

**Diseases Resulting from Trematode Infestations in
Oreochromis niloticus and *Clarias gariepinus* in Ismailia
Governorate.**

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Abstract:

The present study was carried out on 360 of two species of freshwater fishes (200 *Oreochromis niloticus* and 160 *Clarias gariepinus*, which were collected randomly and seasonally from different localities in Ismailia governorate from December 2017 to November 2018. Naturally infested fishes revealed no pathognomonic clinical signs except dark coloration and ulcers on skin in *O. niloticus* and *Cl. gariepinus*. Postmortem examination showed hemorrhages, abrasions in the skin, presence of yellow cysts embedded in musculature and enlargement of liver with yellowish discoloration in both *O. niloticus* and *Cl. gariepinus*. The total infestation rate was 70%; in *O. niloticus* (62%) and in *Cl. gariepinus* (80%). The isolated monogenetic trematode was *Cichlidogyrus tilapiae* from gills of *O. niloticus*. The isolated digenetic trematodes were *Orientocradium batrachoides*, *Astiocrema reniferum* and *Afromacroderoides lazera* from the intestine of *Cl. gariepinus*. *Eumesenia aegypticus* was isolated from the intestinal lumen of *O. niloticus*, while encysted metacercariae (EMC) were isolated from different organs of infested *O. niloticus*. Confirmation of trematode infestation was done by using molecular diagnosis (PCR). Seasonal distribution and histopathological examinations were also recorded and discussed.

INTRODUCTION:

Egypt has large areas of fresh, brackish and marine water bodies suitable for fishing. Moreover, the climatic conditions are optimum for fish farming, with high growth rates for the fish that are raised, which constitute as a cheap source of animal protein for Egyptians (**Ishak et al. 1982**). The freshwater fish *O. niloticus* is of high economic value because of its rapid growth rate. It tolerates a wide range of environmental conditions and can be farmed easily (**Altun et al., 2006**). *Cl. gariepinus* inhabits in fresh waters which has a great importance for fish farming in Africa due to its wide geographical spread, its resistance to handling and stress, high growth and well appreciated in wide range of African countries (**Ikechukwu et al., 2017**).

Trematodes are most common parasites of all types of vertebrates and may inhabit as adults, juvenile worms and EMC. Digenean trematodes are considered the largest group of all internal parasites as they include about 18000 nominal species (**Kostadinova and Pérez-del-Olmo, 2014**). Monogeneans are external parasites of fish, which are hermaphrodite with direct life cycle (**Valigurová et al., 2011**). The aim of this study was to investigate diseases resulting from trematode infestations affecting *O. niloticus* and *Cl. gariepinus*.

Materials and Methods:**Fishes:**

A total number of 360 freshwater fishes of two species (200 *O. niloticus* and 160 *Cl. gariepinus*) of different body weights and lengths were randomly collected alive and freshly dead from fishermens in Ismailia governorate. They were collected in different seasons between Dec.2017 to Nov. 2018. Both fish species were transported to the Lab of Fish Diseases and Management Department, Faculty of Veterinary Medicine, Suez Canal University. Live fishes were transported in polyethylene bags containing 1/3 of its volume water and 2/3 was filled with air, while the freshly dead samples transported in ice box covered by ice.

Clinical examination:

Live fishes and dead ones were examined by using magnifying lens for detection of external abnormalities, according to **Amlacker (1970)**. Internal abnormalities were detected according to **Lucky (1977)**.

Parasitological examination:**1-Macroscopic examination:**

Examination was done by naked eyes and hand lens for detection of external and internal trematodes after fish scarification.

2-Microscopical examinations:

Gills and gill mucus were examined immediately for detection of monogeneans. They were removed with a fine needle and prepared for slides according to **Justine (2005)**. Skin and fins were examined by

scraping them with scalped blade from just behind operculum to the tip of the tail. Scales and mucus were transferred to a cover slide with a drop of distilled water to avoid drying and examined microscopically. Small pieces of the internal organs (liver, spleen, kidneys, gonads and heart) and musculature were taken for wet mount preparation and examined under binocular microscope for detection of EMC. EMC were fixed in formalin solution 5% for 24 hours then examined under light microscope. Stomach and intestine were scrapped then the parasites were removed with a fine brush and adequately washed in physiological saline and prepared permanently according to **Lucky (1977)**.

Preparation of permanent slides and staining:

A-Monogenetic trematodes:

In a clean petri dish containing physiological saline, gills were placed till monogenea detached then transferred in a clean petri dish and fixed with 5% formalin for 24 hours, washed in tap water, staining with borax carmine for 5 minutes then washed carefully with tap water. Differentiation occurred in acid alcohol (1 HCL in 70% ethyl alcohol) using dissecting microscope till reaching appropriate staining then washed with tap water, dehydration occurred by serial grades of ethyl alcohol (30, 50, 70, 80, 90 and 100%), clearance by using clove oil, xylene for removing excess oil and mounted

by using canada balsam then left to dry in a horizontal manner in hot air oven. (**Negm El-Din et al., 1995**).

