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Histopathological Study on Some Parasitic Diseases in Fish

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Abstract

FISH are an important source of protein with low cholesterol, unfortunately fish are liable to many parasites, which have an important effect on fish aquatics, besides their zoonotic importance. Our main objective in this study is to evaluate parasitic infection in African catfish and Nile tilapia and their histopathological effects on infected fish. Two hundred fish samples, 150 catfish and 50 tilapias were collected from the River Nile of Damietta governate, Badr city market, Manzalla city market and Menoufia governate market from January 2022 to February 2024.

The identified parasites in catfish were Encysted metacercaria with prevalence rate (20%), which affected skin and muscles. Microscopically, Encysted metacercaria appeared as parasitic cysts surrounded by fibrous connective tissue capsule, *Polyonchobothrium sp*. with prevalence rate (20%), affected the intestine. Microscopically, intestine showed desquamation of mucosa and goblet cell hyperplasia and *Dactylogyrus sp.* with prevalence rate (10.5%), caused marbling appearance of gills. Microscopically, gills showed hyperplasia and telangiectasis. The identified parasites in tilapia were Encysted metacercaria with prevalence rate (22%), caused many hemorrhagic patches in liver. Microscopically, it showed hydropic degeneration and fatty change, *Chilodonella sp.* with prevalence rate (6%), caused necrosis of gills. Microscopically the gills showed desquamation of epithelium, *Gyrodactylus sp.* with prevalence rate (4%), caused dark discoloration of the skin. Microscopically, the skin revealed many alarm cells and abundant melanomacrophages. and Cymothoid Isopods with prevalence rate (2.8%), caused hemorrhage in gills. Microscopically, the gills showed hemorrhage and loss of gill filaments.

Key words: Nile tilapia, African catfish, Histopathology, Parasites.

Introduction

Fish have a notable impact on a large number of people and societies worldwide, mostly because fish are reasonably inexpensive and accessible source of necessary animal protein [1]. fish immunity may be compromised by wastewater or industrial contaminants [2]. Increasing intensification of fish and lack of health management measures lead to many disease problems in fish and about 80% of fish diseases are parasitic [3]. some parasites may be highly pathogenic and cause serious economic losses or threaten the abundance of native fish species [4]. Parasitic infections can decrease the reproductive performance and affect the feed conversion rate leading to mortality and unmarketability of cultured fish. Degree of harm may range in severity depending on the age and size of fish and parasite, as well as parasitic activity and the intensity of parasites [5]. Endo- and ectoparasites, especially helminths and protozoans can affect fish, causing high mortality rates [6] When water submerge gills, it may carry ectoparasites and contaminants to them. so, ectoparasites become attached to, or penetrate the gill epithelium and obtain adequate nourishment and multiply creating new populations[7].Ectoparasites contain variety of taxa, as protozoans, monogeneans and digenean metacercariae. [8]. The cichlids, harbor most of the infections including, the adult digenean which infect many organs of fish flesh; trematode

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metacercariae encysting in tissue; and adult monogenea of the families, Dactylogridae and Gyrodactylidae infecting gills and skin of the fish [9]. *Dactylogyrus sp.* destructs the efferent vessels causing extensive hemorrhage, ischemia and necrosis in some parts; It may cause the marbling appearance of the gills [10]. *Gyrodactylus sp.* causes dark or pale discoloration of the body, scales loss and excessive mucous secretion [11]. Cymothoids (Crustacean, isopoda) belonged to Family Cymothoidae, are obligatory parasites infecting many fish species. They are bloodsuckers, that reside on the skin, gills, or in the tongue of infected fish. These parasites resulting in malnourishment and eventually death [12]. Cymothoids affect the gill chamber, usually they cause anemia and stunted gill conditions [13]. Freshwater fish have a notable role in transmitting parasites to humans [14]. Zoonotic diseases resulted from ingestion of raw or undercooked fish [15].

The consumption of Encysted metacercaria developed diseases to human being [16] In Egypt, the endemic of encysted metacercarial infection in tilapia and catfish were reported [17]. Fish parasite incidence is correlated with water quality characteristics and stocking density, so, younger fish being more prone to infection than older fish [18]. *Polyonchobothrium clarias* (gastrointestinal helminth worm) is one of Cestodes, which infect pyloric stomach of *Clarias gariepinus* [19]. Its scolex embedded deeply into the tissue so, it is destroyed and causes hemorrhage, congestion [20]. Ciliates of the genus *Chilodonella* cause pigmentation of the skin, ulceration, loss of scales and gill lesions [21].

