

FIELD STUDIES ON PARASITIC DISEASES CAUSED BY ENCYSTED METACERCARIAE AMONG SOME WILD AND CULTURED FRESHWATER FISHES

Eissa, I. A. M.; Badran, A. F.; Mona Abd El-Wahab* and Walaa El-Shaer*

Dept. of Fish Diseases and Management, Fac. of Vet. Med. Suez Canal Univ.

*Animal Health Research Institute, Ismailia branch.

ABSTRACT

A total number of 275 freshwater fish (100 of *Clarias gariepinus*, 100 of wild *Oreochromis niloticus* and 75 of cultured *O. niloticus*) were randomly collected alive from Nile branches and a private fish farm in Ismailia governorate. The fishes were investigated for encysted metacercariae. There were no pathognomonic clinical signs or abnormalities on the external body surface. The isolated encysted metacercariae are identified as *Cynodiplostomatid*, *Heterophid*, *Prohemistomatid*, *Euclinostomum* and *Clinostomum metacercariae*. The results revealed total prevalence of 50, 47 and 13.3% in *C. gariepinus*, wild *O. niloticus* and cultured *O. niloticus* respectively. Such metacercariae prevailed in spring in wild *O. niloticus* and *C. gariepinus* while cultured *O. niloticus* prevailed in summer. The site specificity showed high prevalence in musculature of *C. gariepinus*, in kidney of wild *O. niloticus* and in spleen of cultured *O. niloticus*. In addition, it is revealed that the prevalence in both males and females are nearly equal in *C. gariepinus* and cultured *O. niloticus* while females of wild *O. niloticus* are highly prevailed than males.

الملخص العربي

دراسات حقلية عن الأمراض الطفيلية الناتجة عن الإصابة باليرقات المتحوصلة في بعض
أسماك المياه العذبة الحرة والمستزرعة

إسماعيل عبدالمنعم عيسى أحمد فكري حسين بدران
منى عبدالوهاب شريف ولاء الشاعر

قسم أمراض الأسماك ورعايتها، كلية الطب البيطري، جامعة قناة السويس
معمل بحوث صحة الحيوان بالإسماعيلية*

شملت الدراسة ١٠٠ من الأسماك القطبية و ١٠٠ من البلطي النيلي الحر التي تم جمعها من نهر النيل بالإضافة إلى ٧٥ من البلطي النيلي المستزرع من مزرعة صمكية خاصة بمحافظة الإسماعيلية، وفحصها لم تلاحظ أي من العلامات المرضية المميزة في الأسماك المصابة، وقد تبين من الصفة التشريحية وجود يرقات متحوصلة في الأعضاء الداخلية وتم التعرف عليها بوهيموستوماتيد، هنجورفيد، سينوديلوستوماتيد، أبوكليينوستوماتيد و كليينوستوماتيد. وقد تم تسجيل نسب الإصابة باليرقات المتحوصلة على النحو التالي ٥٠، ٤٧، ٣، ١٣٪ في القرموط الإفريقي والبلطي النيلي الحر والمستزرع على التوالي، وقد تبين أن نسب الإصابة تزيد في القرموط الإفريقي والبلطي النيلي الحر في فصل الربيع بينما تسود في البلطي النيلي المستزرع في فصل الصيف. أما أماكن التواجد فكانت المصلات في القرموط الإفريقي والكلبي في البلطي النيلي الحر والطحال في البلطي النيلي المستزرع كما سجلت إناث أسماك البلطي النيلي الحر النسبة الأعلى في الإصابة بينما تساوت نسب الإصابة في ذكور وإناث القرموط الإفريقي والبلطي النيلي المستزرع.

INTRODUCTION

The presence of encysted or excysted metacercariae are very common especially in freshwater fishes as that may cause public health importance when infested fish eaten with insufficient cooking or light salting (Elssa, 2002).

Also, it leads to great economic losses via decreasing body gain as well as they lead to fish unmarketable for human consumption (Elssa, 2002, Floyd, 2003; and Samaha, 2004).