B-Digenetic trematodes:

Digenean adult trematodes which obtained from intestines and stomach were placed into test tubes containing distilled water and with vigorous shaking, worms were washed, relaxed and be ready for fixation by using 5% neutral buffered formalin for 24 hours, then preserved in vials contain 70% ethyl alcohol and stained, according to **Lucky (1977)**.

C- EMC:

Fixed EMC from infested organs and musculature were stained and mounted according to **Lucky (1977)**.

Detection of *Orientocradium batrachoides* by using PCR:

Samples of DNA were obtained from parasites infested fishes following clearance with SDS 1%. DNA was extracted with QIA amp DNA mini kit (GIAGEN, USA) Ref. No (51304) according to instructions of manufacture. To ensure a good-quality input of DNA, the isolated DNAs were investigated for proper concentration and integrity using agarose running gel assay. To ensure that the isolates were belong to trematode, two sets of universal primers representing variable regions on ITS2 gene were selected according to **Arya et al. (2016)**, and gave amplification bands of 539bp (Table, 1). All PCR amplifications were performed using commercial

Emerald Amp GT PCR Master mix (Takara) Ref. No (RR310A). In all PCR experiments, DNA from pure cultures of trematode were included as a positive control, whereas molecular biology water was used as negative control. Amplified products were then detected by horizontal 1.5% (w/v) agarose gel electrophoresis for 30 min at 1-5 volts/cm. After gel separation, the amplified products were visualized using a Polaroid MP-4 camera and computer digitized (Gel Doc100, Bio-Rad). A 100bp ladder gel Pilot

(QIAGEN, USA) was used as a molecular mass marker.

Histopathological examination:

Small specimens of the suspected organs (skin, intestine, stomach, ovary, tests, brain, eyes, spleen and kidney) that showed lesions were fixed in 10% neutral buffered formalin for 48 hrs., dehydrated in serial grades of ethyl alcohol (70, 80, 90 and 100%) for 1 hr., prepared and stained with Hematoxylin and Eosin (H&E) according to Carleton (1976).

Table (1): Primers sequences and amplified segment (bp) of ITS2 gene.

Target gene	Primers sequences	Amplified segment (bp)	Reference
Trematodes ITS2	GGTACCGGTGG AGTTTCTTTTCC GTG	539bp	Arya et al., 2016
	GGGATCCTGGT AGTTTCTTTTCC TCCGC		

Results:**Clinical examination of the naturally infested fishes:**

Most of the naturally infested fishes (*O. niloticus* and *Cl. gariepinus*) showed no pathognomonic clinical signs. Some cases of *O. niloticus* revealed hemorrhages on the skin, (Plate 1.a) and cataract were also observed, (Plate 1.b). While in *Cl. gariepinus* there were hemorrhages on the ventral aspect, (Plate 1.c) and lot of ulcers all over the body, (Plate 1.d).

Postmortem examination:

Gills of *O. niloticus* showed marbling appearance with excessive mucous secretions with erosions of gill tips, (Plate 2.a). Liver; in infested *O. niloticus* showed enlarged size, (Plate 2.b). Musculature showed yellowish cysts in *O. niloticus*, (Plate 2.c). Ovaries showed large size and firm nodules in *O. niloticus*, (Plate 2.d). Brain of infested with EMC of *O. niloticus* showed severe congestion, (plate 2.e). Kidneys; in *Cl. gariepinus* showed yellowish embedded cysts, (plate 2.f)

Parasitological examination:**A-Monogenetic trematodes:****1-Cichlidogyrus tilapiae:**

The parasite was isolated from the gills of *O. niloticus*, it is a flat worm has two elliptical projections at the anterior end. The posterior end (haptor) has two pairs of anchors and several marginal hooklets. From the morphological and parasitological characteristics, it was related to phylum

Platyhelminthes class *Trematoda*, order *Monogenia*, family *Ancyrocephalidae* and genus *Cichlidogyrus*, (Plate 3.a).

B-Digenetic trematodes:**1-Eumesenia aegypticus:**

It was isolated from the intestine of *O. niloticus*, it was pyriform in shape, and the cuticle covered with spines. The anterior sucker is a cub-shaped. It has two oblique testes situated at the posterior. The ovary is nearly oval in shape. The veteline follicles are clusters situated laterally. From the morphological and parasitological characteristics, it was related to family *Maseniidae* and genus *Eumesenia*, (Plate 3.b).

2-Orientocredium batrachoides:

It was obtained from the intestine of *Cl. gariepinus* and had a lanceolated body armed with a tiny spine. The testes were large in size may be separated or in contact. The ovary is oval in shape, located near to acetabulum. The uterus was a narrow-coiled tube. From the morphological characteristics, it was related family *Orientocreadiidae* and genus *Orientocredium*, (Plate 3.c).