The aim of the study is to evaluate parasitic infection in freshwater Nile tilapia *(Oreochromis niloticus)* and African catfish *(Clarias gariepinus)* and the histopathological impacts of these parasites on the infected fish during the period between January 2022 and Febrauary2024.

Material and Methods

Sampling

From January 2022 to Febrauary2024, 200 infected fish samples were collected from different localities at Damietta, Menoufia Governorate, Badr and Manzalla city. Samples collected, included 150 of catfish *(Clarias gariepinus)* and 50 of tilapia *(Oreochromis niloticus).* Fish samples were collected monthly from these governates and transported alive to Parasitology and Pathology Lab at Faculty of Veterinary Medicine, Menoufia University, Menoufia, Egypt.

Post mortem Examination

The skin was examined for presence of ulcers, erosions, hemorrhage, patches and discoloration. The gills and the gill cover were inspected for the existence of large worms, abnormalities of gills, hemorrhage, congestions, nodules, erosions and presence of slim according to [22]. The liver was examined for any abnormality in color, texture and for presence of nodules or hemorrhagic patches. Intestine was examined for presence of worms, ulcers and nodules. The stomach was inspected for any abnormality in color. Muscles were examined for presence of cysts, worms or hemorrhagic patches.

Parasitological Examination

External Examination

External parasitological examination was done grossly. Isopods were removed from gills of infected fish then preserved in (alcohol formalin acetic acid) AFA according to [23]., then categorized according to their morphological characters according to [24]. *Gyrodactylus sp.* was removed from infected skin and examined under light microscope and categorized according to their morphological characters.

Wet Mount Preparation

Wet smears of gills were prepared and inspected to investigate the presence of different parasites, by using a drop of normal saline and then covered by a clean cover slip then examined microscopically

Iodine-Stained Preparation

Gills Specimens were mixed with a drop of iodine solution, then put on a clean slide and covered with a clean cover slide according to [25].

Internal Examination

Was done carefully on the flesh, wall of the stomach, intestine, muscles, liver, to detect the presence of cestodes, nematodes and/ or trematodes

1.cestodes *(Polyonchobothrium sp.),* isolated then washed in saline solution and preserved in 10% neutral buffered formalin, stained in acetic carmine, then dehydrated and mounted by Canada balsam and covered by cover slide.

Compression Technique

used for detection of encysted metacercaria which were lodged in different tissues including the liver, skin, gills and muscles. Each piece was compressed between two microscopic glass slides and examined for the presence of encysted metacercaria according to [26].

Identification of The Recovered Parasites

Recovered parasites were classified according to taxonomic keys and descriptions according to [27].

Histopathological Examination

Samples fixed at 10% neutral-buffered formalin for 24 hours, washing after tissue fixation Then, the samples were passed in ascending grades of alcohol for dehydration, and then embedded in paraffin wax blocks. Then sectioned at 5 µm thickness, mounted

onto glass slides and stained with Hematoxylin & Eosin (H&E) then examined microscopically [28].

Result

Parasitological Examinations

The Morphological Description of Recovered Parasites

Dactylogyrus sp.

Characterized morphologically by possessing a single pair of anchors pointed to the dorsal side of the haptor, one dorsal bar in some groups, or ventral and dorsal bars in others. They have 4 eyespots, equidistant, Accessory granules which are small, generally elongate ovate and sometimes sparse in anterior region. (Fig. 1(A))

Cymothoid Isopoda

Cymothoa exigua

Females with body length 8-30 mm and 4-15 mm width, dorsal surface without scattered chromatophores. Cephalon is moderately immersed into pereonite

1. Eyes are well developed. Antenna 1 not reach to the end of anterior third of pereonite 1, antenna 2 reaching to half of pereonite (Fig. 1(B))

Renocila thresherorum

Body: Is dark in color with marked concentrated scattered chromatophores on the posterior aspect of segments. It is dorsoventrally compressed measuring 16 -22 (19) mm in total length and 12-16(9) mm in Width.

Posterior border of cephalon: is weakly enclosed in pereonite

I. Eyes are well developed.

The First and second antennae are of 8 articles. (Fig. 1(C))

Chilodonella sp.

The morphology of Chilodonella sp. as follows: Large, flattened, ovoid or heart shaped ciliates with bands of cilia along the long axis of organisms. A single oval to round macronucleus as well as round micronucleus. (Fig. 1(D))

Gyrodactylus sp.