Different data dealing with such infections in various areas and fishes were recorded (Shaapan 1997, Maather El-Aml, 2001, Salah et al., 2005 and Sawan Elsheshnagui et al., 2006).

The present study was planned to investigate the problems in some cultured and wild freshwater fishes (*Clarias gariepinus* and *Oreochromis niloticus*) associated with the most prevailing encysted metacercariae regarding season, sex and site specificity in such fishes.

MATERIAL AND METHODS

Fish:

A total number of 275 of freshwater fishes of different body weights were represented as 100 *Clarias gariepinus*, 100 wild *Oreochromis niloticus* and 75 cultured *O. niloticus* were collected randomly in different seasons. The collected fishes were transported alive to the Fish Diseases Laboratory, Faculty of Veterinary Medicine, Suez Canal University.

Clinical picture and gross examination

The clinical examination of examined fishes was performed according to the methods adopted by Conroy and Hermann (1981) for detecting abnormalities or presence of any cysts externally. The abdominal wall of examined fish was removed; the internal organs and musculature were exposed and examined macroscopically for any gross abnormalities.

Parasitological examination

A - Microscopical examination:

The microscopical examination was carried out using the compression technique according to Morishita et al. (1965) for the detec-

tion of encysted metacercariae (EMC) which may lodged in/or attached on different organs and tissues of the examined fishes.

B- Fixation, staining and mounting of the EMC:

The procedures were done according to the techniques described by **Kruse and Pitchard, (1982)**. It was carried out by keeping the EMC between two slides. By gentle pressure, they were fixed in small vials containing (10%) formalin or AFA (alcohol formaline acetic acid), left overnight then washed with tap water to get rid traces of formalin solution and stained with alum carmine stain (**Lucky, 1977**) and Semichon's acetocarmine stain (**Kruse and Pitchard, 1982**). They were dehydrated using ascending grades of ethyl alcohol which were changed several times to insure dehydration and cleared in clove oil. The specimens were mounted in Canada balsam and left to dry in a hot air oven at suitable temperature (40°C) and covered with a cover slide. Identification of the revealed EMC is carried out according to **Yamaguti (1958)**.

RESULTS

Clinical Examination:

There were no pathognomonic clinical signs or abnormalities on the external body surface of the examined fishes except slight curvature anomaly and abdominal bulging in wild *O. niloticus*.

Postmortem examination:

In some cases of *O. niloticus* and most cases of *C. gariepinus*, there were white or whitish cysts like nodules varying in size from pin head to pea size were found on gills, heart,

liver, spleen, kidneys, intestinal wall, ovary, testis, brain and musculature (Fig. 1,2).

Parasitological Examination:

The isolated EMC were differentiated into spherical or subspherical in shape, double walled cyst. The outer wall was fragile and easily ruptured but the inner one was difficult to remove without injuring the EMC. The whole cyst measured 0.24-0.29 (mean 0.26 mm) length, 0.17-0.23 (mean 0.20 mm) width. They had well developed suckers. The oral sucker was subterminal. It was identified as *Prohemistomatid* (plate 1. A). Another EMC was spherical encircled by two thin layers. The ventral sucker was larger than oral one. The characteristic features were fine streaks of black coloration (melanin pigment) in the infested musculature. It was identified as *Heterophid* (plate 1. B). Also, EMC revealed cyst wall has two layers where the outer one was thick and the inner was thin and hyaline. Oral and ventral suckers were well developed and nearly equal in size. Intestinal caeca extended nearly to posterior extremity, gonads are easily seen in posterior fourth of the metacercarial body together with the excretory vesicle and excretory pore. It was identified as *Cyanodiplostomatid* (plate 1.C). Large metacercariae were grayish white peas in the tissue of the kidneys. It was leaf shaped and fairly large. It measured 5 to 16 mm in length and 1.5 to 5 mm in width. The ventral sucker was very large (about 5 times of the oral sucker) with a short esophagus has thick muscular bulb like. The intestinal caeca were largely swollen in their anterior parts. In further course they were narrow and very irregular in outline (12 and 15 long branches) with dark pigmentation. The two testes were situated in

the posterior third of the body and the ovary was round, small and located between the testes. It was identified as Euclinostomatid (plate I.D). Another EMC characterized by yellow to orange color, spherical in shape with the cyst wall very thick. It was elongated and large measuring 02.0-02.20 (mean 02.10 mm) in length and 0.32-0.52 (mean 0.42 mm) in width. The suckers were well developed. The oral sucker present subterminally and the ventral sucker was large, spherical in shape. Caeae were very long and extended to the posterior end. The genitalia were ill developed. It was identified as Clinostomatid (plate I.E).