3-Afromacroderoides lazera:

The parasite was isolated from the intestine of *Cl. gariepinus*. The worm had elongated body with uniform width. The cuticle was covered with tiny spines. The oral sucker is sub terminal. In front of the ventral sucker there was genital pore. The testes are situated obliquely in the posterior half of the body. The ovary located at the right

of the mid line. The veteline follicles extend from the ventral sucker to the posterior borders of the anterior testis. From the morphological characteristics, it was related family *Afromacrodium* and genus *Afromacrodoides*, (Plate 3.d).

4-*Astiorema reniferum*:

The parasite was isolated from the intestine of *Cl. gariepinus*. It had elongated body covered with tiny spines. The testes were ovoid in shape and the ovary was squarish. The veteline follicles extended laterally from the acetabulum to behind the posterior testis. From the morphological characteristics, it was related family *Opithorchiidae*, subfamily *Astiotrematinae* and genus *Astiorema*, (Plate 3.e).

5-Unidentified EMC of digenetic trematodes:

EMC were recovered in different organs like, liver, ovaries, brain, gills and musculature of both *O. niloticus* and *Cl. gariepinus*. It was circular in shape with double wall; the outer membrane was thick and the inner was thin (Plate, 4).

PCR results:

The samples were analyzed based on the, trematode ITS2 (internal transcribe spacer gene) PCR products having the sequence (GGT ACC GGT GGA TCA CTC GGC TCG TG) and (GGG ATC CTG GTT AGT TTC TTT TCC TCC GC). PCR amplification and agarose gel electrophoresis expected positive band at 539bp, (Photo, 1) which confirmed that the

parasite is a trematode; *Orientocredium batrachoides*

Histopathological findings of the infested *O. niloticus* and *Cl. gariepinus* tissues:

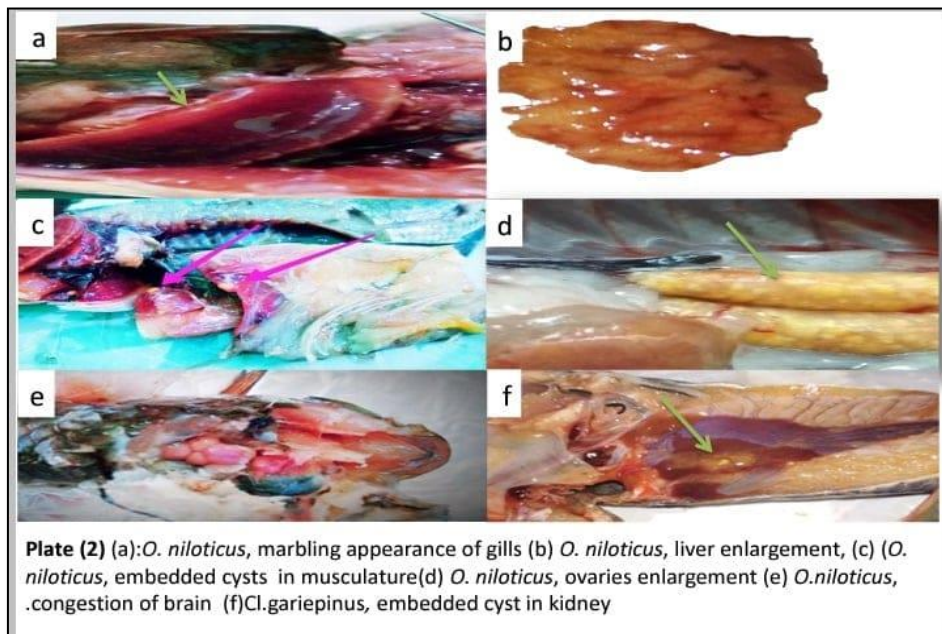
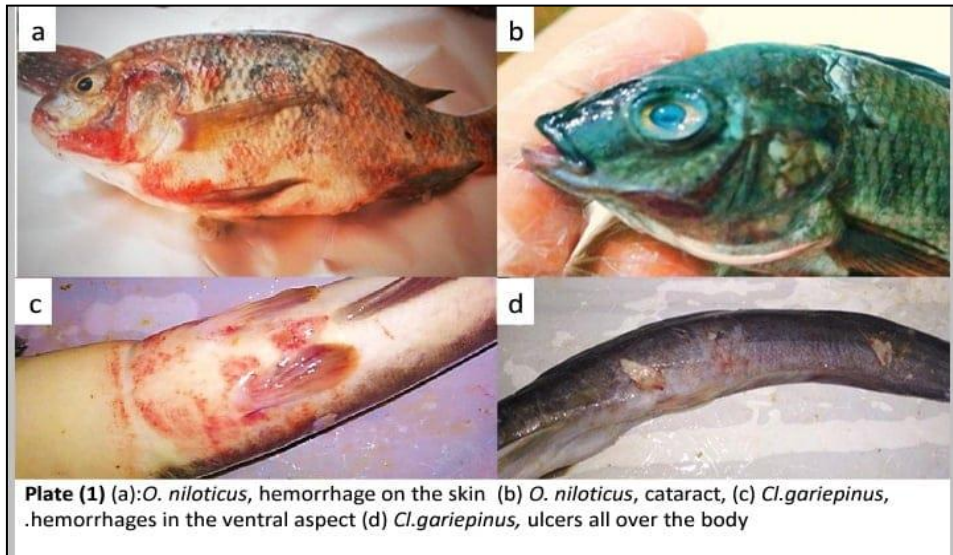
A- *O. niloticus*:

