of Zoology., 72(72): 22–31.
- Menconi, V.; Manfrin, C.; Pastorino, P.; Mugetti, D.; Cortinovis, L.; Pizzul, E.; Pallavicini, A. and Prearo, M. (2020).** First report of *Clinostomum complanatum* (Trematoda: Digenea) in European Perch (*Perca fluviatilis*) from an Italian Subalpine Lake: A Risk for Public Health? International Journal of Environmental Research and Public Health, 17(4): 1389
- Mood, S.M.; Ebrahimzadeh Mousavi, H.A.; Mokhayer, B.; Ahmadi, M.; Soltani, M. and Sharifpours I. (2010).** *Centrocestus formosanus* metacercarial infection of four ornamental fish species imported into Iran. Bulletin of the European Association of Fish Pathologists., 30(30):146–149.
- Mutengu, C. and Mhlanga, W. (2018).** Occurrence of *Clinostomum Metacercariae* in *Oreochromis mossambicus* from Mashoko Dam, Masvingo Province, Zimbabwe. *Scientifica.*, 2018:1-6.
- Noga, E. J. (2010).** Fish disease: diagnosis and treatment. Second Edition, Wiley-Black Well, USA.
- Noga, E. J.(2010).** Fish disease: diagnosis and treatment. Second Edition, Wiley-Black Well, USA
- Park, C.W.; Kim, J.S.; Joo, H.S. and Kim, J. (2009).** A human case of *Clinostomum complanatum* infection in Korea. Korean Journal of Parasitology 47(4):401–404.

- Pouomogne, V. (2008).** Capture-based aquaculture of *Clarias* catfish: case study of the Santchou fishers in western Cameroon. Capture based aquaculture, Global overview, FAO Fisheries Technical Paper, (508): 93-108.
- Saad, S.M.; Salem, A.M.; Mahdy, O.A. and Ibrahim, E.S. (2019).** Prevalence of metacercarial infection in some marketed fish in Giza Governorate, Egypt. Journal of the Egyptian Society of Parasitology., 49(1): 129-134.
- Salem, A.M.; Abdel-Maogood, S.; Abdelsalam, M. and Mahdy, O.A. (2021).** Comparative morpho-molecular identification of *Clinostomum phalacrocoracis* and *Clinostomum complanatum* metacercariae coinfecting Nile tilapia in Egypt. Egyptian Journal of Aquatic Biology and Fisheries., 25(1):.461-476.
- Santos, M.A.; Jerônimo, G.T.; Cardoso, L.; Tancredo, K.R., Medeiros, P.B.; Ferrarezi, J.V.;Gonçalves, E.L.T.; da Costa Assis, G. and Martins, M.L. (2017).** Parasitic fauna and histopathology of farmed freshwater ornamental fish in Brazil. Aquaculture., 470: 103-109.
- Shaheen, A.A.; Abd EL Latif, A.M.; Elmadawy, R.S. and Noor El Deen, A.I. (2017).** Isopodiosis in Some Fishes from Egyptian Qaroun Lake: Prevalence, Identification, Pathology and In Vitro Trials to get rid of it. Research Journal of Pharmaceutical, Biological and Chemical Sciences., 8(1):1973-1978.
- Shaheen, A.A.;El Asely, A.M.;Abd El Gawad, E.A.; Farag, A.F. and Abbass, A.A. (2014).** Diagnosis of some internal parasitic diseases in some freshwater Fishes. Global Journal of Fisheries and Aquaculture Research., 1(2): 01-17.
- Shamsi, S.; Day, S.; Zhu, X.; McLellan, M.; Barton, D.P.; Dang, M. and Nowak, B. F. (2021).** Wild fish as reservoirs of parasites on Australian MurrayCod farms. Aquaculture, 539:736584.
- Shareef, P.A. and Abidi, S.M.A. (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.
- Song, H. B.; Choi, M. and Chung, E. (2018).** Images in clinical tropical medicine, human laryngeal infection by *Clinostomum complanatum*. American Journal of Tropical Medicine and Hygiene, 98(1): 7–8.
- Soror, E.I.; Mahrous, K.F.; Ismail, A.M. and Abbass, A.A. (2012).** Epizootiological studies on proliferative kidney disease in Tilapia (*Oreochromis niloticus*) and African catfish (*Clarias gariepinus*). Benha veterinary medical journal, 23(1): 150-158
- Taher, G.A. (2009).** Some studies on metacercarial infection in *Oreochromis niloticus* in Assiut governorate and their role in transmission of some trematodes to dogs. Assiut University Bulletin of Environmental Research, 12 (1): 63-79.
- Tayel, I.S.; Ahmed, M.N. and Ramadan, A. E. (2020).** Histopathological alterations and parasitic infection in *Oreochromis niloticus* fish inhabiting the river Nile water. Egyptian Journal of Aquatic Biology and Fisheries., 24(1): 233-247.

- Vasemägi, A.; Visse, M. and Kisand, V. (2017).** Effect of Environmental Factors and an Emerging Parasitic Disease on Gut Microbiome of Wild Salmonid Fish. *mSphere.*, 2(6): e00418-17.
- Wang, M.L.; Chen, H.Y. and Shih, H.H. (2017).** Occurrence and distribution of yellow grub trematodes (*Clinstomum complanatum*) infection in Taiwan. *Parasitol. Res.*, 116 pp.1761-1771.
- WHO. (2017).** WHO Factsheet. Foodborne trematodes. WHO. https://www.who.int/health-topics/foodborne-trematode-infections#tab=tab_1.
- Younes, A.M.; Noor Eldin, A. I. and Abd Ellatif. M.A. (2016).** A contribution of crustacean isopoda, bacterial infections and physicochemical parameters in mass mortalities among fishes in Lake Qarun. *Research Journal of Pharmaceutical, Biological and Chemical Sciences.*, 7(2): 1910-1911.
- Younis, A.E.; Hamouda, A.H. and Moustafa, E.M. (2022).** *Euclinostomum heterostomum* and *E. ardeolae* in tilapia species of Aswan Governorate, Egypt: morphological, molecular, and histopathological characterization. *Aquaculture International*, 30(2): 825-844.
- Younis, N.A.; Elgendy, M.Y.; El-Samannoudy, S.I.; Abdelsalam, M. and Attia, M.M., (2023).** *Cyathocotyidae spp* and motile aeromonads co-infections in farmed Nile tilapia (*Oreochromis niloticus*) causing mass mortality. *Microbial Pathogenesis.*, 174: 105897.