Studies on some factors affecting metacercarial infections in African sharp-tooth catfish (*Claris gariepinus*) in Assiut Governorate

Elkamel A. A., Sayed, G. M.*, Ahmed, S. M.,

Arafa, M. I.* , Abd El-Lateif, R. S. A***

Dept. of Parasitology, Animal Health Research Institute, Assiut Lab. *

Dept. of Fish diseases, Animal Health Research Institute, Assiut Lab. ***

Abstract

This work aims to study the effect of some environmental and biological factors on spread encysted metacorcarae (EMC) in catfish (*Claris gariepinus*) at Assiut, Egypt. Out of 240 African catfish were examined, the total prevalence of encysted metacercariae was 56.3%. The high prevalence was observed in young fish (<300 gm) with prevalence 59.2%, while the highest infection rate was recorded in lengths (25-35 cm.) at a rate of 62.3%. The maximum infestation rate was appeared in summer (78.3%), while the lowest one was detected in spring (36.7%). Males had slightly higher prevalence rate (57.5%) than females (54.9%). Two types of E.M.C. were detected in the present work:

Cynodiplostomum E.M.C. (macroscopic cyst) - Prohemistomum E.M.C. (microscopic cyst). Cynodiplostomum E.M.C. was detected in muscular tissue only and their prevalence was 2.5%. While Prohemistomum E.M.C. was detected in muscular tissue, liver, kidney and gills of examined fish and their prevalence was 53.8%, 26.2%, 9.2% and 7.5%, respectively.

Present study reflects the sensitivity of different metacercariae infection in African catfishes the factors tested.

Keywords: *Claris gariepinus*, encysted metacorcarae, Cynodiplostomum, Prohemistomum E.M.C.

Introduction

African catfish (*Claris gariepinus*) is a large group of primarily freshwater fishes which have been widely introduced all over the world (Teugels 1986). African catfishes are widely distributed throughout Africa, inhabiting calm water from lakes, streams, rivers, swamps to floodplains. Some of catfish can survive during the dry seasons as a result of the presence of their accessory air breathing organs (Olufemi et al., 1991).They have a high growth rate and resistant to handling stress (Akinsanya and Otubanjo, 2006).

Various parasites are associated with *C. gariepinus* in the wild and cultured where they cause morbidity, mortality and economic losses in aquaculture practice allover the world (Subashinghe, 1995). These parasites mainly affect the productivity of the fish, growth rate and

reduce the quality of the meat. These factors will result in loss of economic returns and loss of good protein sources (Anderson, 1979). Encysted metacercariae appeared grossly as small rounded white nodules (macroscopic type), were found between muscle fibers, subcutaneous musculature and eyeballs of *Clarias gariepinus* while the other type was detected only by microscopic examination (microscopic type) (El-Mossalami and Sherif, 1994 and Youssef et al., 1993).

Human cases of parasitism transmitted by fish consumption have been reported in several countries especially in those where raw fish dishes are part of their staple diet (Umegai et al., 1990; Barros et al., 2006). Human beings are also liable to get intoxicated through ingestion of dead Anisakids larvae (Nematoda) (Audicana et al., 2002), as well as Clinostomidae digenetic trematode metacercariae (Kifume et al., 2000), lying within the fish's musculature.

Materials and Methods

1-Samples:

Two hundred and forty catfish (*Clarias gariepinus*) of different sexes (113 female & 127 male), were randomly collected from the River Nile in Assiut Governorate. The fishes 2- were collected for parasitological examination during different seasons (20 fish /month over one year). Examined fishes were divided into three classes according to the weight (<300 g, 300-400 g and >400g), also were divided into three classes according to the length; (<25 cm, 25-35 cm. and > 35 cm.)

Parasitological examination:

- The abdominal cavity was opened and the internal viscera: liver, kidney and gills in additionto the muscles were examined for the possible presence of encysted metacercariae (EMC).

(A) Macroscopic examination:

Macroscopic examination was carried out either by naked eye or by the magnifying hand lens (2 X and 4X) for detection of EMC. in musculature and internal organs according to Mahdy et al., (1995).

- The large band muscles of fish body which extended from the anterior part of the fish to the posterior part were divided into 3 equal parts: anterior, middle and posterior, each part subdivided into small pieces then compressed between two slides and examined microscopically (Schaperclaus, 1992).

(B) Microscopic examination:

Tissues of internal organs were screened for the presence of microscopic EMC.

by compression method in which snips were taken from visceral organs (liver, kidney and gills) , compressed between two microscopic glass slides and examined microscopically (Sayasone et al., 2007).