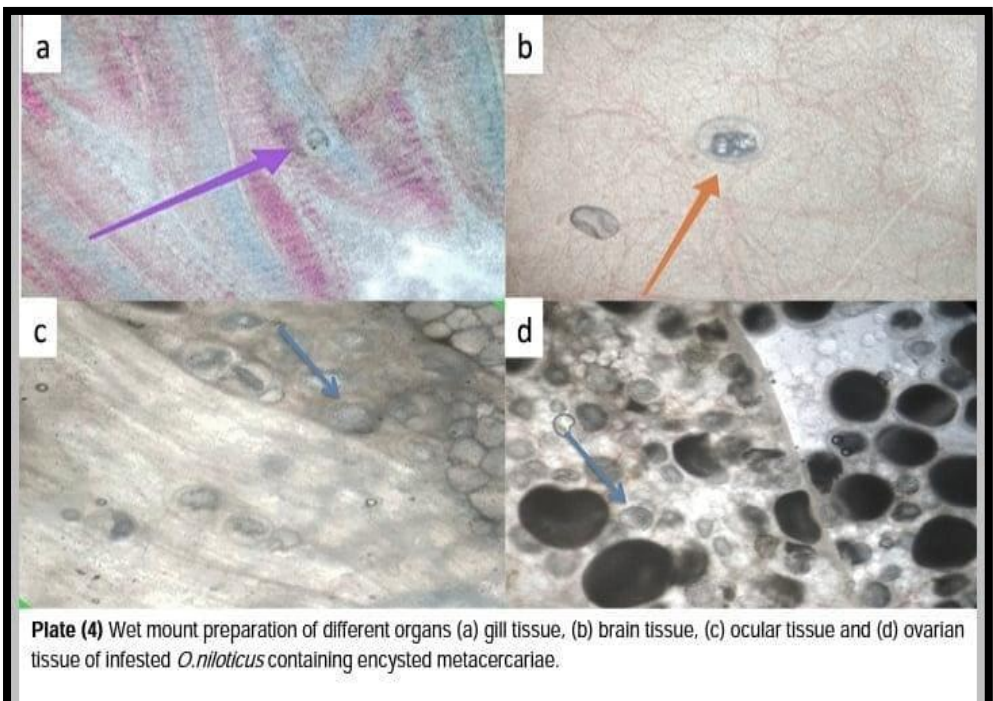
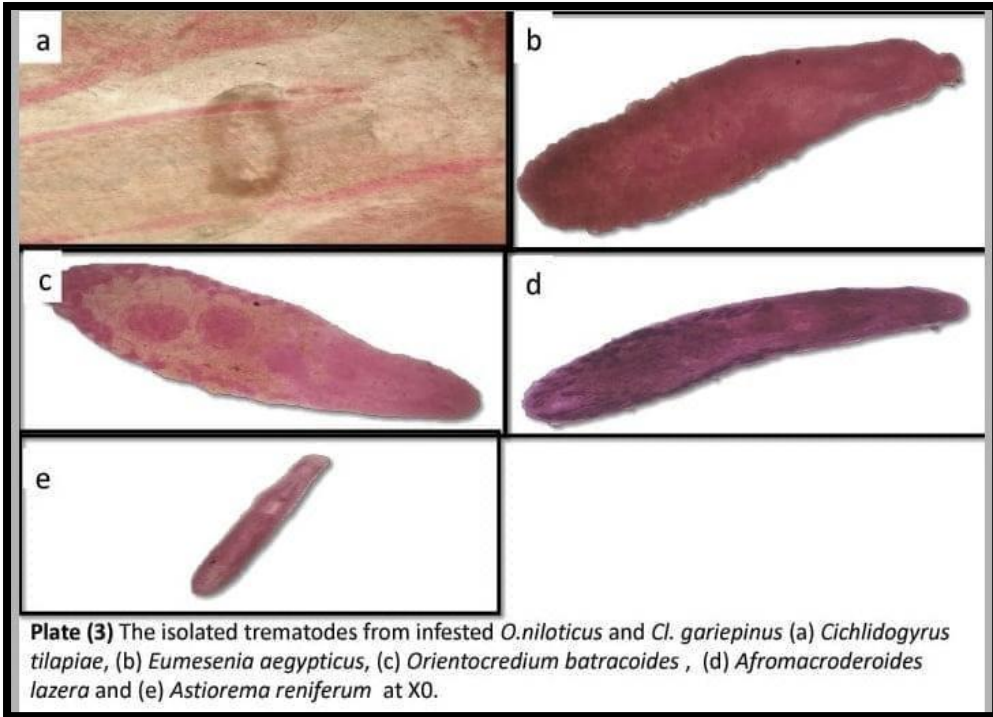
Multiple EMC embedded between the villus epithelium or between serosa of intestinal wall and musculosa in intestine, (Plate 5.a).

