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STUDIES ON SOME CLINOSTOMATID METACERCARIAE FROM *TILAPIA NILOTICA* IN ASSIUT GOVERNORATE

(With 4 Tables and 6 Figures)

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دراسات علي بعض ميتاسركاريا الكلينوستوماتيد من أسماك البلطي بمحافظة أسيوط

محسن ابراهيم عرفة ، مها سيد شاهين ، محمد الصلاحى محمد منيب.

أجريت هذه الدراسة على عدد ١٧٥ من أسماك البلطى النيلى فى محافظة أسيوط لعمل بعض الدراسات على ميتاسركاريا الكلينوستوماتيد التى تصيب تلك الاسماك. بلغت نسبة الاصابة بالانواع المختلفة من الميتاسركاريا ٤٢,٨٦ % وكان التجويف الخيشومى هو أكثر الأماكن تعرضا للأصابة حيث بلغت نسبة الاصابة بة ٢٩,٢٧ % أما فى الكلى فكانت نسبة الأصابة ١٣,٧١% بينما بلغت نسبة الأصابة فى عضلات الفك السفلى ٢,٢٩%. وقد وجدت ١,٧١ % من الميتاسركاريا غير متحوصلة وكانت تتحرك بين العضلات وفى التجويف الخيشومى. وتم تصنيف اربعة أنواع من الميتاسركاريا فى أسماك البلطى وهى: كلينوستومم فالاكروكوراسز ،كلينوستومم كومبلاناتم، كلينوستومم تيلابيا، إيوكلينوستومم اردیولا. وكذلك تم الوصف المورفولوجى لكل نوع من أنواع الميتاسركاريا السابقة. كما تم وصف نويع جديد من ميتاسركاريا الكلينوستومم فالاكروكوراسزلأول مرة فى أسماك البلطى فى أسيوط وكذلك التغيرات المورفولوجيةغير الطبيعية التى وجدت فى احدى ميتاسركاريا الأيوكلينوستوم اردیولا.

SUMMARY

A total number of 175 fishes (*Tilapia nilotica)* were investigated for parasitological studies on some *Clinostomum* metacercariae in Assiut city. The results revealed that 42.86% of examined fishes were infected with different species of metacercariae. The gill chamber was the most common habitat of infection where their infection rate was 29.27% followed by kidneys 13.71% then mandible muscles 2.29%. In 1.71% of infected fishes the metcercariae were detected actively motile in both gill

chamber and between muscle bundles. Four species of metacercariae were detected in the present work: *Clinostomum phalacrocoracis, Cl. tilapiae, Cl. complanatum and Euclinstomum ardeola.* The morphological characters of each species of the detected metacercariae were described. More over a new variety of *Cl. phalacrocoracis* was described for the first time in *Tilapia nilotica* in Assiut. Abnormal morphological changes also were described in one metacercaria of *Euclinstomum ardeola.*

Key words: *Clinostomatid, metacercariae, Tilapia nilotica.*

INTRODUCTION

Fishes could be considered as one of the main source of animal protein and in some countries they constitute the main food stuff beside the other nutritional substances.

Parasitic diseases in warm water fishes are considered serious problems are rather than bacterial diseases (Axelord & Snieszko 1980). They may lead to economical losses in body weight as well as a public health significance in certain circumstances (Stoskopf, 1993). The metacercariae of family *Clinostomatidae* are known to encyst in fish and frogs while their adults are parasites of fresh water fish and frog eating reptiles, birds and mammals (Malek, 1980; Beaver *et al.*, 1984) these metacercariae known as the yellow grub and cause considerable damage to the tissues of the fish host (Katantan *et al.*, 1987). *Clinostomum* metacercariae, like *Fasciola* and *Pentastomes* have been known to cause a clinical syndrome called halzoun or marrara in humans as a result of eating raw or insufficient cooked fresh-water fish (Chung *et al.*, 1995). Human laryngitis as a result of infection with metacercariae of *Clinostomum complanatum* was recorded in many countries as Japan ,India and Israel (Witenberg 1944, Cameron 1945, and Isobe *et al.*, 1994).

The aim of the present work was to estimate the prevalence of clinostomatid metacercariae parasitized *Tilapia nilotica* in Assiut Governorate in addition to identify of the detected metacercariae.

MATERIALS and METHODS

One hundred and seventy five fresh water fishes (*Tilapia nilotica*) were collected from fish markets of Assiut city. Fishes were carefully opened and examined immediately (gills, branchial, pharyngeal regions and kidney) by naked eye for the presence of metacrecarial cysts.