Metacercariae were identified to genus level based on the morphological details, their dimensions, shape of cysts, site of infection and shape and contents of excretory bladder under a light microscope (Elsheikha and Elshazly, 2008; and Sohn et al., 2009).

Results and discussion

Parasitic diseases are considered one of the most important causes of physiological and metabolic changes in fish (Anderson, 1979). So the study of parasitic infection in different fish is considered as an important step in controlling the spreading of parasites as well as maintained the production of fish (Lim, 2003). Encysted metacercariae may affect growth and survival or disfigure fish so that they lose their market value as a food. Some metacercariae are sources of infections for human and domestic animals (Rim et al., 2008).

The obtained results of clinical signs for examined catfish infested with encysted metacercariae were characterized by dark coloration of skin and ulceration in different parts of the body surface. The infested organ had macroscopic large white nodule or small white one as sand paper appearance. The obtained data were nearly in agreement with that recorded by El- Mossalami and Sherif, (1994) also El-Gohary and Samaha (1997) who showed fine streaks of black coloration as a result of melanin pigment in the majority of infested muscles.

It is noteworthy to mention that these signs may be attributed to stress factors on fish caused by metacercarial infestation which leads to lowering the fish resistance and rendering the fish more susceptible to other diseases (Skinner 1982).

Morphological studies:

The identified metacercarae recovered in examined cat fish were belonging to families, Prohemistomatide and Cynodiplostomatide

a- Cynodiplostomum metacercariae:

It is a macroscopic cyst easily seen by naked eye, it appeared grossly as small white nodules scattered in few numbers allover the body. The metacercaria is completely surrounded with thick layer of adipose tissue about 450- 550 µm in thickness takes dark coloration. The metacercaria is oval in shape measured 670 – 830 µm (700 µm) long by 420- 530 µm (480 µm) wide. It is surrounded by two transparent cyst wall measured 12 µm, the outer one is somewhat thicker than the inner cyst wall (Plate 1 Fig 1-3). Metacercariae are immature in the cyst and their shape changes due to body movement.

B

b- Prohemistomum metacercarae :

It is a microscopic cyst detected in muscular tissue, liver, kidney and gills of examined fish ((Plate 1 Fig 4). The encysted metacercarae were of medium sized and of variable colour varied from grayish white to yellowish brown. It was spherical in shape provided with double layered transparent cyst wall, the outer was fragile and easily ruptures and the inner was difficult to be removed. In some encysted metacercariae the parasite was surrounded with a membranous wall containing fluid with dark granules inside. The metacercaria was usually folded inside its cyst wall and it is surrounded at each pole with adipose tissue either completely or partially. The cyst measured 360- 500 µm (420 µm) in diameter.

The morphological features of EMC recovered in the present work were coincided with that previously reported by El- Shahawi (1983), El-Naffar, and El-Shahawi (1986), Hussein (2007). Concerning to the medical importance of the detected metacercarae, Prohemistomum metacercarae well known as parasites of fish

eating mammals, and it was reported by Naser (1941) as a cause of death in human being. Chandler and Read (1961) reported the first case of human infection with Prohemistomum warm in Egypt.

Epidemiological studies:

Table (1) summarizes the over all infection rate of encysted metacercariae in examined fish where it was 56.3 % (135 out of 240). High infection rate with EMC. is usually related to the high prevalence of the intermediate host (snails) of trematodes and the failure of their accurate control. In addition to the contamination of fish collection site with domestic sewage, this might be the source of trematode eggs. In the same trend the suitable water temperature which constitute the main survival factors for these intermediate hosts (Onwuliri and Mgbemena 1989).

Higher metacercarial infection rates were previously mentioned by Olfat (1991), Jehan (1993), Khalifa (1993), Abd El- Hafez (1996), Amany (1997) and Shaban (1997) in *Clarias gariepinus* as 73.33%, 66%, 66%, 76.67%, 85% and 77.37%, respectively. Such variation in prevalence may be related to the difference in the habitat, food supply, abundance of both aquatic snails (the intermediate host) and the aquatic piscivorous birds which play the main role to complete the life cycle of some digenetic trematodes (Taher, 2009).

The relationship between body length and infestation rate with encysted metacercariae in examined catfish was summarized in table (2). The highest prevalence of infestation was detected in the group of 25 cm to 35 cm where their infection rate was 62.34%. While the smallest fish (less than 25 cm) were relatively less infected than the other groups.