The worm characterized by elongated body. Double pointed anterior end and no eye spots. Large opisthaptor consists of two centrally positioned approximately parallel large hooks joined by two connecting bars, a simple dorsal bar and an approximately triangular shaped ventral bar. There are 16 marginal hooks positioned around the periphery of the opisthaptor (Fig. 1(E))

Polyonchobothrium clarias

The morphology of *P. clarias* as follows: The scolex is triangular, bearing a marginal crown of different sizes hooks. The main crown subdivided into two circles by dorsal and ventral indentations of the disc margin. The hooks adjacent to the indentations are smaller than those in the middle of the circles. The ovary is large, compact and bilobed. (Fig. 1(F))

Prevalence of Parasites in Examined Fish Samples Shown in Table (1,2)

Pathological Findings:

The infected gills of *Claris gariepinus* with *Dactylogyrus sp.* macroscopically showed congestion and paleness (marbling appearance), covered with mucoid secretions as showed in (Fig. 2(A)). Erosion, disorientation, bending and partial fusion of gill lamellae. Microscopically, the gills showed hypertrophy of chloride cells, destruction of gill lamellae (Fig. 2(B)), telangiectasia of secondary lamellae, and goblet cells hyperplasia. It also, showed hemorrhage, congestion, inflammatory cells infiltrations, and hyperplasia of the gill epithelium with partial fusion of others to encyst parasitic cysts (Fig. 2(C&D).

The fish infected with crustacean isopoda *(Cymothoa exigua* and *Renocila thresherorum)* showed hemorrhage, pale discoloration of the gills and loss of gill raker as showed in (Fig. 3(A&B)). The histopathological lesions were hemorrhage, loss of gill filaments, and inflammatory cells infiltrations as showed in (Fig. 3(C&D).

Chilodonella sp. caused pale discoloration of the gills and necrosis in some parts as shown in (Fig. 4(A)). Microscopically, gills infected with Chilodonella sp. showed necrosis of some areas, mononuclear infiltration of inflammatory cells, hemorrhage and desquamation of gill epithelium as showed in (Fig. 4(B)).

Gyrodactylus sp. causes darkening and deep pigmentation of the skin, scale loss and scattered hemorrhagic areas on the skin as shown in (Fig. 5(A)). Microscopically, the skin revealed many alarm cells and abundant melanomacrophages, dilated blood vessels, leucocytic infiltration as showed in (Fig. 5(B)).

Encysted metacercaria Affect several organs in fish (skin, liver and muscles). Infected skin showed ulceration, erosion and necrosis(fig6(A&B)). Microscopically, the skin revealed parasitic cysts surrounded by thick fibrous connective tissue capsule, edema and melanomacrophages in dermal layer, with few inflammatory cells (fig6(C&D)). Encysted metacercaria appears in muscles as grayish nodules as sesame seed size. Microscopically, the muscles have different sizes of parasitic cysts surrounded by fibrous connective tissue capsule which caused degeneration and destruction of muscle

fibers (Fig. 6(E)), edema, hyaline degeneration and mononuclear infiltration as showed in (Fig. 6(F)). Liver infected with Encysted metacercaria showed a lot of hemorrhagic areas as showed in (Fig. 7(A&B)). Microscopically, the liver showed hydropic degeneration, fatty change (micro and macro steatosis), necrotic, atrophied, destructed hepatocytes, congestion of portal vein, parasitic cysts surrounded by fibrous connective tissue capsule and inflammatory cells infiltrations as showed in (Fig. 7(C&D)).

Polyonchobothrium sp. in infected fish causes congestion and hemorrhage of the stomach (Fig. 8(A)). Microscopically, the stomach showed dilated blood vessels (Fig. 8(B)). The intestine was invaded by large number of the parasite as shown in (Fig. 9(A)). Infected intestine of catfish microscopically revealed vacuolation of enterocytes, goblet cell hyperplasia as showed in (Fig. 9(B)), leucocytic infiltration (Fig. 9(C)), desquamation of mucosa and hemorrhage as showed in (Fig. 7(D)).