- Encysted metacercariae were collected, excysted and compressed between two glass slides and examined under microscope (Eissa & Halla, 1993). They were fixed, stained with acetic alum carmine and mounted in canada balsam (Kurse & Pritchard, 1982).
- Identification was done according to the keys given by Yamaguti (1958) and Ukoli (1966).
- The detected metacercariae were measured and photomicrographed by using dissecting microscope.

RESULTS

Out of 175 *Tilapia nilotica* examined in Assiut city, 75(42.86%) were infected with different species of clinostomatid metacercariae (Table 1). Metacercariae were detected in both gill chamber, kidneys and mandible muscles, the ratio of infection of each one was 29.71%, 13.71% and 2.29 % respectively. Most metacercariae detected in the present work were seen as a small cyst, but in three cases (1.71%) actively motile metacercariae were found actively moving between gill fibers and muscle bundles. Mixed infection by more than one species of metacercariae was detected in 5(2.86%) of examined fishes. Morphologically four species of metacercariae were identified in the present work (Table 2):

-*Clinostomum phalacrocoracis*& new variety *of Cl. phalacrocorasis.*
- *Cl. complanatum.*
- *Cl. tilapiae.*
-*Euclinstomum ardeola* (normal and abnormal).

The morphological characters and measurements of each one are presented at Tables 3-4 and Fig 1-6.

Table 1: Prevalence of different metacercariae were detected in examined fish.

Examined fish	Infected fishes					
	Encysted metacercariae		Free metacercariae		Total	
	No	%	No	%	No	%
175	72	41.14	3	1.71	75	42.86

Table 2: Prevalence of different species of clinostomatid metacercariae were detected in the present work.

species of metacercariae	No.	%
Clinostomum phalacrocorasis & new variety of Cl. Phalacrocorasis	50	28.6
Clinostomum complanatum	4	2.29
Clinostomum tilapiae	2	1.14
Eiclinostomum aordola	24	13.71

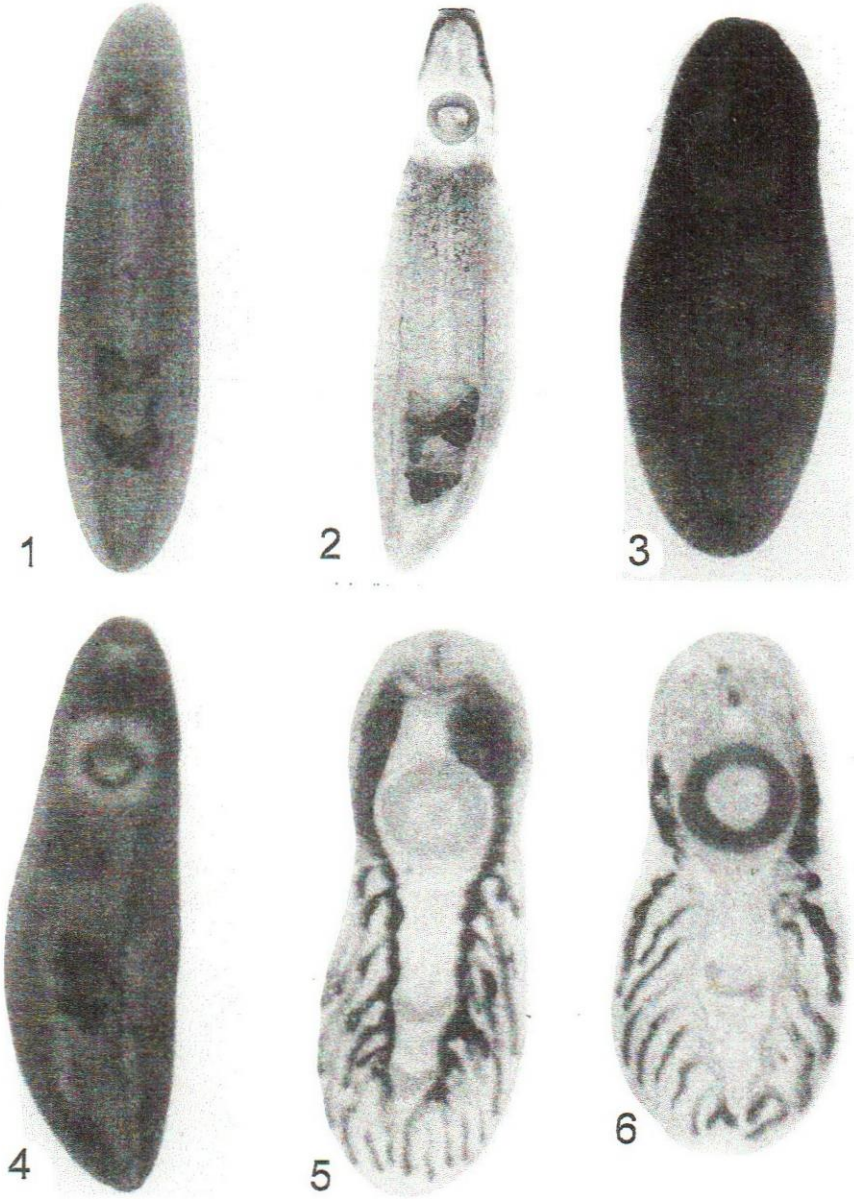
* Mixed infection was detected in five cases.

