

Studies on Some Diseases Caused by Digenetic Flukes and Larvae in Wild African Catfish, "*Clarias gariepinus*"

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Abstract

This study was carried out to investigate some of the internal parasitic diseases of digenetic origin that affect *Clarias gariepinus*. 160 fish were collected randomly and seasonally from different sources in Ismailia governorate. Examined fish showed no pathognomonic clinical signs especially in low parasite load. P.M. lesions were mostly paleness or congestion and enlargement of the liver with pin head spots. Total infestation rate was 75%. The isolated flukes were *Orientocredium* sp., *Astiocrema reniferum*, *Eumesenia* sp. as well as encysted metacercariae. The infestation rate increased with the increase of the body weight and length. Infestation in females were higher than males especially during spring and winter seasons. PCR used for identification of trematodes by using (ITS2) giving positive band at 539bp. The histopathological changes recorded were mostly degeneration, necrosis, sloughing of cells and infiltration.

Introduction

In Egypt, parasitic diseases represent about 80% of fish diseases that leading to reduction of hatchery efficacy, low production and even high mortalities (Eissa, 2002). Fish endoparasites are the main cause of economic losses and most prevalent causative agents of diseases in aquaculture.

Clarias gariepinus Burchell, 2000 is an air breathing catfish so, it can survive severe conditions due to its accessory air breathing organ. *C.gariepinus* inhabits freshwater ranging from lakes, streams, rivers to swamps. Such fish is of great

importance for fish farming due to its wide geographical spread in Africa, its resistance to handling and stress, high growth rate and well appreciated in a wide range of African countries (Ikechukwu et al. 2017). The current investigation was aimed to record the clinical picture associated with diagnosis of the detected internal parasitic Trematodes using classical and advanced methods, recording the total and seasonal prevalence in *C.gariepinus*. Besides, the histopathological alterations were examined.

Material and Methods

Fish:

A total number of (160) live specimens of African catfish (*Clarias gariepinus*) of different body weights and lengths were randomly collected from Ismailia governorate. They were collected in different seasons during in the period from December 2016 to November 2017.

Clinical picture:

Fish specimens were examined clinically for detection of any macroscopic lesions and P.M. examination carried out to detect any internal abnormalities according to *Conroy and Herman (1981)*.

Parasitological examination:

a. First, it was carried out to the fish specimens macroscopically and microscopically.

b. Permanent slide preparation:

The collected flukes were washed in physiological saline then fixed in 5% neutral formalin solution. Then the fixed worms rewashed in physiological saline several times, then staining in alum carmine stain till reach good staining degree in which differentiation can be occur in acid alcohol using dissecting microscope. Then dehydrated in ascending alcohol grades 70,80, 90 and 100% and cleared by clove oil then xylol and finally mounted on glass slide in Canada balsam and covered with cover slide. Then slides dried in an oven at 40°C then examined for microscopical identification (*Lucky, 1977*).

Detection of trematodes using PCR.

Extraction of DNA according to QIAamp DNA mini kit instructions, Preparation of PCR Master Mix according to Emerald Amp GT PCR mastermix, Cycling conditions of the different primers. Temperature and time conditions of the primer differentiated according to the target gene ITS2 during c PCR, DNA molecular weight marker by mixing the ladder gently by pipetting up and down. 6 µl of the required ladder were directly loaded. Finally extraction performed for Agrose gel electrophoreses according to *Sambrook et al. (1989)*.

Histopathological examination

Small pieces of suspected lesions were taken from the naturally infested fish and preserved in 10% neutral buffered formalin for 48 hr. The specimens were dehydrated in ascending grades of ethyl alcohol "70,80,90 and 100%" for 1 hr each then cleared in 50% alcohol-xylol mixture followed by two changes of xylol for 1 hour each. The specimens were embedded in paraffin wax, sectioned at 5µm thickness, then placed on glass slides and stained with Hematoxyline and Eosin (H&E) according to *Carleton (1976)*.