On the other hand, liver showed encysted metacercaria surrounded with a thick fibrous capsule causing atrophy of the surrounding hepatocytes, causing diffuse to multifocal vacuolar degenerations of hepatocytes, (Plate 5.b). Ovary showed massive infestation of the wall and between ovarian follicles with multiple number of encysted metacercariae surrounded by very thick fibrous capsule and adjacent tissues showing atrophy which cause mechanical degeneration, (Plate5.c) Also, brain showed encysted metacercaria embedded in sub-meningeal tissue and surrounded with leukocytic infiltrations, (Plate 5.d).

B- *Cl. gariepinus*

The examined liver of the infested *Cl. gariepinus* showed EMC surrounded with a thick fibrous capsule causing atrophy of the surrounding hepatocytes, (Plate 5.e). Musculature of the infested *Cl. gariepinus* showed EMC between muscle fibers and causing severe degenerative changes, hyalinization, and edema along with leukocytic infiltrations, (Plate 5.f).





Total and seasonal prevalence of trematode disease infestations among examined fishes:

Table (2) and chart (1) showing the total prevalence of *O.niloticus* and *Cl.gariepinus* and table (3) and chart (2) showing the seasonal prevalence of trematode infestation in both of them.

Table (2) prevalence of trematode disease infestations among examined fishes

Fish species	No. of examined fish	No. of infested fish	Prevalence %
<i>O.niloticus</i>	200	124	62
<i>Cl.gariepinus</i>	160	128	80
Total	360	252	70

Chart (1) prevalence of trematode disease infestations among examined fishes

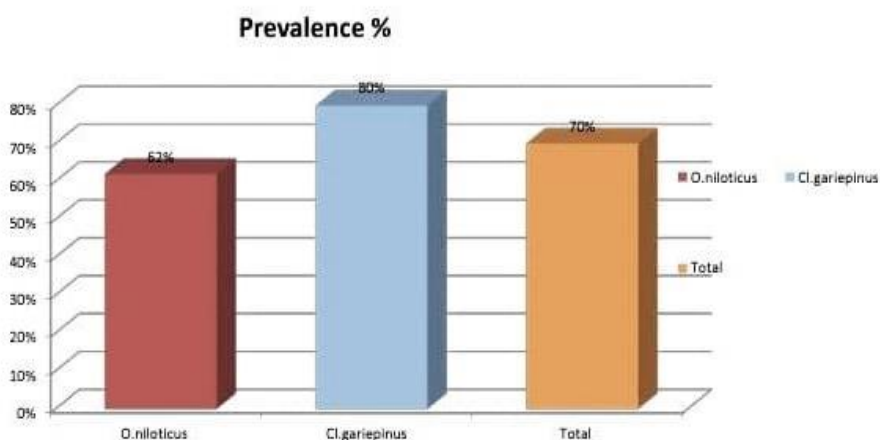


Table (3) Seasonal prevalence of trematodes disease infestations among examined fish species

Fish species	Seasonal prevalence							
	winter		spring		summer		Autumn	
	No.	%	No.	%	No.	%	No.	%
O. niloticus	35 (N50)	70 (N50)	30 (N50)	60 (N50)	27 (N50)	54 (N50)	20 (N50)	40 (N50)
Cl. gariepinus	38 (N40)	95 (N40)	35 (N40)	87.5 (N40)	30 (N40)	75 (N40)	25 (N40)	62.5 (N40)

Chart (2) Seasonal prevalence of trematodes disease infestations among examined fish species