Total and seasonal prevalence: The highest prevalence was found in *C. gariepinus* (50%), followed by wild *Oreochromis niloticus* (47%), then cultured *O.niloticus* (13.30%). Also, the results revealed that EMC prevailed in spring in wild *O.niloticus* and *C.gariepinus* while in cultured *O.niloticus* prevailed in summer, Table, 1. The site specificity showed high prevalence in musculature (*C. gariepinus*) in kidneys (wild *o.niloticus*) and in spleen (cultured *O.niloticus*), Table, 2. In addition, it is revealed that the prevalence in both males and females are nearly equal in *C. gariepinus* and cultured *o.niloticus* while females of wild *o.niloticus* were highly prevailed than males, Table, 3.

DISCUSSION

The current study revealed that in fish metacercariosis, there are no characteristic clinical signs or abnormalities on the external body surface. These results support the findings of Eissa (2002) who recorded that there

no pathognomonic signs in the internal parasitic diseases. The postmortem findings were manifested as emaciation, paleness or hemorrhagic. In some cases, there were enlargement of liver, gall bladder, spleen, and kidneys. In some cases of *O.niloticus* and most cases of *C.gariepinus* there were white or whitish cysts like nodules varying in size from pin head to pea size varying in locations, on most of the internal organs and musculature. These findings are nearly similar to the results given by Woo (1995), Rawla Adway (2000), Eissa et al. (2001), Bassiony (2002) and Osman (2001).

Based on the morphological and parasitological examinations, the isolated encysted metacercariae from the examined fishes are identified as *Cynodiplostomatid*, *Heterophid*, *Prohemistomatid*, *Euclinostomum*, and *Clinostomum*. These results agreed with the descriptions given by Yamagute (1958), Eissa et al. (1996), Shappan (1997), Maather El Lamie (2001) and Noor El-Deen (2008).

In the present investigation, the highest total prevalence was found in *C. gariepinus* followed by wild and cultured *O. niloticus* as 50, 47 and 13.3% respectively. These nearly agreed with Amany Abbass (1997) and Maather El Lamie (2001). These findings may be attributed to that both *C. gariepinus* and wild *O. niloticus* live in a media rich in aquatic snails in nature where such snails nearly rare in *O. niloticus* cultured in fish farms.

Regarding the seasonal variation, it is revealed that EMC prevailed in spring in wild

O. niloticus and *C. gariepinus* while cultured *O. niloticus* prevailed in summer and the lowest in winter. These results nearly agreed with **Shaapan (1997)** and **Tagreed Ibrahim (2000)** who found that the highest prevalence was in summer and the lowest in winter. These findings may be attributed to the water temperature which plays an important role in prevalence. The site specificity showed high prevalence in musculature (*C. gariepinus*), in kidney (wild *O. niloticus*) and in spleen (cultured *O. niloticus*). These results among *C. gariepinus* agreed with **Olfat Mahdy (1991)**; **Tagreed Ibrahim (2000)** and **Maather El Lamie (2001)** who found that the highest prevalence in *C. gariepinus* was in musculature. However, our results disagreed with those given to *O. niloticus* by **Olfat**

Mahdy (1991); who found that the highest prevalence was in musculature. **Tagreed Ibrahim (2000)** and **Maather El Lamie (2001)** who found that the highest prevalence was in gills.

Concerning the prevalence in relation to sex, only females were highly infected than males in wild *O. niloticus*, while both sexes were equally prevailed in both cultured *O. niloticus* and *C. gariepinus*. This may be attributed to the spawning period of wild *O. niloticus* where they are slow, weak and easily exposed to cercarial attack. On the other side, these results disagreed with **Eissa and Hala (1993)** who found that the males were higher than females in case of yellow grub disease in tilapias.