Results

Clinical examination: Most of the examined fish revealed no pathognomonic signs. In some cases

of heavy infestation, digenetic trematodes showed abdominal distention, prolapsed vent, emaciation and skin discoloration. Pale enlarged internal organs (Photo 1), congested and ulcerated intestine. The infested liver with encysted metacercariae revealed rough grayish capsule, firmly attached to the liver which had whitish spots.

Results of parasitological examination:

Digenetic trematodes:

1. Flukes:

a. Orientocreadium species

It was characterized by elongated body shape that became wider at the testicular region. Anterior sucker was small, while the ventral sucker was larger. Short, ovoid pharynx and esophagus, the intestinal bifurcation extended anteriorly until reach to the posterior end surrounded by vitelline glands. (Plate 1)

b. Astiorema reniferum:

The body of *Astiorema reniferum* was looked lanceolated and armed with minute spines. Subterminal spherical oral sucker equal to the ventral sucker. Two intestinal caeca extended to the posterior end where the vitelline glands were found. Testis were ovoid in the posterior part of the body. The ovary was ovoid situated behind cirrus sac. (Photo 2)

c. Eumesenia species:

They were found in the intestine of *C.gariepinus* as pyriform shaped with cuticle covered by spines.

Cub-shaped oral sucker. Intestinal caeca reaching to the posterior end of the body. Two oblique testis overlapping each other at the posterior end of the body. The ovary was oval and anteriolateral to the testis with long uterus filled with numerous eggs. (Photo 3)

2. Encysted metacercariae

Recovered cysts were embedded in the musculature or in the liver tissue. They were circular in shape, double walled, had a thick outer layer (connective tissue capsule), thin inner layer and white creamy in color. (Plate 2,3)

Identification of trematodes using PCR

The trematodes were identified by using target gene **ITS2** (specific primer) have the specific sequence (GGTACCGGTGGATCACTCGGCTCGTG) and (GGGATCCTGGTTAGTTTCTTTTCCTCCGC). PCR amplification of a specific product and agarose gel electrophoresis yielded a positive result of the used sample at 539bp. (Photo 4).

Total prevalence of Digenetic trematode recovered from examined *C.gariepinus*:

Digenetic trematodes were isolated in rates of (40.62%) for intestinal flukes and (34.37%) for encysted metacercariae recovered from musculature and liver. (Table.1_ Fig.1)

Seasonal prevalence of digenetic trematodes in the examined *C.gariepinus*:

The highest prevalence of infestation was detected in spring (92.5%) followed by winter (87.5%) then summer (75.0%) and in autumn (45.0%). Seasonal prevalence of flukes reached the peak of infestation rate in spring (50%) winter (47.5%) summer (40%) and autumn (25%). While, encysted metacercariae were (42.5%), (40%), (35%) and (20%) in spring, winter, summer and autumn respectively (Table.2_ Fig.2).

Prevalence of digenetic trematodes infestation among different body weights of the examined *C.gariepinus*:

The highest prevalence was (94.11 %) in body weights ranged from (900 < 1200 g) and the lowest infestation rate was in weights ranged from (50g _100 g) with a prevalence of (33.3%) (Table.3_ Fig.3).

Prevalence of digenetic trematodes infestation in relation to length of examined *C.gariepinus*:

The prevalence of infestation was the highest with rate of (88.88 %) in lengths ranged from (50 < 60 cm) and the lowest rate was (54.54%) in lengths ranged from (10-20 cm). (Table.4_ Fig.4)

Prevalence of digenetic trematodes in relation to the sex of examined *C.gariepinus*:

The highest prevalence of infestation was in females (77.34%) while, in males were (65.62%). (Table.5_ Fig.5)

Results of histopathological examination of infested fishes:

The intestine of the infested *C.gariepinus* showed cross section of the trematode parasite which causing mechanical destruction of the intestinal villi, extensive necrosis and sloughing of intestinal epithelial. Numerous sections of liver showed multifocal to diffuse vacuolar degeneration of hepatocytes along with discrete necrosis of some hepatocytes. Cross section of encysted metacercaria that was surrounded by thick connective tissue capsule. (Plate 4)

Table (1): Total prevalence of digenetic trematode recovered from examined *Clarias gariepinus*.