Table (1): Prevalence of EMC in relation to weight of examined *C. gariepinus*.

Weight	Microscopic EMC		Macroscopic EMC		Total	
	No.	%	No.	%	No.	%
<300 g (n=120)	68	56.7	3	2.5	71	59.2
300-400 g (n=51)	26	51.1	3	5.9	29	56.9
>400 g (n=69)	35	50.7	-	-	35	50.7

Total (n=240)	129	53.8	6	2.5	135	56.3

The present results indicated that the incidence of metacercaral infection in examined fish increased with the increase of body length, this may be referred to that the heavily infestation with encysted metacercarae result in emaciation associated with low drop in body weight without any changes in their length (Paperna, 1980).

Table (2): Prevalence of EMC in relation to length of examined *C. gariepinus*.

length cm.	Microscopic EMC		Macroscopic EMC		Total	
	No.	%	No.	%	No.	%
< 25 (n=31)	11	35.5	-	-	11	35.5
25-35 (n=162)	95	58.64	6	3.7	101	62.34
> 35 (n=47)	23	48.94	-	-	23	48.94
Total(n=240)	129	53.8	6	2.5	135	56.3

Otherwise, the effect of seasonal variations on the prevalence of metcercariae in examined fish were reported in table (3). The highest infestation rate of microscopic EMC was detected in summer (78.3%) followed by autumn (51.7%), winter (50%) and the lowest one was detected in spring (35%).

It could be explained these results with the finding of El-Naffar and El-Shahawy (1986), Jehan (1993) and Amany (1997) who recorded that the high incidence of encysted metacercaria in fish was detected in summer season. While

macroscopical EMC. was detected only in winter and spring with prevalence rate 8.3% and 1.7% respectively (Table 3).

The highest infestation of EMC. in summer may be attributed to the breeding seasons of the snail (first intermediate host of trematode metacercariae) occured during spring and autumn, which leads to rapid increase of snail populations in summer (EI-Aroussl, 1984).

Moreover Bello et al. (2000) and Simkova et al. (2005) mentioned that the increase in the prevalence of parasitic infections is attributed to the stress of reproductive processes of summer breeding season of cat fish. In addition to difference between the seasons depending on activity of snails where the rise in temperature accelerate the development process of trematodes while a drop in temperature retarded it.

Table (3): Prevalence of EMC in relation to seasonal variations of examined fish.

Season	Microscopic E. M. C		Macroscopic E.M.C		Total	
	No.	%	No.	%	No.	%
Spring (n=60)	21	35	1	1.7	22	36.7
Summer (n=60)	47	78.3	-	-	47	78.3
Autumn (n=60)	31	51.7	-	-	31	51.7
Winter (n=60)	30	50	5	8.3	35	58.3
Total (n=240)	129	53.8	6	2.5	135	56.3

In the current study it was found that the infection rate with encysted metacercariae was relatively similar for both microscopic and macroscopic in males and females fish, in which the prevalence of infection were 55.1% and 2.4% in males and 52.2 % and 2.7% in females respectively (Table 4). This result is supported by El-Naffar and Khalifa (1981) who mentioned the same result in *Tilapia nilotica*. Moreover Thompson and Kavaliers (1994) recoded that there are intrinsic biological differences between host sexes that could lead one sex was more prone to parasitic infections than the other. While, Poulin (1996) concluded that the sex difference is irrelevant and no significant differences between the prevalence and intensity of infection in female and male hosts.

Table (4): Prevalence of EMC in relation to sex of examined fish.

	Microscopic E. M. C	Macroscopic E.M.C	Total

Sex	No.	%	No.	%	No.	%
Female (n=113)	59	52.2	3	2.7	62	54.9
Male (n=127)	70	55.1	3	2.4	73	57.5
Total (n=240)	129	53.8	6	2.5	135	56.3

Various parts of the fish can serve as location (microhabitat) for the metacercariae. The herein results showed that the highest prevalence of microscopic metacercariae was detected in the muscle then liver followed by kidney and gill with the prevalence 53.8, 26.2, 9.2 and 7.5%, respectively. While macroscopic one was detected in muscular tissues only and their prevalence rate was 2.5% (Table 5). Jehan (1993) recorded the infestation rate in *Clarias gariepinus* as 38.48, 71.21, 10.61 and 39.99% in gills, liver, spleen, kidneys, respectively. This difference in percentage of infestation may be due to different localities, from which the fish were collected and water pollution.