Discussion

Parasites are one of the most serious infectious organisms that have a significant impact on fish aquaculture all over the world, In addition to their zoonotic hazards. In our study, we demonstrated the essential information regarding the prevalence of parasites in catfish and Nile tilapia in the River Nile of Damietta governate, Badr city market, Manzala city market and Menoufia governate market and their histopathological impact on the infected fish. Varying host immunity and intermediate hosts development, could be the causes for variations in parasites prevalence [29]. Parasites can threaten the life of fish and decreasing their size so they become more susceptible to various diseases, leading to high mortality rate [30]. Fresh water fish can be infected by various parasites including *Dactylogyrus sp*., Isopoda, *Gyrodactylus sp., Chilodonella sp.,* Encysted metacercaria, *Polyonchobothrium sp.*

In our study, the highest prevalence was reported in the summer (85%) this agree with [31]. who recorded that the occurrence of parasites in examined fish reached the peak in the summer season. The most prevalent parasite in our study was Encysted metacercaria with prevalence rate (20.5%) in different organs. The lesions of Encysted metacercaria in the present study were supported by previous studies showing that parasites can cause serious damage to variety of fish internal organs, including the liver and muscles [32]. In the liver Encysted metacercaria compressed surrounding parenchyma causing atrophy and necrosis of hepatocytes with aggregation of few mononuclear inflammatory cells, these findings were in agreement with [33].

The second most common parasite was *Polyonchobothrium sp*. with prevalence rate (20%), this agreed with [34]. who reported that the prevalence of *Polyonchobothrium sp.* is (22%) in Manzalla Lake, Egypt. The main lesions of infected intestine were vacuolation of enterocytes, leucocytic infiltration, goblet cell hyperplasia and desquamation of mucosa this come in agreement with [35]. The third most common parasite during the summer was *Dactylogyrus sp*. with prevalence rate (10.5%) this agree with [36]. who reported that the rate of infection of (*Gyrodactylus sp.* and *Dactylogyrus sp.*) in *O. niloticus* in Kafr El-Sheikh governorate, reached the peak in summer season. Gills infected with *Dactylogyrus sp.* showed hyperplasia of the gills with partial fusion of others. telangiectasia of lamellae and goblet cells hyperplasia. Also, massive destruction of gill lamellae and hemorrhage, these results were mentioned by [37].

The prevalence of *Chilodonella sp.* was (6%) of infected fish. The main lesions of infected gills were pale discoloration, necrosis and hyperplasia of the gills. This agreed with [38]. who recorded that, infections with *Chilodonella sp.* caused hypertrophy and hyperplasia of gill epithelial, with complete or partial fusion of gill lamellae. The prevalence of *Gyrodactylus sp.* was (4%) of infected fish. The main lesions of infected skin were darkening and deep pigmentation of the skin, scale loss and scattered hemorrhagic areas on the skin. This agree with [35]. who recorded that, tilabia infected with *Gyrodactylus sp.,* showed dark or pale discoloration of the body, scattered hemorrhagic areas and ulceration in the skin.The prevalence of Cymothoid isopoda was (2.8%), severe infections of isopoda caused hemorrhages on the gills, these results were in agreement with what found by [36]. it also caused gill raker loss as mentioned by [39].

Conclusion

On conclusion, the present work provides those parasitic diseases in fish still a major concern to the Egyptian fish sector with sever pathological lesions and a revision of control should be applied

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Ethical approval

Fish collected from lakes and rivers cases and procedures have been approved by the Research Ethics Committee at the Faculty of Veterinary Medicine, Menoufia University, Egypt (MN-VET-Path-24020103).

Competing interests

The authors declare that they have no competing interests.

TABLE 1. Prevalence of Parasites in Examined Fish Samples (Catfish)

Locality	No. infected	*Dactylogyrus sp.*		*Polyonchobothrium sp.*		EMC		Total	
		No. infected	%of infection	No. infected	%of infection	No. infected	%of infection	No. infected	%of infection
Damietta	25	1	4	4	16	2	8	7	28
Menoufia	50	1	2	5	10	18	36	24	48
Manzalla	60	18	30	20	33.3	8	20	46	76.67
Badr	15	1	6.7	1	6.7	2	13.3	4	26.67
Total	150	21	14	30	20	30	20	81	54

TABLE 2. Prevalence of Parasites in Examined Fish Samples (Tilapia)

Locality	No. infected	*Gyrodactylus sp.*		*Chilodonella sp.*		Isopoda		EMC		Total	
		No. infected	% of infection	No. infected	% of infection	No. infected	% of infection	No. infected	% of infection	No. infected	% of infection
Damietta	35	1	2.8	2	5.7	1	2.8	8	22.8	12	34.2
Menoufia	10	1	10	0	0	0	0	2	20	3	30
Manzalla	5	0	0	0	0	0	0	0	0	0	0
Badr	5	0	0	1	20	0	0	1	20	2	40
Total	50	2	12.8	3	25.7	2	2.8	11	62.8	17	34