Total No. of fish examined	Total No. of infested fish	%	Digenetic trematodes			
			EMC		Flukes	
			No	%	No	%
160	120	75	55	34.37	65	40.62

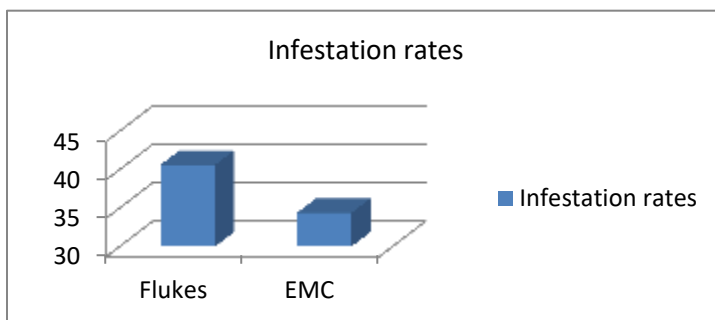


Fig. (1): Total prevalence of digenetic trematode recovered from examined *C. gariepinus*.

Table (2): Seasonal prevalence of digenetic trematodes in the examined *C. gariepinus*

Season	No. of examined fish	No. of infested fish		EMC		Flukes	
		No.	%	No	%	No.	%
Winter	40	35	87.5	16	40	19	47.5
Spring	40	37	92.50	17	42.5	20	50
Summer	40	30	75	14	35	16	40
Autumn	40	18	45	8	20	10	25
Total	160	120	75	55	34.37	56	40.62

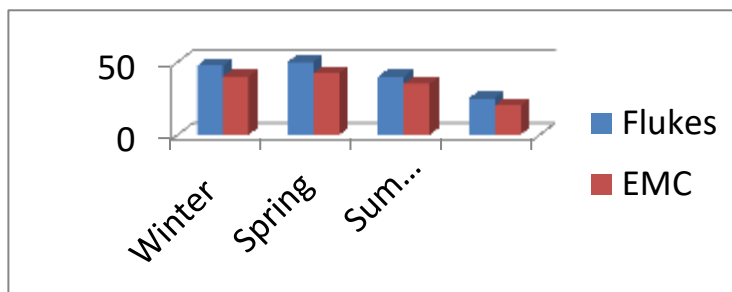


Fig. (2): Seasonal prevalence of digenetic trematodes groups in the examined *C. gariepinus*.

Table (3): Prevalence of digenetic trematodes infestation among different body weights of the examined *C.gariepinus*.

Fish weight (g)	Number of examined fish	Number of infested fish	%
50 >100	30	11	33.33
100 >300	45	36	80
300>600	25	20	80
600 >900	26	22	84.61
900 >1200	34	32	94.11
Total	160	120	75.0

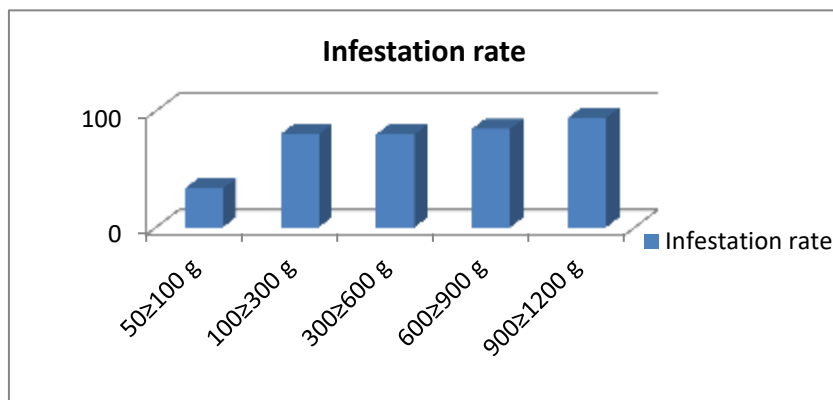


Fig. (3): Prevalence of digenetic trematodes infestation among different body weights of the examined *Clarias gariepinus*.