Table 3: Measurements & characters of different *Clinostomum* metacercariae were detected in the present work. (All measurements are in millimeters)

Characters	Metacercaria of *Cl. pholarcocorasis*	New variety of *Cl. pholarcocorasis*	Metacercaria of *Cl. complanatum*	Metacercaria of *Cl. tilapiae*
Body: shape	Ligniform.	Distinctly divided into two parts.	Tongue-shaped.	Boat shape.
Ends	Rounded ends	Blunt anterior end & pointed postriorlly.	Rounded ends	Rounded anterior end& pointed postriorlly.
L. W.	14.8 -19.6 2.5 - 4.7	16.2 x 4.3	5.1 - 5.9 1.8 - 2.1	4.3–8.1 1.4 – 1.7
Oral sucker : L.×W.	0.6 -0.9 × 0.4 – 0.6	0.6× 0.2	0.2- 0.3 × 0.3 -0.3	0.3 –0.38 × 0.32–0.35
Ventral Sucker: L x W.	1.6 - 1.8 × 1.3 –1.7	1.6 in diameter	0.9- 4.2 × 1.1 – 1.2	0.9–1.2×1.1-1.4
Distance between oral and ventral suckers :	1.52-1.64	2.2	0.64 – 0.74	0.82 – 0.96
Testes:	-Fan shaped. -At the posterior third of the body. -Symmetrical	-Triangular. -At the posterior third of the body. -Asymmetrical	-Triangular. -At the middle third of the body. -Asymmetrical	-Triangular. -At the middle third of the body. -Asymmetrical
Anterior L.× W.	1.58-1.80 ×0.87 -1.44	1.6× 1.2	0.24-0.44×0.55– 0.58	0.32-0.3×0.25- 0.28
Posterior L.× W.	1.5 -2.1×1.2 -1.8	1.8× 1.0	0.3 -0.2 × 0.8-0.9	0.5-0.6× 0.3–0.35
Inter testicular space	1.3 -1.95	1.7	0.3 – 0.4	0.15 - 0.2
Distance between ventral sucker and anterior testes:	5.4 -6.0	6.6	0.8 -1.2	1.7 – 0.9
Ovary :	At the right side between the cirrus pouch and posterior testis.	At the left side near the posterior margin of the anterior testis.	At the right side of inter-testicular space.	At the right side beside the posterior end of the cirrus pouch.
L. x W.	0.5 – 0.4 x 0.15 – 0.3	0.2 x 0.4	0.22- 0.26 × 0.11-0.2	0.2- 0.14 × 0.12-0.16
Cirrus pouch :	At the right side behind the posterior margin of anterior testis	At the left side of Inter-testicular space in-contact with the left caecum.	Near the right margin of the anterior testis.	In the inter-testicular space in-contact with the right caecum.
L.× W.	0.8 -1.1 x 0.5 - 0.7	0.8 x 0.45	0.2 - 0.3 × 0.1– 0.2	0.3 – 0.3 × 0.2 – 0.2
Uterine Sac	5.1 -5.7	5.5		0.7 – 0.8

Table 4: Measurements & characters of *Euclinostomum ardeola* metacercariae (normal & abnormal) detected in the present work. (All measurements are in millimeters)

Characters	Normal metacercaria	Abnormal metacercaria
Body:	Elongated pear shaped.	Pear shaped
L.	8.4-10.5	7.4
W.	3.05- 2.9	3.45
Oral sucker: L. x W.	0.35-0.37 x 0.45-0.62	0.25 x 0.40
Ventral sucker: L x W.	0.87-1.87 x1.5-2.00	1.52 x 1.62
Distance between oral and ventral suckers	1.37-1.75	2.12
Testes: Anterior	Horse shoe shape.	Abnormal shape.
L. x W	0.17-0.35 x 0.68-0.85	0.115 x 0.850
Posterior	detected	
L. x W	0.50-0.62 x 0.55-0.67	Not seen
Inter- testicular space	0.725-0.850	-
Ovary: L.xW.	0.75-0.11 x 0.15-0.22	0.25 x 0.12
Uterine sac:	1.4-1.8	1.00
Intestinal deverticule		
Right	9 –11	9
Left	9 –11	4



1- *Clinostomum phalacrocoracis* ×3.3.
2- A new variety of *Clinostomum phalacrocoracis* ×3.6.
3- *Cl. complanatum* ×8.2.
4- *Cl. tilapiae* ×8.2.
5- *Euclinstomum ardeola* (Normal shape) × 4.4.
6- *Euclinstomum ardeola* (Abnormal shape)× 4.4.