Table (5): Distribution of EMC in different organ in examined *C. gariepinus*.

Organ	Microscopic EMC.		Macroscopic EMC.	
	No.	%	No.	%
Musculature (n=240)	129	53.8	6	2.5
Liver (n=240)	63	26.2	-	-
Kidney (n=240)	22	9.2	-	-
Gills (n=240)	18	7.5	-	-

N.B: Mixed infection in more than one organ was detected in 64 fish.

The distribution of microscopic metacercariae in mussels of examined fish was higher in posterior third (tail region) [6- 289 (105)/ g.] than the middle and anterior thirds [7- 90 (37)/ g. and 3- 72 (27)/ g.] respectively (Table 6), these results are coincided with El- Dally (1988) and El-Gohary and Samaha (1997). Macroscopic metacercariae were scattered in few numbers allover the body.

Table (6): Density of microscopic EMC/ g. of muscular tissue in examined *C. garbense*.

Total	Total		Average number of microscopic EMC. (per gram tissue)

examined fish	Infested fish	%	Head region	Trunk region	Tail region
240	129	53.8	3 - 72 (27)	7- 90 (37)	6 - 289 (105)

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الملخص العربي

دراسات على بعض العوامل المؤثرة على الإصابة بالميتاسركاريا فى أسماك القراميط الأفريقية فى محافظة اسيوط

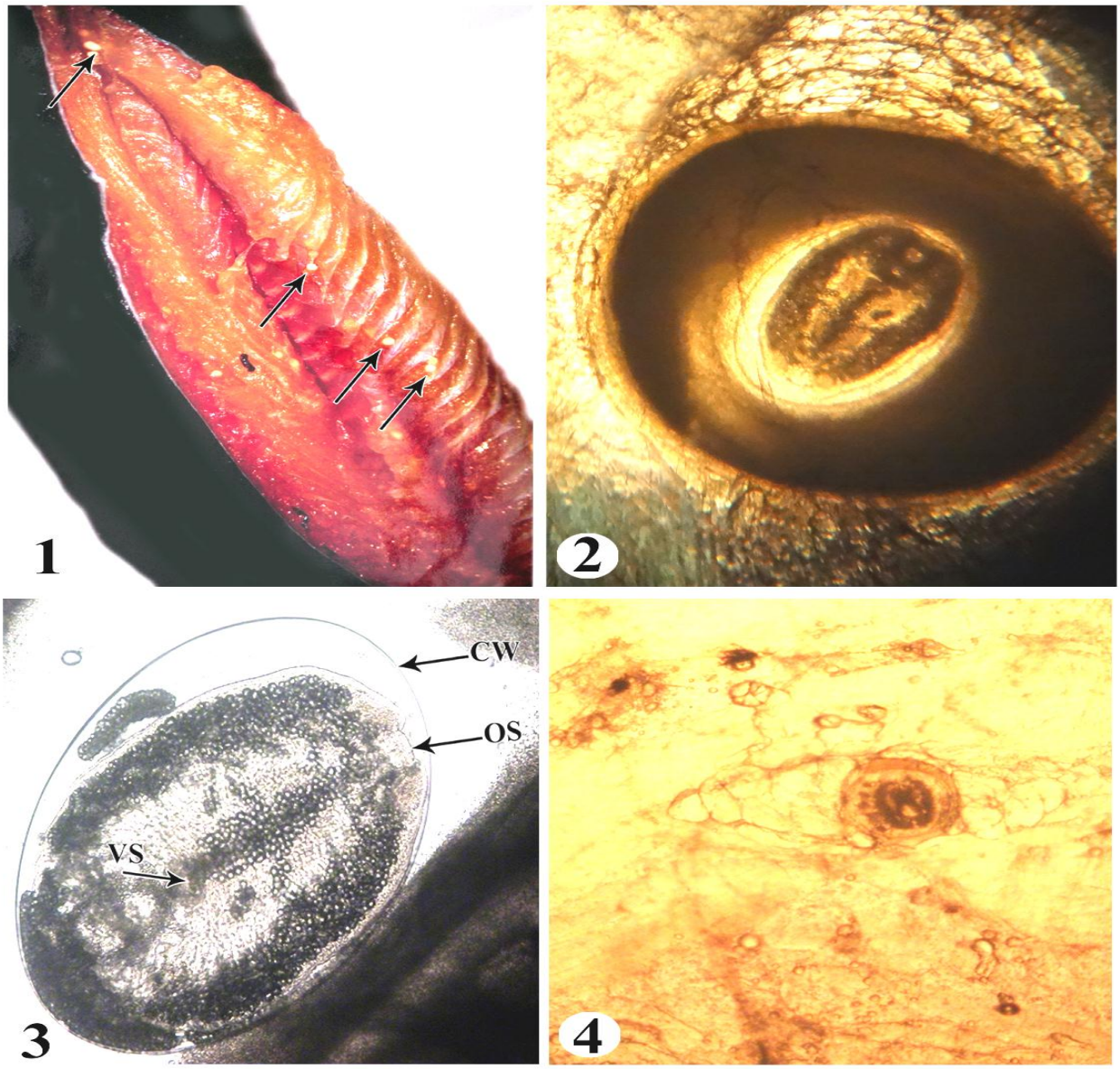
أحمد عبد الهادي الكامل *، جيهان محمد سيد، شعبان محمد احمد***