Table (4): Prevalence of digenetic trematodes infestation in relation to length of examined *Clarias gariepinus*.

Fish length (cm)	No of examined	No of infested	%
10 >20	22	12	54.54
20>30	40	26	65
30>40	34	27	79.41
40 >50	28	23	82.14
50>60	36	32	88.88
Total	160	120	75

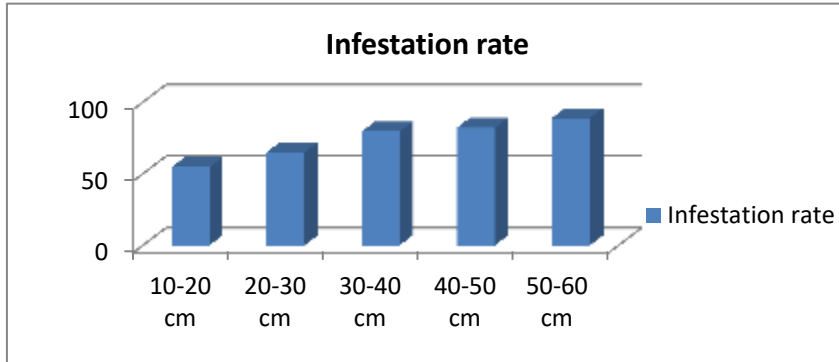


Fig. (4): Prevalence of digenetic trematodes infestation in relation to length of examined *Clarias gariepinus*.

Table (5): Prevalence of digenetic trematodes in relation to the sex of examined *C.gariepinus*.

Fish sex	No. of fish examined	No. of fish infested	%
Females	128	99	77.34
Males	32	21	65.62
Total	160	120	75

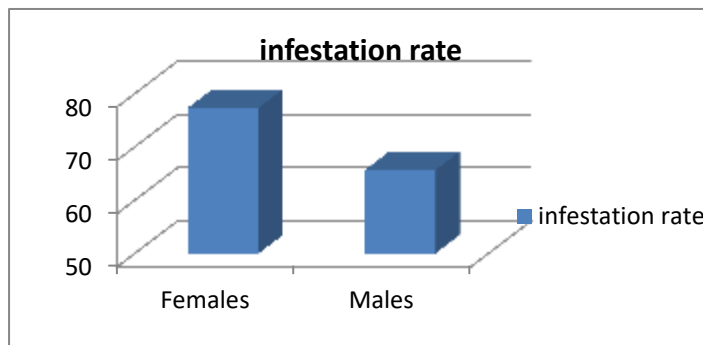


Fig. (5): Prevalence of digenetic trematodes in relation to the sex of examined *Clarias gariepinus*.



Photo (1) Pale enlarged internal organs of infested *C.gariepinus* with trematodiasis