DISCUSSION

This study confirmed that different species of clinostomatid metacercariae have been indigenously distributed in *Tilapia nilotica* in Egypt. Among one hundred and seventy five fish examined in the present work (42.86%) were infected with different species of metacercariae .This result is considered lower than that recorded by: Awad (1992) from River Nile (68.65%) and Khattab (1992) in Giza (87.06%) but is higher than that recorded by Shaheen (1998) in the same locality (29.2%).

Such difference might be attributed to the food supply, the water resources (Nile or canals), and availability of snail intermediate host which play the main role for complete the life cycle of these parasites. Regarding the habitat of metacercariae in examined fishes, it the first time to detect of *Clinostomum* metacercariae in muscle bundles of mandible, but the gill chamber is the most common habitat for them (29.71%). This result might be attributed to that the gill chamber is considered a highly bloody region in the fish body. The same result was recorded by Eissa *et al.* (1996) and Shaheen (1998). Concerning the actively motile metacercariae which were detected in (1.71%) of examined fishes this result may be due to exposure of fishes during marketing to high temperature specially in summer season. Asanji &Williams (1975) mentioned that the optimum temperature of excyctement of *Clinostomum* metacercariae is 37- 42°C.

Morphologically, metacercariae of the present work were differentiated into two genera: *Clinostomum* and *Euclinostomum.* According to key provided by Ukoli (1966) *Clinostomum* metacercaria of the present work were identified as three species: *Clinostomum phalacrocoracis, Cl. complanatum* and *Cl. tilapiae. Cl. phalacrocoracis* metacercaria showed the highest infection rate (28.6%) followed by *Cl. complanatum* (2.29%) and *Cl. tilapiae* (1.14%). This result agree with that recorded by Eissa *et al.* (1996) and lower than that mentioned by Eissa and Hala (1993)

A close study of the morphological characters and measurements of *Cl. phalacrocoracis* metacercariae revealed that one specimen has several morphological variations as the following:

- Their body distinctly divided into two parts where their fore-body is conical shape while the hind-body is elongate lanceolated shape.
- The anterior end is blunt while the posterior end slightly pointed.

- Both testes are triangular (not fan shape) and asymmetrical.
- The ventral sucker is rounded in outline.
- The distance between anterior and ventral sucker is greater.
- Both ovary and cirrus pouch are located at the left side.
- Longer distance between the ventral sucker and anterior testis.

As shown above, the present specimen definitely differentiated from *Cl. Phalacrocoracis* metacercariae for which we considered it as a new variety of *Cl. Phalacrocoracis* waiting for further investigation to confirm the identification and to elucidate their life cycle.

Concerning to the metacercariae of *Euclinostomum* sp. in the present work it was identified as *Euclinostomum ardeola* according to the description of Donges (1974), El-Naffar & Khalifa (1981) and Zedan (1983).

Among *Euclinostomum* metacercariae which were examined in the present work abnormal morphological characters were detected in one specimen as the following:

1- Absence of posterior testis.
2- Abnormal shape of anterior testis.
3-Unequal numbers of intestinal diverticula.
4- The uterine sac is shorter.

These abnormalities may be related to several factors ether internal (way of insemination & senility) or external (environmental factors). Nollen (1988) mentioned that some species of platyhelminths undergo abnormal growth specially their sexual development in case of self- insemination. Thorogood (1997) mentioned that the actual incidence of trematod anomalies may vary according to: host, environmental/teratogenic factors including drugs, polygenic disorders, single gene mutations and others.

CONCLUSION

The infection of *Tilapia nilotica* with *Clinostomum* metacercariae should be seriously considered because it can cause an economic loss and a public health problem. Among these species *Cl. complanatum* metacercariae is necessary to pay more attention, where it causes severe damage of infected fish in addition to human laryngo-pharyngitis as a result of eating of fresh water fish or incomplete cooked fish (Williams and Jones 1976). Previous authors detected few enzootic foci of *Cl. complanatum* in the world. This study is confirm the presence of *Cl. complanatum* in Assiut Governorate, where it was detected

previously by Shaheen (1998). Further studies should be done on other species of fishes and wild birds suspected as natural final host of most *Clinostomum* metacercariae for identification of their life cycle.

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