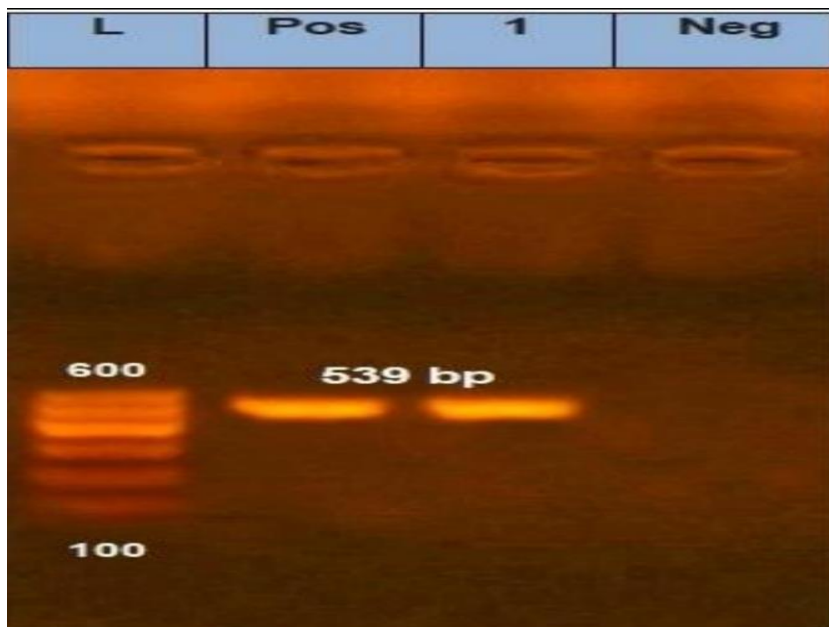
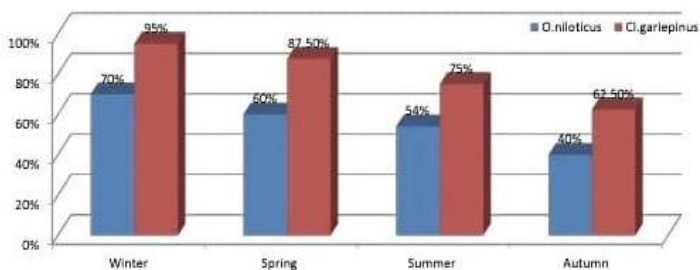
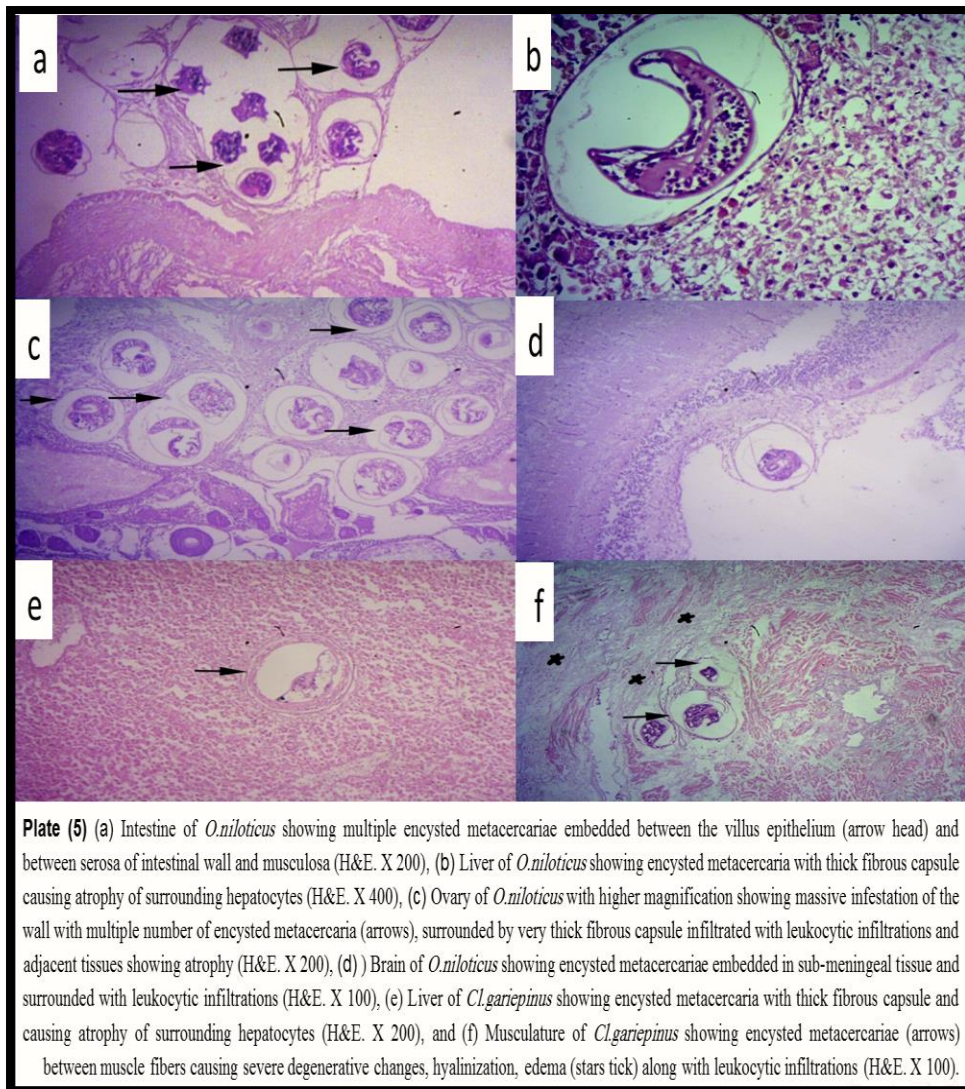


Photo (1): A representative gel displaying analysis of ITS-2 region (internal transcribe spacer gene) from adult trematodes samples.