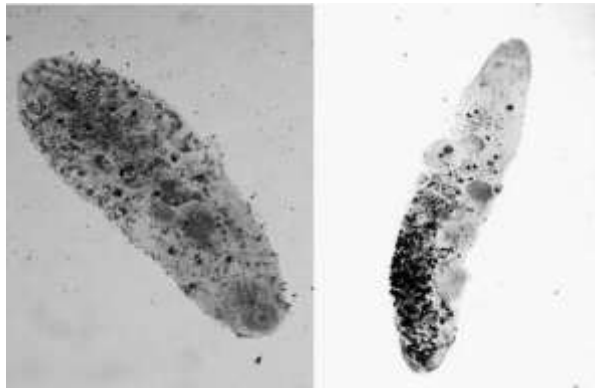


Plate (1) *Orientocredium* species



Photo (2) *Astiorema reniferum*

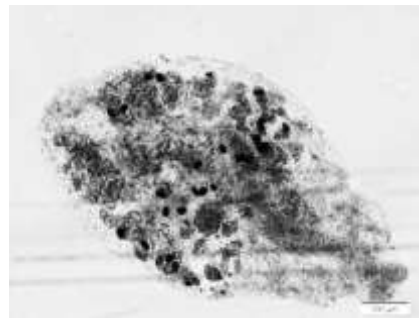


Photo (3) *Eumesenia* species

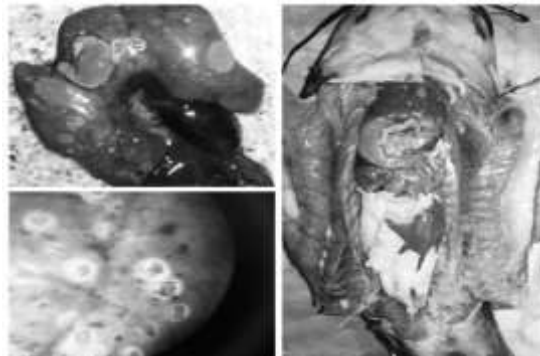


Plate (2): Encysted metacercariae in the liver of infested *C.gariepinus*.

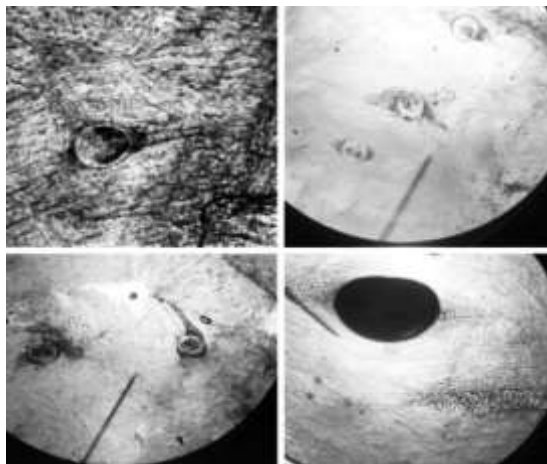


Plate (3): Encysted metacercariae in the musculature of infested *C.gariepinus*.

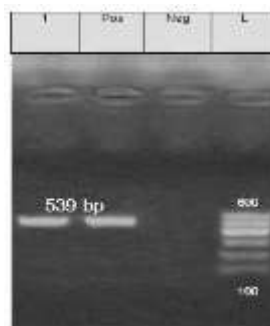


Photo (4): Electrophoretic separation of TrematodeITS2 (Internal transcribe spacer).

*L1: Positive sample; L2: Positive control; L3: negative control; L4: ladder (100bp).

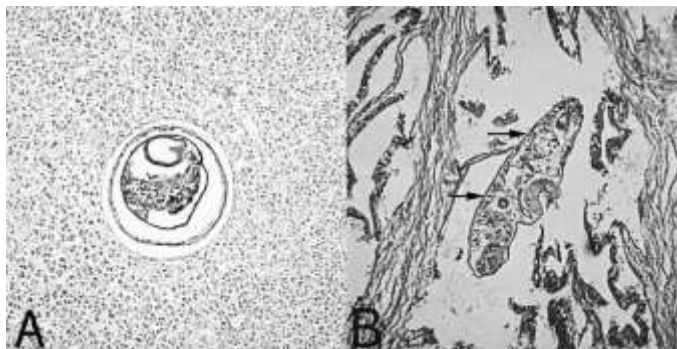


Plate (4): (A). cross section of encysted metacercaria in between liver cells stained with H&E.(B). cross section of trematode parasite in the intestinal lumen.

Discussion

Nowadays, we should pay attention to control the enzootic diseases which affect the fish farming progress in Egypt. Parasitic trematode diseases are one of these diseases which can cause a massive losses in fish cultures..