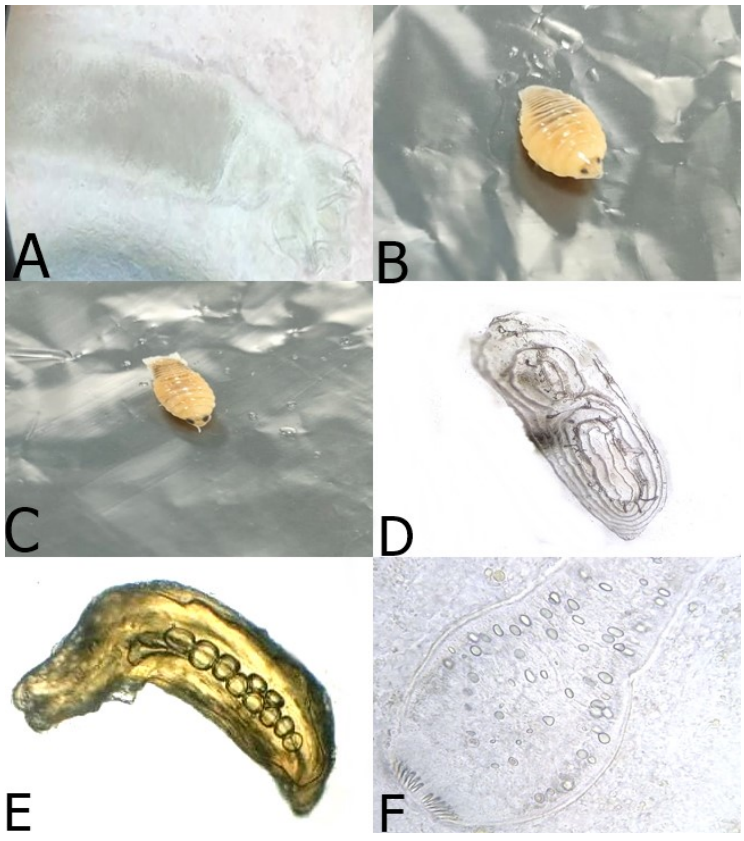

Fig.1. showed gross and microscopic examination of parasites. A. *Dactylogyrus sp.* B. Isopoda of gills (*Cymothoa exigua*). C. Isopoda of gills (*Renocila thresherorum*). D. *Chilodonella sp*. E. *Gyrodactylus sp.* F. *Polyonchobothrium sp*. (larva). (E&D X10; A&F X40).

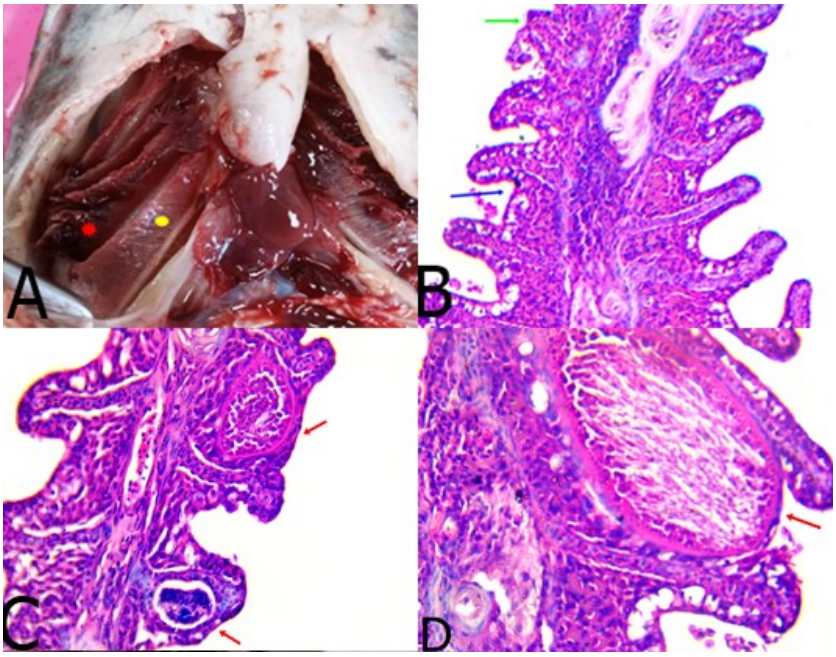

Fig.2. Gills of catfish infected with *Dactylogyrus sp*. A: marbling appearance of the gills, congested area (red star), pale area (yellow star). B: Destruction of the epithelium (green arrow), hypertrophy of chloride cells (blue arrow). C&D: Fusion of gill lamellae to encyst the parasitic cyst (red arrows). (H&E stain B& C:X10; D X40).