Discussion

The present study aimed to investigate some of the parasitic diseases (trematodiasis) affecting *O. niloticus* and *Cl. gariepinus*. The most common clinical signs which detected in naturally infested *O. niloticus* with trematodes were hemorrhages and darkening of the skin, erosion of fins and cataract in the ocular tissue. While in *Cl. gariepinus* there were hemorrhages

and ulcers all over the body and frayed fins. Concerning postmortem examination; there were yellowish cysts embedded in different parts of musculature especially in *O. niloticus*, these results agree with that obtained by **Rawia Adawy (2000)**. Regarding gills of infested *O. niloticus* and *Cl. gariepinus*, it suffered from excessive mucous secretions leading to severe respiratory manifestations; this may

be due to feeding activity of the parasite, locomotion and fixation. Concerning mosaic appearance (marbling) of gills, it may be due to penetration of the parasite to the efferent vessels where there is low blood pressure and the hemorrhage are caused followed by a very hard clotting for closing of these vessels, thrombus is occurred leading to ischemia which resulting in necrosis in some areas and congestion of some areas with progressive degeneration of the other parts of the gill filaments giving this appearance. These results agreed with those reported by **Eissa (2002)**. Also, brain suffered from congestion which revealed heavy infestation of *O. niloticus* which may be due to irritation caused by the EMC. Also, liver, kidneys and gonads of *Cl. gariepinus* were suffered from enlargement. These results were in agreement with that described by **Heba Abdel-Moula (2005)**. Regarding monogenean infestation; the parasite which was isolated from the gills of *O. niloticus*, it was a flat worm, has two elliptical projections at the anterior end. The posterior end (haptor) has two pairs of anchors and several marginal hooklets. According to parasitological examination, the parasite is *Cichlidogyrus tilapiae*. These results agreed with those reported by **Akoll et al. (2012)**. A fluke isolated from the intestine of *O. niloticus* has pyriform shape; the outer surface (cuticle) was covered

with spines. According to the parasitological examination the fluke is *Eumesenia aegypticus*. This description was similar to that described by **Eman Youssef (2001)**. In this study the parasitological examination of *Afromacrodieroides lazera* which was isolated from the intestine of *Cl. gariepinus* revealed that the parasite has an elongated body with uniform width, come to a point at the extremities. These findings were with in agreement with that mentioned by **Eman Youssef (2001)**. Another parasite was isolated from the intestine of *Cl. gariepinus* and characterized by elongated body, covered with tiny spines and the oral sucker is sub terminal and oval in shape. According to the morphological and parasitological examination, the parasite was *Astiorema reniferum*. These descriptions come along with that recorded by **Heba Abd EL-Moula (2005)**. In this study, *Orientocredium batracoides* was obtained from the intestine of *Cl. gariepinus*. It has a lanceolated body armed with tiny spines and the oral sucker was situated sub terminal. These descriptions agreed to that mentioned by **Abbass et al. (2006)**. EMC which were recovered in different organs (liver, ovary, brain, gills and musculature) were circular in shape with double wall; the outer wall is thick and the inner is thin. These results such as mentioned by **Osman (2001)**. Regarding the total prevalence of infestation in *O. niloticus* and *Cl.*

gariiepinus, it was 70%. The total prevalence of infestation in *Cl. gariiepinus* was 80% and in *O. niloticus* was 62%. The difference may be due to the feeding behavior where *Cl. gariiepinus* is a carnivorous bottom feeder, that feed on low valuable food such as mud, mollusks, arthropods and young infested fish, in addition to they are scaleless fish that permit the penetration of the infective stages through the external body surface. Unlike *O. niloticus* which has shortage period to reach the marketable size in addition to the presence of scales which protect its body from some parasitic infestation. These results were near to that mentioned by **Heba Abd El-Moula (2005)** who mentioned that the total prevalence of trematodes infestation in *Cl. gariiepinus* was 40%, in cultured *O. niloticus* was 23.27% and in wild *O. niloticus* was 17% and **Walaa El-Houseiny (2008)** who reported that the infestation rate was 96.55% and 95.83% in both cultured and wild *Cl. gariiepinus*, respectively. While the rate was 62.32% and 43.46% in both cultured and wild sources of Tilapia species, respectively. On the other hand, the results obtained were different from that mentioned by **Akoll et al. (2012)** who mentioned that the total prevalence of parasitic infestation was 89 and 54% in *O. niloticus* and *Cl. gariiepinus*, respectively. These variations may be due to the difference of locations,

environmental conditions, intermediate hosts and final hosts. In the present work, the seasonal distribution was found as, the highest prevalence rates were in winter (70 and 95%) in *O. niloticus* and *Cl. gariiepinus*, respectively followed by spring (60 and 87.5%) in *O. niloticus* and *Cl. gariiepinus*, respectively followed by summer (54 and 75%) in *O. niloticus* and *Cl. gariiepinus*, respectively and the lowest season was autumn (40 and 62.5%) in *O. niloticus* and *Cl. gariiepinus*, respectively. These variations may be due to the difference in seasonal temperature and availability of the intermediate host which increase the feeding activity in these seasons. These results is in agreement with **Bassiony (2002)** who mentioned that the seasonal infestation rate of enteric trematodes was (59.37%) for winter, (50.58%) for autumn, (40.85%) for spring and (22.36%) for summer and **Bichi et al. (2010)** who mentioned that the highest prevalence of trematodes in *O. niloticus* was in winter (36.33%). While our results differ from that obtained by **El-Shahawy (2017)** who reported that the seasonal prevalence of infestation in *O. niloticus* was high in spring (61.1%) then in winter and summer (33.3%) then autumn (16.6%), these variations may be due to differences in locations and environmental conditions. Concerning molecular diagnosis (PCR), the recovered parasites were examined for