The most common clinical signs that recorded from the examination of most of the infested *C.gariepinus* with trematode diseases were emaciation, loss of condition, anemia, abdominal distension and paleness. In the postmortem examination they showed pale internal organs or congested, presence of white pin spots on liver, some white or yellowish cysts seen embedded in the musculature which may lead to low weight gain, immarketability of infested fishes and high mortality which agreed with *Arafa et al.(2014)*.

According to the parasitological examination, the following parasites were identified as *Orientocredium species*, *Astiorema reniferum*, *Eumesenia species* as well as

encysted metacercariae, these results agreed with that mentioned by *Gihan shager (1999)& (2001)*, *Mwita et al. (2004) and Eissa et al. (2010)*. PCR used in identification of trematodes by using (ITS2) gave positive band at 539bp same as mentioned by *Ramy Hassan (2017)*.

The total prevalence of flukes was (40.62%) which was the highest helminthparasite recovered from the intestine of *C.gariepinus*, this result agreed with *Eman yousef (2001)*. On the other hand, the results mentioned by *Al-Bassel (2003) and El-Shahawy (2017)* were in a prevalence lower than our results. Such variations may be attributed to the difference bet the environmental conditions, the presence of intermediate hosts and final hosts.

The prevalence of EMC in this study was (34.37%) which was nearly similar to that mentioned by (*Arafa et al., 2014*). The highest rate was in spring (42.5%) followed by winter (40%) then summer (35%) and the lowest was in autumn

(20%). The results mentioned by *Shaheen et al. (2014)* disagree our findings which may be due to the difference of the fish habitat and the presence of intermediate hosts and final hosts.

The prevalence of flukes was the highest in spring (50%) followed by winter (47.5%) then summer (40%) and the lowest was recorded in autumn (25%). These results come along with that mentioned by *El-Shahawy et al. (2017)* and completely disagreed with *Walaa El-Hossieny (2008)* may be due to the variations in the habitat.

The infestation rate was higher in females than males which agreed with *Eissa et al. (2008)* and *Abdel-Gaber (2015)*. The infestation rate increases with the increase of the body weight and length which agreed with *Abdel-Gaber et al. (2015)* and disagreed with *Eissa et al. (2007)* and *Bari et al. (2014)*.

Concerning the results of encysted metacercaria in the liver of *Clarias gariepinus* were nearly similar to that mentioned by *Nouh et al. (2010)*.

In this study, the histopathological changes of the intestine were nearly similar to that recorded by *Rawia Adawy (2000)* and *Walaa El-Hossieny (2008)*

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 Studies on some Diseases cused by

دراسات عن بعض الأمراض الطفيلية الناتجة عن الإصابة ببرقات وديدان التريماتودا فى اسماك القرموط الافريقى

أجريت هذه الدراسة لتحديد بعض الأمراض الطفيلية الداخلية (برقات و ديدان تريماتودا) التى تصيب أسماك القرموط الأفريقى و قد تم تجميع 160 سمكة فى المواسم المختلفة بطريقة عشوائية من مصادر مختلفة فى محافظة الإسماعيلية. الأسماك التى تم فحصها لم تظهر أي علامات مرضية مميزة واطهرت الصفة التشريحية وجود شحوب أو التهاب وأيضاً وجود تضخم فى الكبد مع ظهور بعض النقط البيضاء على سطحه. كانت نسبة الإصابة الكلية 75%. الديدان التى تم عزلها هى ديدان ثنائية العائل (أورينتوكريديم و الأستوريرما رينيفيرم والايوميزينيا) بالإضافة إلى البرقات المتحوصلة، نسبة الإصابة زادت مع زيادة الطول والوزن و كانت نسبة الإصابة فى الإناث أعلى من الذكور و خصوصاً فى فصلى الربيع والشتاء. تم استخدام تفاعل البلمرة المتسلسل PCR فى التعرف على الديدان المفطحة ثنائية العائل والتي سجلت ظهور قاعدة ثنائية النيروجين عند 539 bp. وتم رصد التغيرات الهستوباثولوجية فى صورة تحطم و تاكل وتساقط للخلايا كما وجد ارتشاح للخلايا الالتهابية.