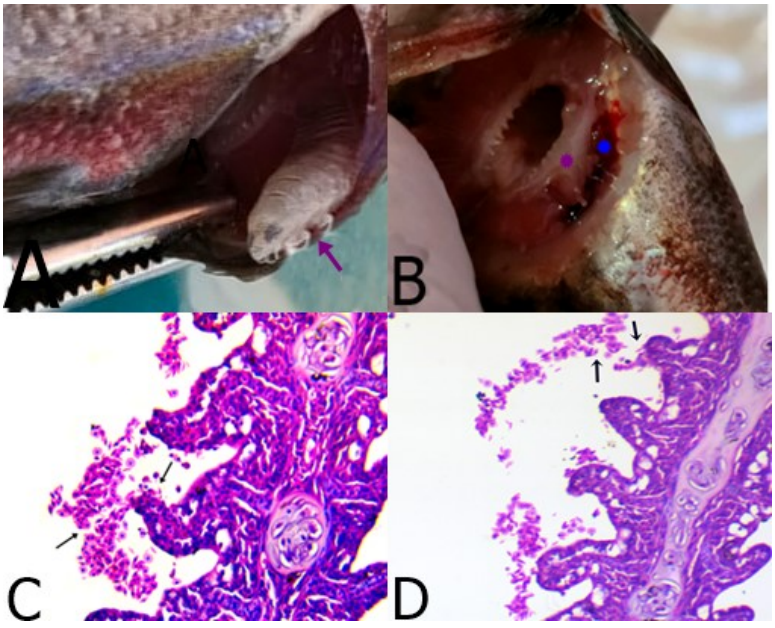

Fig.3. Gills of tilapia infected with Isopoda. A: Isopoda of gills (purple arrow). B: hemorrhage (blue star), loss of gill rakers (purple star). C&D: hemorrhage and desquamation of gill epithelium (black arrows). (H&E stain C& D:X10).

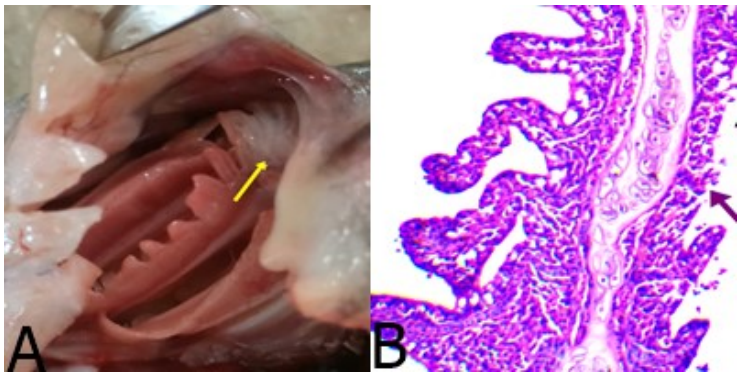

Fig.4. Gills of tilapia infected with *Chilodonella sp*. A. Necrosis (yellow arrow). B. Desquamation of the epithelium (purple arrow). (H&E stain B: X10).

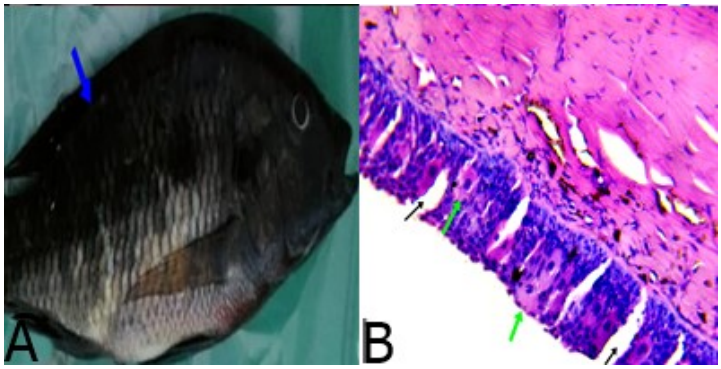

Fig.5. Skin of tilapia infected with *Gyrodactylus sp*. A. Dark discoloration of the skin (blue arrow) B. Erosion, ulceration (Black arrows), increasing alarm cells number (green arrows), abundant melanomacrophages, edema. (H&E stain B: X40)