confirming its relation to trematodes which will help in the future for producing vaccines against trematodes and also, in serological analysis by detection the antigen early. The specimens were examined depending on the presence of trematodes ITS2 (internal transcribe spacer gene). The ITS2 gene gives positive bands at 539bp that confirm that the parasite sample is trematode. These results were similar to that obtained by *Arya et al. (2016)*. In this study the histopathological examination revealed that liver and ovaries of the infested *O. niloticus* and *Cl. gariepinus* showing EMC surrounded with a thick fibrous capsule causing atrophy of surrounding hepatocytes and multifocal vacuolar degenerations of hepatocytes, these results were similar to what mentioned by *Nouh et al. (2010)*.

Conclusion:

- Most of the examined fishes (*O. niloticus* and *Cl. gariepinus*) showed no pathognomonic clinical signs, they were apparently normal. Some cases of *O. niloticus* which were heavy infested with trematodes, revealed hemorrhage on the skin and cataract in the ocular tissue was founded. While in *Cl. gariepinus* a lot of ulcers all over the body, hemorrhage on the skin.
- The highest prevalence of infestation was in *Cl. gariepinus*

(80%) followed by *O. niloticus* (62%).

- The highest prevalence of trematodes infestation was in winter in *O. niloticus* and *Cl. gariepinus*, while the lowest was in autumn.
- *O. niloticus* showed infestation with different worms as *Cichlidogyrus tilapiae*, *Eumesenia aegypticus* and Encysted metacercariae. While *Cl. gariepinus* showed infestation with *Orientocredium batracoides*, *Afromacroderoides lazera*, *Astiorema reniferum* and Encysted metacercariae.
- PCR, the most recent method helped in identification of *Orientocredium batracoides*

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الملخص العربي

الأمراض الناجمة عن الإصابة بالديدان المفلطحة في اسماك البلطي النيلي والقرموط الأفريقي في محافظة الإسماعيلية.

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, مآثر محمد منير طه اللمعي.

قسم أمراض ورعاية الأسماك - كلية الطبيب البيطري. - جامعة قناة السويس

* قسم الپاراسيتولوجيا - كلية الطبيب البيطري. - جامعة قناة السويس

** قسم الباثولوجيا - كلية الطبيب البيطري. - جامعة قناة السويس

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اجريت هذه الدراسة علي نوعين من اسماك المياه العذبة (البلطي النيلي والقرموط الافريقي) والتي بلغ عددها ٣٦٠ سمكه : ٢٠٠ سمكه من نوع البلطي النيلي و ١٦٠ سمكه من نوع القرموط الافريقي حيث تم تجميع العينات من اماكن متفرقة من محافظه الإسماعيلية في مواسم مختلفة خلال فترة الدراسة من ديسمبر ٢٠١٧ الي نوفمبر ٢٠١٨. الاسماك التي تم فحصها لم تظهر اي علامات مرضيه مميزه ولكن اظهرت الصفة التشريحية وجود انزفه علي الجلد وعتامه في العين , وجود حويصلات صفراء علي العضلات, احتقان بالمخ خاصة في اسماك البلطي النيلي مع تضخم الكبد, بينما اظهر فحص القرموط الافريقي وجود انزفه علي الجلد مع وجود بعض القرخ, تضخم في الكبد والكلي مع وجود حويصلات صفراء بها. كان معدل الإصابة الكلي ٧٠ ٪. حيث بلغت نسبة الإصابة بالديدان المفلطحة في اسماك البلطي ٦٢ ٪ وفي القرموط الافريقي ٨٠ ٪. الديدان وحيدة العائل التي تم عزلها هي السيكليدوجيراس من خياشيم البلطي النيلي. كما تم عزل بعض الديدان ثنائية العائل مثل الاورينتوكراديام بتراكويدس والاستيورما رينيفيرام والافروماكروديرويدس لزيروا والتي تم عزلها من امعاء القرموط الافريقي الايموزينيا من امعاء البلطي النيلي . تم استخدام اختبار تفاعل البلمرة المتسلسل PCR في تصنيف احد الديدان ثنائية العائل (الاورينتوكراديام بتراكويدس) . كما تم استخدام الفحص الهستوباثولوجي وذلك للتعرف علي تأثير الإصابة علي بعض الأنسجة مثل الخياشيم والكبد والمخ والعضلات والامعاء والمبيض . كما تم تسجيل معدل الإصابة في المواسم المختلفة.