Table (1) : Total and seasonal prevalence of EMC in the examined fishes.

| Seasons | <i>C. gariepinus</i> | | | Wild <i>O. niloticus</i> | | | Cultured <i>O. niloticus</i> | | |
|---------|----------------------|----|-------|--------------------------|----|-------|------------------------------|----|-------|
| | a | b | % | a | b | % | a | b | % |
| Spring | 25 | 16 | 64.00 | 25 | 18 | 72.00 | 25 | 1 | 4.00 |
| Summer | 25 | 11 | 44.00 | 25 | 14 | 56.00 | 25 | 8 | 32.00 |
| Autumn | 25 | 12 | 48.00 | 25 | 9 | 36.00 | 25 | 1 | 4.00 |
| Winter | 25 | 11 | 44.00 | 25 | 6 | 24.00 | - | - | - |
| Total | 100 | 50 | 50.00 | 100 | 47 | 47.00 | 75 | 10 | 13.30 |

a: No. of examined fish

b: No. of infested fish

Table (2): Comparative prevalence of E.M.C. among different infested organs in infested fishes.

| Organs | <i>C. gariepinus</i> No. & % | | Wild <i>O. niloticus</i> No. & % | | Cultured <i>O. niloticus</i> No. & % | |
|-------------|---------------------------------|--------|--|---------|--|--------|
| Musculature | 44 | (88.0) | 17 | (35.42) | 1 | (10.0) |
| Liver | 18 | (36.0) | 22 | (45.83) | 3 | (30.0) |
| Kidneys | 9 | (15.0) | 28 | (58.33) | 3 | (30.0) |
| Heart | 13 | (26.0) | 13 | (27.80) | - | - |
| Gills | 5 | (10.0) | 20 | (41.0) | - | - |
| Spleen | 5 | (10.0) | 25 | (52.0) | 6 | (60.0) |
| Testis | 3 | (06.0) | - | - | - | - |
| Ovary | 1 | (02.0) | 2 | (04.10) | - | - |
| Brain | - | - | 15 | (31.0) | - | - |

Table (3): Prevalence of EMC in the examined fish in relation to sex.

| | <i>Clarias gariepinus</i> | | | | | | Wild <i>O. niloticus</i> | | | | | | Cultured <i>O. niloticus</i> | | | | | |
|--------|---------------------------|----|-------|----|----|-------|--------------------------|----|-------|----|----|-------|------------------------------|---|-------|----|---|-------|
| | ♂ | | | ♀ | | | ♂ | | | ♀ | | | ♂ | | | ♀ | | |
| | a | b | % | a | b | % | a | b | % | a | b | % | a | b | % | a | b | % |
| Spring | 20 | 12 | 60.00 | 5 | 4 | 80.00 | 16 | 10 | 62.50 | 9 | 8 | 88.77 | 11 | 1 | 09.10 | 14 | - | - |
| Summer | 16 | 7 | 43.75 | 9 | 4 | 44.44 | 16 | 7 | 43.75 | 9 | 7 | 77.77 | 20 | 5 | 25.00 | 5 | 3 | 40.00 |
| Autumn | 18 | 9 | 50.00 | 7 | 3 | 42.85 | 20 | 6 | 30.00 | 5 | 3 | 60.00 | 14 | - | - | 11 | 1 | 09.10 |
| Winter | 23 | 10 | 41.66 | 2 | 1 | 50.00 | 12 | 4 | 33.33 | 13 | 2 | 15.38 | - | - | - | - | - | - |
| Total | 77 | 38 | 49.35 | 23 | 12 | 52.17 | 64 | 27 | 42.18 | 36 | 20 | 55.55 | 45 | 6 | 13.00 | 30 | 4 | 13.33 |



Figure (1): Showing *Clarias gariepinus* infested by large EMC in all musculature regions.



Figure (2): Showing Ovary of *Oreochromis niloticus* infested by *Heterophyed metacercariae*.