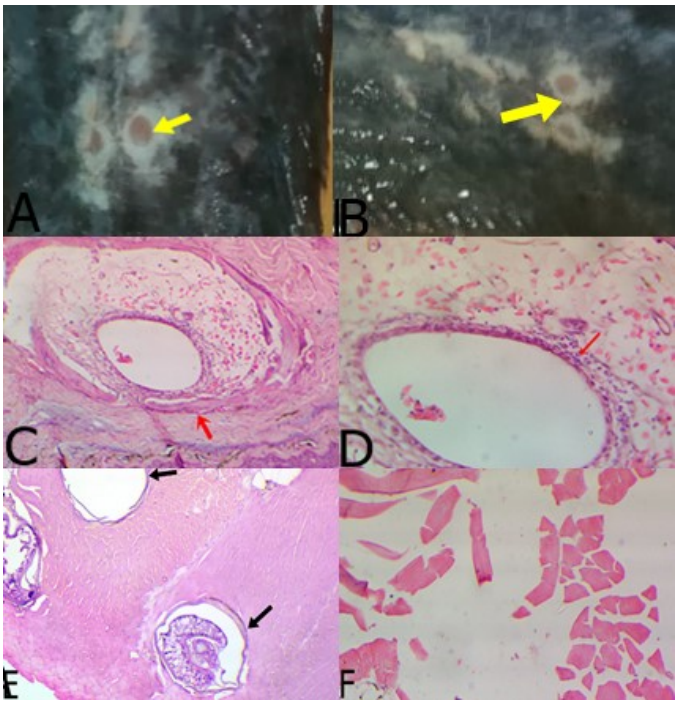

Fig.6. A &B&C&D: Encysted metacercaria in skin and muscles of catfish. A&B: erosion and ulceration (yellow arrows). C&D: parasitic cyst surrounded by fibrous capsule (red arrows). E&F: encysted metacercaria in muscles of tilapia. E: multiple parasitic cysts with different shape and size (black arrows). F: hyaline degeneration, edema and destruction of muscle fibers. (H&E stain C&E&F: X10; D:X40)

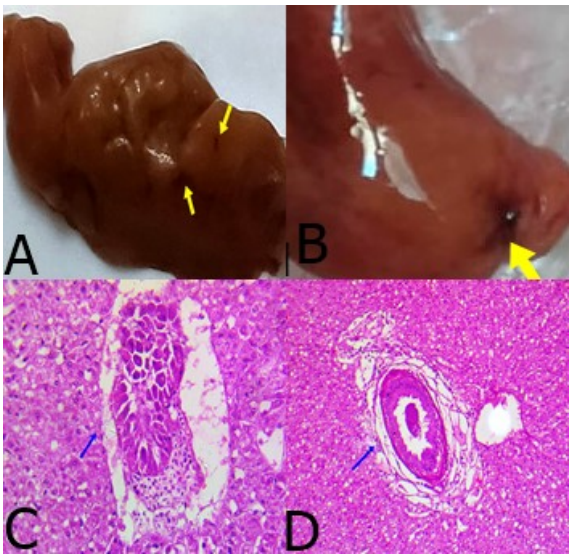

Fig.7. Liver of tilapia A&B. Hemorrhage (yellow arrows) .C& D Parasitic cysts surrounded by fibrous connective tissue capsule and inflammatory cells infiltrations (blue arrows), micro and macro steatosis. (H&E stain C& D X10).

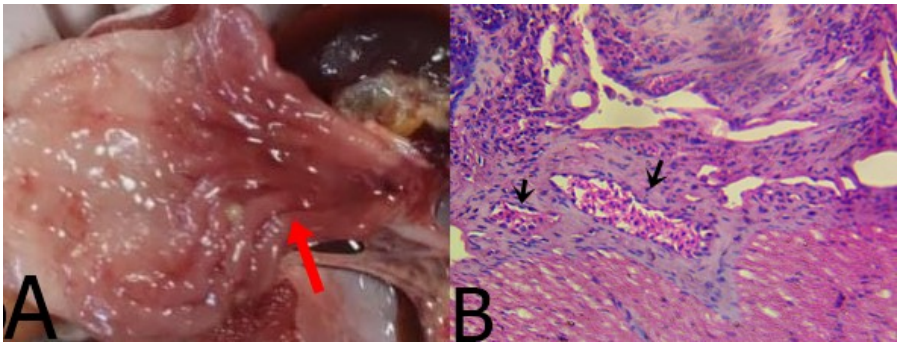

Fig.8. Stomach of cat fish infected with *Polyonchobothrium sp*. A. Congestion and hemorrhage (red arrow). B. Dilated blood vessels (black arrows). (H&E stain B X40)