محسن ابراهيم عرفه ، رشا صالح عبد الفتاح عبد اللطيف****

قسم طب الحيوان (أمراض الأسماك) – كلية الطب البيطري بجامعة أسيوط ، ** معهد بحوث صحة الحيوان – فرع أسيوط

يهدف هذا البحث إلى دراسة تأثير بعض العوامل البيئية والبيولوجية على انتشار الميتاسركاريا فى أسماك القراميط فى محافظة اسيوط. حيث تم تجميع عدد ٢٤٠ سمكة (٢٠ سمكة / شهر) من أسماك القراميط الأفريقية (الكلاريس جاربينيس) مختلفة الجنس والوزن من روافد نهر النيل المختلفة فى محافظة أسيوط خلال عام واحد. وقد خضعت تلك الأسماك للفحص الظاهرى والطفيلى. أسفر الفحص الاكلينيكي للأسماك المصابة عن عدم وجود علامة مرضيه مميزة ما عدا بعض الأسماك المصابة أظهرت إنتفاخ بالبطن و شحوب الأعضاء الداخلية وإحتقان فى الأمعاء. وقد بلغت نسبة الأصابة الكلية بالميتاسركاريا فى اسماك القراميط ٣, ٥٦%. وقد سجلت أعلى نسبة اصابة فى فصل الصيف حيث كانت بمعدل ٧٨,٣% بينما الحد الأدنى فى فصل الربيع ٣٦,٧ %. وقد تم دراسة تأثير اوزان واطوال الأسماك علي نسبة انتشار الميتاسركاريا حيث أظهرت النتائج أن أعلى نسبة أصابة كانت فى الأوزان الصغيرة (أقل من ٣٠٠ جرام) حيث كانت بمعدل ٥٩,٢% بينما كانت أعلى نسبة اصابة فى الأسماك ذات الاطوال من ٢٥– ٣٥ سم بمعدل٦٢,٣%. وتم أيضا دراسة تأثيرالجنس علي وجود الميتاسركاريا حيث أوضحت الدراسة أن ذكر اسماك القراميط يعتبر أعلى نسبيا للأصابة حيث كانت بمعدل ٥٧,٥% مقارنة بالإناث ٥٤,٩ %. بالاضافة الى انه تم التعرف على نوعين من الميتاسركاريا فى أسماك القراميط هما : سينوديبلوستومم (الميتاسركاريا العينية)، بروهيمستومم فيفاكس (الميتاسركاريا المجهرية). وقد وجدت

سينوديبلوستومم ميتاسركاريا فى عضلات الأسماك المصابة فقط بمعدل ٢,٥%. .بينما وجدت بروهيمستومم ميتاسركاريا فى كل من العضلات ، الكبد ، الكلية والخياشيم بمعدل ٥٣, ٨% ، ٢٦,٢% ،٩,٢% ، ٧,٥ % على التوالى. وتعكس هذه الدراسة مدى تأثر العدوى بالميتاسركاريا المختلفة فى أسماك القراميط الأفريقية للعوامل المختبرة.



(Fig 1) - The gross appearance *of Cynodiplostomum metacercariae* as small white nodules (arrow).

(Fig 2) - The *Cynodiplostomum metacercaria* surrounded completely with thick layer of adipose tissue (x 40).

(Fig 3) - Higher magnification of *Cynodiplostomum metacercaria* (CW= cyst wall, OS =oral sucker, VS =ventral sucker) (x100).

(Fig 4) - The characteristic thick double wall *of Prohemistomum metacercaria* which surrounded at each pole with adipose tissue (x 100).