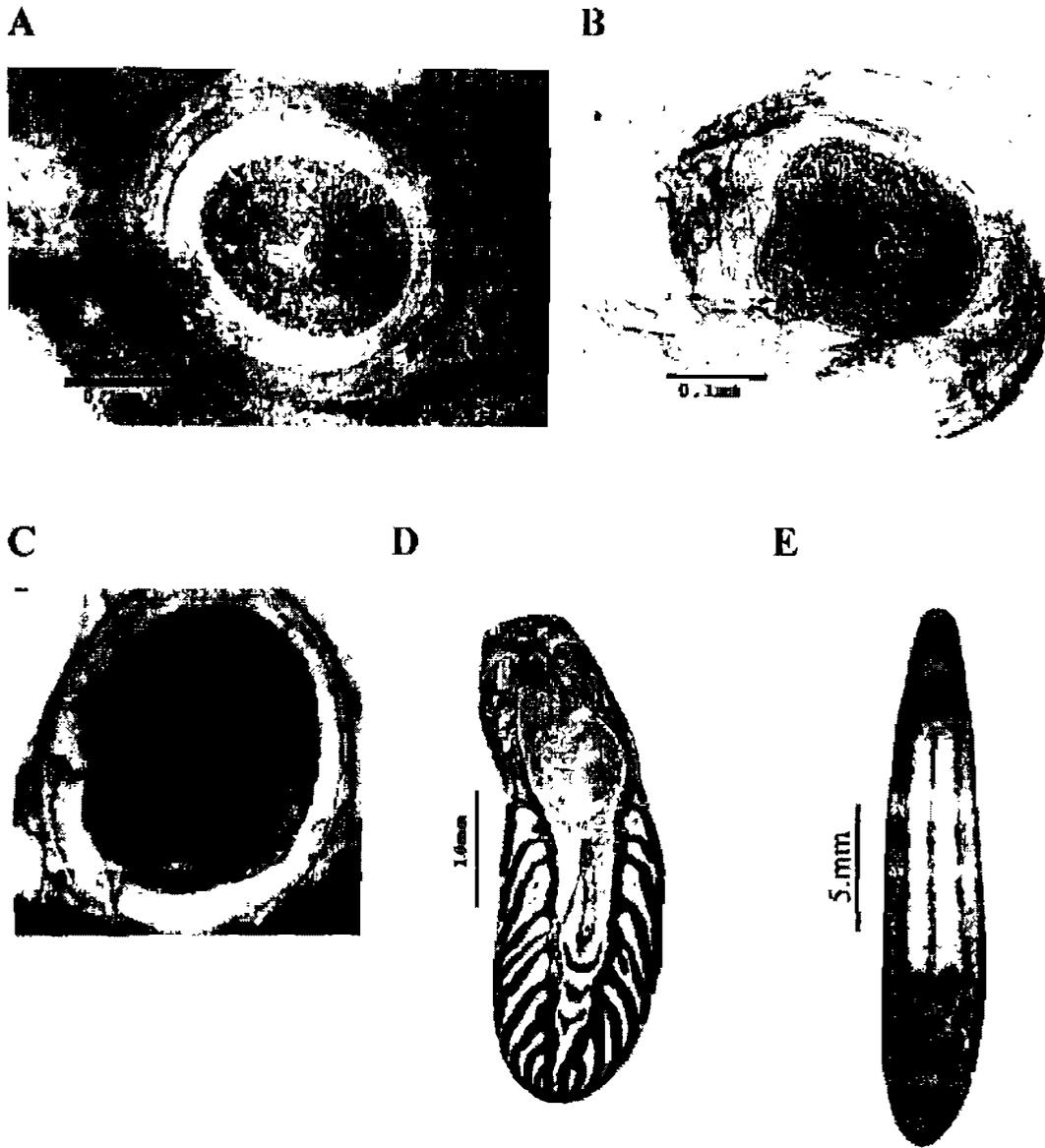


Plate (1) Showing metacercaria which isolated from *C. garlepinus* and *O. niloticus*
A. *Prohemistomatid*, B. *Heterophid* C. *Cynodiplostomatid* D. *Euclinostomum*
E. *Clinostomatide metacercaria*

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