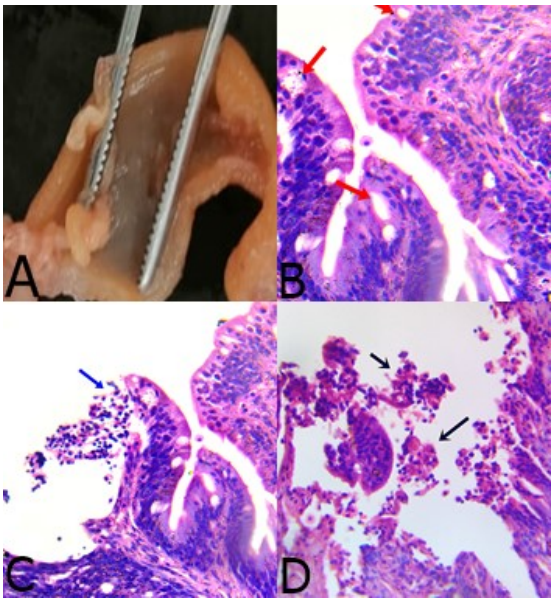

Fig.9. Intestine of cat fish. A. The intestine was invaded by large number of the parasites (*Polyonchobothrium spp.).* B. Goblet cell hyperplasia (red arrows). C. Infiltration of inflammatory cells (blue arrow), D. Desquamation of mucosa and hemorrhage (black arrows). (H&E stain; B&C&D: X40).

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دراسة نسيجية مرضية على بعض الأمراض الطفيلية في الأسماك

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الملخص

تعد الأسماك مصدرًا مهمًا للبروتين عالي الجودة منخفض الكوليسترول، ولسوء الحظ فإن الأسماك عرضة للعديد من الإصابات الطفيلية، والتي لها تأثير كبير على تربية الأحياء المائية، بالإضافة إلى انتقالها للإنسان. هدفنا الرئيسي في هذه الدراسة هو تقييم الإصابة الطفيلية في سمك القرموط الأفريقي وأسماك البلطي النيلي والتأثيرات النسيجية المرضية لهذه الطفيليات على الاسماك المصابه تم جمع مائتي عينة من الأسماك و150 سمكة قرموط الأفريقي و50 سمكة بلطي من نهر النيل بمحافظة دمياط وسوق مدينة بدر وسوق مدينة المنزلة وسوق محافظة المنوفية في الفترة من يناير 2022 إلى فبراير 2024 الطفيليات التي تم تشخيصها في سمك القرموط الأفريقي هي الميتسركاريا المتكيسة بنسبة انتشار (20%) وتصيب الجلد والعضلات مجهريا ظهرت على شكل أكياس طفيلية محاطة بمحفظة ليفية من النسيج الضام واثرت على الامعاء ومجهريا أظهرت تقشر الغشاء المخاطي وتضخم الخلايا الكأسية بنسبة انتشار (20%) *Polyonchobothrium sp.* بنسبة انتشار (10.5%)، مما أدى إلى ظهور رخامي للخياشيم مجهريا، أظهرت الخياشيم تضخم وتوسع الشعيرات *Dactylogyrus sp.* الطفيليات التي تم التعرف عليها في البلطي هي الميتسركاريا المتكيسة بنسبة انتشار (22%) وتسببت في العديد من البقع النزفية في الكبد.

مجهريا أظهرت تغيرا دهنيا بنسبة انتشار (6%) تسببت في نخر الخياشيم مجهريا أظهرت تقشر في الخياشيم *Chilodonella sp.* مما تسبب في تغيير لون الجلد الي اللون الداكن مجهريا، اظهر الجلد العديد من بنسبة انتشار (4%) *Gyrodactylus sp.* الخلايا المنبهة ووفره الخلايا الميلانينيه بنسبة انتشار (2.8%) سببت نزيفاً في الخياشيم من الناحية المجهرية، أظهرت الخياشيم نزيفًا وفقدانًا لخيوط الخياشيم Cymothoid Isopods

الكلمات الدالة: التشريح المرضي، الطفيليات، البلطي النيلي، سمك القرموط الأفريقي.